

Karyological and distributional contributions to Turkish rodent fauna (Mammalia: Rodentia) from islands in Aegean and Marmara Sea

Ege Denizi ve Marmara Denizi'ndeki adalardan Türkiye kemirici faunasının karyolojisine ve yayılışına katkılar

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Abstract

A chromosomal study on five different rodent species (*Mus domesticus*, *Mus macedonicus*, *Apodemus sylvaticus*, *Apodemus witherbyi* and *Rattus rattus*) from Gökçeada and Bozcaada in Aegean Sea and Marmara Island in Marmara Sea was performed to fill the deficiency of karyological information about island populations of these species. Diploid chromosome sets (2n) and fundamental numbers of chromosomal arms (NF) were determined to be 2n = 40, 40, 48, 48 and 38, NF = 40, 40, 48, 48 and 60 for *M. domesticus*, *M. macedonicus*, *A. sylvaticus*, *A. witherbyi* and *R. rattus* respectively. Results showed that there was no difference between karyological values in island and continental populations of those rodents, although Aegean and Marmara seas have served as geographic barriers for a long time between islands and mainland. In addition to the karyotypes of *M. domesticus*, *M. macedonicus*, *A. sylvaticus* and *R. rattus*, their presence in Marmara Islands were firstly determined by current study.

Özet

Beş farklı kemirici türünün (*Mus domesticus*, *Mus macedonicus*, *Apodemus sylvaticus*, *Apodemus witherbyi* ve *Rattus rattus*) Türkiye'deki ada popülasyonlarına ilişkin karyolojik bilgi eksikliğini gidermek amacıyla, bu türlerin Ege Denizi'nde yer alan Gökçeada ve Bozcaada ile Marmara Denizi'nde yer alan Marmara Adası'nda yayılış gösteren popülasyonları üzerine kromozomal bir çalışma gerçekleştirilmiştir. *M. domesticus*, *M. macedonicus*, *A. sylvaticus*, *A. witherbyi* ve *R. rattus* için diploit kromozom sayıları ve temel kromozom kol sayıları sırasıyla 2n = 40, 40, 48, 48 ve 38, NF = 40, 40, 48, 48 ve 60 olarak belirlenmiştir. Ege Denizi ve Marmara Denizi bu türlerin adalarda ve anakarada yayılış gösteren popülasyonları arasında gen akışının kesilmesine neden olan coğrafik bariyerler olarak uzun süredir görev yapmaktadır, buna rağmen, bu çalışma ile bu kemirici türlerinin adalarda ve anakarada yayılış gösteren popülasyonları arasında karyolojik bakımdan herhangi bir farklılaşma olmadığı tespit edilmiştir. Ayrıca, *M. domesticus*, *M. macedonicus*, *A. sylvaticus* ve *R. rattus*'un Marmara Adası'nda yayılış gösterdiği ilk kez bu çalışma ile belirlenmiştir.

INTRODUCTION

Islands have been evaluated as geographical areas possessing special scientific interest for evolutionary studies due to lack of continentally connection with the mainland which prevents gene flow. When considered as "natural laboratories", islands enable to create numerous evolutionary models which can be developed and tested by reducing complexity of the natural world (Whittaker and Fernández-Palacios 2007). Having knowledge about biological features of island populations and comparing them to those of counterparts in the mainland is primarily

need to put into practice this suggested idea. Therefore, it is so clear that detailed information about genetic structure of island populations should be properly obtained.

With surrounded on its three sides by Mediterranean, Aegean and Black Sea, being a peninsula, Turkey has many both large and small islands. Gökçeada, Marmara Island and Bozcaada are respectively three bigger islands among all, according to their surface area (Tunçdilek 1987; Hocaoğlu 1988). Gökçeada and Bozcaada are situated in the northeastern part of Aegean Sea, while,

Marmara Island is located in the southwest of Marmara Sea. All of these three islands have been considered to be the parts of Thrace and Western Anatolia and have recently split from Turkey (Şengör and Yılmaz 1981; Oral et al. 1995). Therefore, these islands have been included in distribution area of Turkish rodents up to date. However, detailed distribution records and features reflecting population structure of them have not been defined accurately. Özkan (1999) has revealed that 7 rodent species from 3 families (Sciuridae, Muridae and Spalacidae) are distributed in Gökçeada and 4 species from 2 families (Muridae and Spalacidae) live in Bozcaada in the first comprehensive study. In addition to this study, presence of the rodent species in Gökçeada and Bozcaada has been proved by different studies (Özkan and Kryštufek 1999; Kryštufek and Vohralík 2005; Kryštufek and Vohralík 2009). Later, Sözen et al. (2013) reported *Nannospalax xanthodon* from Gökçeada and Bozcaada, and indicated that the karyotype of both population is $2n=38$, as the same with blind mole rat population in Aegean Region. Apart from these studies, there has been no study on the island populations of Turkish rodent species and also any information about rodent fauna in Marmara Island has not been known.

