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Comparative Skull and Mandible Geometric Morphometrics of Two Species of Mice, *Mus domesticus* and *Mus macedonicus* (Muridae, Rodentia) in Turkey

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Research Article

ABSTRACT

Using a geometric morphometrics approach, we examined shape and size variations of skull and mandible bone of two evolutionarily distantly related mice from Turkey: *Mus domesticus* and *Mus macedonicus*. PCA analyses revealed overlap in dorsal cranium and mandible shapes of both species, consistent with previous traditional morphological methods. The skull of *M. macedonicus* seems to be larger in size than *M. domesticus* according to box-plot analyses of centroid size values, however there is no obvious difference for the mandible. No difference was observed between sexes in either of the characters. We suggest that future studies focus on dental characteristics and also consider the variation among local populations and ecological variables.

Keywords: House mouse, Macedonian mouse, Geometric morphometrics, Systematics, *Mus* sp.

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Introduction

The genus *Mus* (Clerck, 1757) includes numerous subgenera, species and subspecies, and is one of the most well-known and widely distributed murid genera in the mammalian world [1,2]. The genus is represented by two species in Turkey: *Mus domesticus* (Rutty, 1772), commonly known as the western European house mouse, and *Mus macedonicus* (Petrov & Ruzíc, 1983), which is commonly known as the eastern Mediterranean short-tailed mouse or Macedonian mouse [3]. These species are widespread in both the European part (Thrace) and Asian part of Turkey (Anatolia) [2,4]. The house mice usually occupy areas that are in close association with humans, such as houses, barns, granaries, fencerows and cultivated fields. The short-tailed mouse, on the other hand, prefers cultivated fields, grasses, bushes and mixed forests away from human settlements [5]. Traditional morphological studies have often used ZI (zygomatic index) and H+B/T (the index of head plus body length/tail length) as well as external body, tooth and baculum measurements to distinguish between two species of mice in Turkey [4,6,7]. However, despite the apparent success of this method in distinguishing between the two species, the usability of the index is limited due to the range of ZI values for each species being large and slightly overlapping when the species are compared. In addition, these studies do not provide detailed statistical data in terms of shape and size analyses related to the morphometry of the skull and the entire mandible of the species.

The use of geometric morphometrics technique has increased rapidly in the field of zoological data analysis over the past two decades. The geometric morphometrics approach is useful for investigating shape variation and morphological transformation when species are difficult to differentiate using traditional methods [8]. Digitized

geometric data are compared and differences are calculated by using various mathematical and statistical tests. The technique based on a landmark method is widely used and accepted in studies of animals, especially muroid rodents, that are hard to identify and differentiate with standard morphometric approaches [9,10]. Landmark coordinates describe specific, evolutionarily homologous points located on body parts of the specimens subjected to morphometric analysis, such as the skull and the teeth [11].

In the present study, the geometric morphometrics approach was used to evaluate the shape and size differences of the mandible and the dorsal side of the cranium between *M. macedonicus* and *M. domesticus* specimens from Thrace and Anatolia regions of Turkey, which were previously subject to molecular species identification using the control region of mitochondrial DNA [12-15]. In addition, shape and size variations between males and females of two species were compared to clarify ambiguous reports on sexual dimorphism proposed based on traditional morphometrics.

Materials and Methods

Study Specimens

We assessed skull and mandible shape, and size variation in two species of mice, *Mus domesticus* (33 males, 30 females) and *Mus macedonicus* (95 males, 52 females), using two-dimensional geometric morphometrics [11, 16] (Figure 1 and Table 1). Sample sizes for the skull and the mandible data set were: *M. domesticus* skull = 54, mandible = 51; *M. macedonicus* skull = 137, mandible = 102). All specimens used in this study were obtained from museum collections of İslam Gündüz and Sadık Demirtaş (subcollection IG/SD, Department of Biology, Faculty of Sciences, Ondokuz Mayıs University, Samsun, Turkey).

Table 1. Number of samples per collection locality of *Mus domesticus* and *Mus macedonicus* specimens used in this study. Location numbers correspond to map locations shown in Figure 1.

