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NUCLEAR AND ORGANELLE GENES BASED PHYLOGENY OF *DRYOMYS* (GLIRIDAE, RODENTIA, MAMMALIA) FROM TURKEY

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Molecular phylogeny, phylogeography and genetic structure of the genus *Dryomys* Thomas, 1906 from Turkey were identified by using partial sequences of beta-fibrinogen intron 7 and mitochondrially encoded 12S ribosomal RNA genes and also combined data of two genes. Within *Dryomys nitedula* species, both, nuclear and mitochondrial genes coherently separated the Thrace lineage from the other lineages in Anatolia. Contrary to this, complex and incomprehensible phylogenies were recovered for Anatolian populations of this species. The analysis of the combined data of these two genes resolved mentioned complexity and incongruity and made phylogeny compatible with the results of past studies for the relative position of the Anatolian lineages. Thus, the presence of four different lineages (one in Thrace and three in Anatolia) within *D. nitedula* in the localities exemplified across Turkey was confirmed. Genetic differentiation (K2P distances) between the lineages were moderate at the level of intraspecific diversity. In addition to this, genetic distance (K2P = 5.5%) determined between *D. nitedula* and *D. laniger* conformed the distance suggested for the separate species of mammals. Evolutionary divergence time estimations demonstrated that the probable divergence between *D. laniger* and *D. nitedula* and among its detected lineages started in the border of Late Miocene and Pliocene (5.3 Mya) and lasted to the beginning of the Calabrian Stage of Pleistocene (1.8 Mya) in line with the previous results obtained from fossil and molecular data.

Key words: *Dryomys*, phylogeny, beta-fibrinogen intron 7, 12S ribosomal RNA, Turkey

INTRODUCTION

Turkey is placed at the junction of Europe, Asia, the Arabian Peninsula, and the Caucasus. Biogeographically, its location enables species movements and faunal exchange between the geographies above. Besides, the variable topographic and climatic characteristics as well as being inclusive of floristic

elements from Irano-Turanian, Euro-Siberian and Mediterranean phytogeographical regions, has resulted in the formation of rich biological diversity in Turkey (Akman 1982, Bilgin 2011). Moreover, several geographic barriers such as Western Anatolia Mts, Black Sea Mts, Taurus Mts, Eastern Anatolia Mts, known as "The Anatolian Diagonal" as well as the "Turkish Straits System" cause allopatry and thus both species diversity and intraspecific genetic diversity increase (Bilgin 2011, Korkmaz *et al*. 2014, Şeker *et al.* 2018). Both species diversity and intraspecific diversities observed in mammalians in Turkey properly represent two major levels of present biodiversity concept addressed at three levels, with the approximately 170 species and numerous subspecies (Primack 1993, Wilson & Reeder 2005, Yiğit *et al.* 2006). New mammal species have also been discovered recently, in studies focusing on, especially, intraspecific variations, and using different methodologies, such as morphology, geometric morphometry, and genetics (Gündüz *et al.* 2007, Yiğit *et al.* 2016).

One of the mammalian genus, *Dryomys* Thomas, 1906, is presented by two rodent species *D. nitedula* (Pallas, 1778) and *D. laniger* Felten et Storch, 1968, in Turkey. *Dryomys laniger* only lives in the Taurus Mountains and is an endemic species to Turkey. Intraspecific variations in this species have not been yet studied. On the contrary, it has been documented that *D. nitedula* involves numerous subspecies in Palaearctic, three main genetic groups in Russia and the Caucasus and also four main lineages in Turkey identified by the traditional methods (morphology, karyology, and allozyme) and also by the mitochondrial DNA (Wilson & Reeder 2005, Grigoryeva *et al.* 2015, Kankiliç *et al.* 2018, Bisconti *et al.* 2018). These data make it clear that highly complex intraspecific variability exists within this species. To shed light on, especially, complex intraspecific variations in *D. nitedula*, phylogeny and phylogeography of the genus *Dryomys* in Turkey were contributed by current work using partial sequences of one mitochondrial (12S ribosomal RNA, 12S rRNA), one nuclear gene (beta-fibrinogen intron 7, Fib7) and by the combined data of both gene sequences. The mitochondrial DNA is inherited as a single linkage group and supplies just one independent estimate of the species tree. Therefore, obtained phylogenies based on solely mtDNA can create a mismatch between the gene tree and species tree because of lineage sorting. The way to exceed this mismatch is to use several genes from different linkage groups like nuclear DNA, thus obtained gene trees ensure independent estimates of the species tree. For the same purpose, gene trees constructed by nuclear-encoded introns have been extensively used in conjunction with the mitochondrial DNA trees to make stronger inferences about species trees. In this context, the beta-fibrinogen intron 7 gene has been regarded as a potentially valuable marker for resolving phylogenies of relatively recently evolved groups. Intron sequences in nuclear genome neutrally evolve and include highly variable nucleotide sequence compared to exons (Prychitko & Moore 1997).

MATERIAL AND METHODS

Sequence analyses of mitochondrial 12S ribosomal RNA (12S rRNA) and beta-fibrinogen intron 7 nuclear gene (Fib7) of *D. nitedula* and *D. laniger* samples from Turkey were examined. A detailed description of studied samples and additional samples employing as outgroups from GenBank was in Figure 1 and Table 1. The CTAB isolation protocol was

Table 1. Sampling sites of the specimens in the study. In collection numbers column, (*) indicates the samples involved in only Fib7 data set, while, (**) points out the outgroup sequences employed in the only 12S rRNA data set. The combined data set does not include two samples from Fib7 data set with the collection number of 5160 and 5612.

