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Electrophoretic Aspects of Blood Serum Proteins of the Genus *Apodemus* in the Black Sea Region

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Abstract: Globulin and albumin proteins of *Apodemus* populations in the Black Sea region were examined using SDS-PAGE (sodium dodecyl sulphate - polyacrylamide gel electrophoresis). There were 7-11 bands in the globulin region and 1-3 bands in the prealbumin zone. It was determined that there were variations in both the globulin region and the prealbumin zone in populations of this genus. A band was observed in the postalbumin zone with different electrophoretic mobilities including slow and fast. This different mobility in the postalbumin zone separated *Apodemus* specimens into two different populations in sympatry. These findings showed that at least two populations of the genus *Apodemus* live in the Black Sea region instead of one.

Key Words: *Apodemus*, albumin, globulin, SDS-PAGE, Black Sea region

Karadeniz Bölgesi'ndeki *Apodemus* Cinsi (Mammalia:Rodentia)'nin Kan Serum Proteinlerinin Elektroforetik Özellikleri

Özet: Bu çalışmada, Karadeniz Bölgesindeki *Apodemus* popülasyonlarının albumin ve globulin proteinleri SDS-PAGE (sodium dodecyl sulphate - polyacrylamide gel electrophoresis) tekniğine dayanarak incelendi. Globulin bölgesinde 7-11 bant ve prealbumin zonunda ise 1-3 bant gözlemlendi. *Apodemus* cinsi popülasyonlarında hem globulin bölgesinde hem de prealbumin zonunda varyasyonlar belirlendi. Postalbumin zonunda yavaş ve hızlı olmak üzere farklı elektroforetik mobiliteye sahip tek bir bantın varlığı ortaya çıkarıldı. Postalbumin zonundaki bu farklı mobilite *Apodemus* örneklerini simpatrik olarak yaşayan iki farklı popülasyona ayırdı. Elde edilen bulgular Karadeniz bölgesinde *Apodemus* cinsine ait bir popülasyonun değil iki popülasyonun yaşadığını ortaya koydu.

Anahtar Sözcükler: *Apodemus*, albumin, globulin, SDS-PAGE, Karadeniz Bölgesi

Introduction

Serum proteins and their clinical importance in both humans and mice (*Mus musculus*) have been studied intensively (1,2). Electrophoretic techniques have permitted the separation of major groups such as albumin and globulin in serum (2).

Four species of the genus *Apodemus* (*Apodemus mystacinus*, *Apodemus sylvaticus*, *Apodemus flavicollis*, and *Apodemus agrarius*) are reported from Turkey (3,4). On the basis of their morphological, biometric and allozyme aspects, *Apodemus hermonensis* and *Apodemus uralensis* were recently recorded by Filippucci et al. (5) from western Anatolia. Fraguédakis-Tsolis et al. (6), and Fraguédakis-Tsolis and Chondropoulos (7) have examined the electrophoretic aspects of the blood serum proteins of *A. sylvaticus* in Greece and reported that this species is different from *Mus musculus* and *Pitymys tatraicus* based

on different electrophoretic bands. In respect to blood serum proteins, Verimli et al. have determined differences between *A. mystacinus* and *A. agrarius* (8), and also separated *A. flavicollis* from *A. hermonensis* in Turkey (9). Electrophoresis has permitted the verification of the validity of morphological characters to be diagnostic (10). Electrophoretic analysis has also allowed the detection of sibling species and the clarification of the taxonomic position of several *Apodemus* taxa.

Because of biogeographical aspects, the Black Sea region, especially the eastern part, is a very important region of Turkey. Plant elements of Caucasia occupy the area ranging from Giresun to Caucasia; European ones live from Giresun towards the west (11). This condition may have affected the faunistic structure of the Black Sea region. It is very difficult to distinguish morphologically specimens of *Apodemus* in the eastern Black Sea region.

Therefore, this study was conducted on the genetic structure of populations of the genus *Apodemus* that have adapted to forest areas of the Black Sea region, except *A. mystacinus*, which inhabits rocky areas.

This paper is one of the works focused on blood serum proteins of Turkish rodents, and presents electrophoretic aspects of blood serum proteins, and contributes to the genetic structure of the genus *Apodemus* (excluding *A. mystacinus*) in the Black Sea region.