Karyological studies, which explain number and morphology of chromosomes in a simple sense, have been widely used to be a cytogenetic marker in rodent systematics. To disclose variations in number, shape and banding patterns of chromosomes both within and among populations of numerous rodent species has been considered to be an important step for solving of problems in the speciation processes and thus, taking this step can enable to introduce better taxonomic evaluations (Dobigny et al. 2004). For those reasons, to date, numerous studies have widely used karyological differences and similarities found in rodent populations which are distributed in different geographical areas in Turkey, including *M. domesticus*, *M. macedonicus*, *A. sylvaticus*, *A. witherbyi* and *R. rattus* (Yiğit et al. 1998; Çolak 2003; Gözcelioğlu et al. 2005; Çolak et al. 2005; Kankılıç et al. 2006).

The genus *Mus* includes 4 subgenera which have morphological, morphometric and biochemical

differences, and is represented by 38 species all over the world (Wilson and Reeder 2005). Middle East has been known as origin of the genus and it is suggested that expansion of *Mus* species to the Western Europe and Northern Africa has occurred by anthropogenic effects (Auffray et al. 1990). That two species within the genus; *M. domesticus* and *M. macedonicus* are distributed in Turkey has been reported by morphological, karyological and molecular studies (Özkan 1999; Gözcelioğlu et al. 2005; Çolak et al. 2006). The standard karyotype, $2n=40$ of *M. domesticus* from Turkey was first given by Gündüz et al. (2000a). One another subsequent karyological study with regard to *M. domesticus* and *M. macedonicus* in Turkey confirmed this case (Çolak et al. 2006). However, Gündüz et al. (2000b) found a population having morphological and mitochondrial DNA characteristics of *M. domesticus* from Denizli in Turkey including individuals with 38 chromosomes holding metacentric, contrary to the standard karyotypes of *M. domesticus*. In addition to all karyological information, only distribution records and morphological features of *M. domesticus* from Bozcaada and *M. macedonicus* from Gökçeada were firstly given by Özkan (1999).

Systematic of the genus *Apodemus*, which is widely distributed in Palearctic region, seems highly complex and controversial. Numerous studies that reflect morphological, karyological, biochemical and molecular aspects of this genus have tried to remove this complexity (Özkan and Kryštufek 1999; Macholán et al. 2001; Michaux et al. 2002; Filippucci et al. 2002; Çolak et al. 2007). However, there has been no agreement on this subject to date, because the genus has been studied by dividing some subgenera such as *Apodemus*, *Slyvaemus*, *Alsomys*, *Karstomys*, *Argentaus*, *Agrarius*, *Gurkha* and *Slyvaticus* by many researchers (Zimmerman 1962; Musser et al. 1996; Serizawa et al. 2000; Bellinvia 2004). Wilson and Reeder (2005) remarks that a comprehensive revision that must be inclusive of complete *Apodemus* group is needed for such a division on subgenera rank. Therefore, they suggest that the genus *Apodemus* involves 20 species in the Palearctic without considering any subgeneric division. Of those, 6 species live in different part of Turkey have been reported (*A. flavicollis*, *A. sylvaticus*, *A. mystacinus*, *A. uralensis*, *A. witherbyi*, and

A. agrarius). The species nowadays known as *A. witherbyi* is previously considered to be *A. iconicus* or *A. hermonensis*. $2n=48$ standard karyotype value have been determined in numerous studies for all living *Apodemus* species except for *A. uralensis* in Turkey (Çolak 2003; Kefelioğlu et al. 2003; Çolak et al. 2004; Çolak et al. 2005; Yiğit et al. 2006). In the study performed on island populations of *Apodemus*, Özkan (1999) has detected that *A. flavicollis* and *A. sylvaticus* live in Gökçeada while only *A. witherbyi* inhabits in Bozcaada.

