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Morphometric, Karyotypic and Electrophoretic Analysis of the Genus *Apodemus* Kaup, 1826 (Mammalia: Rodentia) in Thrace

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Abstract: Thirty-two specimens of *Apodemus* in Thrace were evaluated. Ten specimens of *A. flavicollis* and *A. sylvaticus* were karyotyped. Eighteen specimens were electrophoresed to determine patterns of the esterase enzyme system. Morphometric analysis showed that *A. flavicollis* and *A. sylvaticus* were the closest species in a comparison between *A. agrarius*. *A. flavicollis* and *A. sylvaticus* with the same karyotypic values of 2n = 48, Nfa = 46, and NF = 48. Patterns of the esterase enzyme system clearly separated *A. flavicollis*, *A. sylvaticus* and *A. agrarius*.

Key Words: *Apodemus*, Morphometry, Karyotype, Esterase, Thrace

Trakya'daki *Apodemus* Kaup, 1826 (Mammalia: Rodentia) Cinsinin Morfometrik, Karyolojik ve Elektroforetik Analizi

Özet: Trakya'dan toplanan 32 *Apodemus* örneği bu çalışmada değerlendirilmiştir. *A. flavicollis* and *A. sylvaticus*'a ait 10 örnek karyolojik çalışmalarda kullanılmıştır. Esteraz enzim sistemi özelliklerinin ortaya konması için 18 örnek elektroforez çalışmalarında kullanılmıştır. Morfometrik analizler *A. flavicollis* ve *A. sylvaticus*'un *A. agrarius*'a göre yakın türler olduğunu ortaya koymuş olup *A. flavicollis* ve *A. sylvaticus* 2n = 48, Nfa = 46, ve NF = 48'lik aynı karyotipik değerlere sahiptir. Esteraz enzim sistemi özellikleri *A. flavicollis*, *A. sylvaticus* ve *A. agrarius*'u belirgin olarak ayırmaktadır.

Anahtar Sözcükler: *Apodemus*, Morfometri, Karyotip, Esteraz, Trakya

Introduction

Six species of the genus *Apodemus* were recorded from Turkey: *A. sylvaticus*, *A. flavicollis*, *A. mystacinus*, *A. iconicus*, *A. uralensis*, *A. hermonensis* and *A. agrarius* (Ellerman and Morrison-Scott, 1951; Corbet, 1978; Musser and Carleton, 1993; Filippucci et al, 1996; Macholán et al, 2001; Kryštufek and Vohralík, 2001, Kry štufek, 2002). Distribution of *Apodemus* species, especially *A. sylvaticus* and *A. flavicolis*, in both Anatolia and Thrace is problematic. Özkan and Kryštufek (1999) recorded *A. sylvaticus* from Thrace, along with *A. flavicollis*, in accordance with morphological aspects. According to Filippucci (1992), morphological characters do not allow identification of *A. sylvaticus* and *A. flavicollis*, because of the morphological convergence of the 2 species, but electrophoretic analysis allows the detection of sibling species. Although Filippucci et al. (1996) recorded *A. sylvaticus* and *A. flavicollis* from Anatolia with morphological and biochemical identification, Macholán et al. (2001) indicated that the distribution of *A. sylvaticus* is doubtful in Anatolia. Although morphological identification of *A. flavicollis* and *A. hermonensis* is difficult, Verimli et al. (2001) separated *A. hermonensis* from *A. flavicollis* with respect to electrophoresis of blood serum proteins. According to Macholán et al. (1999), the esterase enzyme system shows different patterns in *Sorex satunini* (Turkey) and *S. araneus* (Balkan). The esterase enzyme system may be a distinguishing molecular character for the sibling species *A. flavicollis* and *A. sylvaticus*.

The aim of this study is to contribute to taxonomy, population genetics of the genus *Apodemus* species in Thrace.