Materials and Methods

A total of 56 live specimens collected from Hopa and Ardanuç (Artvin); Ayder and İkizdere (Rize); Altındere and Sümela (Trabzon); Bulancak and Tirebolu (Giresun); Akkuş and Efirli (Ordu); and Samsun were used for electrophoretic analysis (Fig. 1).

Blood was taken by cardiac puncture from the animals, which were anaesthetised with ether. After blood clotting, the sera were separated and centrifuged at 12000 rpm for 3 min. The sera were then mixed with a buffer containing 0.0625 M Tris Cl, pH 6.8, 2% SDS, 10% Glycerol, 5% 2-Mercaptoethanol and 0.01% bromophenol blue (12), and the final concentration of sera was adjusted to 5%. Samples were boiled for 3 min and stored at -70°C until electrophoresis. The amount of protein loaded to gel was determined qualitatively following the method of Esen (13). Samples of 12 to 20 μl were applied to gels in different experiments. Electrophoresis was carried out using a Consort E 863 model vertical slab gel electrophoresis apparatus. SDS-polyacrylamide denaturing gels, separating gels (7.5%) and stacking gels (4%) were prepared as described by Sambrook *et al.* (14). Electrode buffer solution contained 0.025 M Tris, 0.192 M Glycine, and 0.1% SDS at pH 8.3 (12). Molecular Weight Marker (Sigma MW-SDS-200) consists of carbonic anhydrase (29,000), egg albumin (45,000), bovine albumin (66,000), phosphorylase B (97,400), and β -galactosidase (116,000), myosin (205,000).

Constant voltage (8 V/cm) was applied for the stacking gel. After the tracing of dye reached the separating gel, the voltage was adjusted to 15 V/cm and the gel was run until the bromophenol blue reached the bottom of the resolving gel (about 3 hours). After electrophoresis, gels were stained with 0.25% Coomassie

Brilliant Blue R250 in 90 ml of methanol: water (1:1 v/v) and 10 ml glacial acetic acid and destained in methanol:water:acetic acid (45:45:10) (14).

Results

Two different populations of the genus *Apodemus* were determined in the Black Sea region using SDS-PAGE aspects of blood serum proteins, especially fast and slow bands of postalbumin. The allele frequencies of fast and slow bands in the postalbumin zone are shown in Table 1. These populations inhabit in sympatry in many localities in the Black Sea region.

Population I: The postalbumin zone showed the slow pattern of the electrophoretic band of *Apodemus* specimens collected from Artvin, Rize, Trabzon, Giresun, Ordu and Samsun. The molecular weight of slow postalbumin protein was 87,000 (electrophoretic mobility $R_f = 0.38$). The number of bands in the globulin zone of population I ranged from 7 to 10. Prealbumin showed 1-3 electrophoretic bands (Table 2, Figs. 2-7).

Population II: The postalbumin zone showed the fast pattern of the electrophoretic band of *Apodemus* specimens collected from Artvin, Rize, Trabzon, Ordu and Samsun. The molecular weight of the fast postalbumin protein was 83,000 ($R_f = 0.35$). The band number in the globulin zone of population II ranged from 7 to 11. Prealbumin showed 1-3 electrophoretic bands (Table 2, Figs. 2-6).

Both populations live in the localities Artvin, Rize, Trabzon, Ordu and Samsun. In Giresun only population I is found. Two male specimens were caught in Giresun (Bulancak). Two electrophoretic bands in the postalbumin zone of one (no: 2313) of these specimens were observed (Fig. 7). This was observed only in one out of 56 specimens examined. Morphological comparisons also showed a significant difference between two specimens from Bulancak. This indicates heterozygosity.

Discussion

No albumin polymorphisms were observed among 56 individuals of *Apodemus* populations studied in the Black Sea region. However, Fragedakis-Tsolis *et al.* (6) reported that there is one band in the prealbumin zone and two bands in the albumin zone in *A. flavicollis*, one prealbumin band and one albumin band in *A. sylvaticus*,

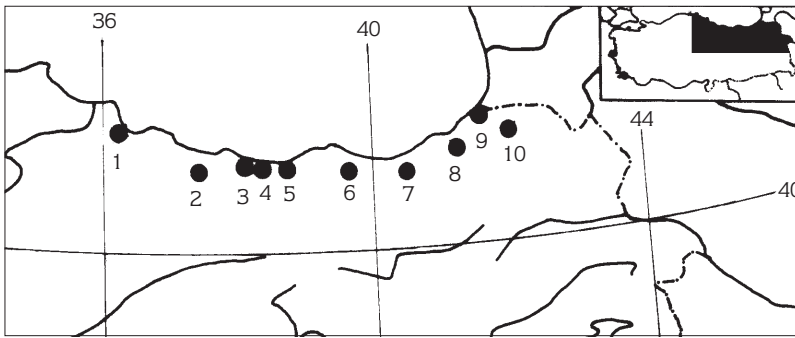


Figure 1. The map showing the localities of the specimens examined.

