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MORPHOMETRIC AND BIOCHEMICAL VARIATION AND THE DISTRIBUTION OF THE GENUS APODEMUS (MAMMALIA: RODENTIA) IN TURKEY

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A total of 253 specimens belonging to the genus *Apodemus* were examined from 45 localities in Turkey based on morphometric and biochemical analyses. Six different *Apodemus* species were distributed; *A. sylvaticus* was recorded only from Thrace (European part of Turkey), *A. iconicus* throughout Turkey, except Thrace and south-eastern Anatolia, *A. flavicollis* from Turkey (rare in central and eastern Anatolia), *A. uralensis* from Marmara and Black Sea Region, *A. agrarius* from the northern parts of Thrace, and *A. mystacinus* from Asiatic Turkey. Analysis of Variance (ANOVA) of the data showed a high heterogeneity among *Apodemus* species. Out of 28 morphometric variables, 27 displayed significant heterogeneity among groups ($p < 0.001$). The first three discriminant functions explained 96.6% of the total variation. *Sylvaemus* species (*A. flavicollis, A. iconicus, A.uralensis* and *A. sylvaticus*) showed overlapping distribution with each other and two other species *A. mystacinus* and *A. agrarius* were formed a separate clusters. Based on ten loci, it was determined that *Idh–1, Mdh-s,* α*-Gpdh* and *Me* were distinguishing characters for *A. sylvaticus, A. uralensis, A. flavicollis* and *A. iconicus. Idh-1, Mdh-S,* α*-Gpdh*, and *Me* were polymorphic loci, and deviated from Hardy-Weinberg equilibrium. The greatest genetic differentiation was originated from *Me*, but the *Idh* had the smallest genetic differentiation among polymorphic loci. UPGMA dendrograms showed that *A. flavicollis* was genetically the closest to *A. iconicus*, but the most distant to *A. mystacinus*.

Key words: *Apodemus*, Turkey, allozyme, distribution, genetic variation, morphometry

INTRODUCTION

The genus *Apodemus* is distributed in the Palaearctic Region, and divided into three subgenera: *Apodemus* KAUP, 1829, is distributed in an area extending from Central Europe to Eastern Asia, *Sylvaemus* OGNEV et WOROBIEV, 1923, is ranged from Western Europe to Central Asia, and *Alsomys* DUKELSKI, 1829, is present in Central Eastern Asia (ELLERMAN & MORRISON-SCOTT 1951, ZIMMERMAN 1962, CORBET 1978, MUSSER & CARLETON 1993).

Six species of the genus *Apodemus* have been recorded from Turkey: *A. sylvaticus, A. flavicollis, A. mystacinus, A. uralensis, A. hermonensis*, and *A. agrarius* (ELLERMAN & MORRISON-SCOTT 1951, STEINER 1978, FILIPPUCCI *et al*. 1996,

MACHOLÁN *et al*. 2001). On the neighbouring geography, however, MEZHZHERIN *et al*. (1992) and VORONTSOV *et al*. (1992) recorded *A. uralensis, A. ponticus*, and *A. fulvipectus* along with a new species of *A. hyrcanicus* from the Caucasus. Similarly, FILIPPUCCI *et al*. (1989) recorded *A. flavicollis, A. mystacinus* as well as a new species, *A. hermonensis*, from Israel. FILIPPUCCI *et al*. (1996) added the Western Turkey to the distribution area of *A. hermonensis*. ÇOLAK *et al*. (2004) analysed geographic variation of *A. mystacinus* in Turkey and determined some morphological differences between populations of *A. mystacinus* from Black Sea and those from Mediterranean Sea.

According to MACHOLÁN *et al*. (2001), *A. hermonensis* is distributed in Burdur, Amasya, Gümüşhane, Kars, Artvin, Ağrı, and Hakkari. KRYŠTUFEK and VOHRALÍK (2001) reported *A. mystacinus, A. flavicollis, A. sylvaticus, A. uralensis, A. iconicus* and *A. agrarius* to be found in different parts of Turkey. KRYŠTUFEK (2002) has examined four *Apodemus* species from the eastern Mediterranean and the Middle East in the Natural History Museum, London, and stated that *Apodemus sylvaticus iconicus* HEPTNER, 1948 is a senior synonym of *A. hermonensis*, and *A. iconicus* is available name for this species. ÇOLAK (2003) analysed specimens of *A. iconicus* from Ankara, Artvin, Bolu, Bursa, Konya, Muş and Samsun based on karyological, morphological and phallic characteristics and determined that *A. iconicus* has 2n = 48 chromosomes. Although there were a lot of systematic studies on *Apodemus* species distributed in Turkey, there were no detailed work on the morphological, distributional pattern and biochemical variation present in the genus *Apodemus*. According to MACHOLÁN *et al*. (2001), morphological identification of four species (*A. sylvaticus, A. flavicollis, A. iconicus* and *A. uralensis*) of the subgenus *Sylvaemus* of the genus *Apodemus* is very difficulty. Therefore, FRYNTA *et al*. (2001) and FRYNTA *et al*. (2006) identified 78 specimens of *Sylvaemus* from Near East based on allozymic identification of MACHOLÁN *et al*. (2001). FRYNTA *et al*. (2006) separated *A. sylvaticus* and *A. flavicollis* from Balkans using the position of posterior edges of foramina incisive in relation to the anterior roots of M1 (FILIPPUCCI *et al*. 1984, POPOV 1993) and allometry between facial length and length of foramen incisivum (KRYSTUFEK & STOJANOVSKI 1996). Also, VERIMLI *et al*. (2001) separated *A. hermonensis* from *A. flavicollis* based on electrophoresis of blood serum proteins. On the basis of electrophoretic and morphological attributes, ÇOLAK *et al*. (2005) separated *A. flavicollis*, *A. sylvaticus* and *A. agrarius* in Turkish Thrace. Recently, BARČIOVÁ and MACHOLÁN (2006) examined skull shape and size *A. sylvaticus* and *A. flavicollis* using traditional and geometric morphometric approach.

In this study, we used both morphological and allozymic attributes for the identification of four species of the subgenus *Sylvaemus* and two other species of ge-

nus *Apodemus*. The aims of this study were to characterize *Apodemus* species in Turkey in terms of 28 morphometric characters and 10 enzyme loci and to contribute to the taxonomy, distribution and the genetics of the genus *Apodemus* in Turkey.