Table of Localities			
Species	**Locality Number**	**Locality**	**Number of Samples**
Mus domesticus	1	Edirne	3
	4	Bursa	4
	7	Sinop	3
	8	Samsun	6
	11	Trabzon	1
	12	Rize	1
	13	Balıkesir	16
	16	Sivas	3
	18	Iğdır	1
	20	Manisa	1
	23	Kayseri	5
	24	Elazığ	2
	25	Van	2
	28	Muğla	1
	27	Denizli	8
	34	Adana	1
	37	Adıyaman	1
	38	Şanlıurfa	4
			63
Mus macedonicus	2	Tekirdağ	1
	3	Çanakkale	8
	4	Bursa	13
	5	Bilecik	2
	6	Kastamonu	1
	8	Samsun	4
	9	Tokat	10
	10	Ordu	2
	13	Balıkesir	1
	14	Eskişehir	2
	15	Ankara	4
	16	Sivas	7
	17	Erzincan	2
	19	İzmir	5
	20	Manisa	23
	21	Afyonkarahisar	2
	22	Konya	12
	23	Kayseri	2
	25	Van	1
	26	Aydın	11
	28	Muğla	1
	29	Burdur	5
	30	Isparta	2
	31	Antalya	6
	32	Karaman	3
	33	Mersin	3
	34	Adana	1
	35	Kahramanmaraş	4
	36	Gaziantep	2
	38	Şanlıurfa	3
	39	Hatay	4
			147
		Total	210

Figure 1. Capture locations of specimens used in this study. Location names (indicated by numbers) have been listed in Table 1.

Imaging and Landmarks

High resolution digital images of the dorsal cranium and the right mandible of each specimen were obtained using a Nikon D5000 (18-55 mm) camera with skull roof and mandible positioned in parallel with to the photographic plane. The images were scaled, edited, and digitized using TPS (Thin-Plate Spin) software series [17,18] for subsequent analyses. In order to emphasize the local impact of possible shape deformation on different parts of the skull and the mandible, we selected to describe 12 landmarks and 5 semilandmarks for the dorsal cranium and 10 landmarks and 22 semilandmarks for the mandible (Figure 2). The landmarks and semilandmarks were placed in accordance with previous studies [19-21].
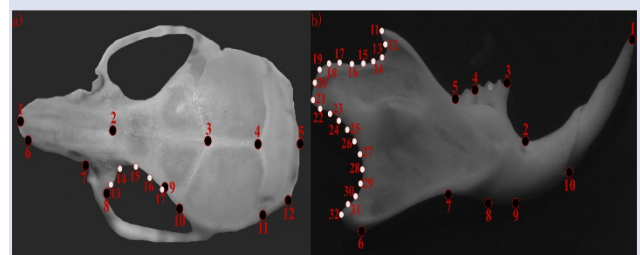

Figure 2. Landmarks (black points) and semilandmarks (white points) used in this study. (specimen: Mus macedonicus, Manisa vicinity, W. Turkey). Landmark locations on dorsal cranium (a): (1) the most rostral point of the nasal bone, (2) intersection of interfrontal and fronto-nasal suture, (3) intersection of the coronal and sagittal sutures, (4) intersection of the sagittal and parietal-interparietal sutures, (5) caudal end of the occipital curve, (6) intersection of the rostral curvature of the nasal process of the incisive bone (Processus nasalis ossis incisivi) and the nasal bone in the dorsal projection, (7) anterior notch on frontal process lateral to infraorbital fissure (8) anterior part of the orbit, (9) the most rostral point of the parietal bone, (10) intersection point of the squamosal and parietal bone, (11) exterior tip of the occipital crest, (12) caudolateral end of the occipital bone in the dorsal projection. The semi landmarks (13-17) surround the frontal bone. Landmark locations on mandible (b): (1) tip of the incisor, (2) mental foramen, (3) distal tip of the first molar, (4) the proximal end point of the first molar, (5) the proximal end point of the second molar, (6) The curve of angular process, (7) superior-most point on inferior border of mandibular ramus, (8) inferior-most point on border of ramus inferior to incisor alveolar, (9) The most inferior point of mental protuberance, (10) inferior-most point on incisor alveolar rim. The semilandmarks (11-32) are placed between coronoid and angular processes of the mandible.

Geometric Morphometric Analysis

After digitalization configuration of landmark and semilandmark coordinates, Generalized Procrustes Analysis (GPA) was applied to superimpose the shape information from each specimen to eliminate the effects of location, orientation, and scaling from the raw data coordinates [22,23]. The resulting projections were displayed on a series of axes and the patterns of inter- and intra-specific shape variation were analysed by using Principal Component Analysis (PCA) and Procrustes ANOVA. Wireframe graphs were used to illustrate the differentiation in shape between two species based on the utilized landmark and semilandmark points.