1. Samsun, 2. Akkuş (Ordu), 3. Efirli (Ordu), 4. Bulancak (Giresun), 5. Tirebolu (Giresun), 6. Sümela, Altindere (Trabzon), 7. Ayder (Rize), 8. İkizdere (Rize), 9. Hopa (Artvin), 10. Ardanuç (Artvin).

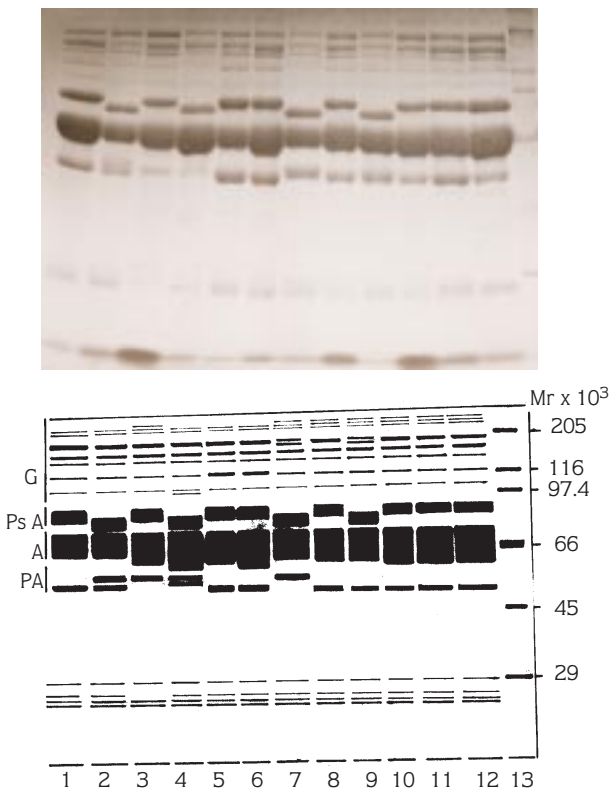


Figure 2. SDS-PAGE patterns of serum proteins of *Apodemus* from Hopa (Artvin, 1-6) and Ardanuç (Artvin, 7-12). G: Globulin, PsA: Postalbumin, A: Albumin, PA: Prealbumin, Mr: Marker (13).

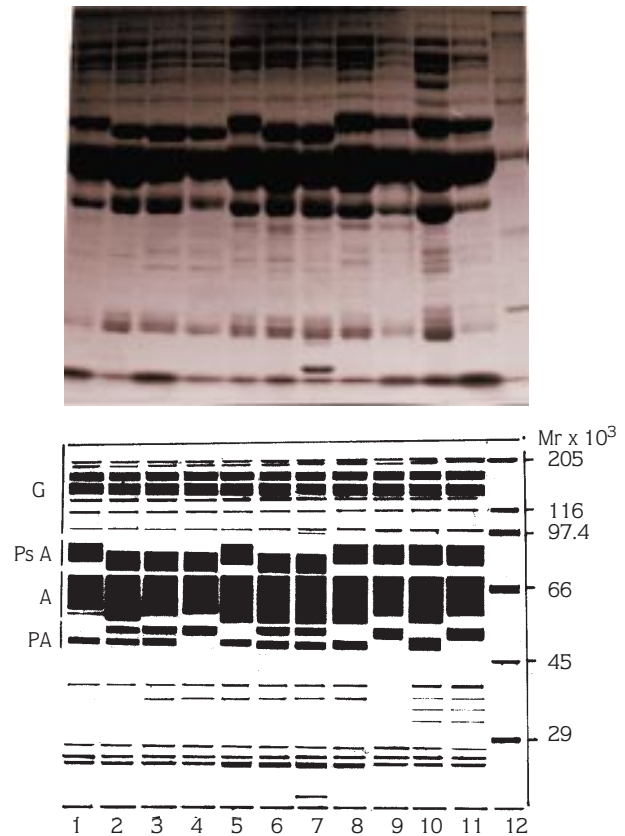


Figure 3. SDS-PAGE patterns of serum proteins of *Apodemus* from İkizdere (Rize, 1-8) and Tirebolu (Giresun, 9-11). G: Globulin, PsA: Postalbumin, A: Albumin, PA: Prealbumin, Mr: Marker (12).

