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# Phenotypic Features of the '*Guentheri*' Group Vole (Mammalia: Rodentia) in Turkey and Southeast Bulgaria: Evidence for Its Taxonomic Detachment

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**Abstract:** The taxonomic status of *Microtus guentheri*, which is distributed in Anatolia, and the related taxa (number of chromosomes 2n = 54) inhabiting Western Anatolia and Southeast Thrace within the territory of Bulgaria and Turkey, were investigated morphologically and statistically. It was determined that the form of the second upper molar in all studied *M. guentheri* specimens belonged to the non-agrestis morphotype as did that in all specimens from Western Anatolia, Bulgarian and Turkish Thrace. The forms of the third upper molar did not separate *M. guentheri* from the specimens of Western Anatolia, Bulgarian and Turkish Thrace, and showed great variations among the normal, simplex, duplicate, and complex forms. In contrast, the marked distinguishing characteristics in dorsal colours, and skull and baculum morphologies were found between these populations. Statistical analyses (Tukey HSD) showed that the mean values of 18 variables in Thrace specimens and *M. guentheri* and 23 variables in the specimens of Thrace and Western Anatolia were significantly ($p<0.05$) different one from another. According to Principal Component Analyses, 75% of the total variance was explained by five components that had eigenvalues higher than 1, and specimens of Bulgarian and Turkish Thrace were clearly separated from *M. guentheri* and the specimens of Western Anatolia in discriminant function analyses. These findings suggested that specimens from Bulgarian and Turkish Thrace cannot be identified as *M. guentheri*. According to the priority rule, the specimens from Bulgarian and Turkish Thrace were assigned to *Microtus hartingi* and the subspecies *martinoi* and *strandzenzis* were considered junior synonyms of this taxon. However, the strong morphological and statistical evidences were provided the population in western Anatolia should be assigned to *Microtus lydius* as a valid species.

**Key words**: Morphological features, Taxonomy, *Microtus*, Bulgaria, Turkey

## Introduction

The subgenus *Sumeriomys* of the genus *Microtus* was subdivided into two groups: *socialis* and *guentheri* (Ellermann, 1941). The voles with number of chromosomes 2n=54 are known as the *guentheri* group (Golenishchev *et al*. 2002), and *Microtus guentheri* (Danford and Alston, 1880), a well known species in this group, ranges from the Balkan Peninsula to Lebanon and Israel (Misonne 1957, Bodenheimer 1958, Ondrias 1966, Atallah 1977, Neithammer and Krapp 1982, Kefelioğlu 1995, Markov *et al.* 1995). However, more recently Yiğit and Çolak (2002) provided clear evidence that *M. guentheri* is a morphologically distinct species from Western Anatolian population, which was considered a valid taxon – *Microtus lydius* Blackler, 1916. Indeed, the morphological differences in the

dorsal colour, tail colour, and shape of the baculum, which were shown by Yiğit and Çolak (2002), were previously reported in original descriptions and in some other papers (Danford and Alston, 1880; Kivanç, 1978; Neithammer and Krapp, 1982; Kefelioğlu, 1995). Apart from these morphological differences, biometric characteristics such as ear length, interorbital construction, mastoid breadth, and height of tympanic bulla in *M. guentheri* were found to be significantly different from those of the nominate subspecies *Microtus lydius lydius*. Thus, based on biometric differences, a new subspecies *Microtus lydius ankaraensis* was also described in Central Anatolia (Yiğit and Çolak, 2002). In Balkan Peninsula the Guenther's vole, whose range also included Macedonia and Former Yugoslavia (Malec and Storch 1963), was represented by three subspecies: *Microtus guentheri hartingi* Barret-Hamilton, 1903, *Microtus guentheri martinoi* Petrov, 1941 and *Microtus guentheri strandzensis* Markov, 1960. Because their biometric characteristics were mostly overlapping (Ondrias 1966), these subspecies were described mainly on the grounds of slight colour differences. Kryštufek *et al.* (2009) stated using the molecular evidence that the specimens of Western Anatolia and Thrace are *Microtus hartingi* not *M. guentheri.* More recently Lemskaya *et al*. (2010) suggested that the ancestral *Microtus* species had a 2n=54 karyotype and *M. guentheri* had no chromosomal rearrangements. They also reported that morphological and molecular data show the divergence time among *Microtus* species lineages may be about 0.5 – 3.5 million years. Therefore, the specimens of the '*guentheri*' group vole known as *M. guentheri* in Western Anatolia and Balkan Peninsula need to be reevaluated taxonomically. Thus specimens of the vole with 2n=54 from Turkey and Bulgaria were morphologically and statistically compared in order to resolve the taxonomic conflict.