To account for the effect of size on shape, a centroid size (CS) analysis was implemented, which was calculated as the square root of the summed squared distances of each landmark from the center of the landmark configuration. The centroid size values generated for the dorsal cranium and the mandible bone were analysed using a Mann-Whitney U test to determine whether there is a significant difference between two species. The CS variation for each species has been visualized with a box-plot graphic.

GPA, PCA, Procrustes ANOVA and wireframe analyses were performed using MorphoJ version 1.07 [24]; Mann-Whitney U test analyses were performed using SPSS version 22.0 [25]; centroid size and box-plot analyses were obtained using PAST version 4.01 [26]. Program outputs were edited and visualized using Inkscape version 0.92 [27].

Results and Discussion

Shape Variation

PCA and comparative wireframe graphs were visualized for the skull and mandible shapes based on PC1 and PC2 values (Figure 3 and Figure 4). The variance was calculated based on the scatter plot along PC1 (explains 23.58% of the variance) and PC2 (explains 16.15% of the variance) for the skull. *M. domesticus* skulls predominantly included positive values in PC1 and negative values in PC2, whereas *M. macedonicus* skulls predominantly contained negative values in PC1 and positive values in PC2, however, the two species were not distinctly separated from each other. According to the wireframe graphs obtained from *Mus domesticus* PC1 and PC2, the variation in shape is mostly explained by the landmarks 3, and 7-10. This indicates that the skull is flattened from the top and narrowed from the orbital area. Similarly, the wireframe analysis for *Mus macedonicus* based on PC1 and PC2 revealed that the points with the most influence on skull shape were landmark numbers 3 and 7-10, resulting in an arched skull shape with a narrow eye socket. Procrustes ANOVA shows significant shape differences between species (F = 16.90, P<0.05), while differences between sexes were insignificant (F=1.21 and P=0.2009 for *M. domesticus*; F=1.0 and P=0.4618 for *M. macedonicus*).

Wireframe graphs revealed morphological differences in the orbital socket and the central part of the skull between the species, which were not clearly supported by the PCA graphs. This discrepancy is probably due to the large variation in breadth of the zygomatic arch within both species [4,6,7]. A wide variety of zygomatic index values have also been previously reported from various European *Mus* populations [28-30]. Recent studies with Quantitative Trait Loci (QTL) mapping methods have shown that many QTLs provide the basis for the genetic architecture of shape variation in *Mus* skulls [31-33]. For these reasons, the use of zygomatic index to distinguish between these species, as well as other *Mus* species, should be approached carefully [34,35].
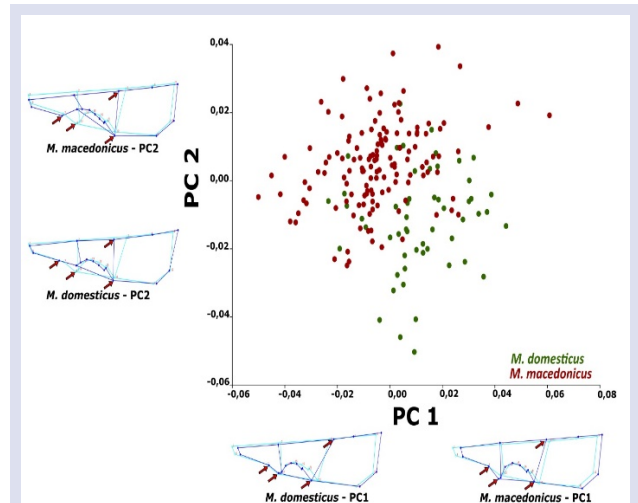

Figure 3. Inter-species principal component and wireframe analyses of skull shapes of *M. domesticus* and *M. macedonicus*. Wireframe diagrams compare the variation in shape with light blue showing the mean shape and dark blue showing the most extreme shape variation explained by each principal component.