Map ID	Taxon	Localities	Collection numbers	N
1	*D. nitedula*	Gelibolu-Çanakkale	7129, 7201, 7164, 7032, 7137	5
2	*D. nitedula*	Edirne	5156, 5157, 5158, 5159, 5160*, 5161, 5162, 5163, 5543, 5609, 5612*	11
3	*D. nitedula*	Orhaniye-Edirne	7163, 7186, 7162*, 7209	4
4	*D. nitedula*	Kumbağ-Tekirdağ	7207	1
5	*D. nitedula*	Elmalı-Antalya	5749, 5756	2
6	*D. nitedula*	Eber-Afyon	5589	1
7	*D. nitedula*	Abant-Bolu	7130	1
8	*D. nitedula*	Yapraklı-Çankırı	508	1
9	*D. nitedula*	Madenköy-Niğde	2322, 2323*	2
10	*D. nitedula*	Hacıbeyköyü-Niğde	353,374	2
11	*D. nitedula*	Pınarbaşı-Kayseri	2929	1
12	*D. nitedula*	Çakallı-Samsun	5357	1
13	*D. nitedula*	Bulancak-Giresun	7208	1
14	*D. nitedula*	Çat-Rize	5358*, 5360*, 5361	3
15	*D. nitedula*	Çamlıhemşin-Rize	5392, 5400, 5404, 5427	4
16	*D. nitedula*	Cankurtaran-Artvin	5713	1
17	*D. nitedula*	Şavşat-Artvin	5712, 5714	2
18	*D. laniger*	Aladağ-Adana	507*	1
9	*D. laniger*	Madenköy-Niğde	1063, 1065, 1066	3
	D. nitedula	–	AJ225119**, KX893545**, KX893546**, KX893547**, KX893548**, KX893549**, KX893550**	7
–	*G. glis*	–	522*	1
–	*M. musculus*	–	HQ675031**, HQ675030**, EF605471*	3
–	*R. norvegicus*	–	FJ919760**, FJ919771**	2

used to obtain total genomic DNA from diverse tissues (Doyle & Doyle 1990). The primer pairs of BFIBR1 and BFBR2 for amplification of Fib7 gene (Seddon *et al.* 2001) and L1091 and H1478 for 12S rRNA gene (Kocher *et al.* 1989) was used in the PCR reaction. PCR reactions were executed in Applied Biosystems® Veriti® 96-Well Thermal Cycler according to the Kocher *et al.* (1989) for 12S rRNA and Seddon *et al.* (2001) for Fib7 genes. After amplification, PCR products were run in 70 V for 90 minutes in 1XTAE on 0.8% agarose gel. Thermo Scientific GeneRuler, 100 bp DNA Ladder marker system, was used to detect the estimated size of each DNA samples on agarose. The agarose gels were stained in ethidium bromide (EtBr) solution for 30 minutes, and later KODAK Gel Logic 100 System was employed for visualization of all gels. The DNA sequencing was performed by Macrogen Europe (Macrogen Inc., Amsterdam, Netherlands).

The BioEdit 7.0.9 (Hall 1999) was used to achieve alignment processes and consensus sequences of both genes. The 12S rRNA, Fib7 and combined data sets included approximately 390 or 391, 614 and 1004 or 1005 bp of sequences, respectively. Table 1 also contains detailed explanations of the samples in the 12S rRNA, Fib7 and combined data sets used in the phylogenetic and network analyses. The maximum likelihood (ML) phylogenetic tree were constructed for 12S rRNA and Fib7 sequences, implemented in MEGA 7 (Kumar *et al.* 2016). The Bayesian Inference accomplished by BEAST was used to estimate phylogenetic relationships based on combined data set (Drummond *et al.* 2012). Divergence time dating was estimated by BEAST v1.8.0 (Drummond *et al.* 2012). The best fit nucleotide substitution models, HKY, K2 and TN93 + G, (Kimura 1980, Hasegawa *et al.* 1985, Tamura & Nei 1993) were respectively selected for 12S rRNA, Fib7 and for the combined data sets accord-

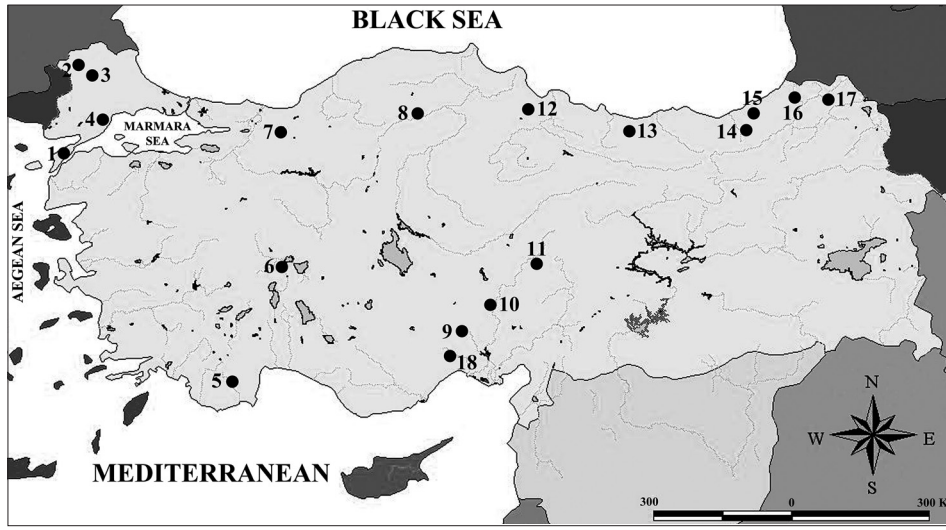

Fig. 1. The map shows collecting sites of the *D. nitedula* and *D. laniger* samples in Turkey. Map numbers and localities; 1. Gelibolu-Çanakkale, 2. Edirne, 3. Orhaniye-Edirne, 4. Kumbağ-Tekirdağ, 5. Elmalı-Antalya, 6. Eber-Afyon, 7. Abant-Bolu, 8. Yapraklı-Çankırı, 9. Madenköy-Niğde, 10. Hacıbeyköyü-Niğde, 11. Pınarbaşı-Kayseri, 12. Çakallı-Samsun, 13. Bulancak-Giresun, 14. Çat-Rize, 15. Çamlıhemşin-Rize, 16. Cankurtaran-Artvin, 17. Şavşat-Artvin, 18. Aladağ-Adana. Numbers in the map correspond to the localities specified in Table 1

