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The importance of shape analysis of the first upper molar in the separation of two subspecies of the Hazel dormouse (*Muscardinus avellanarius* (Linnaeus, 1758)) in Northern Anatolia

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Abstract: Morphological features are important for intraspecific and interspecific variation. Teeth are important taxonomical characteristics because they can differ according to diet. Shape analysis of the first upper molar (M1) was used to determine geographical variations and effects of ecological changes on the population structure of Hazel dormice (*Muscardinus avellanarius*) living in Turkey. Both outline and landmark analysis, as well as a canonical variates analysis, showed significant differences in teeth shape between populations. With this technique, we separated out two subspecies: *M. a. trapezius* and *M. a. abanticus*.

Key words: Anatolia, Fourier analysis, geometric morphometrics, hazel dormouse, *Muscardinus avellanarius*

1. Introduction

The hazel dormouse, *Muscardinus avellanarius*, is distributed throughout Europe and northern parts of Turkey—except Thrace (Kivanç, 1983)—and ranges from Italy to Russia (Wilson and Reeder, 2005; Hutterer et al., 2016) (Figure 1a). In Turkey, this species is arboreal and rare, living in an area extending from Bursa (Uludağ) to Trabzon, covered with deciduous forests including mainly hazelnut trees (*Corylus* sp.) (Yiğit et al., 2006). Within these ranges, they prefer habitats with high biodiversity for feeding, nesting, and avoiding predators (Juškaitis, 2008). They rarely descend to the ground from the bushes where they live, unless it is necessary—for example when hibernating (Bright and Morris, 1991; Capizzi et al., 2002; Morris, 2003; Bright et al., 2006). Sometimes habitats become fragmented, causing loss of the species range, decreasing migration of the species, and increasing predation risk (Amori, 1993; Mortelliti et al., 2010, 2014; Goodwin et al., 2018).

Throughout Turkey, multiple subspecies of *M. avellanarius* have been identified. *M. avellanarius*, specifically, was first recorded by Nehring (1903) from İstanbul (Ümraniye), in the Northwestern region of Turkey. Miller (1908) assigned the specimens from an Eastern region, Trabzon (Coşandere) as *M. trapezius*. Then, Ellerman (1948) evaluated *M. trapezius* as a subspecies of

M. avellanarius, naming it *M. a. trapezius*. Later, Kivanç (1983) recorded *M. avellanarius* in Trabzon (Yomra) and Ordu (Ulubey)—the Northeastern region—and also in Bolu (including Abant-Soğuksu, Yenice, Köseköy, Yiğilca) and Bursa (Yenikonak)—the Northwestern region. Kivanç (1983) described the specimens from the specific region of Bolu, Abant-Soğuksu as a new subspecies, *M. a. abanticus*. This limited data suggests that *M. a. trapezius* is found in the Northeastern region, while *M. a. abanticus* is found in the Northwestern region. However, this data is insufficient to make this conclusion. Karyological aspects would help further identify these groups, but they have only been determined for *M. a. trapezius* by Doğramacı and Kefelioğlu (1992) and Şekeroğlu et al. (2011), while the karyological aspects of *M. a. abanticus* remain limited. Overall, these studies indicate that more information is necessary to justify the taxonomic status at the intrapopulation level of *M. avellanarius* in Turkey.

One new method to determine the taxonomic status of animals at the intrapopulation level is with geometric morphometric analysis of their cranial bone and teeth. Geometric morphometric analysis often represents geographical and ecological variations (Caumul and Polly, 2005; Yamada et al., 2018). For example, Caumul and Polly (2005) investigated changes in skull shape due to environmental factors in marmots (Rodentia). In the