# Materials and Methods

The specimens of '*guentheri'* group were collected from different localities in Turkey and Bulgaria between 1994 and 2007 (Fig. 1). Morphological and biometrical evaluations were performed on both Turkish and Bulgarian specimens with 2n=54. In this connection, the diploid number of chromosomes was checked especially for topotype specimens of *M. guentheri*, the specimens of Western Anatolia (known as *M. lydius*) and Thrace (*Microtus guentheri strandzensis*).

The external, cranial, and os baculum morphologies of more than 200 specimens were morphologically examined. The classification of the molar occlusal pattern of $M^2$ and $M^3$ was performed in accordance with Neithammer and Krapp (1982). Reproductive signs such as lactation, pregnancy, were checked in the field and laboratory to help on determine age. Age was established according to the skull proportions and structure using the criteria described by Bashenina (1953). Only adult specimens were examined in this study. After the age of specimens were determined, 30 characteristics of adult specimens were measured with calipers to the nearest 0.01 mm for statistical comparison.

Adult males and females from each locality are pooled together for further analyses, as the results of the comparison between the mean values (Student's t-test, $p < 0.05$) of the used in this study craniometrical signs did not show any phenotypic difference between skull measurements of the males and females.

These results confirmed the widespread opinion that the metric characteristics in both sexes have almost identical values and corresponded to the algorithm used in the classical morphological studies of phenotypic diversity of voles from the '*guentheri'* group in their original descriptions in some other papers (Danford and Alston, 1880; Markov, 1960; Malec and Storch, 1963; Ondrias, 1966; Kivanç, 1978; Neithammer and Krapp, 1982; Kefelioğlu, 1995; Yiğit and Çolak, 2002), which did not account for sexual dimorphism in studied morphological characteristics.

The specimens from Turkey and Bulgaria were divided into four operational taxonomic units (OTUs) for statistical analyses as follows:

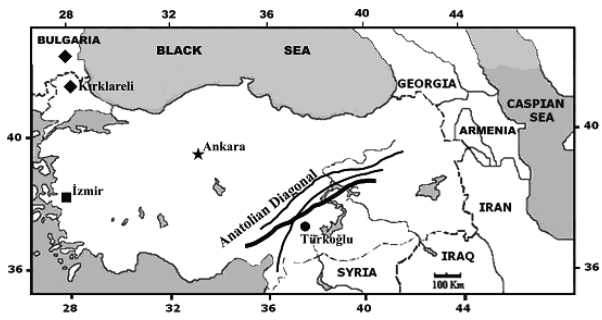


**Fig. 1**. The sampling locations of specimens evaluated under the OTUs
(♦= OTU–1, ★= OTU- 2, ■= OTU-3, ●= OTU-4).

OTU-1: Thrace specimens; Turkish Thrace (n=8) and topotype specimens of *M. g. strandzensis* from Strandzha Mountain Region, Bulgaria (n=23),

OTU-2: Central Anatolia (topotype specimens of *M. lydius ankaraensis* (n=17) from Central Anatolia),

OTU-3: Western Anatolia (topotype specimens of *M. lydius* (n=20) from İzmir, Turkey),

OTU-4: Topotype specimens of *M. guentheri* (n=18) from Türkoğlu/Kahramanmaraş, Turkey.

Pair-wise comparisons of these species by each variable were performed using the *post hoc* Tukey's HSD test with unequal sample size. Measured characteristics were analyzed through ordination analysis (PCA). Discriminant function analysis (DFA) was carried out using SPSS 13. Phenotypic (external and cranial) relationships among groups (OTUs) appeared in neighbour joining (NJ) dendrogram were obtained by distance matrix (Nei, 1978; Rohlf, 2000, NTSYS-pc version 2.1).

Abbreviations used for the characteristics measured were as follows: TBL (total body length), TL (tail length), HFL (hind foot length), EL (ear length), ZB (zygomatic breadth), IC (interorbital constriction), ONL (occipitonasal length), CBL (condylobasal length), BL (basal length), NL (nasal length), FL (frontal length), MAB (mastoid breadth), SH (height of braincase with bullae), OW (occipital width), BW (braincase width), DL (diastema length), PL (palatal length), FI (length of foramina incisiva), HML (height of maxillary), LTB (length of tympanic bulla), MAL (mandible length), MAH (height of mandible), UML (upper molar length), $M^1$ (length of first upper molar), $M^2$ (length of second upper molar), $M^3$ (length of third upper molar), LML (lower molar length), $M_1$ (length of first lower molar), $M_2$ (length of second lower molar), and $M_3$ (length of third lower molar).