ing to results obtained by the employment of the Bayesian information criterion (BIC) and corrected Akaike information criterion (AICC) in MEGA 7 (Kumar *et al*. 2016). Reliability of constructing tree topologies was tested by nonparametric bootstrap (1000 replicates) and by Bayesian posterior probability (BPP). To surpass the threshold of effective sample size (ESS >200), one independent MCMC (Markov Chain Monte Carlo) for 10,000,000 generations with a sampling frequency of every 1000 generations in the Bayesian analysis were accomplished in BEAST v1.8.0 (Drummond *et al.* 2012). Tracer v1.6 (part of BEAST package) was employed to control whether the ESS values were greater than 200 in the independent run. TreAnnotator v1.8.0 (part of BEAST package) was used to summarize the tree file containing 10,000 trees by removing the initial 10% (1000 trees) of the sampled trees as burn-in. To compute the BPPS and divergence times of the tree nodes, the 50% majority rule consensus tree was constructed. The *Mus*/*Rattus* split showing a normal distribution (mean: 11.7 Mya and standard deviation: ±0.4 Mya) was employed as a calibration point in the divergence time analysis (Benton & Donoghue 2007, Voloch & Schrago 2012). The Bayesian tree was visualized and edited by FigTree v1.8.0 (Rambaut 2008). To display among evolutionary relations of clades, a median-joining network was build using Network 5.0.0.3 (Bendelt *et al.* 1999).

For inference of genetic diversity, number of segregating sites (*S*), number of haplotypes (*h*), haplotype (*Hd*) and nucleotide diversity (π) statistics were calculated. Additionally, to determine populations size changes, the neutrality tests for each gene using Tajima's *D* (Tajima 1989) and Fu's *F* statistics (Fu 1997) were performed by DNAsp v6 (Rozas *et al.* 2017). Tajima's *D* statistic is negative when there are high levels of new mutations or recent population expansion. In the case of a small amount of new mutations, a balanced selection or a decrease in the population size, this statistic is positive. Fu's *F* statistics is a robust test used to detect population expansion under hypothesis of neutrality. This statistics takes high negative value when there are large numbers of newly formed haplotypes in the population or recent population expansion. Genetic distance estimations of combined data under Kimura-2 parameter (K2P) model with 1000 bootstrap replicates and basic descriptive statistics of the nucleotide sequences belonging to the two genes were calculated by MEGA 7 (Kumar *et al.* 2016).

RESULTS

In total, for *Dryomys nitedula* and *D. laniger* in order, 11 and two haplotypes in the 12S rRNA and 18 and one haplotypes in the Fib7 gene region were identified (see Table 2 for details). The basic descriptive statistics of the nucleotide sequences belonging to the 12S rRNA and Fib7 genes were separately calculated (Table 3). Total haplotype diversity of two genes was relatively higher in all lineages of *D. nitedula* but moderate in *D. laniger* only for 12S rRNA. In *D. nitedula*, total nucleotide diversity of 12S rRNA in all studied animals was higher than that of the Fib7 gene having a moderate level. As for *D. laniger*, total nucleotide diversity that was able to calculate only for 12S rRNA was at a low level (Table 4) probably because of the small sample size.

Each of the two gene regions and combined data had its own topology and produced three or four lineages within *Dryomys nitedula*. The common

point of these topologies was that within *D. nitedula*, the Thrace lineage (L1) was precisely separated from the other lineages in Anatolia (L2, L3 and L4). The reason for the complexity in the topologies was the exhibition of different geographic distribution patterns of the determined lineages in Anatolia. According to this; (1) the L2 contained only Central Anatolian populations in the

Table 2. 12S rRNA and Fib7 haplotype list of *Dryomys* species from Turkey including outgroup samples from GenBank (H.n. = haplotype number, F. = frequency).

H.n.	F.	Localities and collection numbers	GenBank no.
		12S rRNA	
1	10	Edirne (5156, 5157, 5158, 5159, 5162, 5163, 5609), Orhaniye-Edirne (7163, 7186, 7209)	MN128038
2	1	Edirne (5161)	MN128039
3	5	Edirne (5543), Kumbağ-Tekirdağ (7207), Gelibolu-Çanakkale (7164, 7201, 7137)	MN128040
4	1	Gelibolu-Çanakkale (7129)	MN128041
5	1	Gelibolu-Çanakkale (7032)	MN128042
6	8	Abant-Bolu (7130), Hacıbeyköyü-Niğde (353), Pınarbaşı-Kayseri (2929), Elmalı-Antalya (5749, 5756), Eber-Afyon (5589), Yapraklı-Çankırı (508), Çakallı-Samsun (5357)	MN128043
7	1	Madenköy-Niğde (2322)	MN128044
8	1	Hacıbeyköyü-Niğde (374)	MN128045
9	6	Çat-Rize (5361), Çamlıhemşin-Rize (5392, 5400, 5404, 5427), Cankurtaran-Artvin (5713)	MN128046
10	1	Şavşat-Artvin (5712)	MN128047
11	2	Şavşat-Artvin (5714), Bulancak-Giresun (7208)	MN128048
12	1	*D. laniger*: Madenköy-Niğde (1063)	MN128049
13	2	*D. laniger*: Madenköy-Niğde (1065, 1066)	MN128050
14	3	*D. nitedula*	KX893550, KX893549, KX893548
15	2	*D. nitedula*	KX893547, KX893546
16	1	*D. nitedula*	KX893545
17	1	*D. nitedula*	AJ225119
18	2	*M. musculus*	HQ675031, HQ675030
19	2	*R. norvegicus*	FJ919760, FJ919771