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analysis of the molars, they found significant differences in the intraspecies and interspecies populations of marmots. Yamada et al. (2018) morphologically evaluated the geographical differences of pig (*Sus scrofa*) populations (*Artiodactyla*) and analysed the lower molars as morphological characters, finding significant differences between subspecies. Dentition patterns can be used from both fossil and actual specimens (Dayan et al., 2002). They reflect the peculiarity of divergent lineages and reflect ecological changes (Cardini, 2003; Renaud and Michaux, 2007; Ledevin et al., 2010) because adaptation to ecological niches can be observed as morphological features leading to taxonomic diversifications (Heard and Hauser, 1995; Kaya and Kaymakçı, 2013). Thus, geometric morphometrics techniques are a tool to analyse such effects of ecological changes on the morphological aspects of populations (Echeverry et al., 2020; Parés-Casanova et al., 2020). The most important morphological aspects to investigate are patterns of molars. Shape analysis, specifically, may reflect changes in dentition to food habits. For example, the cusp pattern on the occlusal surface must be adapted to break food up into smaller parts during chewing (Kaya and Kaymakçı, 2013). Moreover, molars are useful in distinguishing geographic populations because they show less ecophenotypic variation (Caumul and Polly, 2005). Also, the first molars often give more significant results, since the third molar shows extreme variation among the three molars, and the second molar is stuck between the first and third (Renaud et al., 1996; Renaud, 2005; Renaud and Michaux, 2007). There are also studies showing that climatic changes cause differentiation in tooth morphology, in this context, the expansion of open and step areas resulted in the evolution of animals with hypsodont teeth (Cerling et al., 1997; Fortelius et al., 2002; Tapaltskyan et al., 2015). Morphological characters may be directly related to the ecological characteristics of each taxon, such as habitat or diet (Auffray et al., 2009). Some researchers have shown that there is an interesting relationship between tooth characteristics and grazing diets in rodents with traditional morphometrics methodologies in lateral view (hypsodont) or occlusal modelling (Williams and Kay, 2001). Notably, the actual composition of the diet is determined by seasonal availability (Freudenthal et al., 2014); thus, diet can change throughout the seasons. Today geometric morphometrics methodologies are of great interest because they enable researchers to quantify variations in shape and size to develop a morphospace that facilitates their ecological and evolutionary implications (Renaud et al., 1996; Michaux et al., 2007; Samuels, 2009; Ledevin et al., 2010). For example, in a study with fossils of *Apodemus*, *Maxomys*, *Mus*, etc., the geometric morphometrics comparison of the first upper molar scratches suggested ecological preferences in the diet based on tooth morphology, and these studies, along

with phylogenetic studies, helped to map the evolutionary history of feeding habits (Gomez Cano et al., 2013). Moreover, in the Northwest and Northeast Anatolia region, the accumulation of the ecological changes from the past to the present within *Glis* and *Apodemus* was established by geometric morphometric techniques (Helvacı et al., 2012; Helvacı and Çolak, 2021). For example, Helvacı et al. (2012) found that *Glis glis* populations differ in first upper molars due to geographical and anthropogenic factors.

M. avellanarius distributed in the North and Northwest Anatolia region also has a long evolutionary history (Kaya and Kaymakçı, 2013). Today, North and Northwest Anatolian regions are one of the regions most affected by geological and tectonic events as well as Pleistocene climatic changes during a period that lasted from the late Miocene to the present day (Popov et al., 2004, 2006; Dubey et al., 2006; Okay and Topuz, 2017). However, the subspecies specification of the *M. avellanarius* is problematic. In this context, we aim in this study to present shape variations of the first upper molar of *M. avellanarius*, adding knowledge about this species to the literature. Considering the impact of ecological factors on morphological variations, we investigated whether there are morphological variations between the first upper molars of *M. avellanarius* living in rare populations.

The aim of this study was to evaluate the importance of the first upper molar in the differentiation of the subspecies of *M. avellanarius*.

2. Materials and methods

This study determined the shape analysis of the first upper molars (M1) from 56 specimens of the hazel dormouse (*Muscardinus avellanarius*). A total of 33 specimens from collections and 23 live specimens were taken from Ankara University-Faculty of Science-Vertebrate Research Laboratory and Ankara University Mammalian Research Collection (AUMAC), respectively. Only adult specimens were used, and they were trapped between 1979 and 2017 in six localities in Northern Anatolia (Figure 1b).

Each of the specimen's skull images was photographed with a stereomicroscope-connected camera. For discriminant analysis, canonical variate analysis was done by Mstat 12.

