



G-banded Karyotypes of Some Species in Gliridae (Mammalia: Rodentia) from Turkey

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Abstract

The results of a cytogenetic study on some representatives of Gliridae from Turkey were introduced. The G-, C-, and AgNOR banded karyotypes of *Muscardinus avellanarius abanticus* from Abant (Bolu), and the G-banded karyotype of *Myomimus roachi* from Thrace were presented for the first time. Additionally, the G-banded karyotypes of *Dryomys nitedula*, and *Myoxus glis* were analysed. Because of not including a secondary constriction and the smallest chromosome being metacentric instead of acrocentric in the autosomal set, the karyotype of *M. a. abanticus* was different from that of *M. a. trapezius*. With the comparison of obtained G-banded patterns belonging to the other Glirid species, it was detected that obtained karyotypes displayed consistency at a great extent with the previously determined karyotypes.



Keywords: Gliridae; G-banding; Karyotype; Turkey.

Türkiye’de Yayılış Gösteren Gliridae (Mammalia: Rodentia) Familyasındaki Bazı Türlerin G-bantlı Karyotipleri

Öz

Bu çalışmada Türkiye’de yayılış gösteren bazı Gliridae temsilcileri üzerinde yapılan bir sitogenetik çalışmanın sonuçları sunuldu. Abant’tan (Bolu) *Muscardinus avellanarius abanticus*'un G-, C- ve AgNOR bantlı karyotipleri ile Trakya'dan *Myomimus roachi*'nin G-bantlı karyotipi ilk kez sunuldu. Ek olarak, *Dryomys nitedula* ve *Myoxus glis*'in G-bantlı karyotipleri analiz edildi. Otozomal sette ikincil bir daralma olmaması ve en küçük kromozomun akrosantrik yerine metasantrik olması nedeniyle, *M. a. abanticus*'un karyotipinin *M. a. trapezius*'un karyotipinden farklı olduğu tespit edildi. Farklı lokalitelerde yaşayan Glirid türlerinin elde edilen G-bantlı karyotiplerinin daha önceki çalışmalarda belirlenen karyotiplerle büyük ölçüde tutarlılık gösterdiği tespit edildi.

Anahtar Kelimeler: Gliridae; G-bantlama; Karyotip; Türkiye.

1. Introduction

Gliridae Thomas, 1897 is one of the oldest extant families of order Rodentia. Members of this family are known for their long-term (5-6 months) hibernation. They can occupy many different habitats; such as woods, thickets, orchards, rocky areas in the forest, steppes, and deserts. Species within this family are mostly found in Europe and Asia Minor, although some of them live in Africa, Russia, India, China, and Japan [1, 2]. The family consists of 9 genera including 28 living species [2]. There are 11 species in the family whose distribution range is limited to the western Palearctic; seven of them (*Myoxus glis*, *Dryomys nitedula*, *Dryomys laniger*, *Eliomys melanurus*, *Myomimus roachi*, *Muscardinus avellanarius*, and *Myomimus setzeri*) lived in Turkey [3]. Among all, the most common dormouse species in Turkey are *M. glis*, *D. nitedula*, and *M. avellanarius*. The remaining species have a more limited geographical distribution range. *M. roachi*, also known as Roach’s mouse-tailed dormouse, is present only in a limited area in the western part of Thrace and Turkey. Another *Myomimus* species, *M. setzeri*, also known as Setzer’s mouse-tailed dormouse, has been recorded from eastern Anatolia adjacent to Iran. Despite *E. melanurus* is found in Egypt, Iraq, Israel, Jordan, Lebanon, Libya, Saudi Arabia, Syria and Turkey (Şanlıurfa), *D. laniger* that is an endemic hibernator species has a limited distribution in the Taurus Mountains [3].

