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COMPARATIVE BIOMETRIC AND MORPHOLOGICAL ANALYSIS OF SUBGENUS *TERRICOLA* (RODENTIA: *MICROTUS*) IN EASTERN BLACK SEA REGION FROM TURKEY

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ABSTRACT. In the scope of current study, 41 specimens of the subgenus *Terricola* collected from nine different localities in Trabzon, Rize and Artvin provinces in Eastern Black Sea Region were subjected to detailed morphological and comparative biometric analyses. Based on those analyses; presence of two species, *Microtus* (*Terricola*) *subterraneus* and *Microtus* (*Terricola*) *majori* were determined in the region. Evaluation of the 12 specimens belonging to *M.* (*T.*) *subterraneus* showed that there is no intrapopulational variation within this species. Two different populations of *M.* (*T.*) *majori* were determined in the region studied, based on morphological differentiations in enamel cusp patterns of 29 specimens. Additionally, as a result of the evaluations made, it was determined that both species could be found in the similar habitats throughout study area and therefore that these two species can be coexist as sympatric within the same geographic area.

1. INTRODUCTION

The genus *Microtus* is distributed in Holarctic and contains a large number of species. About 65 species are present in this genus throughout its distribution range. Distribution map of the *Microtus* species includes various types of habitats such as meadows, pastures, forests and highlands. In this regard, by having quite different habitat types shaping by the influence of diverse ecological conditions, including mentioned habitats, Turkey is host to 13 *Microtus* species three of them are endemic. [1, 2].

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The phylogenetic relationships within this genus are unclear, and there are difficulties in identifying species boundaries within the genus and in identifying subgenus [3]. At the same time rapid diversification and speciation processes which originated from mentioned complex relationships having determined by based on morphology, karyology and even mitochondrial DNA in the genus are known to be still continued [1]. This situation has recently led to the need for increase in the number of species described and even updates in the relationships at the subgenus or the genus level [2, 4]. As a natural consequence of this, with the support of the results of a subsequent molecular study too, the fact that *Chionomys* should be treated as a separate genus from *Microtus* was reinforced more. In addition to this, Palearctic species with characterized by the pitymoid condition of the first lower molar of *Microtus* were incorporated into *Terricola* subgenus, while, those with the similar molar teeth morphology in Nearctic were included in *Pitymys* subgenus as well [3].

The subgenus *Terricola* is one of the seven subgenus of *Microtus* and is represented by three species in Turkey [5-9]. *Microtus* (*Terricola*) *majori* and *Microtus* (*Terricola*) *subterraneus* along with the *Microtus* (*Terricola*) *daghestanicus* are closely related three species of *Terricola* and are known to be the members of pine voles group [10]. Distribution range of *M.* (*T.*) *subterraneus*, known as European pine vole, includes mostly Central and South Europe, northwestern Russia and the northern parts of Turkey. Its distribution in Turkey starts from Thrace in the west and lasts up to near Trabzon, which is type locality of *M.* (*T.*) *majori* in the east. Another species, mostly known as Caucasian pine vole, *M.* (*T.*) *majori*, is distributed in the northeastern parts of Turkey, Caucasus and northern parts of Iran. Although it has been previously suggested by some authors that this species has distributed in Europe including Thrace [6-9], Kryštufek et al. [11] has later showed that this species does not live in Europe. The moist forests on the southern coast of the Black Sea in northland of Turkey are the main habitats of these two small subterranean rodent species involved in *Terricola* subgenus. Previous studies has proposed that the distribution ranges of these two species in Turkey are not overlap and thus these species are not in sympatry event [1, 12, and 13]. Apart from these, although not enough data on the distribution of *M.* (*T.*) *daghestanicus*, Kryštufek and Vohralík [1] suggested that this species lives to a limited extent in the distribution range of *M.* (*T.*) *majori* in Turkey.

M. (*T.*) *subterraneus* and *M.* (*T.*) *majori* are morphologically quite similar species to each other and have been well adapted to the subterranean life with short toe and flat skull. Morphological distinction of these species is often not easy, and the distinction between these two species is mostly based on variations in molar tooth morphology

along with the external morphological characters' size. Except for slight differences, the karyotypes of both species are similar. The karyotype of *M.* (*T.*) *majori* consists of 54 chromosomes as it is in the Anatolian populations of *M.* (*T.*) *subterraneus*. Karyotype of European *M.* (*T.*) *subterraneus* populations is different from that of Anatolian populations and contains 52 chromosomes. These two species are found to be genetically very close and the low genetic distance based on allozyme data between them indicates that they have recently diverged [13]. It has later been suggested that this result is to be in conflict with the results of a subsequent DNA study including only one *M.* (*T.*) *majori* sample [3].

Among the representatives of the subgenus *Terricola* from Turkey, *M.* (*T.*) *subterraneus* and *M.* (*T.*) *majori* were more detailed examined. In the limited number of past studies, taxonomical assessments belonging to the subgenus *Terricola* were made by considering the variations in the fur coloration, molar tooth morphology, skull, baculum and karyology and biochemical [1, 12]. Although these studies provide valuable results, biochemical and karyotype studies among them were considered to have more satiable results for distinguishing both species [13], however, outcomes of those based on morphology were thought that they were not sufficient because they could not fully eradicate the complexities in their taxonomy. In addition, all those studies included either a limited number of samples or a limited number of localities from the area where the both species could be coexist. In this context, when the above-mentioned problems were considered, in particular on the representatives of this subgenus living in the localities from Eastern Black Sea Region, it was seen that there was no detailed study comprising morphological examination along with the comparative biometry employing multivariate statistics. Therefore, by present study, it was aimed to examine the morphological aspects of the populations of these two species living in the Eastern Black Sea Region by performing comparative biometric analysis, and thus to contribute to the taxonomic status and distribution of both species in this area.

2. Materials and Methods

A total of 41 samples of *M.* (*T.*) *subterraneus* and *M.* (*T.*) *majori* collected from nine different localities in the Eastern Black Sea Region were evaluated by morphological and biometric methods (Figure 1, Table 1). Samples were collected by the field studies performed between 2000 and 2008 years. In addition, a small number of museum samples were used as well. All samples used in the study were adult and there was no sexual dimorphism in individuals of the species of this subgenus. For detecting adult samples, the uterus and lactating status for female samples and the

testis status for male samples were considered and data recorded during preparation of the samples. All samples were firstly made into standard museum material. Then, in the morphological examinations, the skull, tooth and fur characteristics of the samples were taken into consideration. After treated at 70 °C for 15 minutes by 10% solution of ammonia (NH3) for removing the soft tissue remnants, the skulls were prepared for morphological examination. The skulls and teeth were detailed examined and photographed under the Scienscope SSZ Trinocular Stereo Zoom Microscope (Scienscope International Corporation, Chino, CA, USA). All skulls and skins were stored in the Ankara University Mammalian Research Collection (www.mammalia.ankara.edu.tr, AUMAC) for subsequent investigations.