MATERIAL AND METHODS

Morphometry

A total of 253 specimens from 45 localities were examined (Fig. 1 and Appendix 1). Six species; *A. flavicollis, A. sylvaticus, A. uralensis, A.mystacinus, A. agrarius*, and *A. iconicus* were identified based on morphological characteristics such as pectoral spot, the posterior end of palatal bone, pterygoid process, fronto-parietal suture, upper molar cusp patterns, and tympanic bullae given by FILIPPUCCI *et al*. (1996) and KRYŠTUFEK (2002). We referred specimens of *A. hermonensis* in Turkey to *A. iconicus* based on the previous study (KRYŠTUFEK 2002).

Age determination of all specimens was performed and adults were evaluated. In the morphometric analysis, 28 metric characters were measured (total length: TL, tail length: T, hind foot: HF,

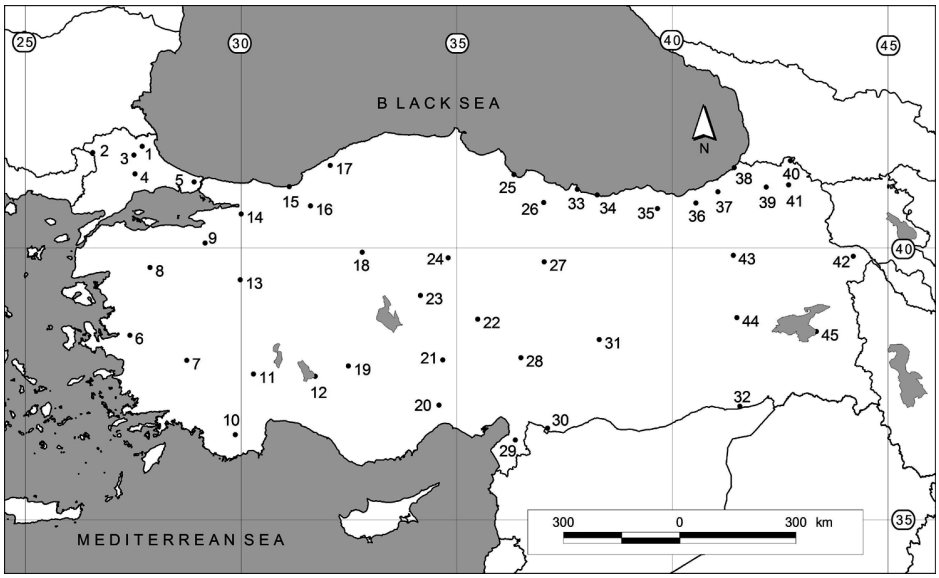

Fig. 1. Distribution map of the analyzed populations of the genus *Apodemus* in Turkey. 1 = Edirne, 2 = Velikaköprüsü (Kirklareli), 3 = Pınarhisar (Kirklareli), 4 = Büyükkarıştıran (Tekirdağ), 5 = Istanbul, 6 = Kemalpaşa (İzmir), 7 = Buharkent (Aydin), 8 = Balıkesir, 9 = Uludağ (Bursa), 10 = Çığlıkara (Antalya), 11 = Burdur, 12 = Beyşehir (Konya), 13 = Kütahya, 14 = Kocaeli, 15 = Akçakoca (Bolu), 16 = Bolu, 17 = Çaycuma (Zonguldak), 18 = Ankara, 19 = Konya, 20 = Sebil (Mersin), 21 = Niğde, 22 = Kayseri, 23 = Kırşehir, 24 = Yozgat, 25 = Samsun, 26 = Akkuş (Ordu), 27 = Sıvas, 28 = Göksun (K = Maraş), 29 = Kırıkhan (Hatay), 30 = Kilis-Gaziantep, 31 = Malatya, 32 = Nusasbin (Mardin), 33 = Efirli (Ordu), 34 = Bulancak (Giresun), 35 = Sümela (Trabzon), 36 = İkizdere (Rize), 37 = Ayder (Rize), 38 = Hopa (Artvin), 39 = Kutul (Artvin), 40 = Posof (Ardahan), 41 = Ardahan, 42 = Iğdır, 43 = Erzurum, 44 = Muş, 45 = Van

ear: E, weight: W, 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, length of lower toothrow cusp: LLT).

23 metric characters of skull were taken with a calliper to the nearest 0.01 mm or under a binocular. These characters were grouped into the size and skull measurements, respectively. The sexual dimorphism was tested with one-way ANOVA for each species. After eliminating the individuals with missing data, the final data matrix was adjusted according to Mosimann method (MOSIMANN 1970) to rule out the effect of growth and size (see FRYNTA *et al*. 2006). To evaluate morphometrical differences among six *Apodemus* species, we computed Mahalanobis distances using Canonical Vector Analysis (CVA) based on pooled variance-covariance matrices (NTSYS-pc version 1.21, ROHLF 2000) and the significance was tested by Hotteling test as described previously (FRYNTA *et al*. 2006). UPGMA clustering was used to construct phenetic relationships. Later the data set was also used in multiple Discriminant Function Analysis using SPSS statistical package (SPSS Inc., 1999).

Isozymes

Electrophoretic analysis was performed on the same 253 *Apodemus* specimens from 45 localities in Turkey (Fig. 1 & Table 1). We combined some localities due to small number of specimens and thus the number of localities was reduced to 31. The following enzyme loci were screened (abbreviation and E.C. numbers are given in parentheses): α-glycerophosphate dehydrogenase (α-*Gpdh*, E.C. 1.1.1.8), malate dehydrogenase (*Mdh-s, Mdh-m*, E.C. 1.1.1.37), malic enzyme (*Me*, E.C. 1.1.1.40), isocitrate dehydrogenase (*Idh-1*, E.C. 1.1.1.42), glyceraldehyde-3-phosphate dehydrogenase (*Gpdh*, E.C. 1.2.1.12), hexokinase (*Hk*, E.C. 2.7.1.1), phosphoglucomutase (*Pgm*, E.C. 2.7.5.1), aldolase (*Ald*, E.C. 4.1.2.13), adenylate kinase (*Adk*, E.C. 4.1.2.13).

Kidney extracts were used for screening *Hk* while muscle extracts were used for the other loci. Tissues were kept at –70°C until use. Electrophoresis was performed on gels composed of 11% starch hydrolyzed. Buffer systems, staining procedures and the electrophoretic running conditions followed previously published studies (SELANDER *et al*. 1971, AYALA *et al*. 1972, HARRIS & HOPKINSON 1976, HILLIS & MORITZ 1990). Alleles were designated alphabetically by their relative mobility, the allelic variant migrating furthest anodal denoted as A.