The genus *Rattus* is widely distributed in the world by reason of living commensal with humans and it is considered to be a taxonomically complex group. Since representatives of the genus have high morphological variabilities, species of this genus are evaluated in six species groups (*R. norvegicus*, *R. exulans*, *R. rattus*, *R. fuscipes*, *R. leucopus*, *R. xanthurus*) involving 66 species (Wilson and Reeder 2005). Of those species groups, presence of two species in Turkey, *R. rattus* and *R. norvegicus*, has been confirmed by revealing their morphological features along with the karyotypes; $2n=38$ for *R. rattus*, $2n=42$ for *R. norvegicus* (Yiğit et al. 1998). In addition to this, a chromosomal polymorphism arising from pericentric inversion and b chromosome has also been detected in Thracian populations of *R. rattus* (Kankılıç et al. 2006). By giving the first distribution record and revealing morphological features of these two species from two Aegean islands, Özkan (1999) reported that while only *R. rattus* distributed in Bozcaada, both of two species live in Gökçeada.

Gökçeada, Marmara Island and Bozcaada which are located in the near of Thrace and Western Anatolia; have been included in potential distribution areas of Turkish rodents. Although distribution records and morphological features of *M. domesticus*, *M. macedonicus*, *A. sylvaticus*, *A. witherbyi* and *R. rattus* in Gökçeada and Bozcaada have

previously been announced, there has not been such information from Marmara Island. Apart from that, any other extensive data related to these rodent populations from these islands, such as karyology, is currently not available. Because of these reasons, present study primarily aims to investigate karyological features of five different rodent species from three islands in Aegean and Marmara Sea in Turkey and to provide distributional data for mentioned rodent species in Turkish islands.

Material and Methods

A total of 62 samples belonging to five different rodent species (*Mus domesticus*, *Mus macedonicus*, *Apodemus sylvaticus*, *Apodemus witherbyi* and *Rattus rattus*) were collected in six different parts of Gökçeada, Bozcaada and Marmara Island for karyological analysis (Table 1 and Figure 1). Before starting karyological analysis, discrimination of morphologically similar species within both two genus, *Mus* and *Apodemus*, were performed by their mitochondrial DNA sequences (cytochrome *b* and control region) instead morphological differences. Karyotype preparations were carried out from bone marrow of colchicine applied animals consistent with the method of Ford and Hamerton (1956). A total of 10 or 20 slides were prepared for each sample. All slides were leaved for drying up by air and then stained by Giemsa. At least 10-20 well spread and non-overlapping metaphase plate was evaluated for each sample. Diploid number ($2n$) and fundamental numbers of chromosomes (NF) together with the sexual chromosomes (X and Y) were marked as metacentric, submetacentric, subtelocentric and acrocentric. All chromosomes were arranged from bigger to smaller, according to their shapes in accordance with Levan et al. (1964). All karyotype preparations, skulls, skins and variable tissues belonging to the samples were deposited in Ankara University Mammalian Research collection (AUMAC, www.mammalia.ankara.edu.tr).