Table 2 (continued)

H.n.	F.	Localities and Collection Numbers	GenBank no.
		Fib7	
1	12	Edirne (5156, 5157, 5158, 5159, 5162, 5163, 5609, 5160, 5612), Orhaniye-Edirne (7163, 7186), Gelibolu-Çanakkale (7032, 7137)	MN128051
2	6	Edirne (5159), Orhaniye-Edirne (7163), Gelibolu-Çanakkale (7201, 7129, 7164), Kumbağ-Tekirdağ (7207)	MN128052
3	1	Edirne (5543)	MN128053
4	1	Edirne (5161)	MN128054
5	2	Abant-Bolu (7130), Çamlıhemşin-Rize (5404)	MN128055
6	2	Eber-Afyon (5589), Bulancak-Giresun (7208)	MN128056
7	1	Yapraklı-Çankırı (508)	MN128057
8	2	Elmalı-Antalya (5749, 5756)	MN128058
9	5	Hacıbeyköyü-Niğde (353, 374), Madenköy-Niğde (2322, 2323), Pınarbaşı-Kayseri (2929)	MN128059
10	2	Çakallı-Samsun (5357), Çamlıhemşin-Rize (5427)	MN128060
11	1	Çat-Rize (5358)	MN128061
12	1	Çat-Rize (5360)	MN128062
13	1	Çat-Rize (5361)	MN128063
14	1	Çamlıhemşin-Rize (5392)	MN128064
15	1	Çamlıhemşin-Rize (5400)	MN128065
16	1	Şavşat-Artvin (5712)	MN128066
17	1	Şavşat-Artvin (5714)	MN128067
18	1	Cankurtaran-Artvin (5713)	MN128068
19	4	*D. laniger*: Aladağlar-Adana (507), Madenköy-Niğde (1063, 1065, 1066)	MN128069
20	1	*G. glis*: Çanakkale (522)	MN128070
21	1	*M. musculus*	EF605471

trees constructed by 12S rRNA and by the combined analysis of the 12s rRNA and Fib7 sequences. However included were some Northeastern Anatolian populations in the Fib7 tree in addition to the Central Anatolian populations; (2) the L3 consisted of only Northeastern Anatolian populations in 12S rRNA tree, but some of the populations constituting this lineage joined in either the Central Anatolia lineage (Fib7 tree) or left the Şavşat population (BI tree). In general, intraspecific nodes in all tree topologies were well supported by at least 70% bootstrap value in ML and at least 0.83 posterior probability in BI. There were no common haplotypes between the lineages of *D. nitedula* and

Table 3. Summary and descriptive statistics of the nucleotide sequences related to the two genes used to study the genus *Dryomys*. Abbreviations: n = sample size, NS = length of nucleotide sequences, I = invariable sites, V = variable sites, P = parsimony informative sites.

Species	Gene									
D. nitedula	12S rRNA					Fib7				
Groups	n	NS	I	V	P	n	NS	I	V	P
All	37	390	370	20	16	42	614	599	15	13
L1	18	390	384	6	2	20	614	611	3	1
L2	10	391	389	2	1	19	614	605	9	8
L3	9	391	382	9	8	3	614	609	5	0
D. laniger	3	389	388	1	0	4	612	612	0	0

Table 4. Genetic diversity statistics and neutrality tests of the genus *Dryomys* from Turkey Abbreviations: n = sample size, *S* = number of segregating sites, *h* = number of haplotypes, *Hd* = haplotype diversity with standard deviations, π = nucleotide diversity with standard deviations, *D* = Tajima's *D* statistics, F = Fu's *F* statistics, *P > 0.10.

Groups	n	*S*	*h*	*Hd*	π	*D*	*F*
				12S rRNA All			
D. nitedula lineages	37	19	11	0.851±0.031	0.01396±0.00093	0.45729*	0.42363*
L1	18	6	5	0.641±0.097	0.00297±0.00104	–1.10898*	–1.57573*
L2	10	2	3	0.378±0.181	0.00142±0.00073	–0.69098*	–0.42293*
L3	9	8	3	0.556±0.165	0.00853±0.00332	0.03044*	0.91144*
D. laniger	3	1	2	0.667±0.314	0.00171±0.00081	–	–
				Fib7 All			
D. nitedula lineages	42	15	18	0.890±0.034	0.00550±0.00068	–0.09837	0.50462
L1	20	3	4	0.574±0.090	0.00111±0.00024	–0.52640*	–1.21388*
L2	19	9	11	0.918±0.045	0.00453±0.00069	0.28262*	0.81543*
L3	3	5	3	1±0.272	0.00543±0.00211	–	–
D. laniger	4	0	1	0	0	–	–

the network analysis indicated a relatively compatible pattern with the phylogenetic trees made for both genes (Figs 2–4).

When the dating analysis was taken into account, a node with the age of 8.05 Mya (95% HPD: 4.63–12.22 Mya), corresponding the Late-Miocene origin, was inferred between *D. nitedula* and *D. laniger*. The diversification of the lineages within *D. nitedula* occurred at the same time as a period ranging from

Table 5. Divergence time estimations and genetic distances among main *Dryomys* clades using the combined data based on Bayesian Inference in millions of years before present (Mya). Nodes are designated as in Fig. 4.

Node	Explanation	Age of node (Mya)	%95 HPD (Mya)	BPP	K2P (%)
A*	*Mus/Rattus*	11.66	10.86-12.43	1	-
B	*D. laniger-D. nitedula*	8.05	4.63-12.22	1	0.55
C	L1-(L2+L3+L4)	2.79	1.6-4.45	1	1.3
D	L3-(L2+L4)	2.16	1.19-3.44	0.83	1.4
E	L2-L4	1.33	0.7-2.16	1	0.6

the border of Late Miocene and Pliocene to the Calabrian stage of Pleistocene (Fig. 4 and Table 5).