Materials and Methods

In this study, 28 morphometric characters of 32 specimens of the genus *Apodemus* from 5 localities in

Thrace were analysed, and 18 specimens from 4 localities were electrophoresed (Table 1). Three species *A. flavicollis*, *A. sylvaticus* and *A. agrarius* were identified based on morphological characteristics such as the pectoral spot, the posterior end of the palatal bone, pterygoid process, fronto-parietal suture, upper molar crown patterns, and tympanic bullae given by Filippucci et al. (1996) and Kryštufek (2002). Ten specimens of *A. flavicollis* and *A. sylvaticus* were karyotyped in accordance with Ford and Hamerton (1956). Adult specimens were used in morphometrical evaluations. In the morphometric analysis measurements of 28 metric characters were used. Measurements of 23 metric characters of the skull were taken with calipers to the nearest 0.01 mm, or under a binocular microscope. NTSYS-pc (Version 1.8) package was used for statistical analyses (Rolf, 1994). The characters are: Total length (TL), Tail length (T), Hind foot (HF), Ear (E), Zygomatic breadth (ZB), Interorbital constriction (IC), Condylobasal length (CBL), Occipito-nasal length (ONL), Basilar length (BL), Nasal length (NL), Nasal width (NW), Facial length of braincase (FLB), Braincase length (BCL), Mastoid breadth (MB), Height of braincase with tympanic bulla (HBB), Height of braincase without tympanic bulla (HB), Occipital width (OW), Braincase width (BW), Diastema (D), Palatal length (PL), Foramina incisive length (FI), Tympanic bulla length (TB), Mandible length (ML), Length of upper toothrow alveoli (LUTa), Length of upper toothrow cusp (LUT), Length of lower toothrow alveoli (LLTa) and Length of lower toothrow cusp (LLT).

The esterase enzyme system (EST, E.C. 3.1.1.1) was screened. Muscle extracts were used for this enzyme system. Tissues were kept at –70 °C until use. Electrophoresis was performed with gels composed of 11% hydrolysed starch. References of buffer systems and staining procedures used and electrophoretic running conditions are listed in Table 2.

Results

Thirty-two specimens of *Apodemus* in Thrace were evaluated (Table 1, Figure 1). Specimens of *A. flavicollis* and *A. agrarius* were collected from deciduous forests at Velikaköprüsü (Demirköy) and İğneada. *A. sylvaticus* was recorded from destroyed areas at the edge of forests, and from cultivated areas.

Morphometry

Twenty-eight character measurements (Table 3) of 32 *Apodemus* species were used in morphometrical evaluations. NTSYS-pc formed 2 groups. The first group is *A. flavicollis-A. sylvaticus* and the second group is *A. agrarius* (Figure 2)

Table 1. Number of specimens of *Apodemus* analysed in Thrace.

Species	Locality	Morphometric Evaluation (n)	Electrophoretic Evaluation (n)
Apodemus sylvaticus	Edirne	7	1
	Büyükkarıştıran (Tekirdağ)	2	2
	Pınarhısar (Kırklareli)	3	
Apodemus flavicollis	Velikaköprüsü (Demirköy)	11	10
	Edirne	2	
Apodemus agrarius	İğneada (Kırklareli)	6	5
	Velikaköprüsü	1	

Table 2. Buffer and staining procedure of esterase enzyme analysis.

Electrode buffer	Gel buffer	References of buffer	Electrophoretic conditions	References of histochemical stain
300 mM boric acid, 60 mM NaOH, pH: 8.2	76 mM Tris, 5 mM Citric acid pH: 8.65	Ayala et al. 1972	8 V / cm 5 h	Hillis and Moritz, 1990