Summary of genetic parameters, genetic distances (NEI 1978), and the genetic differentiation (F_{ST}) were obtained using the BIOSYS-1 package (SWOFFORD & SELANDER 1981). Neighbour joining clustering based on NEI (1978) genetic distance was constructed using NTSYS-pc (ROHLF 2000)

RESULTS

Morphometry

The mean values of the characters measured, minimum, maximum and the standard errors have been shown in Appendix 2, together with the total number of individuals in each *Apodemus* species.

Analysis of Variance (ANOVA) of the data showed high heterogeneity among *Apodemus* species. Out of 28 morphometric variables, 27 displayed significant heterogeneity ($p < 0.001$) except tail length (TL) ($p > 0.05$). Phenetic comparisons using Mahalanobis distance based on the size adjusted data revealed clear distinctions between *Sylvaemus* species and the other two species of *Apodemus*. Four *Sylvaemus* species were grouped together and *A. agrarius* and *A. mystacinus* were later added to this cluster (Fig. 2). The distances between *Sylvaemus* species and *A. mystacinus* were significantly different ($p < 0.001$), however the significance was not determined for the comparisons between *Sylvaemus* species and *A. agrarius* ($p > 0.05$). Then we carried out Canonical Variate Analysis using pooled variance-covariance matrix and the CVA scores on the scatter-plot showed similar groupings (Fig. 3). The first three canonical variates explained 91.6% of the total variation. The first, the second and the third canonical variate explained 67.5%, 13.7% and 10.4% of the variation, respectively. When *A. mystacinus* and *A. agrarius* were clearly separated from four *Sylvaemus* species, these two species were eliminated and the new data set was subjected to a second CVA for the discrimination of four *Sylvaemus* species. The second CVA yielded much better separation among *Syl-*

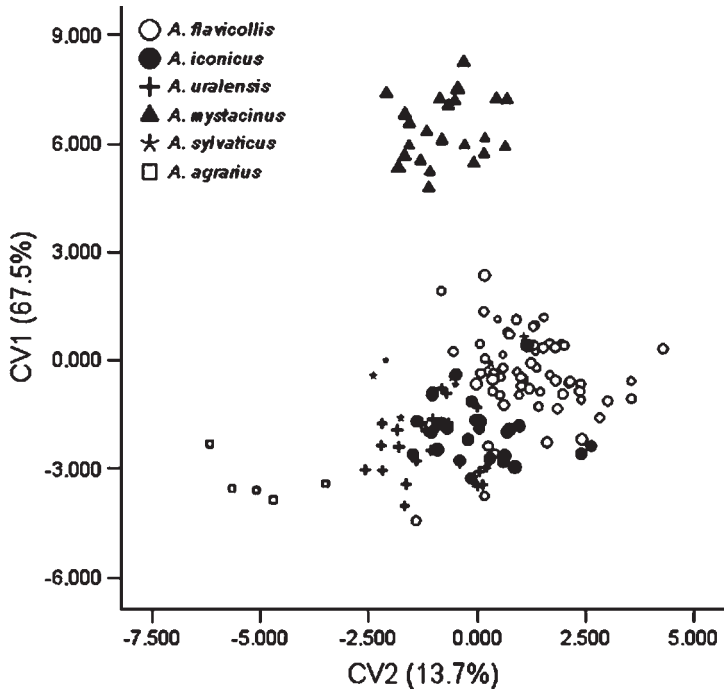

Fig. 2. A scatterplot of six *Apodemus* species based on CVA analysis on the pooled variance covariance matrix

vaemus species (Fig. 4). *A. sylvaticus* was clearly separated from the other three species. On the other hand, 3% of *A. flavicollis* overlapped with *A. iconicus* and 6% of *A. flavicollis* overlapped with *A. uralensis*. In addition, *A. iconicus* and *A. uralensis* were overlapping 10%. The six species were subsequently analyzed with a multiple discriminant function analysis (DFA) on the size adjusted data of the 28 morphological characters. The three axes obtained in the DFA explained 96.6% of the total variation. The proportions of variation explained by the first, the second and the third axis were 76.6, 11.5, and 8.5%, respectively. *A. mystacinus* formed a group distant from the other species. Another well separated species appeared to be *A. agrarius* whereas the remaining *Sylvaemus* species formed a single cluster (Fig. 5). BW, FI and LLT were the variables with the highest loadings on the first canonical axis, whereas, IC, TB and E were loaded highly on the second canonical axis. In the third axis, NW, TB and LLT were the variables contributing to the separation of the groups.

Isozymes

Of the 10 loci, four were found to be polymorphic; *Idh-1,* α*-Gpdh*, *Mdh-s*, *Me*, whereas six loci (*Mdh-m, G3pdh, Pgm, Ald, Hk*, and *Adk*) were found to be monomorphic, and fixed for the same allele in all *Apodemus* species studied. Allele frequencies of the six *Apodemus* species were tabulated (Table 1). A summary

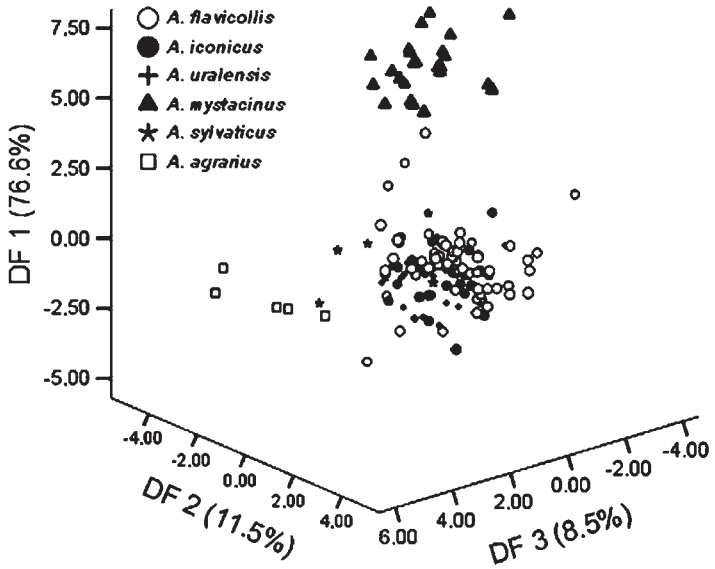

Fig. 3. A scatterplot of six *Apodemus* species based on DFA of the Mosiman adjusted data matrix