# Results

Topotype specimens of *M. guentheri*, which have uniform dark brownish dorsal fur, are somewhat reminiscent of the dorsal colour of *Microtus rossiaemeridionalis* (*Microtus levis*). In terms of appearance, this vole is easily distinguishable from the specimens of Turkish Thrace and Bulgaria (OTU-1) and *M. lydius* (OTU-2, 3) because *M. lydius* and Thrace specimens have a more yellowish coloration on the sides. Habitat preference is also somewhat different among *M. guentheri*, *M. lydius*, and Thrace specimens. Especially the type locality of *M. guentheri* is east of a natural geographical barrier known as the Anatolian Diagonal in Southeastern Turkey (Fig. 1) and lives in meadows and clover fields, and on river banks rather than the arid and semi-arid steppe.

Cranial morphology was similar among these populations. Differences, however, were found in the frequencies of molar occlusal patterns on $M^2$ and $M^3$. Except for *M. lydius*, which has 10% agrestis and 90% non-agrestis morphotype, all taxa have the non-agrestis occlusal pattern. $M^3$ patterns were not found to be taxon specific and were mostly observed in normal and duplicated forms (Table 1). In addition, the shape of the os baculum was found to be distinctive between OTUs with the main difference in the base shape of the os baculum. The base in OTU-4 is triangular and protrudes upward and there is a deep concavity on the underside. In contrast, it is smooth and disk-shaped in OTU-1, 2, and 3, with a recess on the posterior tip of the base in some specimens. However, the base of the os baculum in Thrace specimens is somehow prismatic, the recession is not well marked, and the posterior tip of the base is almost smooth but not oval. Two os baculum samples were shown in Fig. 2.

Craniological and somatological characteristics of both species, show similar absolute variabil-

**Table 1.** Frequencies (%) of molar cusps type in OTUs of *Microtus,* N= number of specimens, A= Agrestis, NA= Non-agrestis.

| Species | N | $M^2$ | | $M^3$ | | | |
|---|---|---|---|---|---|---|---|
| | | A | NA | Normal | Simplex | Duplicate | Complex |
| OTU-4 | 18 | - | 100 | 61 | 17 | 11 | 11 |
| OTU-1 (Turkish Thrace) | 11 | - | 100 | 36 | - | 64 | - |
| OTU-1 (Bulgaria) | 23 | - | 100 | 78 | - | 13 | 9 |
| OTU-2 and 3 | 20 | 10 | 90 | 70 | 10 | 20 | - |

ity of their corresponding parameters into the four investigated operational taxonomic units (OTUs) (Table 2).

Pair-wise comparisons performed with Tukey's HSD test using 30 variables showed significant differences in 25 variables between four OTUs of the genus *Microtus*. Thrace specimens were found to differ from *M. guentheri* (OTU-4) by the following characteristics: HFL, ZB, IC, ONL, CBL, FL, MAB, SH, BW, DL, PL, FI, HML, LTB, MAL, MAH, $M_2$, and $M_3$ ($p<0.05$). In comparing the first OTU with *M. lydius* (OTU 2 and 3) some variables (IC, FL, MAB, PL, FI, MAL, $M^1$, $M_2$, and $M_3$) distinguished OTU-1 from OTU-2 and 3. Pair-wise comparisons showed that OTU-1 was statistically the most distinct among the OTUs. These findings were also supported by cluster analyses. The specimens of OTU-1 were separately entered into the analyses as Bulgarian and Turkish Thrace specimens, and the NJ dendrogram summarizes the phenetic relationship found among the OTUs in Fig. 3. In this cluster, the specimens of Bulgaria and Turkish Thrace formed the first subcluster, and OTU-3 (*M. l. ankaraensis*) was connected to this sub-cluster. OTU-2 and 4 separately formed a sub-cluster and clearly diverged from Thrace specimens (Fig. 3). The NJ dendrogram also showed gradual differentiation in biometrical characteristics from north to south in the areas of OTUs.

In the Principal component analysis (PCA), the Kaiser-Meyer-Olkin Measure of Sampling Adequacy showed a high level of sampling adequacy, with the value 0.918. In order to test for homogeneity of variances, Bartlett's test was first used to test if our samples have equal variances across OTUs. In the Bartlett test statistic, the upper critical value of the chi-square distribution (approx. chi-square value: 2753.053) corresponded to a significance level of 0.01. Thus the null hypothesis was rejected and the data set was assumed to fit the PCA statistic. PCA yielded five PCs that explained 75.7% of the total variance (Table 3). The first three PCs explain 67.1% of the total character variation (49.7%, 9%, and 8.3%, respectively). The initial eigenvalues of the components that explain the total variance were moderate in discriminating between OTUs of *Microtus*. All 30 morphometric characters were found to have some contribution to this variance but the three highest contributions to PC1 were attributed to ONL (0.947), PL (0.936), and CBL (0.932). Other significant contributions to PC1 were HML, MAL, ZB, DL, NL, MAH and SH with values above 0.800. The characters that contribute to PC2 mostly had values less than 0.743.