For the combined data, K2P distances revealed a moderate genetic differentiation among the four main lineages, as indicative of intraspecific variation (Table 5). The results of the neutrality tests (Tajima's *D* and Fu's *F* statistics) for both genes indicated that *D. nitedula* populations were steady in time.

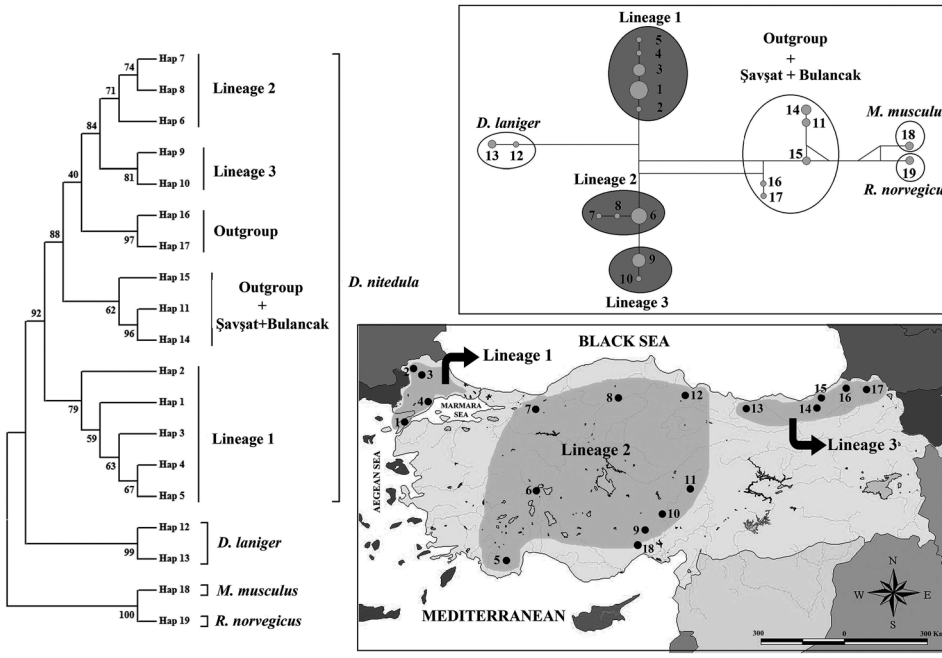

Fig. 2. Haplotype network and ML phylogenetic tree arising from the analysis of 12S rRNA data set. Size of the circles in the haplotype network reflects the haplotype frequencies explained in detail in Table 2. In ML tree, the bootstrap statistics were identified on major nodes. The map shows possible geographic distribution of major *D. nitedula* clades in Turkey determined by 12S rRNA data set

Although Tajima's *D* statistic was negative and significant for the Fib7 gene region of all *D. nitedula* populations, this statistic both had a small value and Fu's *F* statistics were positive indicating stability of population's size (Table 4).

DISCUSSION

Past studies on *D. nitedula* taxonomy were chiefly based on morphological differentiation, which contributed to description of numerous subspecies and thus led to the complexity in its taxonomy (Yiğit *et al.* 2003, 2011, Wilson & Reeder 2005). To reduce this complexity and to reach more precise taxonomic judgments for this species, initially mtDNA sequence variations have been started to use, and then nuclear genes have been used in conjunction with the mt DNA analysis (Grigoryeva *et al.* 2015, Kankiliç *et al.* 2018, Bisconti *et al.* 2018). The present study is the first, which comprise both mitochondrial and nuclear DNA sequences in the genus *Dryomys* in Turkey. The results obtained in the current study and the past molecular studies have shown that there are

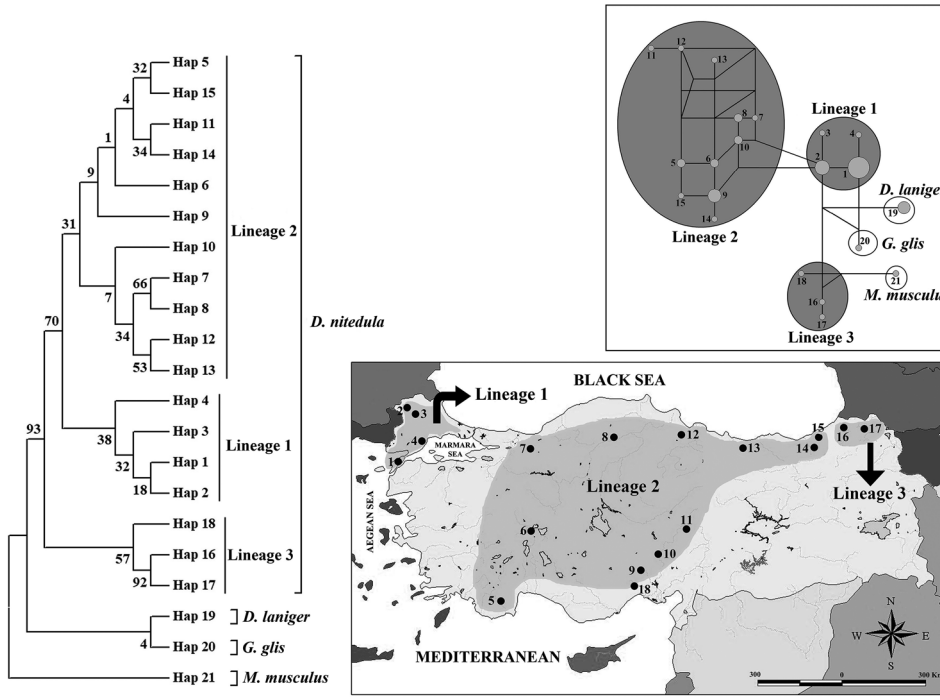

Fig. 3. Haplotype network and ML phylogenetic tree arising from the analysis of Fib7 data set. The circles in the network are proportional to the haplotype frequencies detailed explained in Table 2. Bootstrap statistics were indicated for major nodes in the ML tree. The map on the right side in the figure shows possible geographic distribution of major *D. nitedula* clades in Turkey determined by Fib7 data set

significant intraspecific genetic differentiations in *D. nitedula* compatible with the genetic species concept (Bradley & Baker 2001).