Until this time, there have been numerous studies that examine the characteristics of karyological of Glirid taxa in Europe and Turkey. [4, 5]. Beyond that the conventional karyotypes of most species in Gliridae from Turkey have been determined by previously performed studies [6, 7]. Notwithstanding, there is still lack of information about the banded and even conventional karyotypes of many taxa in this family. Heretofore, two subspecies of *M. avellanarius*, also called the hazel dormouse, in Turkey have been identified based on the morphological differences between geographically very distant populations of this species [8, 9]. The first one, *M. a. trapezius*, has been described from Coşandere (Trabzon), while, the second one, *M. a. abanticus*, has been identified from Abant (Bolu). Various karyotype studies including conventional, C- and G- banding techniques have often been focused on the European populations of *M. avellanarius* [10-16], and little has been known about the karyology of the Turkish populations [17, 18]. The C- and G-banded karyotypes in the populations of *M. a. trapezius* from Trabzon and Ordu have been previously reported [17, 18]. On the other hand, even conventional karyotype of the *M. a. abanticus* is still unknown. The conventional and AgNOR- banded karyotype of *M. roachi* has been studied by Civitelli et al. [19]. However, the C- and G-banded karyotypes of *M. roachi* from Turkey have not been reported up to the present. The conventional stained and banded karyotype of *D. nitedula* has been detected from various parts of its geographic distribution range in Turkey [6, 7, 19, 20]. In contrast to this, the C- and G- and AgNOR-banded karyotype of this species has been studied on the limited number of specimens from the restricted number of areas in Turkey [7, 19, 20]. The conventional karyotype of fat dormouse, *M. glis*, has been investigated in specimens from both Anatolia and Thrace [21, 19]. The C- and G- banded karyotype of this species have been studied from different parts of Black Sea regions [20, 22].

On the members of the Gliridae family from Turkey, the conventional karyotype studies have been performed more often, however, karyotype banding studies have been limited. The increase in the number of banding studies conducted on the members of this family, which has a wide range in our country, will make it easier to determine possible intraspecific variations within the species of this rodent family; and thus, this will enable us to directly contribute to the understanding of our country's biodiversity and hence global biodiversity. Based on this thought, the current study primarily presented the banded karyotypes of the representatives of Gliridae from Turkey (*M. a. abanticus*, *M. a. trapezius*, *M. roachi*, *D. nitedula*, and *Myoxus glis*) to fill the gaps in our knowledge about chromosome banding patterns of dormouse species. In addition, the G-, C-, and AgNOR-banded karyotypes of *M. a. abanticus* were introduced for the first time.

2. Materials and Methods

Karyotype analyses were carried out on 51 specimens belonging to *M. a. abanticus*, *M. roachi*, *D. nitedula*, and *M. glis* in the Gliridae family. The number of specimens, sample designations, and collecting sites for each species was identified in Table 1 and Fig. 1. Animal samples were captured by the fieldwork performed in accordance with the legal permission (no: 72784983-488.04-150036) given by the Republic of Turkey Ministry of Agriculture and Forestry, General Directorate of Nature Conservation and National Parks. Experimental processes were conducted according to the animal experiments local ethics committee decision provided by the Animal Experiments Local Ethics Committee of Ankara University (no. 2015-6-105).

Karyotype preparations were obtained from the fresh bone marrow of colchicine treated animals following the method of Ford and Hamerton [23]. To define autosomal and sexual chromosomes pairs, the C-, G- and AgNOR staining techniques were employed. The G-banding processes of the chromosomes were carried out in accordance with the process introduced by Seabright [24]. Constitutive heterochromatin and nucleolus organizer regions (NORs) were detected by following the staining techniques of Sumner [25] and Howell and Black [26], respectively. A total of 10 slides were made from each sample and nearly 20 well-spread metaphase plates were examined. The slides were photographed with the Nikon DS-Ri2 stereo binocular microscope. Chromosome numbers were determined by attentively counting of well-spreading metaphase plates. The most frequently observed chromosome counts were regarded as valid karyotypes. The diploid number of chromosomes ($2n$), the total numbers of chromosomal arms (NF), and the numbers of autosomal arms (NFa), as well as the X and the Y chromosomes, were classified. All chromosomes were arranged from bigger to smaller and noted to be the metacentric, submetacentric and acrocentric according to their centromere positions, consistent with the Levan et al. [27]. The skins, skulls, and karyotype preparations of all examined specimens were deposited at the Niğde Ömer Halisdemir University, Niğde, Turkey.

3. Results

The G-, C-, and AgNOR-banded karyotype of *M. a. abanticus* were studied for the first time. The karyotype of three populations from Abant (Bolu) - type locality of *M. a. abanticus*-, Uludağ (Bursa) and Yığılca (Düzce) in Turkey had $2n = 46$, $NF = 92$ and $NFA = 88$ values. All the autosomal chromosomes were bi-armed (meta- and submetacentric or subtelocentric pairs) in gradually decreasing size. The X chromosome was a medium-sized metacentric, while the Y chromosome was a small-sized acrocentric. AgNOR staining revealed that the nucleolar organizer regions positioning in the secondary constrictions were localized in the 20th and 22nd autosomal pairs. All detected NORs were heteromorphic and observed only in one of the homologs. Most of the autosomal chromosomes enjoyed apparent C-positive bands in pericentromeric regions, while,

C-bands in some chromosomes were nebulous. The sexual chromosomes also had dark C-bands. (Fig. 2).