Before starting the biometric analyses, the morphological criteria suggested by Ognev [14] and Osborn [15] were taken into consideration in the morphological diagnosis of both species. According to that, samples with an extra marked protrusion in the posterior of second upper molar tooth in the lingual side were grouped as *M.* (*T.*) *majori*, while, samples with no such protrusion were considered to be *M.* (*T.*) *subterraneus*. Also, *M.* (*T.*) *majori* samples were divided into two different groups as *M.* (*T.*) *majori* 1 and *M.* (*T.*) *majori* 2 based on the morphological variation determined in the third molar teeth of upper jaw (an extra protrusion in the labial and an additional recess). Ognev [14] has previously proposed that *M.* (*T.*) *majori* samples having such an extra protrusion in the labial and an additional recess from Sümela (Trabzon), where the type locality of this species is, were the nominate subspecies of this species. Therefore, *M.* (*T.*) *majori* samples used in the study were divided into two groups in the statistical analyses by taken into consideration this taxonomic rationale. Thus, all statistical analyses were performed on three different groups together with *M.* (*T.*) *subterraneus* samples.

The data set including four standard external, 22 cranial and eight dental measurements taken from the samples were used in the multivariate statistical analyses (TABLE 2). While preparing the data set, biometric characters frequently used in previous studies were taken into consideration [11, 12, 16 and 17]. For minimizing the measurement error, all the measurements were taken by the same person using the same digital caliper in the same laboratory conditions considering the applications in previous studies [18]. In the first place, the mean and standard error values of the internal and external character measurements of the populations belonging to the three groups were determined as descriptive statistics. Then, one-way analysis of variance (one-way ANOVA), minimizing the type I error in the multivariate data set, was used to determine whether there was significant difference between the group means. A multiple comparison test, Hochberg's GT2 that takes

into account the unequal sample size, were carried out to compare the means of the groups. With the similar purposes of one-way ANOVA, the multivariate analysis of variance (MANOVA) was used to determine if differences in biometric characters had significant effects on the mean vectors of the investigated groups, also whether there was any interaction among both groups and biometric characters. One another multivariate statistical method, the Discriminant Function Analysis (DFA), was carried out; (1) to estimate the relationships between groups and biometric characters, (2) to predict group membership of samples, (3) to test whether samples are classified as predicted and (4) to determine how much of the observed total variance among the groups can be explained by biometric characters.

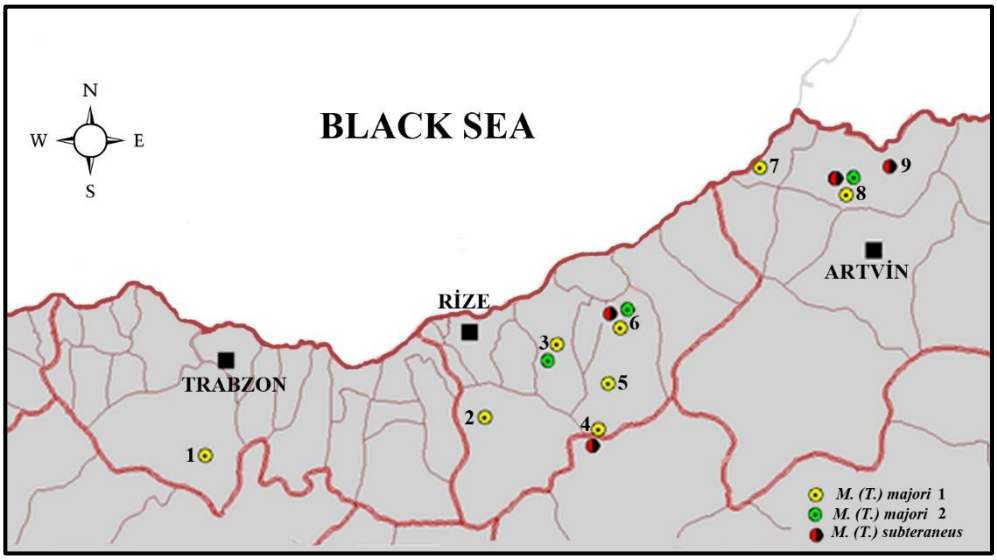

FIGURE 1. Collection sites of the samples; 1. Sümela (Trabzon), 2. İkizdere (Rize), 3. Kaptanpaşa (Rize), 4. Verçenik (Rize), 5. Çat (Rize), 6. Çamlıhemşin (Rize), 7. Hopa (Artvin), 8. Borçka (Artvin), 9. Karagöl-Borçka (Artvin).

In addition, the Principal Component Analysis (PCA), which is a size reduction method, was used to explain total variations among groups by fewer principal components that includes load of those biometric characters rather than a large number of correlated biometric characters. Before applying PCA, whether the data set was suitable for the analysis was checked by the Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy and Bartlett test of sphericity tests. All those analyses

were performed implemented in SPSS 15.0 for Windows [19]. Lastly, a clustering analysis was performed based on the Manhattan distance produced from averages of biometric characters of three groups and the unweighted pair group mathematical averages dendrogram (UPGMA) was created by NTSYSpc 2.2 [20].

TABLE 1. Collection site and sample size of examined populations

Population	**Locality**	**n ♂**	**n ♀**
M. (*T.*) *majori* 1	Sümela	3	7
	Ikizdere	1	-
	Kaptanpaşa	2	-
	Verçenik	-	1
	Borçka	1	1
	Hopa	-	1
	Çat	-	1
	Çamlıhemşin	-	2
M. (*T.*) *majori* 2	Kaptanpaşa	4	-
	Çamlıhemşin	1	1
	Borçka	2	1
M. (*T.*) *subterraneus*	Verçenik	2	1
	Çamlıhemşin	1	-
	Borçka	3	3
	Karagöl	1	1