External and cranial characteristics of the four OTUs were subjected to DFA. Of the 86 specimens, 84 (98%) were classified correctly. Total variation

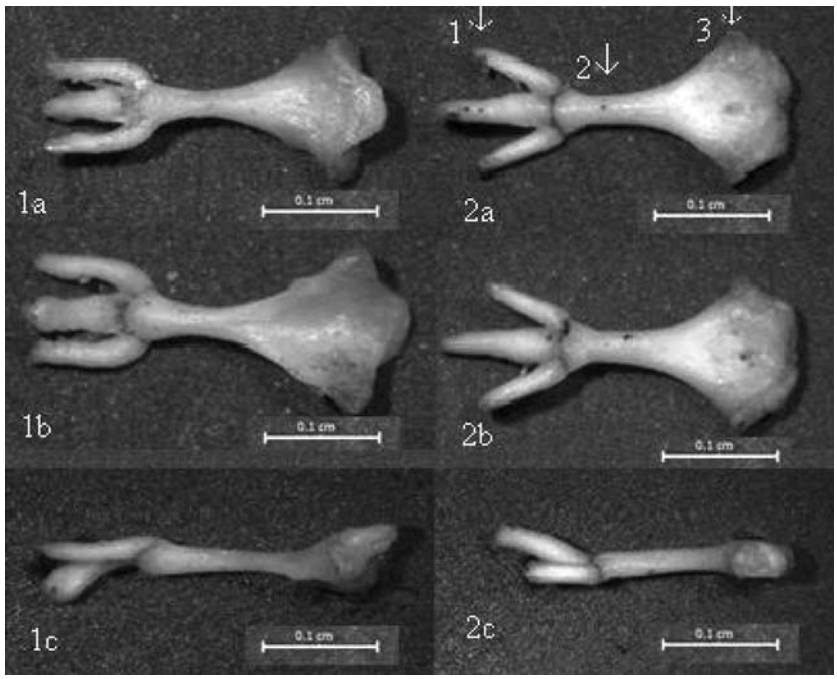


**Fig. 2**. *M. guentheri* (left), *M. lydius* (right), 1a, 2a=dorsal, 1b, 2b=ventral, 1c, 2c=lateral views (1=cartilagous part of baculum, 2=shaft, 3=base).

**Table 2.** Basic descriptive statistics of the somatometrical and craniometrical features (mm) taken in specimens of the '*guentheri*' group vole (Mammalia: Rodentia). Description of abbreviations of the features are given in Material and Methods.