Table 1. Allelic frequencies of four polymorphic loci of six *Apodemus* species in Turkey (Monomorphic six loci was not shown)

Alleles	*A. flavicollis*	*A. mystacinus*	*A. sylvaticus*	*A. agrarius*	*A. uralensis*	*A.iconicus*
	N = 83	N = 49	N = 7	N = 5	N = 50	N = 59
IDH						
A	0.928	–	–	–	0.020	0.941
B	0.072	1.000	1.000	–	0.980	0.059
C	–	–	–	1.000	–	–
α-*GPDH*						
A	0.036	1.000	0.071	–	0.950	0.042
B	0.958	–	0.357	–	0.050	0.958
C	0.006	–	0.572	1.000	–	–
MDH-S						
A	1.000	1.000	0.929	–	1.000	1.000
B	–	–	0.071	–	–	–
C	–	–	–	1.000	–	–
ME						
A	–	1.000	1.000	–	0.020	–
B	1.000	–	–	–	0.980	1.000
C	–	–	–	1.000	–	–

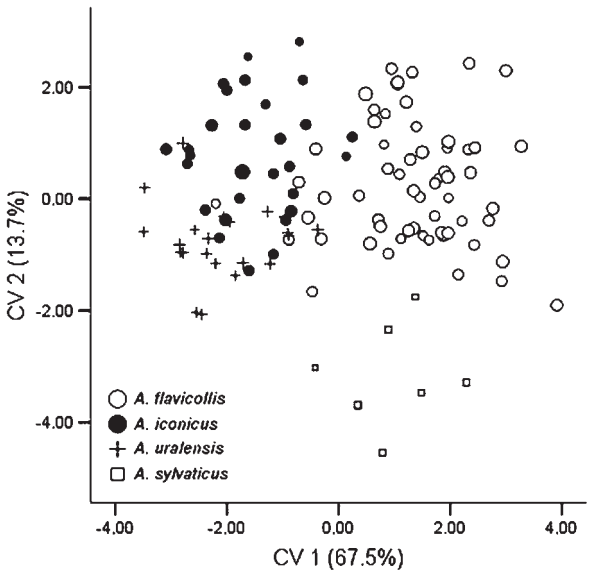

Fig. 4. A scatterplot of *Sylvaemus* species based on CVA analysis on the pooled variance covariance matrix

Table 2. Mean values of genetic variability at 10 loci in *Apodemus* species. *H*: Mean heterozygosity; H_o: observed heterozygosity; H_e: expected heterozygosity; *A*: average number of alleles per locus; *P*: proportion of polymorphic loci (standard deviations in parentheses)

Species	Specimen numbers	A	P	H	
				Ho	He
A. flavicollis	83	1.3 (0.2)	10.0	0.004 (0.004)	0.022 (0.015)
A. mystacinus	49	1 (0.0)	0.0	0.000 (0.000)	0.000 (0.000)
A. sylvaticus	7	1.3 (0.2)	20	0.029 (0.019)	0.073 (0.058)
A. agrarius	5	1 (0.0)	0.0	0.000 (0.000)	0.000 (0.000)
A. uralensis	50	1.3 (0.2)	10.0	0.002 (0.002)	0.018 (0.01)
A. iconicus	59	1.2 (0.1)	10.0	0.003 (0.002)	0.019 (0.013)

of genetic variation was shown in Table 2. *Idh-1,* α*-Gpdh, Mdh-s*, and *Me* deviated from Hardy-Weinberg equilibrium ($p < 0.01$) in favour of homozygotes. According to Nei's genetic identity matrix, the highest genetic similarity (0.986) was found between *A. iconicus* and *A. flavicollis*, and the lowest similarity (0.600) was between *A. agrarius* and *A. mystacinus*.

The highest genetic differentiation was due to the *Me* locus, and the least differentiation was due to *Idh-1* based on F_{ST} values (data not shown). Neighbour joining dendrogram showed that populations of *A. iconicus* from Posof and Ardahan

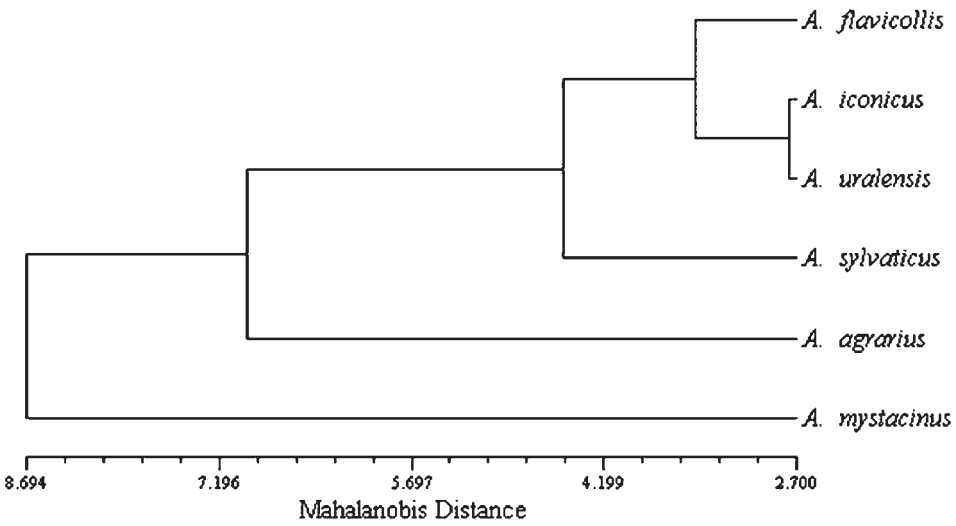

Fig. 5. Morphometric relationship of six *Apodemus* species based on Mahalanobis distance

were closer to *A. flavicollis*, populations of *A. flavicollis* from Sümela and Sürmene were closer to *A. iconicus*, and populations of *A. iconicus* from Ulukışla and Madenköy were closer to populations of *A. uralensis*. Nevertheless, based on the ten loci studied, four *Apodemus* species (*A. sylvaticus, A. uralensis, A. mystacinus* and *A. agrarius*) were clearly separated in Turkey. However *A. flavicollis* and *A. iconicus* showed a close affinity and formed a single cluster in the dendrogram (Fig. 6).