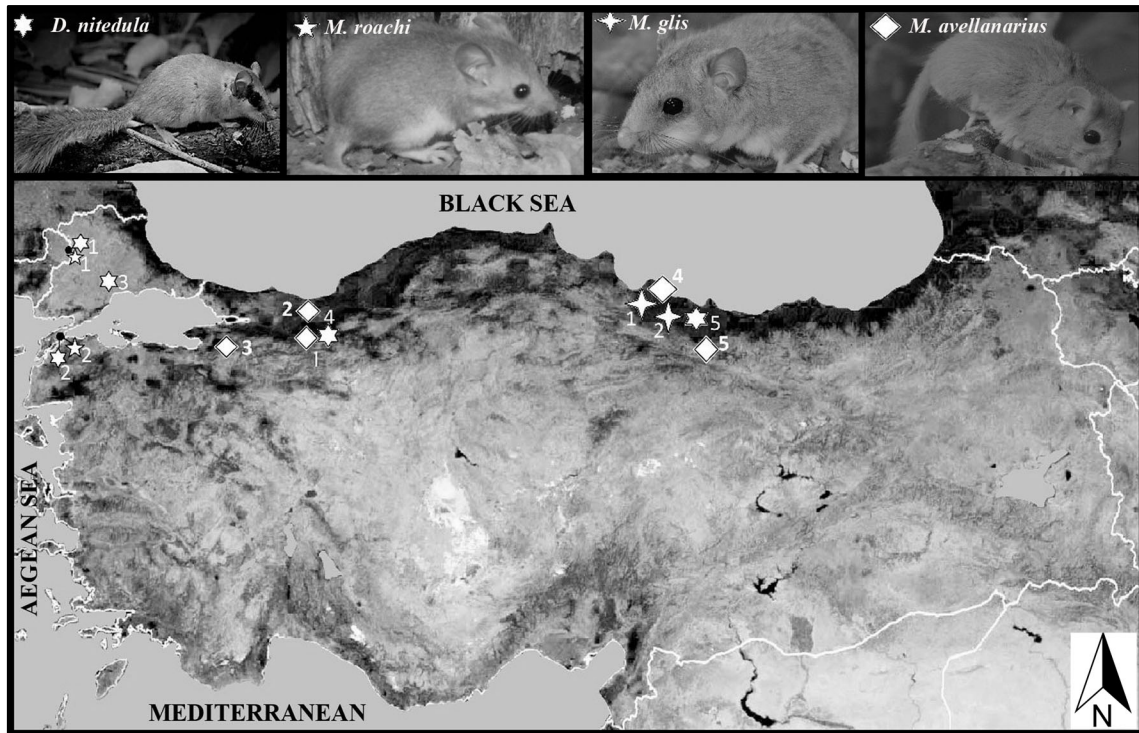


Figure 1: The map showing collecting sites of the samples, the numbers and symbols in the map correspond to the localities and species given in Table 1

Additionally, the G- banded karyotypes of *M. a. trapezius* from Ordu-Ulubey and Giresun-Bulancak were presented. The detected karyotype consisted of $2n = 46$, $NF = 90$, and $NFa = 86$ values. The X chromosome was larger metacentric and Y chromosome was acrocentric. This karyotype was different from the karyotype of *M. a. abanticus*, because of including a secondary constriction in the 20th chromosome pair and the smallest chromosome being acrocentric instead of metacentric in the autosomal set (Fig. 3).

The G- banded karyotype of *M. roachi* was detected for the first time. The karyotype of three specimens from Edirne and Çanakkale from Thrace in the European part of Turkey had the karyotype including $2n = 44$, $NFa = 84$ and $NF = 88$ values. All the autosomal chromosomes were bi-armed (meta- or submetacentric pairs) of decreasing size. In the karyotypes, the X chromosome was a medium-sized submetacentric, while the Y chromosome was a medium-sized metacentric (Fig. 4A).