3. Results

3.1 Morphology

3.1.1 Cranial characteristics

Although the skull was smaller in *M.* (*T.*) *majori* 1 than others were, it was virtually in the same structure in each populations. All the skulls had the entirely delicate structure and were flat looking from the posterior of the nasal bone to the occipital bone. The rostrum region was short and curved downwardly in the anterior. The anterior of the nasal bone was not exceed the anterior of the incisors. The parietal bone was widen to the edges and, in its anterior, indented into the frontal bone in different forms. This indentation seemed like a spearhead in *M.* (*T.*) *subterraneus*, contrary to this, it was in the form of a slight arc in both populations of *M.* (*T.*) *majori*. The brain capsule was wide and flat. In addition, compared to the entire skull, the brain capsule was the most occupant part of the skull in the ventral view. Interorbital region was relatively narrow and long in *M.* (*T.*) *subterraneus*. The same region was relatively wider and shorter in the *M.* (*T.*) *majori* 1 population than in the

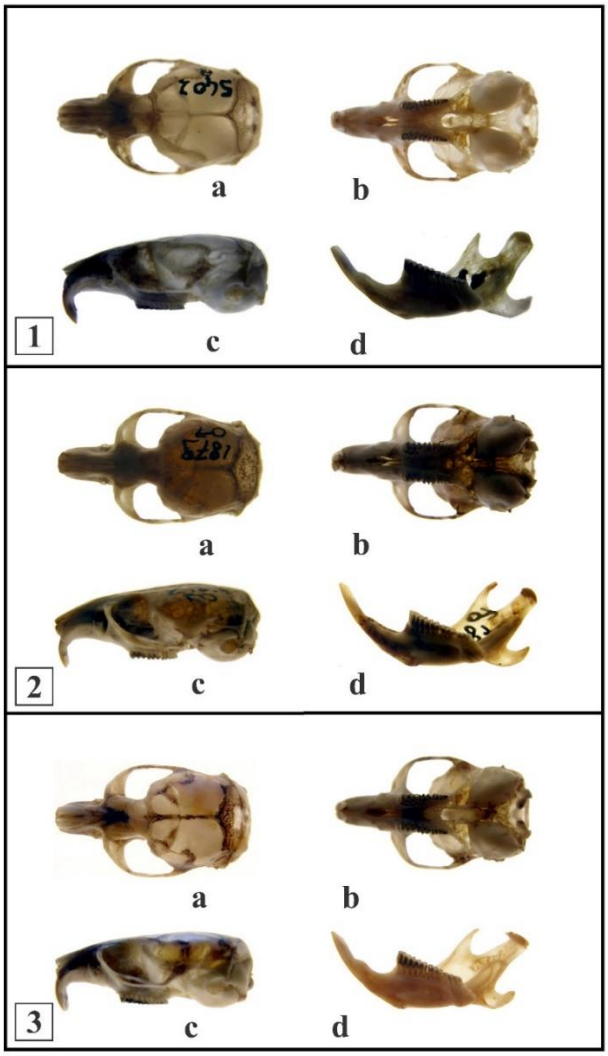

FIGURE 2. Dorsal (a), ventral (b), lateral (c) view of the skulls and right mandibles (d) of *M.* (*T.*) *subterraneus* (1), the first (2) and second (3) populations of *M.* (*T.*) *majori*.

M. (*T.*) *majori* 2 population. The zygomatic arch was fragile and relatively wide. The ventral part of the skull was narrow and triangular. In the ventral, the skull has a flat structure from the middle of the rostrum to the tympanic bullae. The posterior of the foramen incisive was in line with the anterior of the first upper molar teeth, and its

anterior was well behind the incisors. The pterygoid bones extended parallel to each other and ended by inclining towards the lateral from behind the anterior of the tympanic bullae. The tympanic bullae were of normal size compared to the skull. Mandibles were in a sensitive structure in all populations (FIGURE 2).

3.1.2 Dental characteristics

In all three populations, the incisors are orthodont (FIGURE 2, 1c, 2c and 3c). In all of the twelve specimens examined of *M.* (*T.*) *subterraneus* the crown of the first lower molar tooth (M_1) had a triangular like appearance, formed by 6 protrusions and 5 indentations in the lingual side, while, 5 protrusions and 4 indentations in the labial side (FIGURE 3, 1). A similar view was found in 19 of the 20 samples in the first population and in all of the samples of the second population of *M.* (*T.*) *majori* (Fig 3, 2 and 3). In one sample of the first population of *M.* (*T.*) *majori* (Sümela, Trabzon), the protrusion on the labial of the anterior lobe was unclear and thus four protrusions and three indentations were identified in M_1 (Fig 3, 2c). In addition, the same region of M_1 had a rather small and unclear additional protrusion and thus an indentation in three samples of the first population (Sümela: 1, Çamlıhemşin: 2) and in one sample of the second population of *M.* (*T.*) *majori* (FIGURE 3, 2a, 2b and 3a). On the other hand, this structure was not observed in the other samples of the same populations.

The appearance and number of the triangular like closed areas in the anterior lobe of the crown in M_1 was highly variable in all populations (FIGURE 3). This structure or appearance converged narrowly in seven samples of *M.* (*T.*) *subterraneus* (FIGURE 3, 1a and 1b), 13 samples of the first population (FIGURE 3, 2a, 2b and 2e) and seven samples of the second population of *M.* (*T.*) *majori* (FIGURE 3, 3b, 3c, 3d and 3e), whereas, in the remaining samples of each population, they were relatively broadly joined. The number of the triangular like closed areas in M_1 changed between four and six in all populations. A total of six triangular like closed areas in the crown of M_1 were detected in eight samples of *M.* (*T.*) *subterraneus*, twelve of the first population and six samples of the second population of *M.* (*T.*) *majori*. In the three samples of *M.* (*T.*) *subterraneus* (Borçka, Artvin), 12 samples of the first and three samples of the second population of *M.* (*T.*) *majori* (Kaptanpaşa: 2, Borçka: 1), five triangular like closed areas were determined in M_1 (FIGURE 3, 1a, 2c, 2d, 3a, 3b, 3c and 3d). This appearance was the result of the combination of the closed areas in the crown causing to form both the third protrusion in two sides and fourth labial, fifth lingual protrusions. In one sample of *M.* (*T.*) *subterraneus* (Borçka, Artvin), the closed area in the anterior lobe was unambiguously associated

with the closed area in the third lingual and labial protrusions and therefore the number of closed areas was determined as four (FIGURE 3, 1c).