2.1. Outline analysis

We sampled 64 points as equal along the occlusal surface of the left first upper molar using TpsDig. The start point was digitized at the junction point between the premolar and the first upper molar (Figure 2). Using the first nine harmonics for the M1, an elliptic Fourier analysis was applied to the M1 outlines (Figure 3).

2.2. Landmark analysis

Nine landmarks were digitized to the left first upper molar using by TpsDig. Number 1 and 2 are junction points

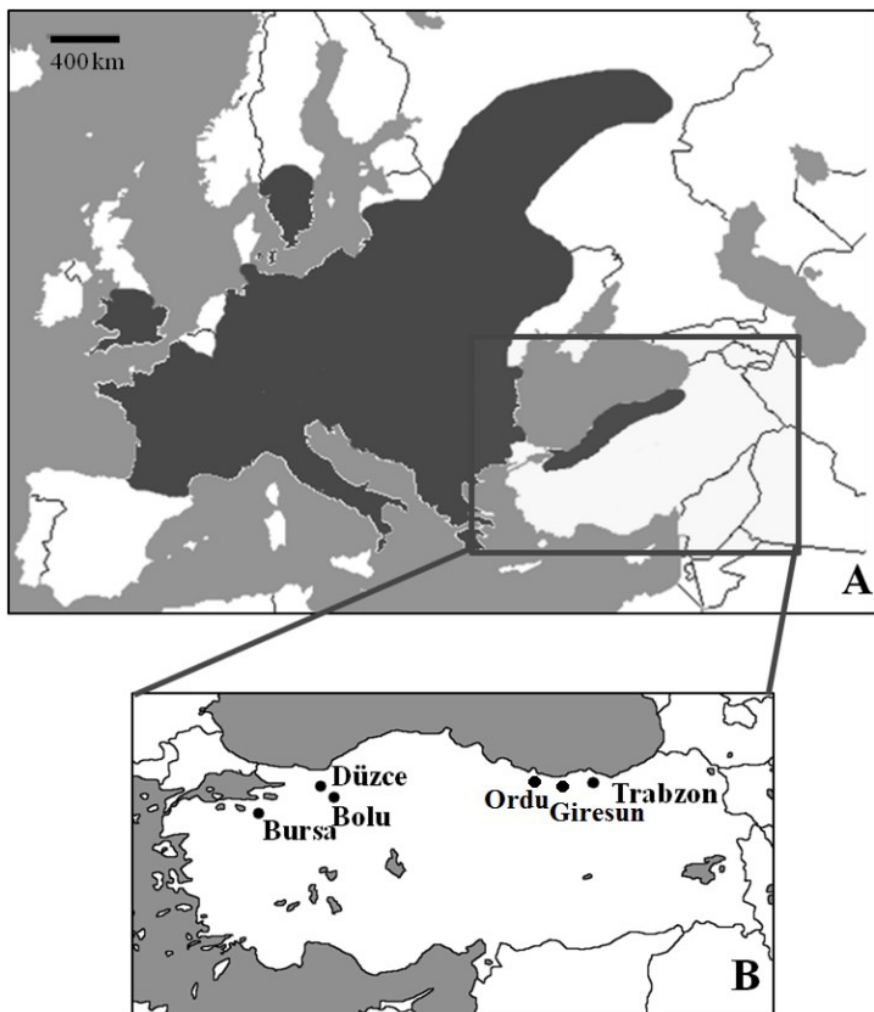


Figure 1. Worldwide distribution of *Muscardinus avellanarius* (a) and trapped localities in Northern Anatolia, Turkey (b) [Number of specimens: in the West; Bolu = 23, Bursa = 3, Düzce = 6, and in the East; Giresun = 4, Ordu = 6, Trabzon = 14].

between the premolar and the first upper molar, number 3, 4, 5, and 6 are the maximum curvature of the outline, number 7 and 8 are junction points between the first upper molar and the second upper molar, number 9 is the maximum cavity of the outline along the lingual side (Figure 4). All of the landmarks were superimposed using MorphoJ.