Table 1: The number of specimens, sample designations, collecting sites and karyological characteristics for each species. (NFa: autosomal fundamental number NF: chromosome arm numbers)

Species	Map Number	Localities (Latitude/Longitude)	Total	♂	♀	2n	NFa	NF
<i>Myoxus glis</i> (✦)	1	Giresun-Bulancak (40° 56' 08" N / 38° 13' 51" E)	1	1	-	62	120	124
	2	Ordu-Ulubey-Yukarıkızılen (40° 46' 38" N / 37° 42' 39" E)	2	1	1	62	120	124
<i>Dryomys nitedula</i> (☆)	1	Edirne-Orhaniye-Bağlık (41° 31' 07" N / 26° 39' 09" E)	1	-	1	48	92	96
	2	Çanakkale-Gelibolu-Sütlüce (40° 20' 35" N / 26° 36' 03" E)	1	-	1	48	92	96
	3	Tekirdağ-Kumbağı-Naip (40° 52' 25" N / 27° 25' 31" E)	1	-	1	48	92	96
	4	Bolu-Abant (40° 35' 41" N / 31° 16' 57" E)	1	-	1	48	92	96
	5	Giresun-Bulancak (40° 55' 12" N / 38° 12' 56" E)	1	1	-	48	92	96
<i>Myomimus roachi</i> (★)	1	Edirne-Orhaniye-Bağlık (41° 29' 50" N / 26° 38' 49" E)	1	-	1	44	84	88
	1	Edirne-Azatlı (41° 29' 38" N / 26° 42' 03" E)	1	-	1	44	84	88
	2	Çanakkale-Gelibolu-Sütlüce (40° 20' 54" N / 26° 36' 47" E)	1	1	-	44	84	88
<i>Muscardinus avellanarius</i> (◇)	1	Bolu-Abant-Soğuksu (40° 36' 49" N / 31° 17' 56" E)	7	4	3	46	88	92
	2	Düzce-Yığılca (40° 57' 31" N / 31° 27' 04" E)	4	3	1	46	88	92
	3	Bursa-Uludağ (40° 07' 06" N / 29° 07' 11" E)	2	1	1	46	88	92
	4	Ordu-Ulubey (40° 52' 36" N / 37° 45' 32" E)	6	3	3	46	86	90
	5	Giresun-Bulancak (40° 55' 57" N / 38° 14' 52" E)	4	2	2	46	86	90