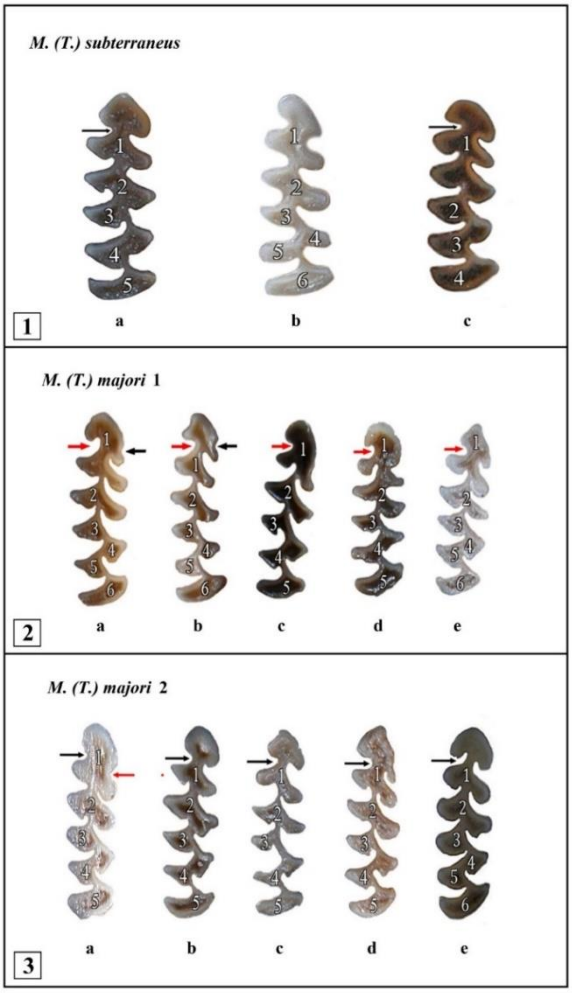

FIGURE 3. Variations of the first molar tooth (M_1) of the lower jaw in the subgenus *Terricola*. Numbers ranging from 1 to 6 show the number of closed areas in the crown of the teeth. In *M.* (*T.*) *subterraneus*, the black arrows show the narrow (1a) and wide (1c) junctions of the closed areas in the anterior lobe. In the samples of the first population of *M.* (*T.*) *majori*, the black arrows indicate an ambiguous indentation in the labial of anterior lobe (2a and 2b). The red arrows show two different types of convergence of narrow spaces (2a, 2b and 2e) and wide (2c and 2d). In the samples of the

second population of *M.* (*T.*) *majori*, black arrows show two different convergence of narrow (3b, 3c, 3d and 3e) and wide (3a) closed areas.

Despite the fact that there was an extra marked protrusion in the posterior of second upper molar tooth (M^2) in the lingual side in the samples from the two populations of *M.* (*T.*) *majori*, while, none of *M.* (*T.*) *subterraneus* samples had such a protrusion (FIGURE 4). This protrusion was unclear in eight samples of the first population and in three samples of the second population of *M.* (*T.*) *majori*. This was quite evident in the other samples of both populations. Triangular like closed areas in the crown of the M^2 formed by the second labial and lingual protrusions were discrete in two samples, completely unified in six samples and combined with a thin line in other samples of *M.* (*T.*) *subterraneus*. Similar morphological variations, in the same order, were detected in three, 13 and four samples of the first population, while, they were determined in three, five and one samples of the second population of *M.* (*T.*) *majori*.

In ten samples of *M.* (*T.*) *subterraneus*, the crown of the third upper molar tooth (M^3) had a triangular like appearance, formed by four protrusions and three indentations in the lingual side, while, three protrusions and two indentations in the labial side. In two samples (Borçka, Artvin), an unclear protrusion was observed in the labial near the posterior end of the M^3. The morphological structure of the M^3 varied considerably in the first population of *M.* (*T.*) *majori*. This structure was as in the *M.* (*T.*) *subterraneus* in 14 samples. The number of triangular like closed areas in the crown of 12 of these samples was three. In one of the remaining two samples, the number of triangular like closed areas was four (Sümela, Trabzon) and the other was two (Borçka, Artvin). The labial of the six samples had three protrusions and two indentations, while the lingual had a fifth an ambiguous protrusion and a fourth indentation in the posterior. In the second population, an extra protrusion and an indentation were observed in the posterior of the labial in the M^3, unlike the first population. Therefore, in all of the samples examined in this population, four protrusions and three indentations in the labial, a fifth ambiguous protrusion and a fourth indentation in the lingual near the posterior of the tooth were determined. In all of the samples of each populations, the number of closed areas in the crown of the M^3 was three (FIGURE 4).

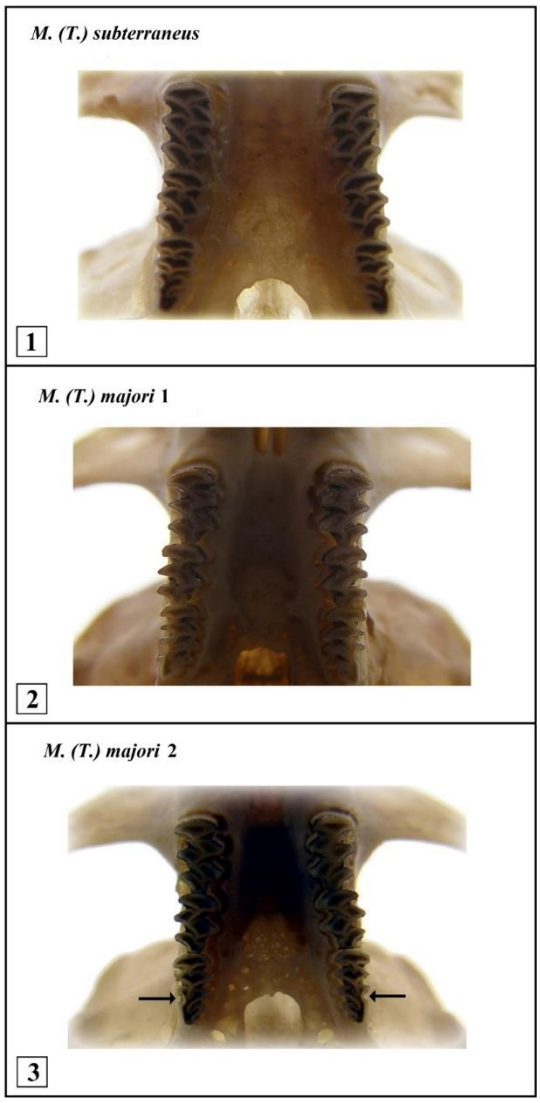

FIGURE 4. Variations in the second and third molar teeth (M^2 and M^3) of the upper jaw in the subgenus *Terricola*. In the samples of the second population of *M*. (*T*.) *majori* (3), the arrows in the right and left lower corners show the different structure formed by an extra protrusion-leading occurrence of a recess in the labial.