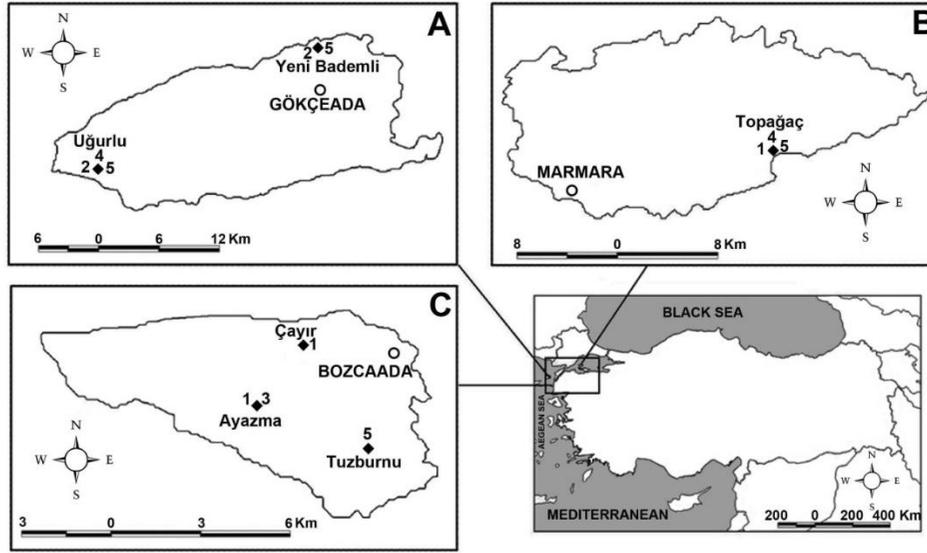


Figure 1. The map showing localities of collected samples in study areas. (A) Gökçeada, (B) Marmara Island, (C) Bozcaada. 1: *M. domesticus*, 2: *M. macedonicus*, 3: *A. witherbyi*, 4: *A. sylvaticus*, 5: *R. rattus*.

Table 1. Sampling locations of the specimens used in the study

Localities	Species	Sample size	Map number
Uğurlu-Gökçeada	<i>Mus macedonicus</i>	15	2
Uğurlu-Gökçeada	<i>Apodemus sylvaticus</i>	2	4
Uğurlu-Gökçeada	<i>Rattus rattus</i>	4	5
Yeni Bademli-Gökçeada	<i>Mus macedonicus</i>	5	2
Yeni Bademli-Gökçeada	<i>Rattus rattus</i>	1	5
Topağaç-Marmara Island	<i>Mus domesticus</i>	13	1
Topağaç-Marmara Island	<i>Apodemus sylvaticus</i>	2	4
Topağaç-Marmara Island	<i>Rattus rattus</i>	5	5
Ayazma-Bozcaada	<i>Mus domesticus</i>	4	1
Ayazma-Bozcaada	<i>Apodemus witherbyi</i>	5	3
Tuzburnu-Bozcaada	<i>Rattus rattus</i>	5	5
Çayır-Bozcaada	<i>Mus domesticus</i>	1	1

RESULTS

Diploid chromosome number and fundamental number of chromosomal arms in all island populations of *Mus domesticus* and *Mus macedonicus* was observed as $2n = 40$ and $NF = 40$. A total of 19 pair of acrocentric autosomal chromosomes in different size accompanied by sexual chromosomes X and Y, was consisted of diploid chromosomal set. X chromosome was always bigger than Y in all populations and both of them was also acrocentric (Figure 2).

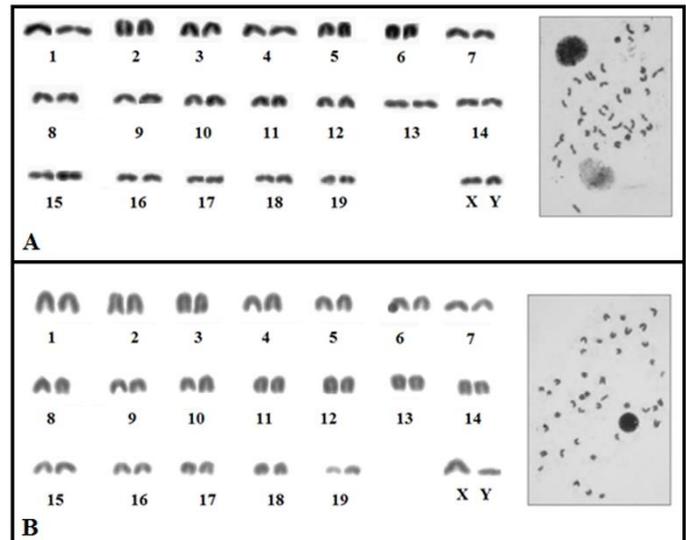


Figure 2. Karyotypes of *M. domesticus* from Bozcaada (A) and *M. macedonicus* from Marmara Island (B).

Karyotype of *Apodemus sylvaticus* from Gökçeada and Marmara Island and *Apodemus witherbyi* populations in Bozcaada included $2n = 48$ chromosomes. Fundamental number of chromosomal arms of two species was $NF = 48$. All of diploid chromosomal set involving sexual chromosomes was formed by both large and small acrocentric chromosomes. Of the sexual chromosomes, X was larger than Y in all populations (Figure 3).