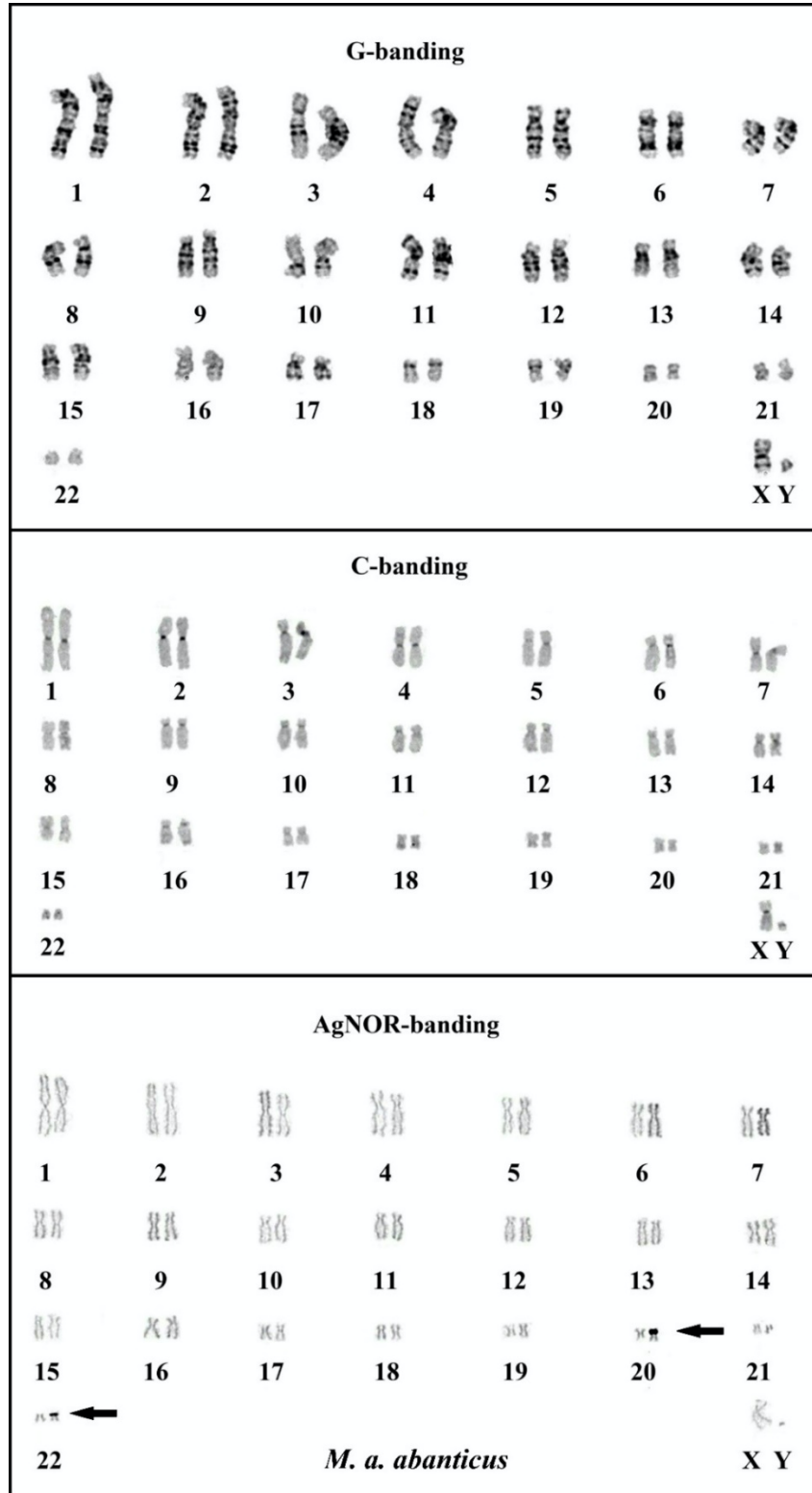


Figure 2: The G-, C- and AgNOR- banded karyotype of *M. a. abanticus* from Abant-Bolu. The arrows indicate the position of the heteromorph active AgNOR regions

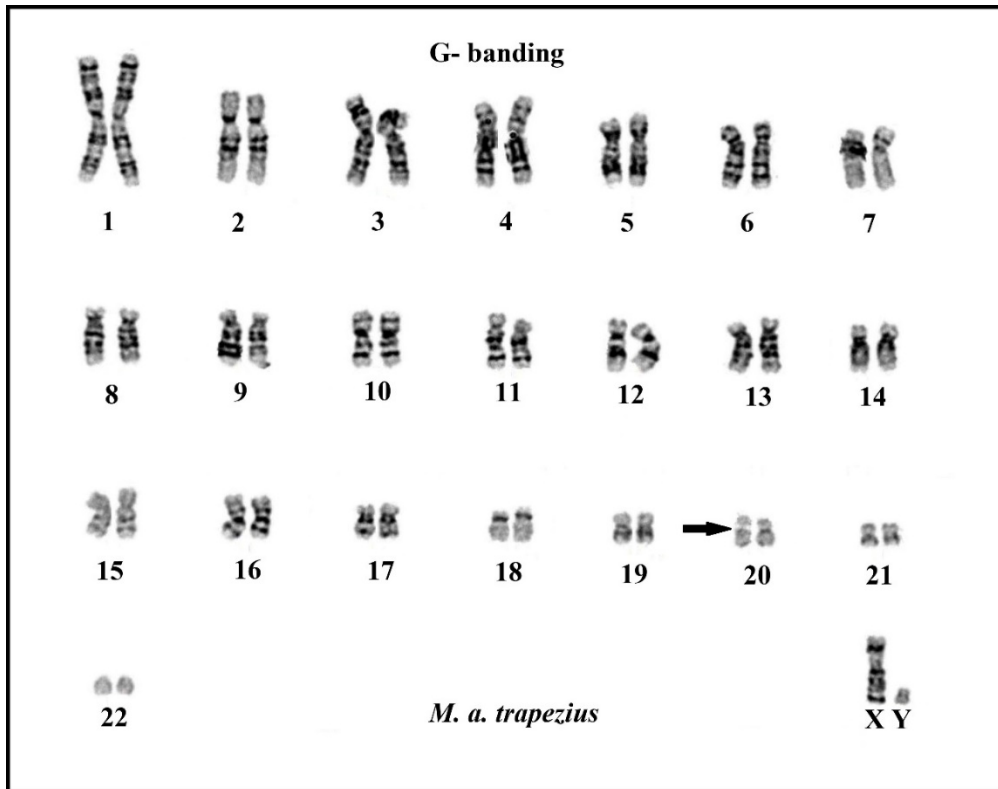


Figure 3: The G- banded karyotype of *M. a. trapezius* from Ulubey-Ordu. The arrow indicates the secondary construction