Similarly, the variance was calculated along with PC1 (explains 34.68% of the variance) and PC2 (explains 18.57% of the variance) based on the scatter plot graphic for the mandible. *M. domesticus* showed intensity on the negative side of the graph for both PC1 and PC2 (Figure 4). In contrast, *M. macedonicus* showed an intensely positive distribution for PC1 and showed both positive and negative distribution for PC2. Both species showed the greatest amount of shape variation in the ramus region between the coronoid process and the angular process illustrated by the wireframe analyses, however, this difference was not reflected in the PCA graphs. Therefore, our results failed to provide support for the use of jaw morphometry for the differentiation of these two species of *Mus*. The main reasons for this discrepancy can be explained by the attachment styles of the temporalis and masseter muscle groups, as well as the mandible being a malleable bone. While masseter muscles help molar mastication activity, temporal muscles serve a role in gnawing [36]. Therefore, the development of these muscles is related to both nutrient content and ecological environment of the species [37]. Another factor that may have a role in the observed lack of differentiation can be due to the pleiotropic

effects of QTLs, which also function in the modular organization of both the skull and the mandible [38]. Procrustes ANOVA shows significant shape differences between species (F = 31.32, P<0.05), while differences between sexes were insignificant (F=1.15 and P=0.1974 for *M. domesticus*; F=0.63 and P=0.9879 for *M. macedonicus*).

Our results demonstrate that skull and jaw morphometry fail to provide a clear distinction between two species, and support that dental morphometry has the potential to provide more superior data for distinguishing between these species, as previously proposed by Macholán (2006) for various species of *Mus*.
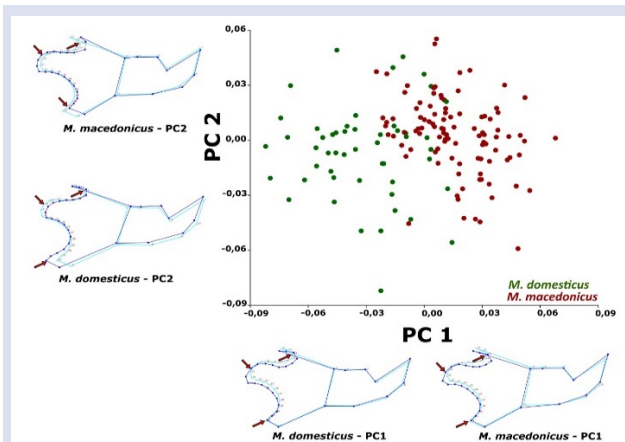

Figure 4. Inter-species principal component and wireframe analyses of mandible of *M. domesticus* and *M. macedonicus*. Wireframe diagrams compare the variation in shape with light blue showing the mean shape and dark blue showing the most extreme the shape variation explained by each principal component.

Finally, there was no difference between sexes in the amount of variation explained by PC1 or PC2 for skull or mandible shape in either of the species (Figure 5 and Figure 6). Principal component values were distributed uniformly among sexes in terms of both species and characters.
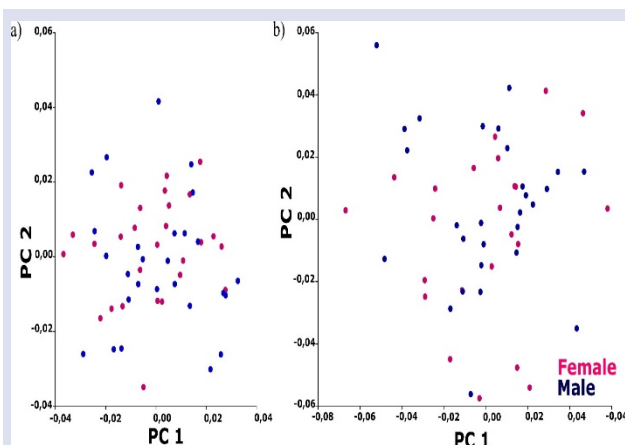

Figure 5. Intra-specific sex-linked principal component analysis of the skull shape (a) and mandible shape (b) of *M. domesticus*.
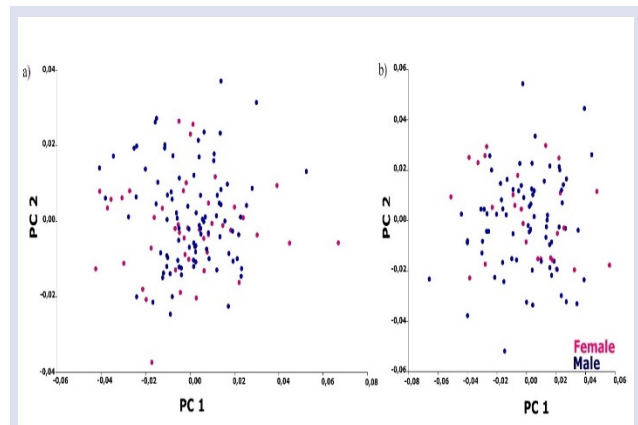

Figure 6. Intra-specific sex-linked principal component analysis of the skull shape (a) and mandible shape (b) of *M. macedonicus*.