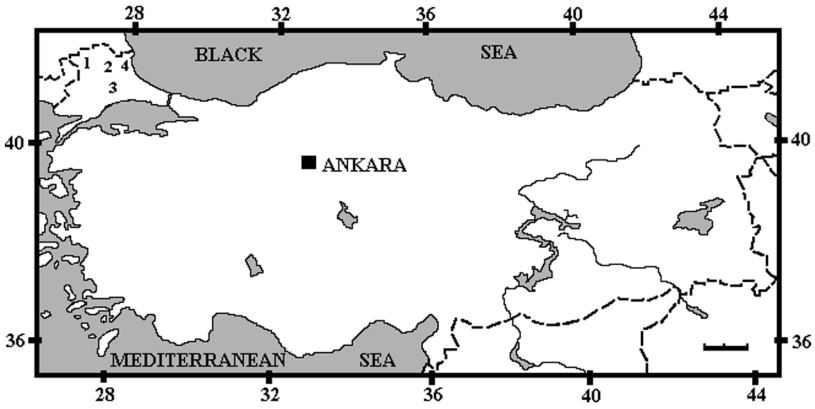

Figure 1. Map showing the localities of *Apodemus* specimens examined in Thrace. 1. Edirne, 2. Pınarhisar, 3. Büyükkarıştıran, 4. Velikaköprüsü and İğneada.

Table 3. Cranial and body measurements of *A. flavicollis, A. sylvaticus* and *A. agrarius* in Thrace. See text for abbreviations.

	A. flavicollis			*A. sylvaticus*			*A. agrarius*		
Characters	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
TL	195	231	204	172.0	202.0	183	171	181	174.00
T	93	112	103	66.0	100.0	84.67	75.0	80.0	77.25
HF	24	27	25	24.0	25.0	24.71	21.0	22.0	21.40
E	17	21	18.5	16.0	20.0	17.57	14.0	15.0	14.20
W	21	38	28.7	18.0	35.0	25.43	20.0	30.0	25.20
ZB	12.4	14.7	13.4	12.0	13.8	13.00	12.0	12.4	12.20
IC	4	4.9	4.35	4.1	4.5	4.29	4.2	5.0	4.62
CBL	22.8	26.3	24.3	22.2	24.8	23.41	23.0	24.2	23.45
ONL	25.4	29.9	27.1	25.8	27.4	26.47	25.1	26.1	25.35
BL	20.4	24.5	22.4	21.0	23.1	21.74	21.0	22.0	21.55
NL	8.9	11	10.04	9.5	10.5	9.96	9.0	9.9	9.56
NW	2.7	3.8	3.25	3.0	3.4	3.11	3.2	3.9	3.56
FLB	12.8	15.1	14	12.5	14.3	13.50	13.3	13.8	13.52
BCL	11.6	13.2	12.25	11.5	12.2	11.91	11.8	12.2	11.98
MB	6.1	7.2	6.6	6.1	6.9	6.64	6.1	6.2	6.15
HBB	9	10.1	9.5	8.8	9.4	9.17	8.4	9.0	8.70
HB	7.9	8.9	8.24	7.5	8.1	7.86	7.5	7.8	7.68
OW	11	12	11.33	10.6	11.5	11.21	10.5	11.2	10.90
BW	11.4	12.3	11.8	11.1	12.3	11.73	11.0	11.2	11.10
D	6.4	8	7.17	6.6	7.6	7.10	7.0	7.5	7.16
PL	10.8	12.8	11.7	11.0	12.3	11.54	11.0	11.5	11.22
FI	5.13	5.94	5.52	5.1	5.9	5.44	4.7	5.0	4.89
TB	4.86	5.4	5.22	4.6	5.4	4.94	4.7	4.7	4.73
ML	14.8	16.2	15.3	13.9	15.0	14.46	14.2	15.5	14.56
LUTa	4.32	4.86	4.57	4.1	4.6	4.36	4.3	4.3	4.30
LUT	3.78	4.05	3.96	3.5	3.8	3.59	3.5	3.7	3.62
LLTa	4.05	4.59	4.29	4.1	4.3	4.17	4.0	4.0	4.00
LLT	3.78	4.32	4.09	3.8	4.1	3.92	3.7	3.7	3.70

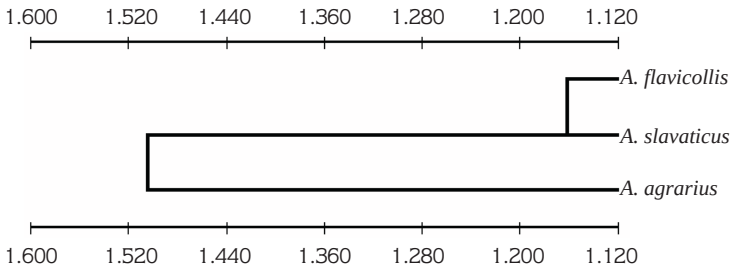

Figure 2. UPGMA dendrogram generated by NTSYS-*pc* based on 28 morphometric characters of 29 specimens of *Apodemus*.