3.1.3 Fur coloration

In *M.* (*T.*) *subterraneus*, the fur color was in light brown tones in the dorsal part. The colour of the hairs in the dorsal fur was yellowish brown, gray and pale yellow. Mostly, the colorations on the lateral sides were lighter and grayer than the dorsal, while in some samples yellowish brown close to orange (Borçka: n = 3). There was a borderline separating the dorsal and ventral fur coloration in all samples. The ventral fur was dirty gray color including dominant white tones. In some samples, yellowish tones were observed in the ventral fur (Verçembek: n = 2, Borçka: n = 5, Karagöl: n = 1). The tail was two-color; the dorsal part was brownish gray and the ventral was whitish gray.

In the first population of *M.* (*T.*) *majori*, the dorsal fur colour of the samples changed from yellowish dark brown and gray to reddish. The dorsal fur of the samples obtained from the type locality was markedly reddish. In addition, the dark brown and dark greyish brown tones close to the cinnamon colour were seen in the samples with darker fur other than the type localities. The fur colour was lighter on the lateral sides than in the dorsal and varied from reddish to yellowish brown and gray. Dorsal and ventral fur separated by a distinct line. The ventral part was generally whitish-dirty gray. However, the white tones were seen intensely in the ventral fur of some samples, while, those of others included reddish yellow tones close to orange (Sümela: n = 3, İkizdere: n = 1, Çamlıhemşin: n = 1, Borçka: n = 2, Hopa: n = 1). The tail was in two colors, brown in dorsal part and white in ventral.

In the second population of *M.* (*T.*) *majori*, the dorsal fur colour was matte and varied. Both lighter and darker tones were observed than that of the first population of *M.* (*T.*) *majori*. It usually ranged from light and dark yellowish brown and blackish dark gray to dark brown close to cinnamon colour, even reddish. The fur on the lateral sides consisted of grey and reddish yellow hairs. The ventral fur was dirty greyish white and in some samples (Çamlıhemşin: n = 1, Borçka: n = 2) was markedly orange-yellow. The tail was two-color; greyish brown close to the black in dorsal and dirty white in ventral (FIGURE 5).

FIGURE 5. Dorsal and ventral colour variations of the fur in *M.* (*T.*) *subterraneus* (1a and 2a), the first population (1b and 2b) and the second population (1c and 2c) of *M.* (*T.*) *majori*.

3.1.4 Biometry

Mean and standard errors, as descriptive statistics, of the 34 biometric characters belonging to the three populations in the subgenus *Terricola* were indicated in TABLE 2. The one-way ANOVA results showed that statistically significant differences ($P<0.05$) were found between mean of the five (hind foot length, cranium length, upper molars alveoli length, M^2 length and M_1 length) of the total 34 biometric characters (TABLE 3). Hochberg's GT2 results demonstrated that mean of hind foot length was significantly differed between *M.* (*T.*) *subterraneus* and first population of *M.* (*T.*) *majori*. It was also determined that the mean of the other remaining four biometric characters were statistically different between *M.* (*T.*) *subterraneus* and second populations of *M.* (*T.*) *majori* by the same multiple comparison test. The MANOVA results showed no significant difference ($P> 0.05$) between group mean vectors of three groups (TABLE 4).

TABLE 2. Mean and standard error values of biometric characters in the subgenus *Terricola*

	M. (T.) subterraneus		***M. (T.) majori* 1**		***M. (T.) majori* 2**	
Biometric characters	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Total body length	135,2725	3,10691	141,2105	1,82321	143,4444	4,03840
Tail length	39,8183	1,57547	41,1055	0,72509	44,7778	2,38501
Hind foot length	16,3325	0,60302	18,1580	0,23241	16,8322	0,45389
Ear length	9,6675	0,53654	10,0525	0,43814	10,1678	0,42219
Zygomatic Breadth	13,5158	0,19176	13,7820	0,13058	14,0267	0,31796
Rostrum Breadth	3,8375	0,03289	3,8455	0,02805	3,9467	0,04272
Interorbital Breadth	4,1075	0,07145	4,0340	0,05807	4,2122	0,04542
Condylobasal Length	21,7408	0,23728	22,2275	0,15846	22,4267	0,38932
Condylonasal Length	23,2067	0,24986	23,7230	0,19287	24,0344	0,39311
Occipitonasal Length	22,8925	0,24912	23,3775	0,18100	23,6911	0,34280
Basal Length	20,5917	0,24820	21,0610	0,16132	21,0822	0,39444
Nasal Length	6,8025	0,11112	6,9270	0,08620	7,0378	0,13308
Nasal Breadth	2,9208	0,03331	2,8350	0,05206	2,9178	0,03789
Frontal Length	11,8808	0,17517	12,0530	0,10955	12,0611	0,18375
Parietal Length	3,5383	0,07248	3,6985	0,11406	3,4978	0,07940
Facial Region Length	14,4508	0,15946	14,8645	0,15327	14,9522	0,28418

Cranium Length	9,1550	0,15831	9,3760	0,09416	9,7144	0,19476
Mastoid Breadth	7,0025	0,08408	7,0225	0,05498	7,0789	0,08613
Cranium Depth	8,0675	0,10623	8,0580	0,05859	8,3144	0,11329
Cranium Breadth	11,3967	0,13270	11,4295	0,10550	11,7422	0,10460
Diastema Length	6,9383	0,12516	7,1575	0,07458	6,9511	0,18336
Incisive Foramen Length	3,8992	0,14170	3,9845	0,05220	3,8300	0,13995
Incisive Foramen Breadth	0,9733	0,03803	1,0375	0,02323	1,11267	0,10918
Tympanic Bulla Length	6,1042	0,09352	6,1480	0,04475	6,1956	0,13052
Mandible Length	13,2558	0,12697	13,4060	0,11239	12,0756	1,35466
Mandible Height	6,2742	0,10096	6,4650	0,11976	6,2244	0,12443
Upper Molars Alveoli Length	5,3892	0,07103	5,5615	0,03975	5,6667	0,08660
Lower Molars Alveoli Length	4,9400	0,06748	5,1215	0,04887	5,0856	0,05786
M^1 Length	1,8033	0,01920	1,8460	0,01177	1,8533	0,02682
M^2 Length	1,3700	0,03119	1,4145	0,01863	1,5200	0,05292
M^3 Length	1,7017	0,03128	1,7160	0,01210	1,7311	0,04373
M_1 Length	2,3675	0,03635	2,4540	0,03042	2,5289	0,05208
M_2 Length	1,2617	0,02081	1,2910	0,01591	1,2667	0,02261
M_3 Length	1,2050	0,02816	1,2775	0,01981	1,2833	0,03659

TABLE 3. One-way ANOVA results among three populations of the subgenus *Terricola*