| | **Operational taxonomic units (OTUs)** | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **OTU-1 (Turkey)** | | | **OTU-1 (Bulgaria)** | | | **OTU-2** | | | **OTU-3** | | | **OTU-4** | | |
| | **N** | **X** | **SD** | **N** | **X** | **SD** | **N** | **X** | **SD** | **N** | **X** | **SD** | **N** | **X** | **SD** |
| TBL | 8 | 141.00 | 10.97 | 23 | 138.00 | 9.21 | 17 | 146.53 | 15.57 | 20 | 139.80 | 16.29 | 18 | 138.72 | 10.19 |
| TL | 8 | 29.00 | 2.27 | 23 | 28.50 | 4.72 | 17 | 29.24 | 5.07 | 20 | 26.85 | 3.75 | 18 | 27.39 | 3.29 |
| HFL | 8 | 21.88 | 0.84 | 23 | 20.80 | 1.39 | 17 | 21.82 | 1.74 | 20 | 20.00 | 1.12 | 18 | 20.11 | 1.02 |
| EL | 8 | 13.69 | 0.46 | 23 | 13.22 | 0.52 | 17 | 14.00 | 1.32 | 20 | 12.40 | 1.05 | 18 | 13.11 | 0.76 |
| ZB | 8 | 16.17 | 0.85 | 23 | 17.00 | 0.90 | 17 | 16.45 | 1.29 | 20 | 15.19 | 1.12 | 18 | 15.43 | 0.73 |
| IC | 8 | 3.69 | 0.13 | 23 | 3.61 | 0.20 | 17 | 3.84 | 0.14 | 20 | 3.83 | 0.11 | 18 | 3.84 | 0.21 |
| ONL | 8 | 27.64 | 1.41 | 23 | 28.10 | 0.91 | 17 | 28.06 | 1.51 | 20 | 25.88 | 1.64 | 18 | 26.64 | 0.91 |
| CBL | 8 | 28.09 | 1.40 | 23 | 28.00 | 1.02 | 17 | 28.42 | 1.88 | 20 | 26.22 | 1.92 | 18 | 26.85 | 1.10 |
| BL | 8 | 26.46 | 1.50 | 23 | 25.34 | 1.01 | 17 | 26.70 | 1.83 | 20 | 24.91 | 1.70 | 18 | 25.23 | 1.06 |
| NL | 8 | 8.46 | 0.56 | 23 | 8.00 | 0.46 | 17 | 8.28 | 0.69 | 20 | 7.54 | 0.75 | 18 | 7.73 | 0.52 |
| FL | 8 | 9.18 | 1.18 | 23 | 9.60 | 0.46 | 17 | 10.48 | 0.66 | 20 | 9.98 | 0.69 | 18 | 10.04 | 0.53 |
| MAB | 8 | 10.15 | 0.34 | 23 | 10.77 | 0.32 | 17 | 9.27 | 0.52 | 20 | 9.00 | 0.68 | 18 | 9.80 | 0.35 |
| SH | 8 | 11.1 | 0.30 | 23 | 10.58 | 0.28 | 17 | 10.61 | 0.41 | 20 | 9.84 | 0.40 | 18 | 10.07 | 0.41 |
| OW | 8 | 13.52 | 0.54 | 23 | 12.36 | 0.65 | 17 | 13.49 | 0.79 | 20 | 12.49 | 0.78 | 18 | 12.99 | 0.48 |
| BW | 8 | 12.85 | 0.41 | 23 | 12.65 | 0.37 | 17 | 12.69 | 0.44 | 20 | 12.62 | 0.38 | 18 | 12.24 | 0.32 |
| DL | 8 | 8.51 | 0.49 | 23 | 9.10 | 0.40 | 17 | 8.79 | 0.58 | 20 | 8.03 | 0.74 | 18 | 7.93 | 0.42 |
| PL | 8 | 14.57 | 0.75 | 23 | 14.84 | 0.82 | 17 | 14.05 | 0.98 | 20 | 12.94 | 1.04 | 18 | 13.50 | 0.53 |
| FI | 8 | 4.63 | 0.32 | 23 | 5.17 | 0.24 | 17 | 4.64 | 0.49 | 20 | 4.13 | 0.46 | 18 | 4.57 | 0.33 |
| HML | 8 | 8.33 | 0.54 | 23 | 8.70 | 0.53 | 17 | 8.42 | 0.67 | 20 | 7.54 | 0.71 | 18 | 7.56 | 0.33 |
| LTB | 8 | 8.51 | 0.55 | 23 | 9.42 | 0.43 | 17 | 8.77 | 0.51 | 20 | 7.66 | 0.68 | 18 | 8.27 | 0.44 |
| MAL | 8 | 17.94 | 1.05 | 23 | 17.48 | 0.79 | 17 | 16.43 | 1.15 | 20 | 15.07 | 1.17 | 18 | 16.37 | 0.82 |
| MAH | 8 | 9.77 | 0.60 | 23 | 9.82 | 0.47 | 17 | 9.43 | 0.78 | 20 | 8.96 | 0.60 | 18 | 9.02 | 0.54 |
| UML | 8 | 7.27 | 0.31 | 23 | 6.67 | 0.36 | 17 | 6.74 | 0.44 | 20 | 6.49 | 0.41 | 18 | 6.63 | 0.28 |
| $M^1$ | 8 | 2.62 | 0.29 | 23 | 2.45 | 0.14 | 17 | 2.33 | 0.19 | 20 | 2.29 | 0.18 | 18 | 2.42 | 0.18 |
| $M^2$ | 8 | 2.33 | 0.11 | 23 | 2.00 | 0.15 | 17 | 1.99 | 0.16 | 20 | 2.01 | 0.15 | 18 | 1.97 | 0.13 |
| $M^3$ | 8 | 1.98 | 0.41 | 23 | 2.32 | 0.19 | 17 | 2.15 | 0.18 | 20 | 2.00 | 0.14 | 18 | 2.23 | 0.22 |
| LML | 8 | 7.21 | 0.37 | 23 | 6.56 | 0.44 | 17 | 6.77 | 0.43 | 20 | 6.51 | 0.40 | 18 | 6.49 | 0.33 |
| $M_1$ | 8 | 2.82 | 0.46 | 23 | 3.41 | 0.23 | 17 | 3.22 | 0.19 | 20 | 3.22 | 0.31 | 18 | 3.22 | 0.23 |
| $M_2$ | 8 | 2.28 | 0.21 | 23 | 1.80 | 0.14 | 17 | 1.59 | 0.15 | 20 | 1.71 | 0.14 | 18 | 1.67 | 0.18 |
| $M_3$ | 8 | 2.02 | 0.17 | 23 | 1.70 | 0.13 | 17 | 1.61 | 0.19 | 20 | 1.63 | 0.10 | 18 | 1.58 | 0.17 |