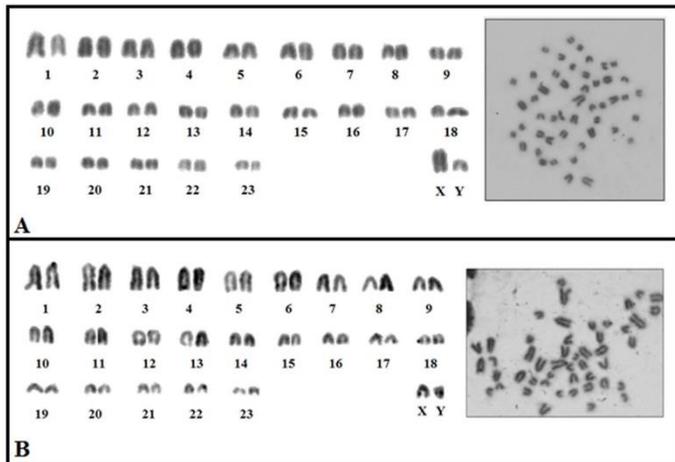


Figure 3. Obtained karyotypes of *A. sylvaticus* from Gökçeada (A) and *A. witherbyi* from Bozcaada (B).

All samples examined of *Rattus rattus* from Gökçeada and Marmara Island was male, while, samples from Bozcaada were female. A total of $2n = 38$ chromosomes and $NF = 60$ constructed the diploid chromosomal set in *R. rattus* populations from three islands. Diploid chromosome set could be investigated in three section due to having morphologically different shape and centromeric site of chromosomes. The first section consisted of 18 metacentric and submetacentric chromosomes (marked with the numbers 1, 3 and from 13 to 17). 4 submetacentric chromosomes (number 2 and 10) found in the second section. Remaining 16 acrocentric chromosomes (referred to the number from 4 to 9 and 11 to 18) created the third section. X chromosome was bigger acrocentric, while Y was smaller acrocentric in the populations examined from Gökçeada and Marmara Island (Figure 4). As to Bozcaada populations, X chromosomes were smaller acrocentric (data not shown).

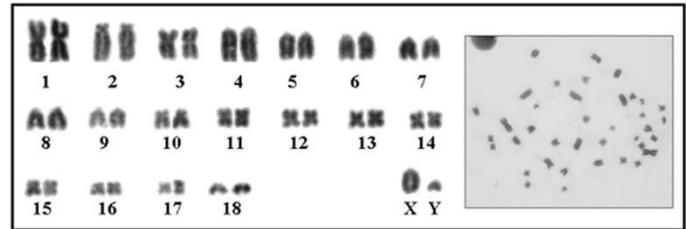


Figure 4. Standard karyotypes of *R. rattus* from Marmara Island.

DISCUSSION

This report revealed karyological features and distributional records of five different rodent species from Gökçeada, Marmara Island and Bozcaada, which were respectively the three bigger islands of Turkey with regard to their surface and these islands are included potential distribution areas of Turkish rodents. Results showed that island populations of these rodent species had the same karyotype values with the continental populations and there was no intrapopulational variation. Also, presence of *M. domesticus*, *M. macedonicus*, *A. sylvaticus* and *R. rattus* in Marmara Islands were firstly determined by present study and thus, karyological and distributional contributions were provided for Turkish rodent fauna.

$2n=40$ diploid set involving wholly acrocentric chromosomes is determined as a characteristic karyotype for *Mus domesticus* and *Mus macedonicus* in different parts of their distribution areas, although Robertsonian fusion leads to some karyological variations in this genus (Zima and Kral 1984; Gündüz et al. 2000a, Arslan and Zima 2014). Standard karyotype including 40 acrocentric chromosomes has been reported for *M. domesticus* and *M. macedonicus* from Central Anatolia, Western Black Sea and Eastern Anatolia in Turkey (Gündüz et al. 2000b; Gözcelioğlu et al. 2005; Yiğit et al. 2006). However, the karyotype with 38 chromosomes including a metacentric chromosome pair arising from Robertsonian fusion has been detected in a population of *M. macedonicus* from Western Anatolia (Gündüz et al. 2000a). Arslan and Zima (2014) have stated that elaborative researches based on large scale geographic sampling are required to corroborate or refuse possible chromosomal variations in the karyotype of the house mouse. When this statement is considered, this study focusing on island populations of these two species are important in terms of providing

karyological data. The karyotypes revealed by this study of island populations of *M. domesticus* and *M. macedonicus* are consistent with the previously reported karyotypes of Anatolian and Thrace populations belonging to the these two species and intrapopulation variation is not observed, although those islands have been separated from the mainland about 11-7 kyr ago (Şengör and Yılmaz 1981; Oral et al. 1995, Gökaşan et al. 1997) and Aegean and Marmara seas have function as geographical barriers. This may mainly be resulting from an interaction between island and mainland populations by lasting transport of this rodent by human to those islands.