Biometric characters	**S.S. (among groups)**	**S.S. (within groups)**	**F**	**d.f. (among groups)**	**d.f. (within groups)**	**P**
Total body length	404,219	3711,562	2,069	2	38	0,140
Tail length	133,693	936,981	2,711	2	38	0,079
Hind foot length	27,882	83,360	6,355	2	38	0,04*
Ear length	1,585	123,781	0,243	2	38	0,785
Zygomatic Breadth	1,365	18,612	1,393	2	38	0,261
Rostrum Breadth	0,077	0,573	2,543	2	38	0,092
Interorbital Breadth	0,200	2,104	1,806	2	38	0,178
Condylobasal Length	2,800	27,887	1,907	2	38	0,162
Condylonasal Length	3,791	33,503	2,150	2	38	0,130
Occipitonasal Length	3,489	29,101	2,278	2	38	0,116
Basal Length	1,925	29,222	1,252	2	38	0,298
Nasal Length	0,290	5,729	0,963	2	38	0,391
Nasal Breadth	0,073	1,280	1,087	2	38	0,347
Frontal Length	0,259	11,042	0,446	2	38	0,643

Parietal Length	0,331	6,091	1,034	2	38	0,365
Facial Region Length	1,698	18,098	1,782	2	38	0,182
Cranium Length	1,613	9,408	3,258	2	38	0,049*
Mastoid Breadth	0,032	2,616	0,230	2	38	0,796
Cranium Depth	0,450	3,718	2,299	2	38	0,114
Cranium Breadth	0,750	7,342	1,941	2	38	0,157
Diastema Length	0,469	6,602	1,349	2	38	0,272
Incisive Foramen Length	0,160	5,096	0,597	2	38	0,556
Incisive Foramen Breadth	718,467	7358,479	1,855	2	38	0,170
Tympanic Bulla Length	0,043	3,142	0,261	2	38	0,772
Mandible Length	11,573	139,055	1,581	2	38	0,219
Mandible Height	0,474	7,911	1,138	2	38	0,331
Upper Molars Alveoli Length	0,425	1,806	4,473	2	38	0,018*
Lower Molars Alveoli Length	0,254	1,750	2,762	2	38	0,076
M^1 Length	0,017	0,153	2,168	2	38	0,128
M^2 Length	0,120	0,462	4,925	2	38	0,013*
M^3 Length	0,004	0,323	0,264	2	38	0,769
M_1 Length	0,137	0,721	3,609	2	38	0,037*
M_2 Length	0,008	0,190	0,770	2	38	0,470
M_3 Length	0,047	0,350	2,554	2	38	0,091

TABLE 4**.** MANOVA results

Effect		**Value**	**F**	**Hypothesis df**	**Error df**	**Sig.**
Groups	Pillai's Trace	1,818	1,758	68	12	0,140***
	Wilks' Lambda	0,007	1,640	68	10	0,200***
	Hotelling's Trace	24,953	1,468	68	8	0,294***
	Roy's Largest Root	18,297	3,229	34	6	0,072***

Eigenvalue statistics were found to be significant for two canonical discriminant functions determined by DFA (Wilk's Lambda = 0.457, P < 0.001). As a result of DFA, the first canonical discriminant function explained the 81.4% of the total observed variations among three groups, while, the second one clarified 16.8% of the total variations. In the classification matrix, it was determined that 75.6% original group cases correctly classified (TABLE 5).

TABLE 5. Classification matrix obtained by DFA

GROUPS	**ACCURACY (%)**	**Predicted Group Membership**		
		1	2	3
1. *M. (T.) majori* 1	80	16	3	1
2. *M. (T.) subterraneus*	75	3	9	0
3. *M. (T.) majori* 2	66,7	2	1	6

According to the obtained canonical scores, the relative positions of the groups to each other was shown in the scatter plot. Pursuant to the scatter plot, the two populations of *M.* (*T.*) *majori* were spread closer to each other, while they were relatively more distant than the *M.* (*T.*) *subterraneus* (FIGURE 6).

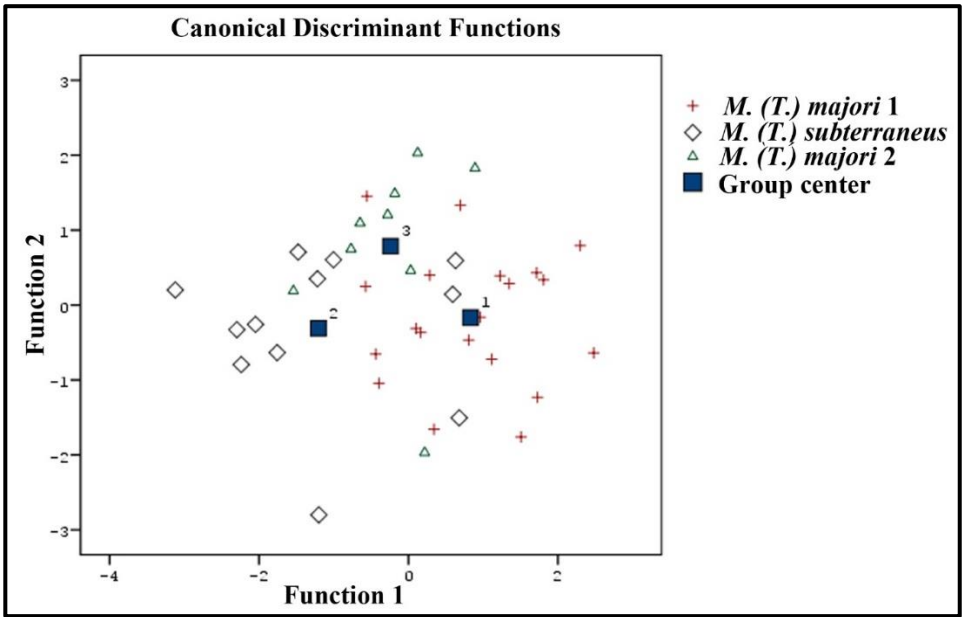

FIGURE 6. Scatter plot showing relative positions of three populations of *Terricola*

KMO measure of sampling adequacy was 0.645 and Bartlett's test of sphericity was significant at P<0.001 level, which indicated the data set was highly suitable for PCA. The first seven principal components, having eigenvalues greater than 1, explained a cumulative 74.487% of the total variation of the full data in the PCA. The loads of all biometric characters in the principal components were shown in the rotated component matrix (TABLE 6). According to the matrix, 13 cranial characters having loads whose absolute value greater than 0.5 were determined under the first principal component. Similarly, the total body length and the two dental characters (M^3 and M_3 Length) also contributed to the first principal component (explained variance: 41.956%). The second principal component included four cranial and one dental character (explained variance: 7.717%). As for the third principal component, the loads of dental characters, which were considered discriminative characters, predominantly contributed that (explained variance: 7.268%). While the fourth

principal component composed of the loads of external characters (explained variance: 5.877%), three cranial characters had the load on the fifth and sixth principal components (explained variance: 4.811% and 3.614%, respectively). Only hind foot length had the load on the last principal component (explained variance: 3.244%).