was described by four components in the DFA. Wilks' lambda values assume that the characteristics with low values are more discriminative between the subpopulations. Wilks' lambda values, which explain the tests of equality of group means, were significant for most of the characteristics ($p<0.01$). Canonical discriminant function analysis based on three components from 1 through 3 yielded a highly significant Wilks' lambda of 0.004 ($\chi^2$= 378,462, DF= 90, $p<0.001$). The first canonical variable (CV) explained 72.7% of the variance; the first and second CVs explained 89% and both could clearly separate the OTUs of *Microtus*. When considering the OTUs of *Microtus* analyzed in this study, the first CV markedly separated OTU-1 from OTU-2, OTU-3, and OTU-4. However, the second CV distinguished OTU-4 from the other OTUs. OTUs from Bulgaria and Turkish Thrace (OTU-1) and Türkoğlu

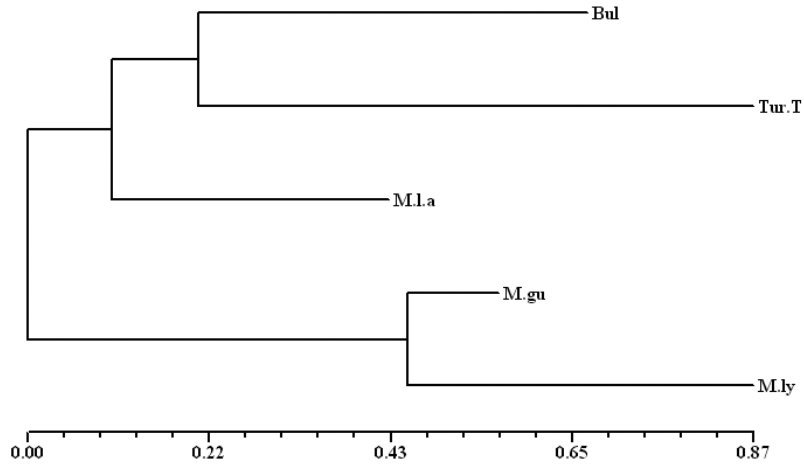


**Fig. 3**. Neighbour joining dendrogram (Nei 1978) indicating phenetic relationship of OTUs (OTU-1: Bul.-Tur.T., OTU-2: M. ly., OTU-3: M. l. a., OTU-4: M. gu.).

**Table 3.** PCA of OTUs extracted five principal components that explain 75.7 % of total variance.

| Component | Initial Eigenvalues | | |
|---|---|---|---|
| | Total | % of Variance | Cumulative % |
| 1 | 14.931 | 49.772 | 49.772 |
| 2 | 2.711 | 9.038 | 58.810 |
| 3 | 2.506 | 8.352 | 67.162 |
| 4 | 1.537 | 5.124 | 72.285 |
| 5 | 1.027 | 3.423 | 75.709 |

Extraction Method: Principal Component Analysis.

(OTU-4) were perfectly classified (100% correct classification), followed by OTU-2 (95%) and OTU-3 (94.1%). According to the results obtained from the DFA, *Microtus* from Bulgaria and Turkish Thrace are markedly diverged from the Anatolian OTUs (Fig. 4). When we considered just Anatolian OTUs, they were also separated from each other but the classification rate was slightly lower.

As a result, morphological characteristics and statistical analyses showed that the specimens from Thrace (OTU-1) represent a separate taxon from *M. guentheri* and OTU-2,3. According to the priority rule, the specimens of both Turkish Thrace and Bulgaria were assigned to *Microtus hartingi*, which is considered a valid taxon. The subspecies *martinoi* and *strandzensis* remain synonyms of this species. The taxonomic status of OTU-2, 3 which are clearly different from *M. guentheri* remains unclear. However our findings from OTU-2, 3 support the thought that the population in Western Anatolia can be evaluated as valid taxa '*Microtus lydius'*. To the certain assaying this taxa a separate species that we need a bit more evidence especially from the molecular systematics.

# Discussion

*M. guentheri* was first described from Türkoğlu (Kahramanmaraş), located in Southeastern Turkey, by Danford and Alston (1881), and *M. lydius* was recorded from İzmir in the Western part of Turkey, about 900 km from Türkoğlu. Yiğit and Çolak (2002) stated that the main factor that prevents *M. guentheri* from penetrating into Western Anatolia is the mountainous barrier called the Anatolian Diagonal. In addition, striking differences in dorsal coloration, baculum morphology, and biometric characteristics were also reported (Kivanç 1978, Neithammer and Krapp 1982, Kefelioğlu 1995, Yiğit and Çolak 2002, Golenishchev *et al.* 2002). Our results supported previous reports describing differences among the specimens of Thrace, Western Anatolia, and *M. guentheri*.