3. Results

Shape analysis of the first upper molar of *M. avellanarius* populations displayed significant differences. The canonical variates analysis showed two canonical values for 76% of the total variation of the samples (CA1: 41%, CA2: 35%, $p < 0.01$). Figure 5 exhibited the morphological variations of the first upper molar at the first two canonical axes.

Bolu, Bursa, and Giresun populations displayed positive values on the CA1 axes, whereas Bursa, Bolu, Düzce, and Trabzon populations showed positive values on the CA2 axes. The Bursa population tended to show positive values along both CA1 and CA2 axes.

The canonical variates analysis showed two canonical values for 70% of the total variation of the samples (CA1: 42%, CA2: 28%, $p < 0.01$) for landmark analysis. Figure 6 shows the morphological variations of the first upper molar at the first two canonical axes.

Ordu, Giresun, and Trabzon populations displayed positive values on the CA1 axes, whereas Bursa and Trabzon populations showed positive values on the CA2 axes. The Düzce population tended to show negative values along the CA1 and CA2 axes. While the Bolu population indicated positive and negative values on the CA2 axes.



Figure 2. Occlusal surface of the left first upper molar and start position of digitisations.

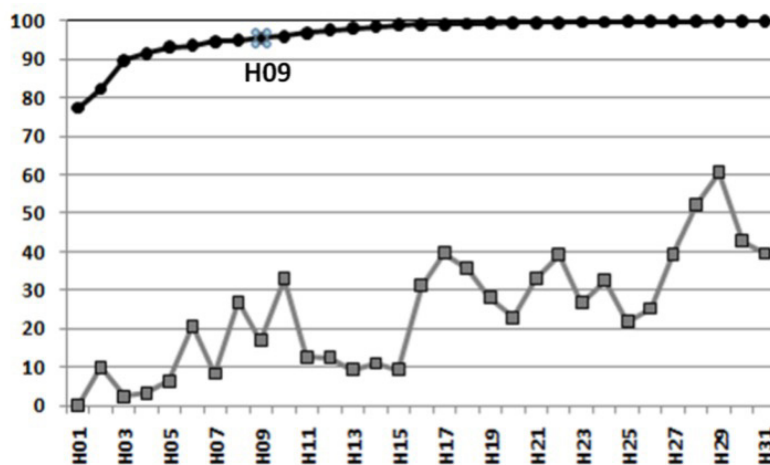


Figure 3. Harmonic order for the left first upper molar of *M. avellanarius*.

4. Discussion

The hazel dormouse, *M. avellanarius* is distributed across Northwest Anatolia and Northeast Black Sea regions. These regions have some formations, which can affect population structure, such as different vegetation, climate, glacial refugia, topography, and some physical barriers; the Kızılırmak River and the Melet River. Kıvanç (1983) analysed a wide

range of samples based on morphological characteristics and described new subspecies, *M. avellanarius abanticus* from Abant (Soğuksu) and *M. avellanarius trapezius* based on traditional morphometrics analysis. Kıvanç (1989), Kıvanç and Yardımcı (2000), and Kankılıç et al. (2021) testified the validity of two subspecies based on tooth variations and karyological aspects, respectively.

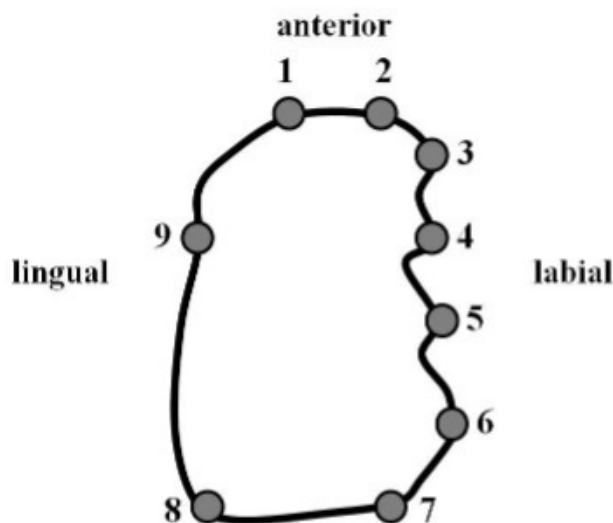


Figure 4. Positions of nine landmarks for the left first upper molar (Definitions are in the text).