DISCUSSION

Apodemus species showed high levels of morphometric and biochemical variation in Turkey. Of the 28 morphometric characters studied, 27 were found to be sig-

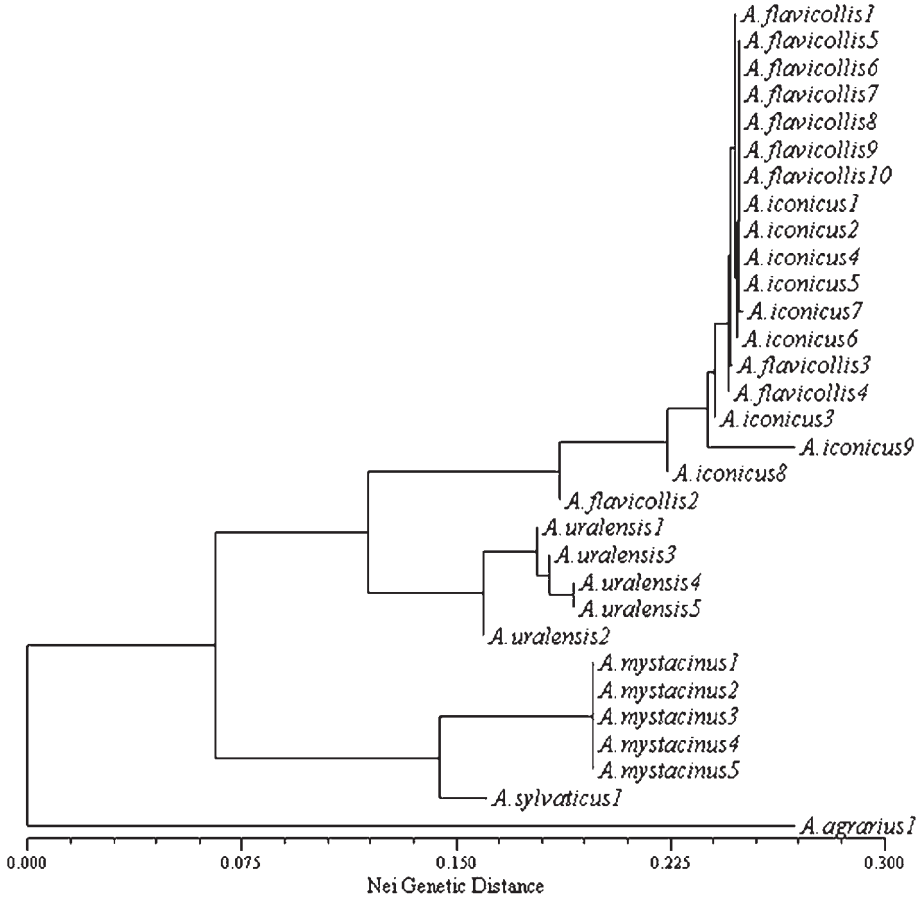

Fig. 6. Genetic relationship of *Apodemus* species based on Nei genetic distance of 10 enzyme loci

nificantly different between the six species studied ($p < 0.001$), except ear length ($p < 0.05$). In addition to morphometry, 10 enzyme loci were also utilized to find out the extent of biochemical variation present in *Apodemus* species in Turkey. Of the 10 loci examined, four were found to be polymorphic among the six species and six of the loci showed invariant banding pattern in six *Apodemus* species.

Distributional pattern

Apodemus species show allopatric and sympatric distribution in Turkey. Although FILIPPUCCI *et al*. (1996) recorded *A. sylvaticus* from Çaycuma, the samples that were collected for this study showed that they are *A. flavicollis* based on morphological characteristics and 10 enzyme loci. Seven specimens from Edirne (close to Bulgarian border), steppe area closer to Çorlu (Tekirdağ), had the same morphological characteristics (pectoral spot and dentition pattern) as in *A. sylvaticus*. According to KRYŠTUFEK and VOHRALÍK (2001), *A. sylvaticus* is distributed in Thrace, possibly Marmara and western Black Sea Mountains. Similarly our findings confirmed the results of MACHOLÁN *et al*. (2001) who mentioned that *A. sylvaticus* is virtually absent from the entire area of the Middle East.

In this study *A. flavicollis* and *A. uralensis* were recorded from Sümela, as it is previously reported (MACHOLÁN *et al*. 2001). Similarly, the distributional pattern of *A. uralensis* determined in this study was the same as it is previously given (FILIPPUCCCI *et al*. 1996, MACHOLÁN *et al*. 2001). The distributional range of *A. flavicollis* was extended to SE Turkey (Kilis, Nizip, and Nusaybin), on the other hand, *A. iconicus* was not recorded from the same geography in the present study.

Biometric variation and multivariate analysis

A wide range of biometric variation occurs in a geography extending throughout Turkey as well as European part (Thrace). Almost all of the morphometric variables were significantly different among six *Apodemus* species ($p < 0.001$) except tail length ($p < 0.05$), this was well reflected the importance of measured morphometric characters. *A. sylvaticus, A. flavicollis, A. iconicus* and *A. uralensis* were similar in appearance and measurements so that they formed the largest cluster in multivariate analysis (CVA, DFA). *A. agrarius* and *A. mystacinus* could be differentiated morphometrically in this study as would be expected (Figs 3 & 5). On the basis of morphometric analysis, FRYNTA *et al*. (2006) determined that samples of the subgenus *Sylvaemus* were divided into three groups; *A. flavicollis–A. hyrcanicus, A. sylvaticus* and *A. uralensis–A. hermonensis* (*A. iconicus*). In size-adjusted data, *A. sylvaticus* was basal branch and *A. uralensis* was a group, and *A. flavicollis–A.*

hyrcanicus–A. hermonensis (*A. iconicus*) was another group (FRYNTA *et al*. (2006). According to FRYNTA *et al*. (2006), the position of *A. hermonensis* (*A. iconicus*) in the phenetic dendrogram is sensitive to the clustering method used. The results of present study (Fig. 5) are similar to those of FRYNTA *et al*. (2006).