Table 1. Allel frequencies of postalbumin (PsA) of the genus *Apodemus* populations in the Black Sea region. N = number of specimens

		Samsun (n = 4)	Ordu (n = 6)	Giresun (n = 5)	Trabzon (n = 14)	Rize (n = 13)	Artvin (n = 14)
PsA	Fast	0.50	0.17	0.10	0.50	0.77	0.29
	Slow	0.50	0.83	0.90	0.50	0.23	0.71

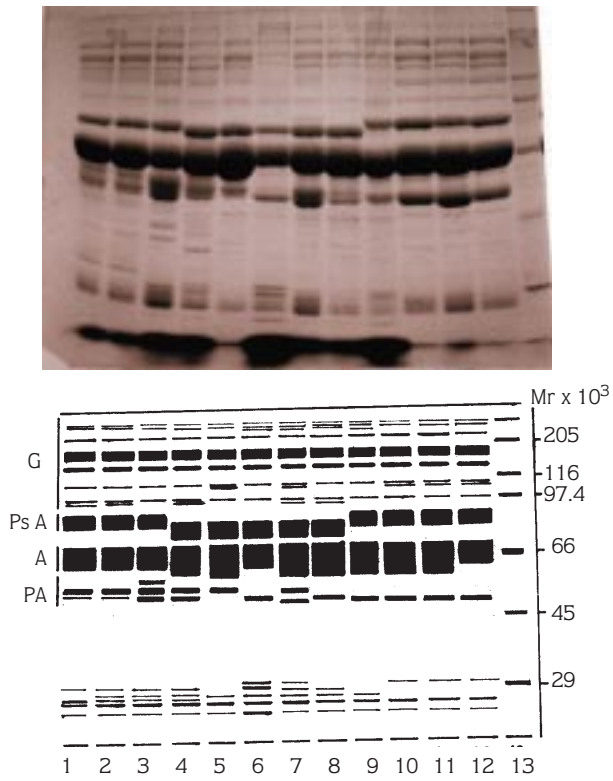


Figure 4. SDS-PAGE patterns of serum proteins of *Apodemus* from Sümela (Trabzon, 1-8) and Efirli (Ordu, 9-12). G: Globulin, PsA: Postalbumin, A: Albumin, PA: Prealbumin, Mr: Marker (13)

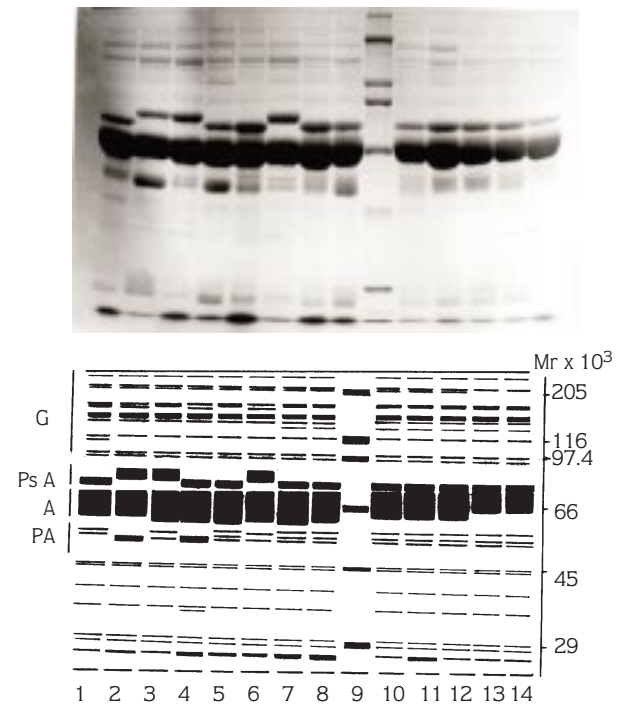


Figure 5. SDS-PAGE patterns of serum proteins of *Apodemus* from Samsun (1-4), Akkuş (Ordu, 5-6), Sümela (Trabzon, 7-8), Ayder (Rize, 10-11) and İkizdere (Rize, 12-14). G: Globulin, PsA: Postalbumin, A: Albumin, PA: Prealbumin, Mr: Marker (9).