In a general frame of phylogenetic positions of the lineages within *D. nitedula*, the reconstructed phylogenies collectively demonstrated that Thrace lineage is a separate genetic group differing from the other lineages in Anatolia. This conclusion contrasts with the allozyme data (Yiğit *et al.* 2011). Contradictory phylogenies of Anatolian populations separately shown by one mitochondrial and one nuclear gene may be attributed to the limited variations in the partial sequence of 12S rRNA, to the slow evolution rate of Fib7 and also relatively little sampling site and sample size. The combined data analysis eliminated this contradictory situation and supported the existence of three genetic lineages in Anatolia, which was congruent with the results of a previous study (Kankiliç *et al.* 2018).

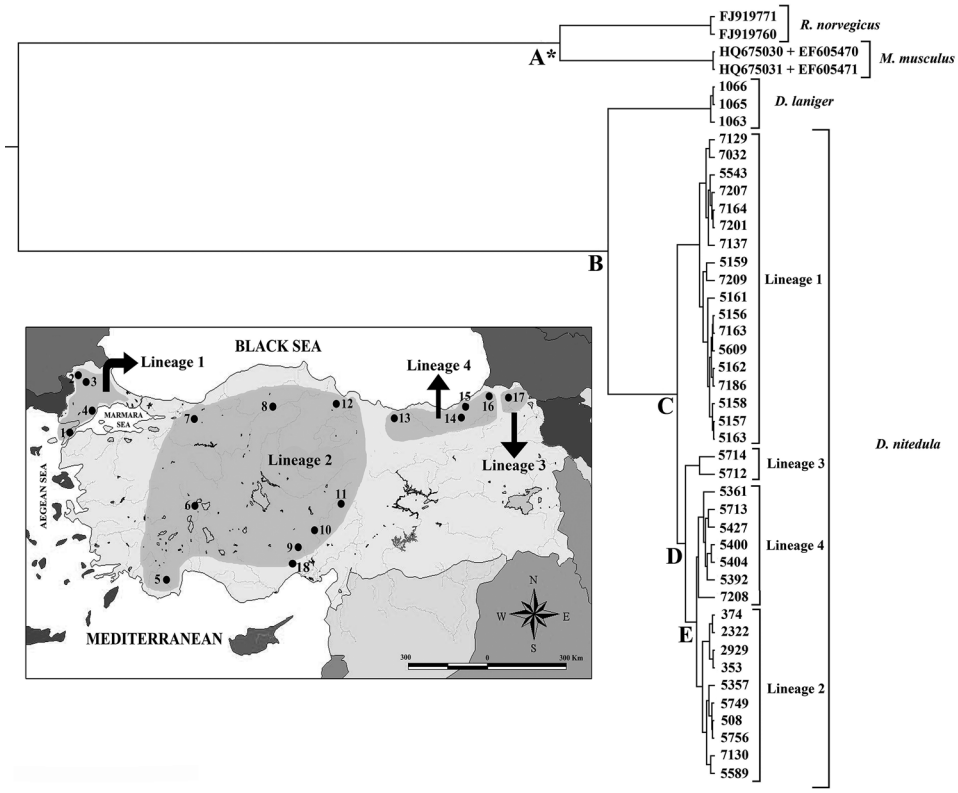

Fig. 4. Time-calibrated phylogenetic tree obtained from the Bayesian Inference of combined data set. Marks on nodes (from A to E) correspond to the nodes explaining evolutionary divergence times of the clades listed in Table 5. Shaded areas in the map included in the figure display possible distributions of major *D. nitedula* clades in Turkey reconstructed by combined data set

The level of genetic divergence (K2P distance; ranging from 0.6 to 1.3) calculated for the combined data between all lineages was at a moderate level compared to the previous results of ND*1* gene sequence variation (Kankiliç *et al.* 2018). Nevertheless, indicated intraspecific diversification (K2P < 2%) within *D. nitedula* is in accordance with the genetic species concept (Bradley & Baker 2001). Confidence intervals of divergence time estimations indicated the time period corresponding to the border of Late Miocene and Pliocene to the Calabrian stage of Pleistocene. Mentioned time period was coherent with the occurrence time of some geographic barriers such as Turkish strait system, North Anatolia Mountains in Anatolia and Lesser Caucasus in the northeastern of Turkey (Şengör & Yilmaz 1981, Kerey *et al.* 2004, Popov *et al.* 2006). Impacts of those geographic barriers on the genetic differentiation of Turkish forest dormouse populations which were previously documented by Kankiliç *et al.* (2018) could be considered as the main reason for the lineage differentiation determined in this study.

The other representative species of the genus, *D. laniger* endemic to Turkey, was positioned as a basal group in all phylogenetic trees. The genetic distance of this species with *D. nitedula* (K2P: 5.5%) was high and indicated the presence of the separate biological species (Bradley & Baker 2001, Baker & Bradley 2006). Divergence time estimation between these two species referred to the end of the Miocene, which was the acceptable divergence time for *D. laniger* and *D. nitedula,* according to the data obtained from both molecular markers and fossils (Franzen & Storch 1975, Daams 1981, Montgelard *et al.* 2003, Kankiliç *et al.* 2018).