The overlapping distribution of *A. uralensis* with *A. flavicollis, A. sylvaticus* and *A. fulvipectus* (*A. iconicus*) was also shown in an earlier study (HILLE *et al*. 2002). The results of the present study showed similar groupings based on 28 skull and body measurements (FILIPPUCCI *et al*. 1996). However, some of the character ranges and the diagnostic characters were not in agreement with the present study. FILIPPUCCI *et al*. (1996) reported that the hind foot length was 22.5–23 mm in *A sylvaticus* where as the same character was smaller than that of *A. sylvaticus* from Turkish Thrace. Similarly the most distinctive measurements between *A. uralensis* and *A. hermonensis* (*A. iconicus*) is the crown length of upper molars. The range of the values according to FILIPPUCCI *et al*. (1996) was 3.59–4.02 mm for *A. hermonensis* (*A. iconicus*) and 3.22–3.73 mm for *A. uralensis*. However in this study the same characters were found to be 3.2–3.8 mm for *A. uralensis* and 3.28–3.8 mm for *A. iconicus*.

Isozyme variation

Among 10 loci studied four showed polymorphism in six *Apodemus* species used in this study. However the polymorphic loci were not showed variation in all species. *Idh* was polymorphic only in *Apodemus flavicollis*, *A. uralensis* and *A. iconicus* whereas α-*Gpdh* was polymorphic in all *Sylvaemus* species. *A. sylvaticus* was the only variable species in terms of *Mdh-S* locus and similarly *A. uralensis* was the single variable species based on *Me* locus.

Detailed examination of polymorphic loci had distinguishable power among species. *Idh C* allele was the diagnostic allele for *A. agrarius*. α-*Gpdh* on the other hand was monomorphic for *A. mystacinus* and *A. agrarius* in which α-*Gpdh A* allele and α-*Gpdh C* allele were fixed for these species respectively. In addition α-*Gpdh C* allele was also present in *A. flavicollis* and *A. sylvaticus*, however, this allele was rare in former species but was the most common allele in the latter species. *Mdh-S A* allele was fixed in four of the species except *A. sylvaticus and A. agrarius*. However, *A. agrarius* was monomorphic but for the allele *C* of *Mdh-S* which could be diagnostic for *A. agrarius*. The only variable species for *Mdh-S* locus was *A. sylvaticus*. Five of the *Apodemus* species were monomorphic for the *Me* locus and the only variable species was *A. uralensis*. This locus was the most distinguishable locus among other polymorphic loci. *Me A* allele was fixed for *A. mystacinus* and *A. sylvaticus*. Similarly *Me B* allele was fixed for *A. flavicollis* and *A. iconicus* and *Me C* allele is diagnostic for *A. agrarius*.

Genetic variability parameters such as mean expected (H_e) and observed (H_o) heterozygosities, average number of alleles per locus (*A*) and the proportion of polymorphic loci (*P*) in this study were compared with the previously reported studies (FILIPPUCCI *et al*. 1989, BRITTON-DAVIDIAN *et al*. 1991, FILIPPUCCI 1992, FILIPPUCCI *et al*. 1996, MACHOLÁN *et al*. 2001, FILIPPUCCI *et al*. 2002, HILLE *et al*. 2002) and found similarities and differences among different studies. These differences could be attributable to the differences in studied loci and the number of specimens used. Although there seems to be consensus on he constructed dendrogram summarizing the genetic relationships between *Apodemus* species there still some discrepancies among studies in terms of the groupings of different studies. For example *A. flavicollis* was clustered *A. sylvaticus* in one study based on 20 gene loci (BRITTON-DAVIDIAN *et al*. 1991) and it was grouped with *A. hermonensis* (*A. iconicus*) in the dendrogram generated from 20–38 gene loci for *Apodemus* species (FILIPPUCCI 1992, FILIPPUCCI *et al*. 2002). On the other hand *A. sylvaticus* was distinguishable and separated well from the other *Apodemus* species in most of the studies in the UPGMA cluster as in this study (FILIPPUCCI *et al*. 1996, MACHOLÁN *et al*. 2001, FILIPPUCCI *et al*. 2002, HILLE *et al*. 2002). Similarly the position of *A. mystacinus* and *A. agrarius* in this study was in agreement with the previous studies (FILIPPUCCI 1992, FILIPPUCCI *et al*. 2002).

Genetic relationship of the *Apodemus* species as displayed by the phylogenetic trees in the previous studies based on 30–38 loci were similar to what is obtained in the present study based on 10 loci (FILIPPUCCI 1992, FILIPPUCCI *et al*. 1996, MACHOLÁN *et al*. 2001, FILIPPUCCI *et al*. 2002, HILLE *et al*. 2002).

Detailed comparison of such studies (biochemical analysis) was rather difficult with respect to methodologies or the naming of alleles. Thus without comparing the original samples as reference samples, the similarities or the differences between studies were not possible. As MACHOLÁN *et al*. (2001) mentioned for the correct examination of the samples, they should be run on the same gel which would be the best for the sake of the clear conclusions and discussion.

However, the results of the present study and that of MEZHZHERIN *et al*. (1992) showed similarities in terms of α-*Gpdh* loci in which *A. flavicollis* and *A. hermonensis* (*A. iconicus* in this study) share the same alleles with varying allele frequencies, different from *A. uralensis*. This locus, on the contrary, was monomorphic in another study by MACHOLÁN *et al*. (2001). *Idh* locus in this study showed differences with MACHOLÁN *et al*. (2001) in which *Idh-1 113* allele was found in *A. hermonensis* (*A. iconicus*) where as this allele was only found in *A. agrarius* and fixed. *Idh 100* allele is fixed in *A. uralensis* in MACHOLÁN *et al*. (2001) but this allele was (*Idh-1 B* allele in the present study) the most common allele in the present study. Similarly, *Idh-1 108* allele which corresponds to allele *A*

in this study is variable in *A. flavicollis* and *A. iconicus*, whereas, this allele is found to be fixed in *A. flavicollis* by MACHOLÁN *et al*. (2001). In short the findings of this study and the results of preciously published works support each other (FILIPPUCCI 1992, FILIPPUCCI *et al*.1996, MACHOLÁN *et al*. 2001, FILIPPUCCI *et al*. 2002, HILLE *et al*. 2002).

Morphology by itself or biochemical enzyme systems individually was not enough for determining the species-specific categories. In combination and also other characteristics like ecology and geography would be useful for elucidating the taxonomic problems of the species under question. Based on morphological characteristics, *A. flavicollis* and *A. iconicus* from Ardahan, Burdur, Corum, Konya, Muş, Samsun, Sivas and Van were easily distinguishable whereas the identification was very difficult between *A. flavicollis, A. uralensis* and *A. iconicus* from Abant, Uludag, and Eastern Black Sea region. Moreover, some specimens of *A. flavicollis* from Hopa, Bulancak, Ikizdere and Sürmene (below 500 m a.s.l.) had similar alleles of *Idh-1* and α-*Gpdh* as in *A. uralensis*.