Karyology

Apodemus flavicollis and *Apodemus* sylvaticus

The diploid number of chromosomes is 2n = 48, the number of autosomal arms is NFa = 46, and the fundamental number is NF = 48. The autosomal set contains 46 acrocentrics. The X chromosome is a large acrocentric, and the Y chromosome is a small acrocentric.

Patterns of the Esterase Enzyme System

Twelve electrophoretic bands in the esterase enzyme system of *Apodemus* specimens in Thrace were scored. On the basis of esterase banding patterns, 3 different regions were observed on the gel (Figures 3). Specimens of *A. agrarius* formed a separated banding region on the gel, differing from other the specimens. *A. sylvaticus* showed a pattern different from that of *A. flavicollis*. Eighteen specimens of *Apodemus* were analysed by NTSYS-pc, based on banding patterns of electrophoretic gel (Figure 4). The UPGMA dendrogram clearly separated *A. sylvaticus* and *A. flavicolis*. Five specimens of *A. agrarius* were divided into 2 groups. Two specimens (agra14 and agra15) with different alleles were different from other specimens of *A. agrarius*.

Discussion

Character measurements of *Apodemus* species in the present study are consistent with those given by Ellerman (1948), Ondrias (1967) and Doğramacı (1972) for *Apodemus* species.

The karyotype of *A. agrarius* was given by Yiğit et al. (2000) and Kefelioğlu et al. (2003). The karyotype of *A. sylvaticus* and *A. flavicollis* was described as 2n = 48, NF = 48, and NFa = 46 by Kral and Zima (1984) from Yugoslavia and several countries in Europe, by Gagia et al. (1985) for *A. flavicollis* from Thrace, Doğramacı and Kefelioğlu (1991) from Anatolia and Bulatova et al. (1991) from Azerbaijan. These karyological aspects are consistent with those of *A. sylvaticus* and *A. flavicollis* in Thrace. The presence of additional chromosomes seems to be characteristic for the genomes of *A. flavicollis*. Soldatovic et al. (1972) noted a karyotype of 2n = 50 chromosomes from Sara Mountain. Kral and Zima (1984) found an additional small acrocentric chromosome in *A. flavicollis* from Bulgaria. Zima and Macholán (1995) determined a karyotype of 2n = 48 chromosomes for *A. flavicollis* with B chromosomes from Asia Minor. According to Macholán and Zima (1997), specimens of *A. flavicollis* from Zonguldak have 2n=48, and there are no supernumerary chromosomes (B chromosomes) in the karyotype of *A. flavicollis*. This study obtained the same results as those of Macholán and Zima (1997). Zima et al. (1997) recorded 2n = 48, and supernumerary chromosomes in the karyotype of *A. sylvaticus* from the Czech Republic. B chromosomes were not determined in *A. sylvaticus* from Thrace. We determined an additional small acrocentric chromosome in some cells of *A. flavicollis* from Velikaköprüsü, and chromosomes of *A. flavicollis* from Velikaköprüsü were larger than those of *A. sylvaticus* from Pınarhisar.