This study is employing mitochondrial and nuclear genes, in order to provide a new perspective to the molecular phylogenetics and phylogeography of *Dryomys* in Turkey. Fib7 and 12s rRNA genes led to relatively contradictory phylogenetic patterns, because the Fib7 dataset was phylogenetically much less informative and partial sequence of 12s rRNA was used. In contrast to this, the combined data set rather suggested a congruent model with the past results concerning the phylogeny of *D. nitedula* from Turkey. Because of the lack of extensive geographic sampling, the intraspecific variations of the endemic species, *D. laniger*, could not be appropriately determined. Turkey is an important location for the biodiversity of Europe (Hewitt 1996). Even if not directly, this study also aims to emphasize the biogeographic importance of Anatolia in global phylogeographical assessments. However, for such a global phylogeographic assessment specific to *Dryomys* and, to better understand the role of Anatolia in Europe's biodiversity, it is required to use other molecular markers such as widely used cytochrome *b* gene of mitochondrial DNA (Grigoryeva *et al.* 2015, Bisconti *et al.* 2018).

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REFERENCES

Akman, Y. (1982) : Climats et Bioclimats Méditerranéens en Turquie. – *Ecologia Mediterranea* **8**: 73–87.

Baker, R. J. & Bradley, R. D. (2006): Speciation in mammals and the genetic species concept. – *Journal of Mammalogy* **87**: 643–662. https://doi.org/10.1644/06-mamm-F-038R2.1

Bendelt, H., Froster, P. & Rohl, A. (1999): Median joining network for inferring intraspecific phylogenies. – *Molecular Biology and Evolution* **16**: 37–48. https://doi.org/10.1093/oxfordJournals.molbev.a026036

Benton, M. J. & Donoghue, P. C. (2007): Paleontological evidence to date the tree of life. – *Molecular Biology and Evolution* **24**: 26–53. https://doi.org/10.1093/molbev/msl150

Bilgin, R. (2011): Back to the suture: the distribution of intraspecific genetic diversity in and around Anatolia. – *International Journal of Molecular Sciences* **12**: 4080–4103. https://doi.org/10.3390/ijms12064080

Bisconti, R., Aloise, G., Siclari, A., Fava, V., Provenzano, M., Arduino, P., Chiocchio, A., Nascetti, G. & Canestrelli, D. (2018): Forest Dormouse (Dryomys nitedula) populations in southern Italy belong to a deeply divergent evolutionary lineage: implications for taxonomy and conservation. – *Hystrix, Italian Journal of Mammalogy* **29**(1): 75 –79. https://doi.org/10.4404/hystrix-00023-2017

Bradley, R. D. & Baker, R. J. (2001): A test of the genetic species concept: cytochrome-*b* sequences and mammals. – *Journal of Mammalogy* **82:** 960–973. https://doi.org/10.1644/1545-1542(2001)082<0960:ATOTGS>2.0.CO;2

Daams, R. (1981): The dental pattern of the dormice Dryomys, Myomimus, Microdyromys and Peridyromys. – *Utrecht Micropaleontological Bulletins (Special Publication)* **3**: 1–115.

Doyle, J. J. & Doyle, J. L. (1990): Isolation of plant DNA from fresh tissue. – *Focus* **12**: 13–15.

Drummond, A. J., Suchard, M. A., Xie, D. & Rambaut, A. (2012): Bayesian phylogenetics with BEAUTI and the BEAST 1.7. – *Molecular Biology and Evolution* **29**: 1969–1973. https://doi.org/10.1093/molbev/mss075

Franzen, J. F. & Storch, G. (1975): Die unterpliozane (turolische) Wirbeltierfauna von Darn-Durkheim, Rheinhessen (SW-Deutschland). 1. Entdeckung, Geologie, Mammalia: Carnivora, Proboscidea, Rodentia. Grabungsergebnisse 1972–1973. – *Senckenbergiana Lethaea* **56:** 233–303.

Fu, Y. X. (1997): Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. – *Genetics* **147**: 915–925.

Gündüz, İ., Jaarola, M., Tez, C., Yeniyurt, C., Polly, P. D. & Searle, J. B. (2007): Multigenic and morphometric differentiation of ground squirrels (Spermophilus, Sciuridae, Rodentia) in Turkey, with a description of a new species. – *Molecular Phylogenetics and Evolution* **43:** 916–935. https://doi.org/10.1016/j.ympev.2007.02.021

Grigoryeva, O., Krivonogov, D., Balakirev, A., Stakheev, V., Andreychev, A. & Orlov, V. (2015): Phylogeography of the forest dormouse Dryomys nitedula (Gliridae, Rodentia) in Russian Plain and the Caucasus. – *Folia Zoologica* **64**: 361–364. https://doi.org/10.25225/fozo.v64.i4.a12.2015

Hall, T. A. (1999): A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. – *Nucleic Acids Symposium Series* **41**: 95–98.

Hasegawa, M., Kishino, H. & Yano T. (1985): Dating of human-ape splitting by a molecular clock of mitochondrial DNA. – *Journal of Molecular Evolution* **22 (2)**: 160–174. https://doi.org/10.1007/bf02101694

Hewitt, G. M. (1996): Some genetic consequences of ice ages, and their role in divergence and speciation. – *Biological Journal of the Linnean Society* **58**: 247–276. https://doi.org/10.1111/j.1095-8312.1996.tb01434.x

Kankiliç, T., Şeker P. S., Erdik, A. C., Kankiliç, T., Selvi, E., Yiğit, N. & Çolak, E. (2018): Determination of genetic variations in the genus Dryomys Thomas, 1906 (Rodentia: Gliridae) distributed in Turkey using NADH dehydrogenase 1 (ND1) gene. – *Mitochondrial* DNA *Part A* **29**(6): 933–942. https://doi.org/10.1080/24701394.2017.1389915