This is possible due to the geographic structure of these species in which three species of *A. flavicollis*, *A. iconicus*, and *A. uralensis* live in close sympatry. Thus several characteristics might have converged during the evolutionary time scale as it is stated before (FILIPPUCCI *et al*. 1996, MACHOLÁN *et al*. 2001). Also, According to FRYNTA *el al*. (2006), adaptive zones of Near East taxa may be correspondingly reduced and potentially resulting in enhanced morphological similarity among the Near East *Apodemus* species. However, the identification is relatively easier for *A. flavicollis* and *A. iconicus* in Ankara, Ardahan, Ardanuc, Çorum, Konya, Muş, Samsun, Sıvas, and Van in where they have allopatric distribution such that *A. flavicollis* in forest, fragmented forests and shrub and *A. iconicus* in step and field.

Systematic identification of Turkish *Apodemus* specimens based on both morphological and biochemical methods was confirmed the groupings of species and consistent with those published results of previous studies. Ten isozyme loci, in combination with morphometrical characteristics and ecological distribution patterns helped to obscure the taxonomic and genetic relationship of the *Apodemus* species distributed in Turkey. However, support from molecular studies will be helpful in detail and more robust analysis of the phylogenetic relationship of the genus *Apodemus* in the future.

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Appendix 1. List of total *Apodemus* specimens examined morphologically, biochemically and distributional patterns

Species	Population no.	Population name	Localities (number of specimens and cities are in parentheses)
A. *flavicollis*	1	A.flavic1	Kurupelit (3) (Samsun); Akkuş (3) and Efirli (4) (Ordu); Bulancak (2), Tirebolu (3), Kesap (1) (Giresun)
	2	A.flavic2	Sümela (4), Sürmene (3) (Trabzon)
	3	A.flavic3	İkizdere (6) (Rize)
	4	A.flavic4	Ardanuç (4), Hopa (2), Borçka (1), Artvin (1)
	5	A.flavic5	Çaycuma (6) (Zonguldak)
	6	A.flavic6	Bozüyük (3) (Bilecik); Gönen, Manyas (2) (Balıkesir); Beykoz (1) (İstanbul); Abant (1) (Bolu); Uludağ (5) (Bursa)
	7	A.flavic7	Çorum (4); Kurtboğazı (1) (Ankara); Beyşehir (4) (Konya); Akseki (1) (Antalya)
	8	A.flavic8	Maraş (1); Kilis (2); Nusaybin (1) (Mardin)
	9	A.flavic9	Velikaköprüsü, İğneada (11) (Kırklareli)
	10	A.flavic10	Kemalpaşa (2), Bayındır (1), Yamanlar (1) (İzmir); Burdur (1); Buharkent (1) (Aydın)
A.*iconicus*	11	A.iconic1	Kurupelit (3) (Samsun)
	12	A.iconic2	Ardanuç (5), Hopa (1), Borçka (2), Artvin (1)
	13	A.iconic3	Posof (3), Ardahan (4)
	14	A.iconic4	Van (2); Muş (3); Horasan (1) (Erzurum)
	15	A.iconic5	Ankara (3); Bünyan (2), Pınarbaşı (2) (Kayseri); Sıvas (1); Kırşehir (3); Sungurlu (1) (Çorum)
	16	A.iconic6	Ulukışla (3), Madenköy (4) (Nigde)
	17	A.iconic7	Beyşehir (1) (Konya); Burdur (3); Akseki (3) (Antalya)
	18	A.iconic8	Uludağ (4) (Bursa); Bozüyük (3) (Bilecik); Gönen, Manyas (2) (Balıkesir)
	19	A.iconic9	Kemalpaşa (1), Gölcük-Ödemiş (1) (İzmir)
A. *uralensis*	20	A.uralen1	İkizdere (9), Ayder (6) (Rize)
	21	A.uralen2	Sümela (6) (Trabzon); Akkuş (1) (Ordu)
	22	A.uralen3	Hopa (2), Kutul (1) (Artvin)
	23	A.uralen4	Akçakoca (2) (Zonguldak); Abant (5), Bürnük (1) (Bolu)
	24	A.uralen5	Uludağ (13) (Bursa)
A. mystacinus	25	A.mystac1	Sebil, Çamalan (11) (Mersin); Çýdlýkara, Akseki (6) (Antalya); Madenköy (1) (Niđde); Maraţ (1)
	26	A.mystac2	Akçakoca (6) (Zonguldak); Uludađ (1) (Bursa)
	27	A.mystac3	Altındere (2), Sümela (3) (Trabzon)
	28	A.mystac4	Ardanuç (8) (Artvin)
	29	A.mystac5	Kemalpaşa (3) (İzmir); Buharkent (2) (Aydın); Beyşehir (3) (Konya); Burdur (2)
A. sylvaticus	30	A.sylvac1	Edirne (6); Tekirdağ (1)
A. agrarius	31	A.agrari1	5 km South İğneada (5) (Kırklareli)

Appendix 2. Minimum, maximum, mean and standard errors of 28 morphometric characters in six *Apodemus* species