The karyotype of *D. nitedula* from Thrace located in the European Turkey and Anatolia was studied using the G-banding technique. The karyotypes of five specimens collected from five localities had the values of $2n = 48$, $NF = 96$ and $NFa = 92$. It was determined that the karyotypes of all specimens consisted of 23 pairs of large and small meta-, submetacentric and subtelocentric autosomal chromosomes. The first autosomal pair was frankly bigger than the other autosomal pairs in the complement. In conformity with the findings of previous studies, it was detected that 21st autosomal chromosomes were in a heterozygous secondary constriction condition in this karyotype. The X chromosomes were identified to be in the large and submetacentric shape (Fig. 4B).

The G-banded karyotype of *M. glis* from Turkey was investigated. The karyotype having the values of $2n = 62$, $FN = 124$, $NFa = 120$ was found in two *M. glis* specimens from two different localities of Anatolia. Except for the Y- chromosome, all chromosomes, including X- as well, in the karyotype were noted to be bi-armed, meta- and submetacentric chromosomes. The 17th autosomal pair appeared to carry a secondary constriction in the karyotype. The X- chromosome was determined to be a large-sized and metacentric element, while the Y- chromosome was detected to be dot-like and most likely acrocentric element (Fig. 4C).

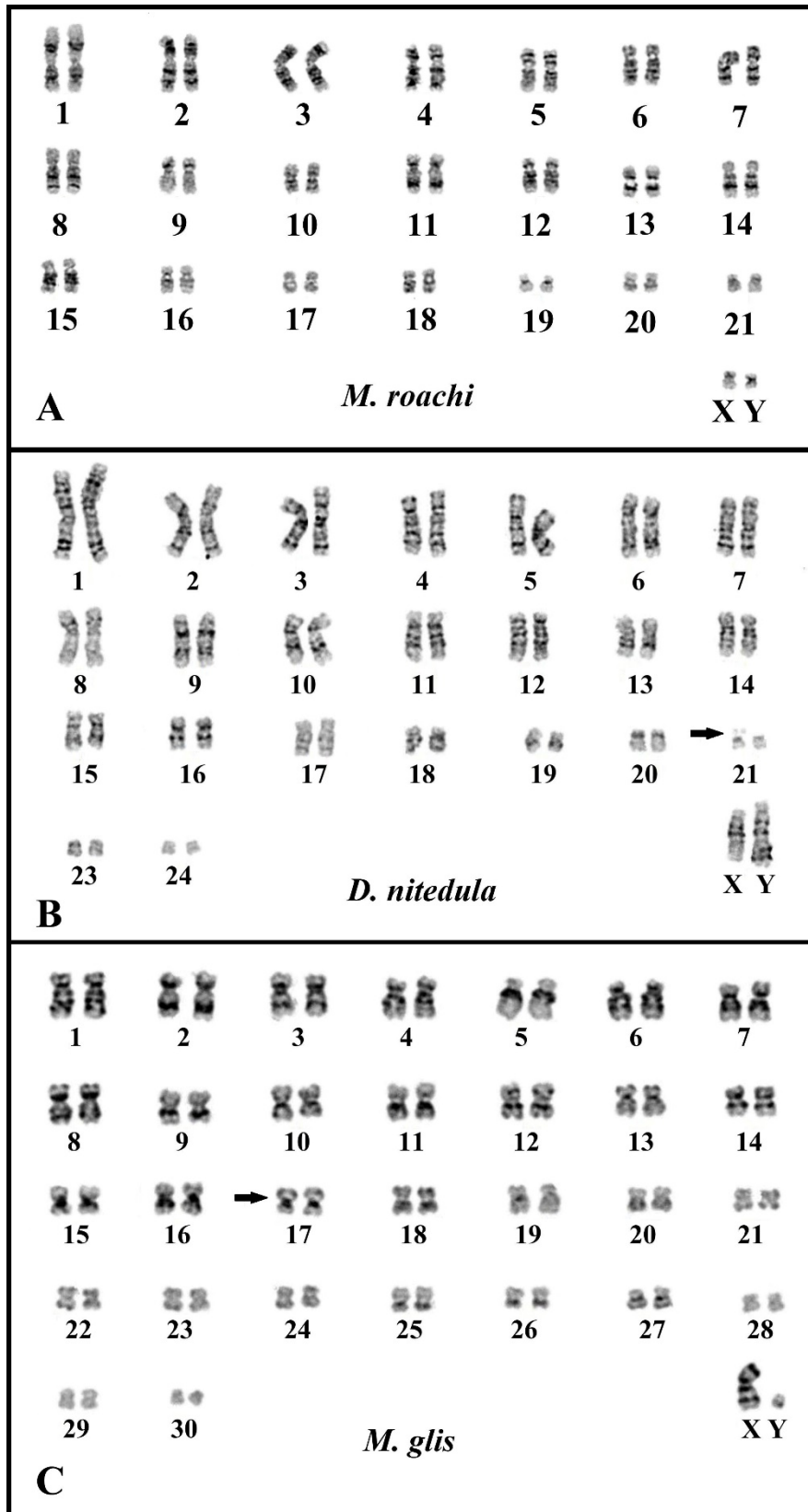


Figure 4: G- banded karyotypes of *M. roachi* from Edirne (A), *D. nitedula* from Tekirdağ (B), and *M. glis* from Ordu (C). The arrows indicate the secondary construction in the karyotypes

4. Discussion

Identifying intra-specific variations is the most fundamental step in documenting biodiversity in a particular region. In this sense, the variations hidden in the genome of both plant and animal species in our country are tried to be revealed by using a wide variety of markers, including karyotype analysis [7, 17, 19, 28, 29]. Karyotype variations are an easy-to-apply method used to determine genetic variation. In this study, carried out for this purpose, the G-, C-, and AgNOR-banded karyotypes of *M. a. abanticus* and the G-banded karyotypes of *M. roachi* from Turkey were presented for the first time. Additionally, the G-banded karyotypes of *M. a. trapezius*, *D. nitedula*, and *M. glis* were submitted as well.