TABLE 6. Rotated component matrix produced by PCA

	Principal components						
Biometric characters	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Facial Region Length	0,924	0,029	0,172	0,123	0,043	-0,034	0,023
Basal Length	0,918	0,147	0,260	-0,057	0,079	0,017	0,061
Condylonasal Length	0,917	0,233	0,184	0,021	0,017	0,029	-0,040
Condylobasal Length	0,912	0,196	0,223	0,030	0,054	0,110	0,018
Occipitonasal Length	0,902	0,272	0,178	0,061	0,047	0,093	0,015
Diastema Length	0,854	-0,161	0,019	0,018	0,169	0,173	0,048
Nasal Length	0,797	0,211	0,153	0,045	-0,010	-0,120	0,200
Cranium Length	0,765	0,404	0,081	-0,193	-0,049	0,010	-0,075
Zygomatic Breadth	0,741	0,399	0,172	-0,026	-0,085	0,299	0,081
Upper Molars Alveoli Length	0,734	0,200	0,424	0,064	0,014	-0,041	-0,193
Tympanic Bulla Length	0,706	0,203	0,060	-0,168	0,056	-0,038	-0,095
Total body length	0,652	0,065	0,243	0,355	-0,071	0,366	0,218
Frontal Length	0,637	0,153	0,067	-0,010	0,159	-0,044	-0,229
Incisive Foramen Length	0,521	-0,081	-0,180	-0,131	0,346	0,450	-0,130
M_1 Length	0,498	0,375	0,424	0,066	-0,107	0,320	0,072
Interorbital Breadth	-0,072	0,840	-0,119	-0,062	0,138	-0,253	-0,086
Cranium Breadth	0,382	0,795	0,115	-0,041	-0,069	0,125	0,004
Cranium Depth	0,477	0,759	0,104	-0,083	-0,096	-0,065	-0,062
Rostrum Breadth	0,368	0,579	0,322	0,259	-0,188	-0,094	0,107
M^1 Length	0,422	0,506	0,415	0,038	0,237	0,077	0,194
M_2 Length	0,026	-0,099	0,727	-0,174	-0,248	-0,092	0,192
M^2 Length	0,379	0,125	0,680	0,206	-0,160	0,177	-0,280
M^3 Length	0,524	0,280	0,608	-0,057	0,148	0,035	-0,253
M_3 Length	0,588	-0,016	0,597	-0,036	0,058	-0,060	0,047
Lower Molars Alveoli Length	0,407	0,233	0,587	-0,006	0,106	0,014	0,252
Ear length	0,093	-0,022	-0,047	0,650	0,258	0,254	-0,006
Tail length	0,450	0,280	-0,019	0,626	-0,292	0,308	0,163
Mastoid Breadth	0,373	0,204	0,143	-0,526	0,136	0,109	-0,103
Mandible Length	-0,131	-0,043	0,373	0,421	0,244	-0,208	0,288
Parietal Length	0,201	0,281	-0,066	-0,036	0,717	0,114	0,100
Nasal Breadth	-0,025	0,278	0,022	-0,130	-0,652	0,091	0,088
Incisive Foramen Breadth	0,086	0,135	-0,013	-0,161	-0,005	-0,811	0,046
Hind foot length	-0,081	-0,036	0,059	0,185	-0,057	-0,064	0,855
Mandible Height	0,275	0,127	0,428	-0,295	0,319	0,157	0,465

The UPGMA dendrogram based on a pairwise matrix of Manhattan distances calculated by biometric differentiation among populations showed similar results to the results of DFA shown by the scatter plot. According to this, the first and second populations of *M.* (*T.*) *majori* were clustered together, and *M.* (*T.*) *subterraneus* was created a separate branch from them (Figure 7).

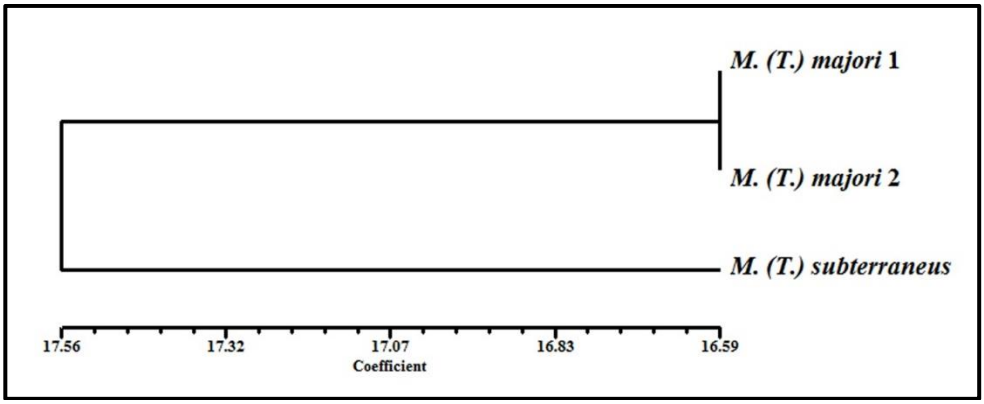

FIGURE 7. The UPGMA dendrogram based on a pairwise matrix of Manhattan distances

4. DISCUSSION

In the scope of this study, morphological and biometric aspects of populations of the subgenus *Terricola* from Eastern Black Sea Region in Turkey were investigated. According to the findings, it was thought that morphological and biometric differences observed in the molar tooth structure of the populations were more useful to some extent, rather than external morphological characters and skull characters to distinguish the populations. Additionally, unlike known distribution of *M.* (*T.*) *subterraneus* in Turkey, it was determined for the first time that this species could be occurred in the localities of Rize and Artvin, which are further east of Trabzon within distribution range of *M.* (*T.*) *majori*. Thus, that *M.* (*T.*) *subterraneus* and *M.* (*T.*) *majori* can be coexist as sympatric within the same geographic area was also detected.