Özkan and Kryštufek (1999) recorded *A. flavicollis* from the Istranca Mountains, and *A. sylvaticus* from Edirne. In this study, specimens from Edirne, Büyükkarıştıran (Tekirdağ), Pınarhisar (Kırklareli), Velikaköprüsü (Istranca Mountains) and İğneada (Kırklareli) were evaluated. *A. flavicollis* was determined from Velikaköprüsü (Istranca Mountains), *A. sylvaticus* from Edirne, Tekirdağ, Kırklareli, and *A. agrarius* from

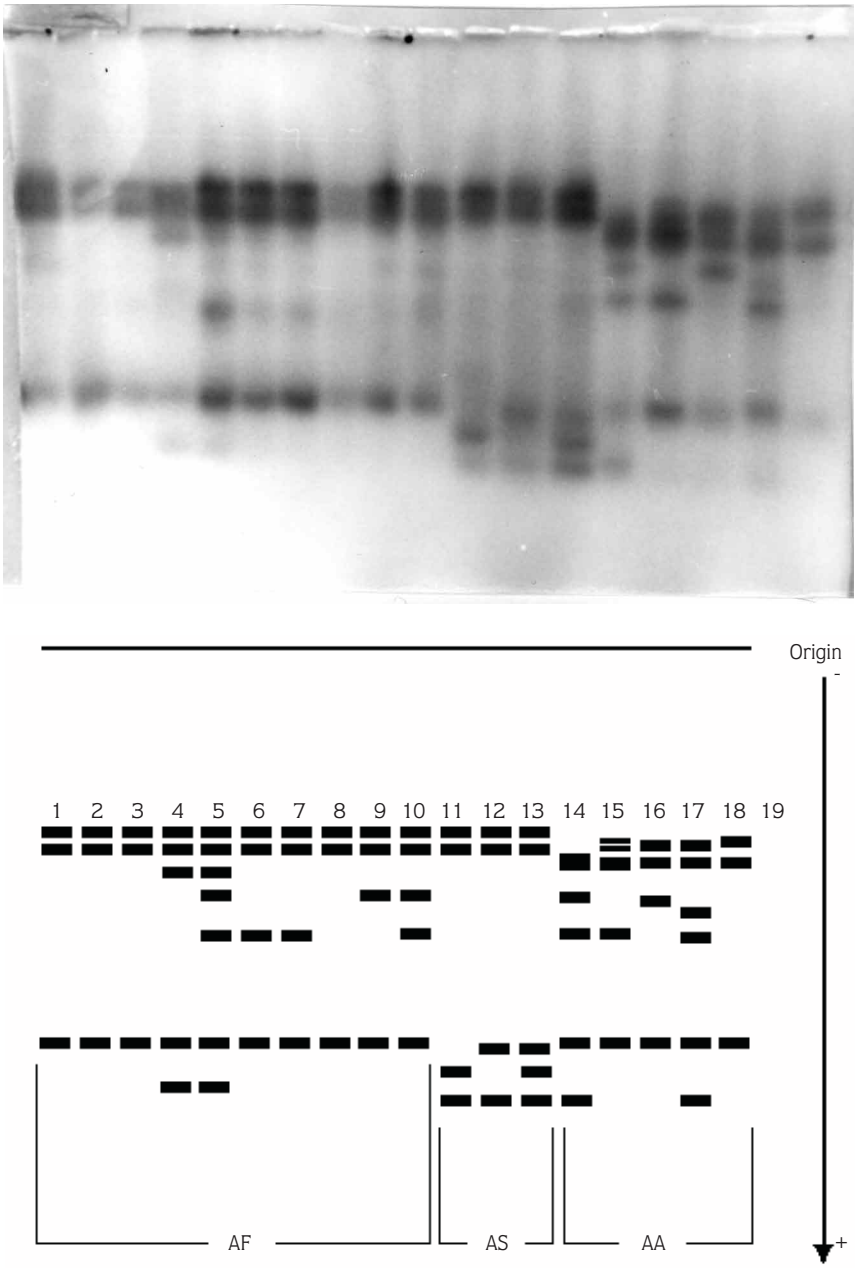

Figure 3. Zymogram (above) and its diagrammatic representation (bottom) of the gel for muscle esterase using α-naphthyl acetate as substrate. AF: *A. flavicollis*, AS: *A. sylvaticus*, AA: *A. agrarius*.

İğneada. This result is consistent with that of Özkan and Kryštufek (1999).