Kefelioğlu and Kryštufek (1999) considered *M. guentheri* and *M. lydius* as conspecific and compared them to social voles (*Microtus socialis*, *Microtus anatolicus*, and *Microtus dogramacii*). They suggested that OTU guentheri appeared to be the most distinct from other social voles in Turkey, and occlusal cusps of the second upper molar of non-agrestis morphotype do not provide categorical species diagnostics. The same morphotype was also found in the topotype specimens of *M. guentheri* but both morphotypes in

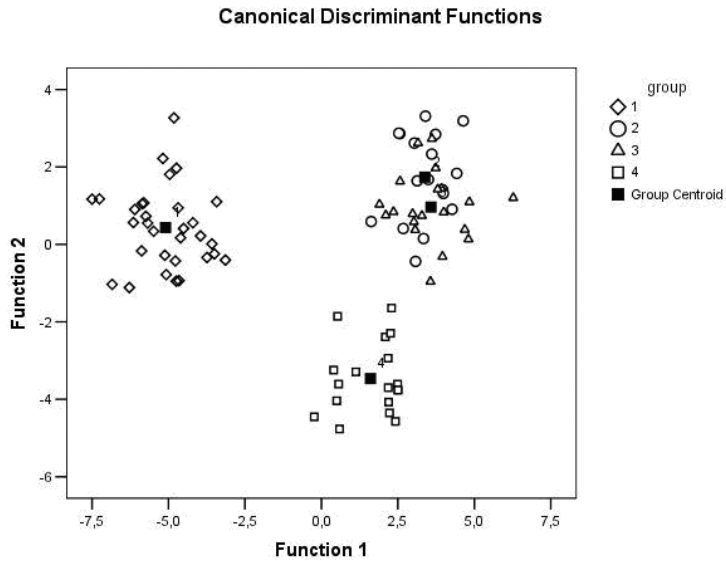


**Fig. 4**. Scatter plot of the discriminant scores for OTUs derived from 86 specimens and 30 variables (1: Specimens of OTU-1, 2: OTU-2, 3: OTU-3, 4: OTU-4).

$M^2$ were seen in OTU-2, 3 (Table 1). Golenishchev *et al.* (2002) reported the agrestis morphotype for $M^2$ and the normal form for $M^3$ in Bulgarian specimens. Our findings are not consistent with those reported by Golenishchev *et al.* (2002) for the $M^2$ pattern but the normal form was the most frequently observed in Bulgarian specimens.

The os baculum was found to be a taxonomically important characteristic separating the OTUs. The typical os baculum morphology was previously reported by Kivanç (1978), Kefelioğlu (1995) and Yiğit and Çolak (2002) for *M. guentheri*. However, Kefelioğlu (1995) suggested that topotype specimens of OTU-3 had the same morphology. In contrast, Yiğit and Çolak (2002) showed differences in os baculum morphology between these taxa; the difference in os baculum among Bulgarian specimens was similarly reported by Golenishchev *et al.* (2002).

The specimens of Balkan Peninsula with geographic proximity to Turkish Thrace were assigned to two subspecies: *M. g. hartingi* and *M. g. strandzensis*. The pelage of *M. g. hartingi* is buff brown to tawny olive along the back, becoming lighter and more yellowish on the sides (Ondrias 1966). Markov (1960) described a new subspecies, *M. g. strandzensis*, based on specimens collected near the village of Gramatikovo, Strandzha Mountain (Eastern Bulgaria). He described the dorsal colour of these specimens as grayish-brown, turning gray-yellowish on the belly without a clear line of demarcation. Moreover, *M. g. strandzensis*, as described by Markov (1960), had longer hind feet than *M. g. guentheri*. The colour descriptions of Bulgarian (Markov 1960) and Greek (Ondrias 1966) specimens show slight differences but the descriptions are generally consistent with each other. In our study, specimens examined from both Bulgarian and Turkish Thrace have the same dorsal colouration, and this colouration of pelage is slightly different from that of OTU-2,3 but clearly different from that of *M. guentheri*.

Ondrias (1966) stated that the dorsal colour of the specimens from Eastern Bulgaria is similar to that of the specimens from Greece as well as those from Former Yugoslavia, and the external and cranial measurements of Greek subspecies are smaller than those of Bulgarian subspecies. However, when comparing the mean and ranges of measurements (total length, tail length, hind foot length, ear length, condylobasal length, basilar length, zygomatic breadth, interorbital constriction, nasal length, maxillary tooth row, and diestema length) of *M. g. hartingi*, *M. g. lydius*, and *M. guentheri* given by Ondrias (1965), the mean values are very similar to each other and remain within the ranges of these subspecies, even in the small number of specimens measured. These findings support the idea that measurements alone are not discriminative without applying complex statistical analysis.