Kryštufek and Vohralík [1] consider the east Anatolian populations of *M.* (*T.*) *subterraneus* to be easily separable from *M.* (*T.*) *majori* in terms of interorbital construction and the dorsal profile of the skull. In addition to this, they have stated that *M.* (*T.*) *subterraneus* has deeper skull than *M.* (*T.*) *majori* has. The skulls of the

examined populations of both species in the scope of current study were nearly in the same structure except for the some slight differences statistically insignificant. A narrower and longer interorbital construction in the skull of *M.* (*T.*) *subterraneus* than that of *M.* (*T.*) *majori* determined by this study could be perhaps considered to be a separator morphological differentiation between the skulls of two species as it was suggested by Kryštufek and Vohralík [1]. It was observed that the dorsal profile of all skulls of both species had no marked differences from each other unlike the findings (concavity) of Kryštufek and Vohralík [1], while, it was compatible with the determinations of Çolak et al. [12]. Additionally, no significant difference were detected too among cranium depth of both species unlike the statement of Kryštufek and Vohralík [1]. Except those, as a slight morphological distinction between the skulls of both species, it was detected by the study that the anterior of the parietal bone was in a spearhead form in *M.* (*T.*) *subterraneus* or slight arc form in *M.* (*T.*) *majori*. However, it is worth mentioning that a sample series is needed to be able to generalize this as a strong diagnostic character.

The dorsal and ventral fur color is highly variable and importantly differ between the two species. Alterations and dissimilarities in the fur color of examined samples is as partly in stated by Çolak et al. [12] and partly in stated by Kryštufek and Vohralík [1]. Accordingly, dorsal fur color can be regarded partly to be a separator character and respectively generalized as light brown in *M.* (*T.*) *subterraneus*, dark brown in the first population of *M.* (*T.*) *majori* and dull including lighter and darker brown tones in the second populations of *M.* (*T.*) *majori*. The color of the ventral fur in the distinct populations of both species includes the diverse intensity of each color tone, but is generally whitish-dirty gray. This coloration observed within all populations create more complexity rather than a distinction between them. Therefore, the color of the ventral fur does not exactly represent a distinctive character feature. Contrary to the observations of Kryštufek and Vohralík [1], there is a clear boundary line on the lateral of the specimens of both species that distinguishes the color of dorsal and ventral fur as previously determined by Çolak et al. [12]. The tail fur is bicolor and mostly incorporates brown above side and grey plume below side. They are also in highly variable tones of brown and grey, creating more complexity rather than a distinction.

All observed variations of the first lower molar, such as number of protrusion and indentation in labial and lingual, shape of the triangular like closed area in the anterior lobe and total number of closed area, were highly variable and shared within both species. Therefore, it is thought that first lower molar were not discriminative in respect to the morphological structure. This is a case determined before by

Kryštufek and Vohralík [1], and individuals with different tooth structures were evaluated as morphotype within each species according to the mentioned variations. In contrast to this complex case observed in morphology of the first lower molar, it was detected by the one-way ANOVA and Hochberg's GT2 that statistically significant differences was found between mean of the first lower molar length of *M*. (*T*.) *subterraneus* and second populations of *M*. (*T*.) *majori* by biometric evaluations. In a way that makes this statistics insignificant, it was seen that the first lower molar tooth length did not contribute to any principal component in PCA. In contradistinction to the complexity arising from the variable structure of the first lower molar, it is believed that the second upper molar is more powerful separator in real terms for distinguishing of both species because of the extra marked protrusion in its posterior. As a matter of fact, this morphological differentiation found in *M*. (*T*.) *majori* populations had formerly been proposed as a distinctive character [14, 15]. In addition to this apparent morphological differentiation in the second upper molar, biometric difference in the mean of the second upper molar length between *M*. (*T*.) *subterraneus* and *M*. (*T*.) *majori* were also found to be statistically significant. Supporting to this, it was detected that the load of mentioned biometric character was contributed to the third principal component that explains 7.26 % of total variations in PCA. In addition, unlike the morphological structure of the third upper molar in the first population of *M*. (*T*.) *majori*, that of the second *M*. (*T*.) *majori* population had a protrusion that leads to an extra indentation in the labial. However, it is useful to state that this morphological structure was found to be statistically insignificant. All specimens with such a tooth morphology were treated as nominate subspecies of *M*. (*T*.) *majori* by Ognev [14]. Since it is clear that additional studies are needed to define a new subspecies, no attempt was made in this sense and mentioned populations of *M*. (*T*.) *majori* was evaluated as two separate populations as first and second.

It has been suggested that the tail length is relatively longer in *M*. (*T*.) *majori* than that of *M*. (*T*.) *subterraneus* by Kryštufek et al. [11] and Çolak et al. [12]. The one-way ANOVA and Hochberg's GT2 results showed that there was no statistically significant difference between group means in terms of this character. Even more the load of this character contributed to the fourth principal component that explains a small percentage (5.87 %) of total variations observed in rotated component matrix produced by PCA. Therefore, it can be said that the relative differences in the tail length, which have been preciously used to be a discriminative character between the populations, was not statistically significant.

According to the results of Kryštufek et al. [11], 12 cranial characters employing in DFA was useful to distinguish the *M.* (*T.*) *subterraneus* and *M.* (*T.*) *majori* by explaining 91.8 % of total variations. By this study, more variables were utilized in DFA (34 morphological characters) and similarly with the findings of Kryštufek et al. [11], the high percentage of variation (81.4%) was detected among three groups. As it was stated by Kryštufek et al. [11], the suggestion that morphological characters used in DFA could be beneficial in separating the two species was well projected by the scatter plot showing relative positions of three populations of *Terricola*. Besides, the samples were grouped with a high percentage of accuracy in the classification matrix. Similar clustering of three populations in the UPGMA dendrogram to the scatter plot is another important result supporting this condition. Moreover, the fact that 12 of the total 21 characters contributing to the first and second principal component yielded by PCA are the same as those in DFA performed by Kryštufek et al. [11] was another finding that coherently supports the results of DFA performed by current study. However, approximate 46% of the total variance in the discriminant scores could not be explained by morphological differences between the groups, according to the Wilk's Lambda statistics revealed by DFA (Wilk's Lambda = 0.457, $P < 0.001$). The results of MANOVA also support this situation. This also could be thought of as a situation that shows that biometric characters used in the study have a not very strong discriminatory power, even though high the percentage of variation was detected.

Morphological evaluations and the results of multivariate statistical analyzes using biometric characters showed that there was a certain degree of morphological and biometric differentiation between the populations of the *Terricola* subgenus living in the Eastern Black Sea Region and that no definitive distinction could be made between these subspecies. Additional research using molecular techniques should be conducted to make a more definitive judgment on the taxonomic status of this subgenus in the study area.

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