Table 2. Distribution of globulin, prealbumin and postalbumin proteins in population I and population II in the Black Sea region. N. Number of specimens, BN. Number of bands, PsA. Postalbumin

Locality	Population I PsA Slow (n)	Globulin		Prealbumin		Population II PsA Fast (n)	Globulin		Prealbumin	
		N	BN	N	BN		N	BN	N	BN
Artvin	10	2	7	9	1	4	1	8	2	1
		6	8	1	2		3	9	2	2
		2	9							
Rize	3	3	7	3	1	10	4	7	1	1
							1	8	4	2
							3	10	5	3
						2	9			
Trabzon	7	1	7	2	1	7	3	8	3	1
		2	8	4	2		1	9	2	2
		3	9	1	3		3	10	2	3
		1	10							
Giresun	4	3	7	3	1	-	-	-	-	
Ordu	5	4	8	4	1	1	1	9	1	3
		1	10	1	2					
Samsun	2	1	9	1	1	2	1	10	1	1
		1	10	1	2		1	11	1	2

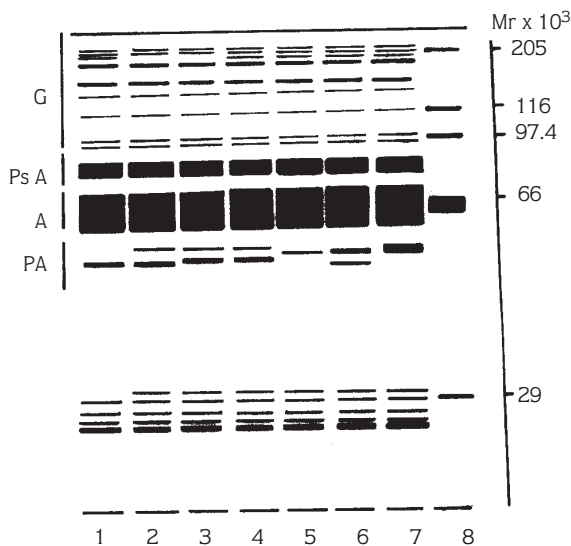
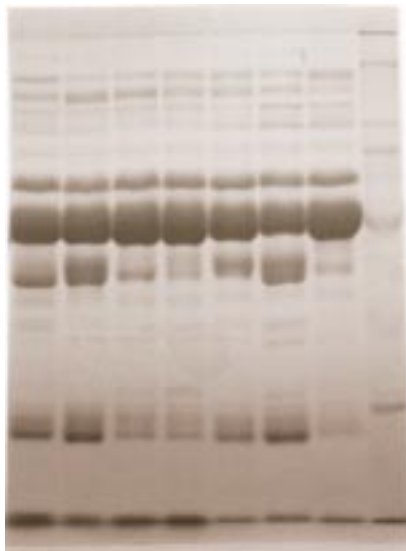


Figure 6. SDS-PAGE patterns of serum proteins of *Apodemus* from Ardanoç (Artvin, 1-2), Altindere (Trabzon, 3-4), Sümela (Trabzon, 5-7). G: Globulin, PsA: Postalbumin, A: Albumin, PA: Prealbumin, Mr: Marker (8).

and a fast prealbumin and two albumin bands in *A. mystacinus*. These results show that the electrophoretic features of the prealbumin and albumin proteins of three *Apodemus* species in Greece are different from those of *Apodemus* populations in the Black Sea region.