The karyotype of *M. avellanarius* was investigated from only two localities in north-eastern Anatolia throughout Turkey until now. Those obtained karyotypes belonged to the *M. a. trapezius*, known subspecies of this species from the region. The smallest autosomal pair in investigated karyotypes was considered to be subtelocentric in the Trabzon population or acrocentric in the Ordu population. Accordingly, two karyotype forms with $NFa = 86$ or $NFa = 88$ values were determined for this subspecies based on the morphological difference of the smallest pair [17, 18]. The G- banded karyotype of *M. a. trapezius* presented by this study included the values of $2n = 46$, $NF = 90$ and $NFa = 86$ to be compatible with the karyotype determined by Şekeroğlu et al. [17]. The karyotype we identified from Ulubey-Ordu included a secondary constriction in the 20th chromosome pair, similar to the karyotype of *M. a. trapezius* revealed by Şekeroğlu et al. [17] and Doğramacı and Kefelioğlu [18]. Also, the autosomal and sex chromosome morphologies in the karyotype of *M. a. trapezius* determined by this study and also those of previously presented one by Şekeroğlu et al. [17] were nearly the same as those of European populations of *M. avellanarius* except for the karyotype introduced by Doğramacı and Kefelioğlu [18] [4, 5]. In this context, it is necessary to open a separate parenthesis to the study of Peshev and Delov [15]. The researchers stated in their study that *M. a. avellanarius* had a value of $NFa = 88$. However, it is clearly seen in the presented idiogram and the text of the study that the smallest autosomal chromosome pair is acrocentric. We think that this ($NFa = 88$) may have arisen from a printing error. Therefore, this species should have an NFa of 86 instead of 88. Considering that, the $NFa = 88$ value is only seen in the populations of *M. avellanarius* from Turkey.

The karyotype of *M. a. abanticus* from Abant-Bolu was described for the first time by the current study and it was detected that the karyotype had $2n = 46$, $NF = 92$ and $NFA = 88$ values. Obtained karyotype was substantially compatible with the karyotype of Doğramacı and Kefelioğlu [18] except to not include a secondary constriction. This karyotype was different from the karyotype of *M. a. trapezius* in two ways. The first was that the detected karyotype of *M. a.*

abanticus did not include a secondary constriction. The second was that the smallest chromosome in the autosomal set was metacentric instead of acrocentric. By using silver-nitrate staining, the nucleolar organizer region was localized in the secondary constrictions in autosomal pair no. 20 and 22. All observed NORs were heteromorphic and occurred in differ homologues (Fig. 2).