Filippucci (1992) determined 1-3 alleles of Est-3 in 25 populations, 1-2 alleles in 14 populations of *A. flavicollis*, and 1 allele in 2 populations of *A. agrarius*. In this study, 4-5 bands in the esterase enzyme system were observed for *A. sylvaticus*, 3-7 for *A. flavicollis*, and 3-6 for *A. agrarius*. This banding pattern in the esterase enzyme system differed in 3 *Apodemus* species in Thrace, as reported by Filippucci (1992). Macholán et al. (2001) found 1-4 alleles in the esterase enzyme system (Est-1, Est-2, Est-3) in 4 populations of *A. flavicollis* (Artvin,

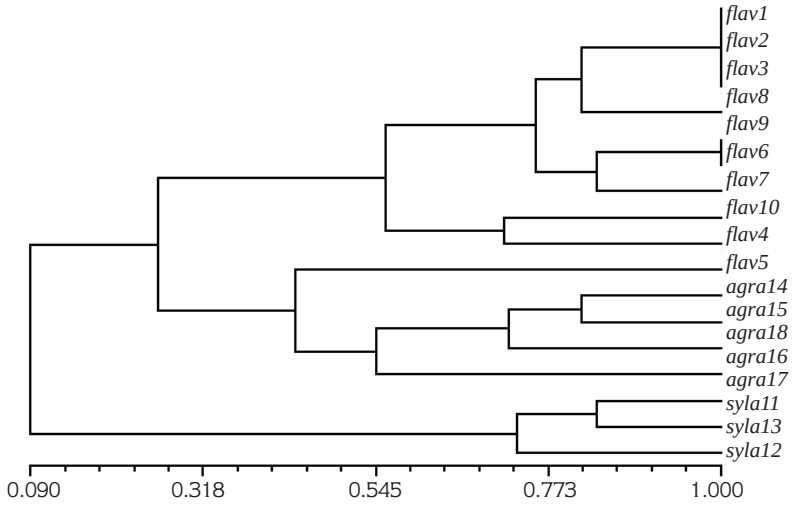

Figure 4. UPGMA dendrogram generated by NTSYS-*pc* based on 18 specimens of *Apodemus* and 12 esterase bands.

Table 4. Nonmetric values of 12 electrophoretic bands detected in esterase gel for 18 specimens of *Apodemus* in Thrace. 1-10: *A. flavicollis*, 11-13: *A. sylvaticus*, 14-18: *A. agrarius*. SN: Specimen No, BN: Band No.

SN BN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
2	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
3	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
4	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	1	0	0	0	1	1	0	0	0	1	0	1	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
7	0	0	0	0	1	1	1	0	0	1	0	0	0	1	1	0	1	0
8	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	1
9	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0
11	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	1	0

Gümüşhane, Ermenia, Iran). In contrast to Macholán et al. (2001), electrophoretic analysis gave 3-7 bands in the esterase enzyme system in the population of *A. flavicollis* from Thrace. Filippucci et al. (1989) determined 3 alleles in Est-3 of *A. sylvaticus* in Israel, and Filippucci et al. (1996) determined 3 alleles in the esterase enzyme system (Est-1, Est-2, and Est-3) in *A. sylvaticus* from Çaycuma, 4 alleles in the esterase enzyme system (Est-1, Est-2, and Est-3) from the Istranca Mountains and 3 alleles in the esterase enzyme system (Est-1, Est-2, and Est-3) in *A. flavicollis* from Anatolia. We determined 3-7 electrophoretic bands in the esterase enzyme system of *A. flavicollis*, and 4-5 bands in that of *A. sylvaticus* in Thrace.

In conclusion, morphometrics did not distinguish *A. flavicollis* from *A. sylvaticus*, whereas patterns of esterase clearly separated the sibling species *A. flavicollis* and *A. sylvaticus* as well as *A. agrarius*. The esterase enzyme system seems to be a distinguishing character for *A. flavicollis*, *A. sylvaticus* and *A. agrarius*.

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