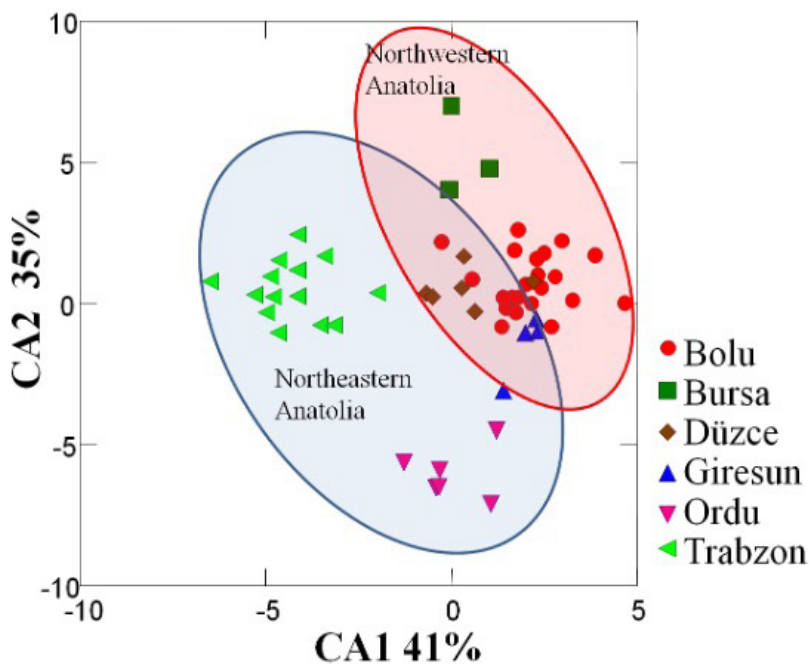


Figure 5. Canonical variate analysis of *M. avellanarius* on the M1 for outline analysis.

M. avellanarius is a hibernator species and needs complex habitat requirements (Foppen et al., 2002; Vilhelmsen, 2003; Bright et al., 2006). Changes in geographical climate and vegetation may allow *M. avellanarius* to have different morphological aspects of its molars by its main range in Turkey as in another

glirid, *Glis glis* (Helvacı et al., 2012). Helvacı et al. (2012) indicated that *G. glis* has diversity based on this eco-region pattern, inferred from the first upper molar shape analysis. We analysed specimens from both the west and the east of the Melet River; however, our findings did not reveal an isolated effect of the Melet River on populations of *M.*

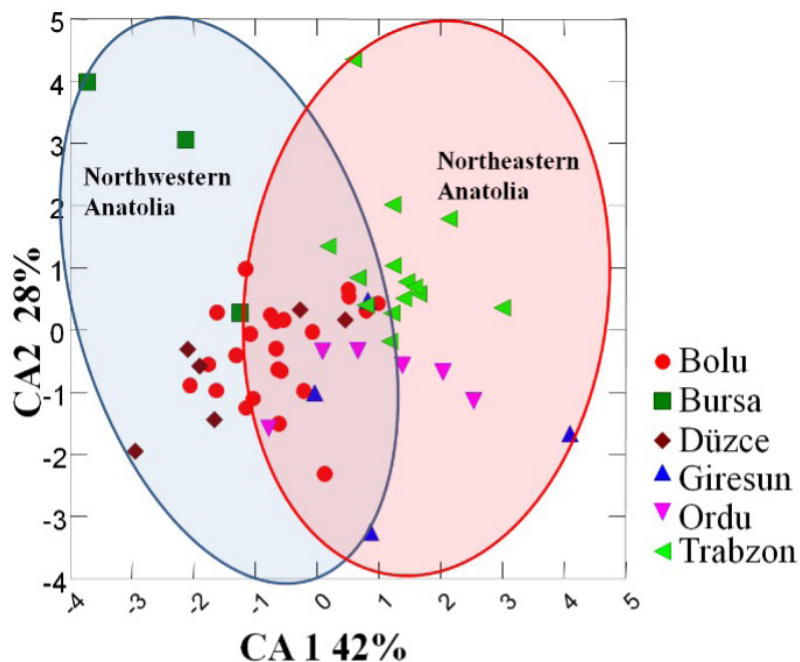


Figure 6. Canonical variate analysis of *M. avellanarius* on the M1 for landmark analysis.