Kerey, E., Meriç, E., Tunoğlu, C., Kelling, G., Brenner, R. L. & Doğan, A. U. (2004): Black Sea-Marmara Sea Quaternary connections: new data from the Bosphorus, İstanbul, Turkey. – *Palaeogeography, Palaeoclimatology, Palaeoecology* **204**: 277–295. https://doi.org/10.1016/S0031-0182(03)00731-4

Kimura, M. (1980): A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. – *Journal of Molecular Evolution* **16***:* 111–120. https://doi.org/10.1007/bf01731581

Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Paabo S., Villablanca, F. X. & Wilson, A. C. (1989): Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. – *Proceedings of the National Academy of Sciences* **86**: 6196–6200. https://doi.org/10.1073/pnas.86.16.6196

Korkmaz, E. M., Lunt, D. H., Çıplak, B., Değerli, N. & Başıbüyük, H. (2014): The contribution of Anatolia to European phylogeography: the centre of origin for the meadow grasshopper, Chorthippus parallelus. – *Journal of Biogeography* **41**: 1793–1805. https://doi.org/10.1111/jbi.12332

Kumar, S., Stecher, G. & Tamura, K. (2016): MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. – *Molecular Biology and Evolution* **33**: 1870–1874. https://doi.org/10.1093/molbev/msw054

Montgelard, C., Matthee, C. A. & Robinson, T. J. (2003): Molecular systematics of dormice (Rodentia: Gliridae) and the radiation of Graphiurus in Africa. – *Proceedings of the Royal Society of London. Series B, Biological Sciences* **270**: 1947–1955. https://doi.org/10.1098/rspb.2003.2458

Primack, R. B. (1993): *An introduction to conservation biology*. – Sinauer Associates Inc, Sunderland, Massachusetts, 320 pp.

Prychitko, T. M. & Moore, W. S. (1997): The utility of DNA sequences of an intron from the β-fibrinogen gene in phylogenetic analysis of woodpeckers (Aves: Picidae). – *Molecular Phylogenetics and Evolution* **8**(2): 193–204. https://doi.org/10.1006/mpev.1997.0420

Popov, S. V., Shcherba, I. G., Ilyina, L. B., Nevesskaya, L. A., Paramonova, N. P., Khondkarian, S. O. & Magyar, I. (2006): Late Miocene to Pliocene palaeogeography of the Paratethys and its relation to the Mediterranean. – *Palaeogeography, Palaeoclimatology, Palaeoecology* **238**: 91–106. https://doi.org/10.1016/j.palaeo.2006.03.020

Rambaut, A. (2008): FigTree, Version 1.2. http://tree.bio.ed.ac.uk/software/FigTree/

Rozas, J., Ferrer-Mata, A., Sánchez-Delbarrio, J. C., Guirao-Rico, S., Librado, P., Ramos-onsins, S. E. & Sánchez-Gracia, A. (2017): DnaSP v6: DNA sequence polymorphism analysis of large datasets. – *Molecular Biology and Evolution* **34**: 3299–3302. https://doi.org/10.1093/molbev/msx248

Seddon, J. M., Santucci, F., Reeve, N. J. & Hewitt, G. M. (2001): DNA footprints of European hedgehogs, Erinaceus europaeus and E. concolor: Pleistocene refugia, postglacial expansion and colonization routes. – *Molecular Ecology* **10**(9): 2187–2198. https://doi.org/10.1046/j.0962-1083.2001.01357.x

Şengör, A. M. C. & Yilmaz, Y. (1981): Tethyan evolution of Turkey: a plate tectonic approach. – *Tectonophysics* **75**: 181–241. https://doi.org/10.1016/0040-1951(81)90275-4

Şeker, P. S, Selvi, E., Kankiliç, T. & Çolak, E. (2018): Geographical distribution pattern of mitochondrial DNA cytochrome b diversity in populations of Arvicola amphibius (Linnaeus, 1758) (Mammalia: Rodentia) in Turkey as determined by PCR-RFLP. – *Acta Zoologica Bulgarica* **70**(1): 19–30.

Tajima, F. (1989): Statistical-method for testing the neutral mutation hypothesis by DNA polymorphism. – *Genetics* **123**: 585–595.

Tamura, K. & Nei, M. (1993): Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. – *Molecular Biology and Evolution* **10**: 512–526. https://doi.org/10.1093/oxfordJournals.molbev.a040023

Voloch, C. M. & Schrago, C. G. (2012): Impact of the partitioning scheme on divergence times inferred from mammalian genomic data sets. – *Evolutionary Bioinformatics* **8**: 207–218. https://doi.org/10.4137/ebo.S9627

Wilson, D.E. & Reeder, D.A.M. (2005): *Mammal species of the world. A taxonomic and geographic reference.* 3rd ed. – John Hopkins University Press, Baltimore, 2142 pp.

Yiğit, N., Çolak, E., Çolak, R., Özkan, B. & Özkurt, Ş. Ö. (2003): On the Turkish population of Dryomys nitedula (Pallas, 1779) and Dryomys laniger Felten et Storch, 1968 (Mammalia: Rodentia). – *Acta Zoologica Academiae Scientiarum Hungaricae* **49**(Suppl. 1): 147–158.

Yiğit, N., Çolak, E., Sözen, M. & Karataş, A. (2006): *Rodents of Türkiye "Türkiye Kemiricileri"*. – Meteksan Yayınevi, Ankara, 154 pp.

Yiğit, N., Çolak, E., Çolak, R., Özlük, A., Gül, N., Çam, P. & Saygili, F. (2011): Biometric and allozymic variations in the genus Dryomys (Rodentia: Gliridae) in Turkey. – *Acta Zoologica Bulgarica* **63**: 67–75.

Yiğit, N., Çolak, E. & Sözen, M. (2016): A new species of voles, Microtus elbeyli sp. nov., from Turkey with taxonomic overview of social voles distributed in southeastern Anatolia. – *Turkish Journal of Zoology* **40**: 73–79. https://doi.org/10.3906/zoo-1404-19

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