Size Variation

The results of the Mann-Whitney U test indicate that the two species significantly differed in dorsal cranium centroid size (U=2181, P=0.05) but there was no significant difference for the mandible (U=2546, P=0.696). Also, we failed to reject the null hypothesis and therefore we inferred that there was no significant difference between sexes of both species for the two characters (P>0.05).

In parallel with the Mann-Whitney U test, the boxplot analysis indicates that *M. macedonicus* is larger in size than *M. domesticus* by log-centroid size values of the dorsal cranium, but, there is no obvious difference for the mandible (Figure 7). Previous morphometric studies revealed that there could be slight variations in size among local populations within both species [20, 39]. Moreover, Macholán (1996) showed that size variation can fluctuate within populations due to different age structure of populations and due to ecological variables. In this study, we did not consider local populations, age structures and ecological variables; we focused solely on the variation of shape and size between two species found in Turkey. Therefore, the number of specimens and localities in our study may not be satisfactory for centroid size analysis. For this reason, future studies should consider repeating the centroid size analysis on a large number of specimens from different local populations, also takes ecological variables into consideration. In other words, further studies are needed to clarify this observed difference in size between two species.
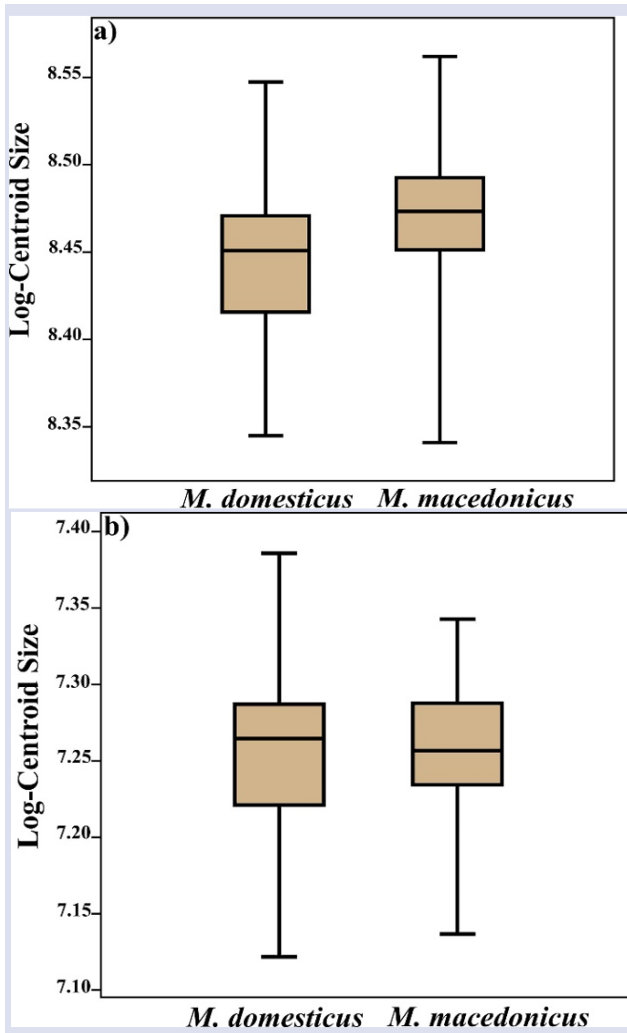
Figure 7. Boxplot graphics showing maximum, minimum and median values of log-centroid size for dorsal cranium (a) and mandible (b) between Turkish mice.

Conclusions

The current study enabled us to obtain morphometric data regarding the analysis of shape and size between two evolutionarily distantly related species, namely *Mus macedonicus* and *M. domesticus*. The results revealed that there was no significant difference in shape of the dorsal side of the skull and the mandible between the two species despite a statistically significant difference in centroid size values for the dorsal cranium. The centroid size values indicate that the dorsal side of the cranium is larger in *M. macedonicus* compared to *M. domesticus* in Turkey. Moreover, there were no morphometric differences between males and females for either of the studied features. Future studies using dental characters, which are frequently used in *Mus* species-subspecies studies, and gene expression analyses such as QTL mapping can identify morphological variations at inter- and intra-specific level in mice of Turkey.

Acknowledgment

Conflicts of interest

There are no conflicts of interest in this work.

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