Karyological aspects of different species of the genus *Apodemus* were examined and $2n=48$ standard karyotypes with different NF values (48 for *A. flavicollis*, *A. sylvaticus*, and *A. witherbyi*, 52 for *A. mystacinus* and 56 for *A. agrarius*) were defined within this genus by several researchers in Turkey (Çolak 2003; Kefelioğlu et al. 2003; Çolak et al. 2004; Çolak et al. 2005, Yiğit et al. 2006). However, karyological information for *A. uralensis* from Turkey has not been available yet. Obtained samples of *A. sylvaticus* from Gökçeada and Marmara Island and *A. witherbyi* from Bozcaada, which are identified by using their mitochondrial DNA sequence data, have also $2n = 48$ and $NF = 48$ standard karyotype values and show no differences from previously investigated samples in others parts of Turkey. Therefore, it seems that there is no chromosomal variation between island and continental populations of these species and it can be said that Turkey including islands hosts chromosomally highly stabile *Apodemus* populations. However, comprehensive and comparative studies using chromosome banding techniques may be more useful for revealing possible chromosomal variations in the genus *Apodemus* as it has been stated by Arslan and Zima (2014).

The karyotype value $2n = 38$ and $NF = 60$ which is considered to be oceanic type (Yosida 1980a; Baverstock et al. 1983) have been given for the *Rattus rattus* populations from both Anatolia and Thrace, comprising İğneada (Kırklareli) (Yiğit et al. 1998). In addition to this, certain variations arising from pericentric inversion and supernumerary b chromosome have been observed in

the Thracian populations of this species, including Gelibolu peninsula (Çanakkale) and Pınarhisar (Kırklareli) (Kankılıç et al. 2006). Results of current study determined that $2n = 38$ and $NF = 60$ karyotype value compose of diploid chromosomal set for *R. rattus* populations distributed in all examined islands. Investigation areas are known to be geographically linked to Western Anatolia and Thrace in the near past (Şengör and Yılmaz 1981; Oral et al. 1995, Gökaşan et al. 1997). However, neither number nor morphological aspects of chromosomes in the populations from three islands show any differences from Western Anatolia and Thrace. Turkey has been situated in the possible expansion route of this rodent from Indian Peninsula, which is known origin center of *R. rattus*, to Western Europe. Although some chromosomal variation may be occurred in Thracian populations which are vulnerable to interaction with other populations in the mainland of Europe, karyological structure of *R. rattus* populations in islands may have been remained invariably because of fixation arising from isolation by the Aegean and Marmara Seas. Such chromosomal variations suggested by Kankılıç et al. (2006) have been not found in Anatolian populations and this situation has increased being fixation probability of *R. rattus* populations in islands.

Variable topography, different climate and geomorphological conglomeration process of Turkey has caused intraspecific variations for both animal and plant species. A total of 68 rodent species live in Turkey and most of them have chromosomal variations connected with their geographic distributions (Yiğit et al. 2006; Gündüz et al. 2007; Yiğit et al. 2016). This study aiming to determination of possible chromosomal variations of island populations of some rodent species indicated that Gökçeada, Marmara Island and Bozcaada populations of those rodents have no differences in their karyotypes, although those islands are disconnected with the mainland of Turkey (both Thrace and Anatolia) and Aegean and Marmara seas served as geographic barriers between these islands and mainland for a long time. Morphologic features, karyological aspects (only *Nannospalax xanthodon*) and distribution records of some rodent species Gökçeada and Bozcaada has been previously reported (Özkan 1999; Kryštufek and Vohralík

2005; Kryštufek and Vohralík 2009; Sözen et al. 2013). However, up to date, there has been no information about the rodents living in Marmara Island. In addition to the their karyotypes, distribution records of *M. domesticus*, *M. macedonicus*, *A. sylvaticus* and *R. rattus* from Marmara Islands were firstly determined by this study.

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