According to paleontological evidence, the guentheri group evolved from the fossil genus *Allophaiomys*, which radiated independently in Northern Eurasia,

Central Asia/Himalayas, and North America during the early Pleistocene (Chaline 1972, Van Der Meulen and Zagwijn 1974, Chaline 1985, Chaline *et al*. 1999). Geographic barriers that separate the Balkan Peninsula from Asiatic Turkey are the Dardanelle and Bosporus straits. Recent evidence suggested that the Dardanelle and Bosporus straits were formed in the late Pliocene and connected Black Sea to Mediterranean Sea (Bacescu, 1985; Tortonese, 1985; Çağatay *et al*. 2000, Yaltirak *et al.* 2000). Although Moureau (1955) suggested that the Bosporus land bridge was in existence during Würm glaciations, it seems unlikely that terrestrial and meadow rodents would cross the straits. Therefore, it is possible that the Dardanelle and Bosporus straits were more influential on the speciation of terrestrial rodent species than arboreal ones and that these straits form a natural boundary between Europe and Asia. 2n=54 was reported as ancestral karyotype for *Microtus* and the research showed that the divergence time between *Microtus* spp lineages are about 0.5-3.5 million years (Lemskaya *et al.* 2010). Our findings also supported this suggestion that the most common karyotype in Anatolia is 2n=54, and *M. guentheri*, specimens of Western Anatolia and Thrace were evaluated as sister species. Our findings are also consistent with those of Kryštufek *et al.* (2009). They reported that specimens of both Thrace and Western Anatolia were assigned to *M. hartingi*. According to cyt *b* sequence analyse, *M. guentheri* was strongly separated from the specimens of Western Anatolia and Thrace which were grouped into the same clade (Kryštufek *et al.* 2009). However considering the Dardanelle and Bosporus straits formed in the late Pliocene even an existence the straits during Würm glaciations, the assigning the population of Western Anatolia as *M. hartingi* is somehow doubtful and needs more clear evidences. Supporting this assumption Central Anatolia was reported to become an evolutionary theater for speciation of *Microtus* species, stating that the genus *Microtus* is ideal for evolutionary studies on the role of Quaternary glacial cycles on diversification (Jaarola *et al.* 2004). Therefore, many rodent species such as *Microtus anatolicus*, *Microtus dogramacii* and *Spermophilus taurosensis* were recently described in and western Anatolia (Kefelioğlu and Kryštufek 1999, Kryštufek and Kefelioğlu 2001, Gündüz *et al*. 2007). The recent specification of closely related species such as *Mesocricetus newtoni – Mesocricetus brandti* and *Spermophilus citellus – Spermophilus torosensis – Spermophilus xanthoprymnus* supports such a scenario (Yiğit *et al*. 2005, Yiğit *et al*. 2006, Gündüz *et al*. 2007). It was also reported that *Erinaceus concolor* specimens of Asiatic Turkey and Israel have haplotypes quite distinct from those of the Balkan population (Hewitt 1999, Seddon *et al*. 2001). Given this and our findings that os baculum morphology, dorsal coloration, and the DFA distinguish *M. guentheri* from OTU-2, 3 and OTU-1, it can be concluded that Anatolian populations (OTU-2, 3, 4) diverged from Thrace population (OTU-1). There is no doubt that OTU-1 is a different species from *M. guentheri*, and specimens (previously known as *M. g. hartingi*) in the Balkan Peninsula were considered a valid taxon, namely *M. hartingi*. However, molecular research is necessary to elucidate the phylogenetic relationship and level of speciation between *M. hartingi* and the specimens of Western Anatolia. As a result, our findings strongly indicate that the specimens of Bulgarian and Turkish Thrace cannot be identified as *M. guentheri* or *M. lydius*. The morphological and biometrical comparisons showed that Thrace specimens previously assigned to *M. g. hartingi*, *M. martinoi* and *M. g. strandzensis* resemble each other in morphological and craniometrical respects, and are quite distinct from Anatolian specimens. Therefore, according to the priority rule, the specimens from Bulgarian and Turkish Thrace were assigned to *Microtus hartingi*, and the subspecies *martinoi* and *strandzensis* remain synonyms of this species, whose taxonomic validation and geographic boundaries need further clarification. The distribution of *M. guentheri* is confined to Southeastern Turkey, and this study supported the findings of Yiğit and Çolak (2002) which provides the strong evidences *M. lydius* for evaluate as valid taxa.

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