	A. flavicollis				*A. iconicus*				*A. uralensis*				*A. mystacinus*				*A.sylvaticus*				*A.agrarius*			
Char.	Min	Max	Mean	SE	Min	Max	Mean	SE	Min	Max	Mean	SE	Min	Max	Mean	SE	Min	Max	Mean	SE	Min	Max	Mean	SE
TL	155.0	243.0	197.75	2.26	165.0	224.0	187.48	2.16	161.0	212.0	186.31	2.61	214.0	261.0	238.75	3.07	173.0	200.0	183.50	5.32	170.0	180.0	175.00	2.08
T	60.0	122.0	96.82	1.54	71.0	114.0	94.50	1.49	71.0	112.0	92.00	1.91	115.0	137.0	125.95	1.65	66.0	100.0	84.67	4.66	75.0	80.0	77.25	1.03
HF	20.0	27.0	24.16	0.16	21.0	26.0	22.89	0.17	20.0	26.0	22.53	0.20	25.0	28.0	26.63	0.15	24.0	25.0	24.71	0.18	21.0	22.0	21.40	0.24
E	13.0	21.0	17.76	0.14	14.0	20.0	16.22	0.20	12.0	18.0	16.27	0.23	18.0	25.0	21.00	0.28	16.0	20.0	17.57	0.65	14.0	15.0	14.20	0.20
W	11.0	37.0	23.92	0.62	13.0	26.0	19.91	0.52	13.0	26.0	19.10	0.62	24.0	52.0	35.80	1.32	18.0	35.0	25.43	2.26	20.0	30.0	25.20	1.93
ZB	11.2	14.7	13.35	0.09	11.6	14.0	12.60	0.10	11.2	13.4	12.59	0.12	13.8	16.2	15.07	0.12	12.0	13.8	13.00	0.53	12.0	12.4	12.20	0.07
IC	4.0	4.9	4.24	0.02	3.5	4.6	4.23	0.03	4.0	4.6	4.24	0.03	4.4	5.0	4.76	0.04	4.1	4.5	4.29	0.07	4.2	5.0	4.62	0.15
CBL	20.8	26.5	23.58	0.16	20.6	24.1	22.26	0.15	20.7	23.9	22.18	0.17	26.1	28.1	27.31	0.10	22.2	24.8	23.41	0.32	23.0	24.2	23.45	0.27
ONL	23.0	29.5	26.36	0.18	23.5	27.8	25.06	0.16	23.5	26.4	24.90	0.15	29.5	32.0	30.82	0.12	25.8	27.4	26.47	0.25	25.1	26.1	25.35	0.25
BL	18.8	24.5	21.58	0.15	19.0	22.4	20.45	0.14	19.0	22.6	20.40	0.16	24.0	26.1	25.08	0.11	21.0	23.1	21.74	0.29	21.0	22.0	21.55	0.26
NL	7.0	11.5	9.64	0.10	7.2	10.1	8.80	0.09	7.2	10.3	9.00	0.13	10.5	12.9	11.70	0.13	9.5	10.5	9.96	0.17	9.0	9.9	9.56	0.16
NW	2.6	3.8	3.15	0.03	2.5	3.5	2.93	0.03	2.7	3.5	3.08	0.04	3.2	4.1	3.85	0.04	3.0	3.4	3.11	0.06	3.2	3.9	3.56	0.13
FLB	11.2	15.1	13.48	0.09	11.4	14.1	12.69	0.09	11.8	14.1	12.91	0.11	15.0	17.2	16.07	0.08	12.5	14.3	13.50	0.25	13.3	13.8	13.52	0.09
BCL	10.0	15.5	11.75	0.11	9.9	13.0	11.06	0.12	9.8	12.1	10.93	0.11	13.2	14.6	13.97	0.07	11.5	12.2	11.91	0.11	11.8	12.2	11.98	0.09
MB	5.5	7.4	6.41	0.05	5.6	6.8	6.24	0.05	5.6	6.8	6.12	0.05	6.5	7.5	7.08	0.05	6.1	6.9	6.64	0.10	6.1	6.2	6.15	0.03
HBB	8.2	10.2	9.26	0.05	8.0	9.4	8.72	0.05	7.9	9.1	8.60	0.07	9.7	11.2	10.39	0.07	8.8	9.4	9.17	0.08	8.4	9.0	8.70	0.13
HB	7.0	8.9	8.05	0.05	7.0	8.3	7.78	0.05	7.2	8.2	7.65	0.06	8.4	9.9	9.25	0.06	7.5	8.1	7.86	0.09	7.5	7.8	7.68	0.07
OW	9.4	11.8	11.07	0.05	10.0	12.0	10.78	0.08	10.1	11.2	10.74	0.06	11.0	12.9	12.01	0.09	10.6	11.5	11.21	0.13	10.5	11.2	10.90	0.15
BW	10.8	13.4	11.78	0.05	10.5	13.0	11.43	0.07	10.6	11.9	11.27	0.05	13.0	14.1	13.50	0.06	11.1	12.3	11.73	0.15	11.0	11.2	11.10	0.06

Appendix 2 (continued)

	A. flavicollis				*A. iconicus*				*A. uralensis*				*A. mystacinus*				*A.sylvaticus*				*A.agrarius*			
Char.	Min	Max	Mean	SE	Min	Max	Mean	SE	Min	Max	Mean	SE	Min	Max	Mean	SE	Min	Max	Mean	SE	Min	Max	Mean	SE
D	5.9	8.0	6.90	0.06	5.8	7.6	6.511	0.06	6.1	7.3	6.71	0.07	7.1	8.4	7.92	0.05	6.6	7.6	7.10	0.15	7.0	7.5	7.16	0.09
PL	9.5	13.7	11.56	0.12	10.0	13.0	11.32	0.14	9.6	12.9	11.03	0.19	12.5	14.2	13.39	0.07	11.0	12.3	11.54	0.21	11.0	11.5	11.22	0.08
FI	4.3	6.0	5.16	0.04	4.3	5.9	4.90	0.05	4.6	5.2	4.83	0.04	6.0	6.8	6.45	0.04	5.1	5.9	5.44	0.11	4.7	5.0	4.89	0.06
TB	4.3	5.7	5.01	0.04	4.1	4.9	4.43	0.03	4.0	4.9	4.39	0.04	4.6	5.5	4.99	0.05	4.6	5.4	4.94	0.11	4.7	4.7	4.73	0.00
ML	12.8	16.5	14.66	0.10	12.5	16.0	13.88	0.11	12.1	15.0	13.72	0.14	16.0	18.0	17.19	0.08	13.9	15.0	14.46	0.16	14.2	15.5	14.56	0.24
LUTa	3.7	4.9	4.25	0.03	3.5	4.9	4.07	0.04	3.2	4.3	3.89	0.04	4.6	5.2	4.89	0.04	4.1	4.6	4.36	0.07	4.3	4.3	4.30	0.00
LUT	3.0	4.1	3.64	0.03	3.2	3.8	3.40	0.03	3.2	3.8	3.27	0.03	4.0	4.7	4.37	0.04	3.5	3.8	3.59	0.05	3.5	3.7	3.62	0.05
LLTa	3.5	4.6	4.11	0.03	3.5	4.6	3.91	0.04	3.5	4.1	3.69	0.03	4.6	4.9	4.73	0.02	4.1	4.3	4.17	0.05	4.0	4.0	4.00	0.00
LLT	3.2	4.3	3.82	0.03	3.2	3.8	3.54	0.03	3.2	3.8	3.38	0.04	4.0	4.9	4.59	0.03	3.8	4.1	3.92	0.06	3.7	3.7	3.70	0.00