avellanarius in the region. The Northern Black Sea shores of Anatolia are covered with humid deciduous forests and the forest ecosystem has plant communities rich in species. Specimens from Ordu, Giresun, and Trabzon overlapped on the range of *M. a. trapezius*. This might have resulted from ecological differentiation. Atalay and Efe (2010) and Atalay et al. (2014) indicated that forests in Giresun, Ordu, and Trabzon (Northeastern Anatolia) are richer in species and understorey than the forest ecosystems in the west. Interestingly, Bursa and Trabzon populations showed positive values on CA2 axes (Figure 6). Bursa has the weakest understorey vegetation among these localities (Ordu, Giresun, and Trabzon) (Atalay and Efe, 2010; Atalay et al., 2014).

In this study, we determined the separation of *M. avellanarius* into a Northwest group corresponding to *M. a. abanticus* and a Northeast Anatolia group corresponding to *M. a. trapezius* based on an outline analysis (Figure 5) and landmark analysis on the first upper molar (Figure 6). These findings supported the possible effect of the Kızılırmak River on the divergence of *M. avellanarius* to two different subspecies, *M. a. trapezius* and *M. a. abanticus*. However, to justify this case, further research is necessary on specimens from the near west and east banks of the Kızılırmak River.

Feeding biology is an important factor to understand an animal's ecology (Kaya and Kaymakçı, 2013). Glirid tooth morphology has taken shape throughout the

Cenozoic period (Daams and De Bruijn, 1995) and tooth diversification arose because of hard food consumption (Daams and Van der Meulen, 1984) across phylogeny (Caumul and Polly, 2005). According to microwear analysis, Kaya and Kaymakçı (2013) noted that dormice had complex diets (e.g., grass, insect, seeds, and fruit) in the Sivas Basin, in eastern Central Anatolia. Similarly, Oliver (2014) indicated that the molar tooth morphology of *Armantomys* (an extinct genus of Gliridae in Spain) depends on its varied diet. Also, teeth characteristics of dormice are often used for their classification (García - Alix et al., 2008). Shape analysis is related to feeding biology and it describes geographic variation in teeth characteristics (Renaud, 2005). According to fossil records, the molar teeth of rodents evolved from brachydont to hypselodont based on vegetation change (Tapaltsyan et al., 2015). It is thought that teeth evolution was related to feeding biology and their abrasive characteristics allowed a wide range of food (Tapaltsyan et al., 2015).

M. avellanarius generally prefers hazel and bramble (Bright et al., 2006) but feeds miscellaneous according to season (Bright and Morris, 1993), also preferring seeds, flowers, and berries (Juškaitis, 2007). The hazelnut is a major source of nutrition for the hazel dormouse in Turkey. On the other hand, Northern Anatolia has many rivers. The division of the distribution area with rivers and the local vegetation difference may have caused this species to be biogeographically different, as seen in our results. Also,

because this species lives in short-range habitats (Mortelliti et al., 2010) and accordingly adapt to these different habitats, populations may cluster discretely like in this study (Figures 5 and 6). However, the addition of hard food, such as oak seeds, to the feeding habits may have caused a differentiation in tooth morphology in Northwest Anatolia.

It is considered low dispersal of this species (Bright and Morris, 1992; Mortelliti et al., 2010), habitat loss, habitat fragmentation, and destroyed forest composition becomes more important.

In this study, both outline analysis (Figure 5) and landmark analysis (Figure 6) results showed significant differences in the first upper molar shape for the hazel dormouse, *M. avellanarius*. Our results showed that populations were grouped as Northwest and Northeast

Anatolia. Consequently, although there appears to be a clear difference between populations, to say that these groups are subspecies for this distinction between the Northwest Anatolia and Northeast Anatolia populations would be an overstatement based on the current study. Perhaps these populations are on their way toward being an ecological race or subspecies, but there is a need for further studies supported by larger and molecular data.

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