According to Fragedakis-Tsolis and Chondropoulos (7), one prealbumin zone is constantly present in *A. flavicollis*, and there are two albumin zones in *A. flavicollis*. A comparison of the albumin and prealbumin

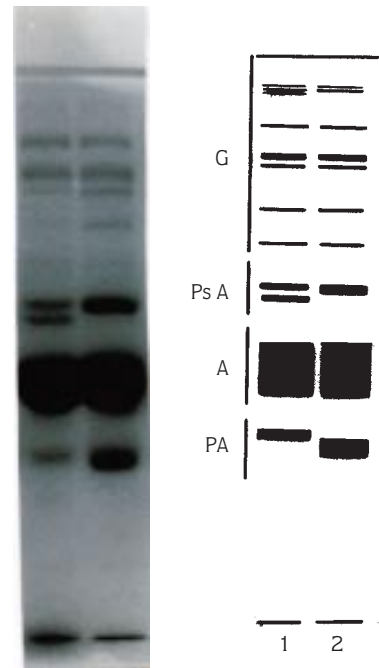


Figure 7. SDS-PAGE patterns of serum proteins of two *Apodemus* specimens from Bulancak (Giresun, 1,2). G: Globulin, PsA: Postalbumin, A: Albumin, PA: Prealbumin.

proteins of *Apodemus* populations in the Black Sea region with those of *A. flavicollis* in Greece showed that our populations differ from *A. flavicollis* in Greece on the basis of both prealbumin and albumin proteins.

Gemmeke et al. (15) have identified 1-5 electrophoretic bands in the postalbumin zone of 21 populations of European *A. sylvaticus*, whereas the present study indicates one electrophoretic band in the postalbumin zone of *Apodemus* specimens in Black Sea region.

Verimli et al. (8) have determined 2-4 bands in the prealbumin zone and 7-8 bands in the globulin zone of *A. mystacinus*. In this study, 1-3 bands in the prealbumin zone and 7-11 bands in the globulin zone of *Apodemus* specimens in the Black Sea region were observed. This variation is consistent with the findings of Verimli et al. (8) for *A. mystacinus*.

According to Verimli et al. (9), *A. flavicollis* differs from *A. hermonensis* on the basis of SDS-PAGE patterns of blood serum proteins of specimens collected from Beyşehir, Burdur, Samsun and Akkuş (Ordu). They also recorded a slow band in the postalbumin of *A. flavicollis* and a fast one in that of *A. hermonensis*. The findings of

our study is the same as those of Verimli et al. (9). These findings showed that two different populations of *Apodemus* live in the Black Sea region. Also, according to Verimli et al. (9), there are 6-9 bands in the globulin zone of *A. flavicollis*, and 7-9 bands in that of *A. hermonensis* in Turkey. In our study, 7-11 bands in the globulin zone were observed in the blood serum of *Apodemus* specimens in the Black Sea region.

According to Reuter and Kennes (16), on the basis of the horizontal starch gel electrophoresis technique, the electrophoretic pattern of female *Mus* is significantly different from that of the males with respect to the prealbumin zone, due to the presence of three bands in the prealbumin zone. In contrast, SDS-PAGE did not reveal any difference between males and females in *Apodemus* specimens in the Black Sea region. Pantelouris and Hale (17) stated that plasma from adult female mice at various stages of pregnancy produced 21 bands on starch gel after electrophoresis. In the present study, proteins with a molecular weight smaller than 45,000 were not fully marked on the gel after SDS-PAGE on the serum of *Apodemus* populations in the Black Sea region. Therefore, these protein bands were not evaluated.

In population I, there is a slow band in the postalbumin zone with electrophoretic mobility $R_f = 0.38$. There are 1-3 bands in the prealbumin zone and 7-10 bands in the globulin zone with different, stronger electrophoretic mobilities. In population II, there is a fast band in the postalbumin zone with $R_f = 0.35$. There are 1-3 bands in the prealbumin zone and 7-11 bands in the

globulin zone with different, stronger electrophoretic mobilities. A comparison of population I with population II shows that these two populations are distinguishable with respect to electrophoretic postalbumin patterns.

Neuhauser (18) described *A. flavicollis saturatus* from İzkidere (Rize), and Dođramacı (3) divided populations of *A. flavicollis* into two subspecies (*A. f. saturatus* ranging from Trabzon to the east, and *A. f. brauneri* extending from Trabzon to the west). Also, on the basis of morphological and biometrical aspects, Dođramacı (3) has separated *Apodemus* specimens collected from İzkidere (Rize) and Meryemana (Trabzon) into three populations (73% hybrid, 10% one group and 17% another group). This study did not indicate any hybrid condition between specimens of *Apodemus* in Black Sea region. However, SDS-PAGE on the basis of blood serum proteins of *Apodemus* specimens in the Black Sea region shows that two populations inhabit İzkidere and Meryemana, as reported by Dođramacı (3). Further studies are required to clarify the taxonomic status of these populations.

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