Although the conventional karyotype of *M. roachi* from Thrace was known, however, no information about the banded karyotype of this species has been imparted so far. This deficiency has been tried to be compensated to a certain extent by this study presenting the G-banded karyotype. The results related to autosomal chromosomal set complied with those of Civitelli et al. [19]. Unlike this study, Y chromosome in the sample from the same locality was identified as metacentric rather than acrocentric.

That the karyotype of *D. nitedula* from various parts of Turkey (both from Thrace and Anatolia) had a steady karyotype value of $2n = 48$ FN = 96 and NFa = 92 consistent with the results of previous studies were determined. The autosomal complement was entirely composed of the variable numbers of large and small, bi-armed chromosomes in the form of meta-, submeta- or partly subtelocentric. In the karyotypes obtained by many previous studies, variations that were thought by us as not obvious in the shape and size of the chromosomes were found. Since it was thought that revealed chromosomal morphologies may have changed according to the researchers' perspective, no grouping attempts with respect to chromosome morphology were made in this study. Our results were found to be consistent with those of Doğramacı and Kefelioğlu [6], Şekeroğlu and Şekeroğlu [20], and Arslan et al. [7] including samples from Anatolia, and Mitsainas et al. [30] including the populations of *D. nitedula* from Greece. The karyotype introduced by the current study also comprised the secondary construction in the 21st chromosome pair, as it was detected by previous studies. Three *Dryomys* species, *D. laniger*, *D. nitedula* and *D. pictus* have been reported from Turkey until this time [2, 3]. From these species, *D. nitedula* populations in Turkish Thrace were mostly assigned to *D. n. wingei* [2]. Another subspecies of this species, *D. n. phrygius*, thought to be an endemic to Turkey, were firstly reported from western Anatolia [31], but so far, their taxonomic status has not been clarified in detail. Our results based on the sampling from both Thrace and Anatolia showed that there was no karyological difference between G- banded karyotypes of two subspecies.

Obtained karyotype having $2n = 62$ diploid chromosome number in the two populations of *M. glis* by the current study was also previously determined in European and Asian populations [12-15, 19, 20, 21, 22, 30]. Some chromosomal differences in the karyotypes of this species have been found before, even if the karyotype structures were homogeneous on a large scale. For example, the presence of a pair of acrocentric chromosomes in the autosomal set has been reported

in Bulgarian populations [15]. However, a similar situation was not observed in the karyotypes presented by the current study; all the autosomal chromosomes were bi-armed, mostly congruent with the results of the previous studies. In addition, the secondary constriction in a small-sized pair has been frequently described in most examined populations of this species from Europe and Turkey [14, 20, 30]. Similar to them, the 17th autosomal pair appeared to carry a secondary constriction in the karyotype determined by the current study. Mitochondrial DNA sequence analyses revealed that there is an amazing genetic homogeneity within the distribution range of the populations of this species not only in Turkey, but also in the whole of Europe [32]. This may be considered to be an indicator that the İstanbul and Çanakkale straits, and the Marmara Sea, obstacles for the terrestrial connection between Europe and Asia continentals, are not an effective geographic barrier for the gene flow between the populations of the edible dormouse. However, it is clear that more evidence is required for the solution of such complex biogeographical events. The karyotype data reported here and supporting the aforesaid genetic homogeneity can be considered as evidence required for the explanation of the mentioned situation.

5. Conclusions

By the results of the current karyotype study on some representatives of Gliridae from Turkey, the G-, C-, and AgNOR banded karyotypes of *M. a. abanticus* from Abant (Bolu), and the G-banded karyotype of *M. roachi* from Thrace were presented for the first time. With the examination and comparison of the determined findings, it was demonstrated that the intraspecific variations can be easily revealed by the karyotype. Available karyological variations in the determined karyotypes should be considered to be the noteworthy steps for the populations' differentiation. Of course, a strong genetic difference at the subspecies level between *M. a. abanticus* and *M. a. trapezius* may not be mentioned at this stage. Therefore, obtained findings by current study and the results of further studies based on additional markers will allow a more accurate assessment. Additionally, those kinds of intraspecific variations should be taken into account as the major indicators the documenting of Turkey's biodiversity. On the other hand, the steady karyotypes that are compatible with the previous findings could indicate a genetic homogeneity for other species examined in the study.

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