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Geometric morphometric analysis of the molars in three species of the genus *Mus* (*Mus*) (Rodentia, Muridae) based on the outline method

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Abstract

The relatively new technique of outline-based geometric morphometrics was applied in a study of the variation in the shape of the upper and lower molars among 122 mice, belonging to one species from Iran (*Mus musculus*) and two species from Europe (*Mus macedonicus, Mus spicilegus*). Differentiation of specimens based on molar shape was highly dependent on the details of the shape information. Among molars, the second upper and first lower molars are better at separating the species. This method provides a useful way to distinguish species based on the outline of their molars.

Keywords: shape variation, upper and lower molars, mice, Iran, Europe.

Introduction

The ancestor of the genus *Mus* appeared first in the Indian subcontinent dated to the late Miocene; it has been present in the Mediterranean area from the Middle Pleistocene (Cucchi 2005). Results of a fully resolved tree supports monophyly of the genus *Mus*, monophyly of the subgenus *Mus*, and division of the subgenus *Mus* into Palearctic (*M. musculus*, *M. macedonicus*, *M. spicilegus*, and *M. spretus*) and Asian (*M. cervicolor*, *M. cookii*, and *M. caroli*) clades (Lundrigan *et al*. 2001). Within the Palaearctic clade, the sister-group relationship between the eastern Mediterranean short-tailed mouse (*M. macedonicus*) and the mound-building mouse (*M. spicilegus*) is also well established (Lundrigan *et al*. 2001, Prager *et al*. 1996). *Mus macedonicus*, *Mus spretus* and *Mus spicilegus* are therefore closely related to and occur sympatrically with *M. musculus* in Europe and the Middle East (Suzuki *et al*. 2004).

Cucchi *et al*. (2002) demonstrate that *M. macedonicus* now live on Cyprus and have been there from at least the 9th millenium BC. The steppe mouse, *M. spicilegus* Petényi, 1882, is presently confined to central Europe (Cucchi 2005), to the European lowlands from Austria and Slovakia in the north, to Bulgaria in the south and the Ukraine in the east, including Serbia, Hungary, Romania, and Moldova (Macholán 1999b).

The house mouse (*Mus musculus*) is the most recent offshoot of the genus *Mus*; the native range of the commensal house mice is all of the Eurasia plus North Africa (Prager *et al*. 1998). It can be divided into three to five taxa that, either as species or subspecies, are designated *domesticus* of West Europe, North Africa and the Middle East, *musculus* of eastern Europe and northern Asia, *castaneus* of southeastern Asia, *bactrianus* of south-central Asia from Iran to N India (Prager *et al*. 1998) and the recently designated *gentilulus* from Yemen.

Several studies have attempted to distinguish these species from each other. For instance, a study of cranial and dental traits tried to distinguish *M. musculus* and *M. spicilegus* from other Western Palearctic mouse taxa (Macholán 1996b); and molar shape distinguishes *M. spicilegus* from European mouse species (Macholán 1996a). Shape analysis is part of geometric morphometrics (Rohlf 2000) that has been quite successful in describing morphological variation.

In this study we looked at the geometric morphometrics of the species *M. macedonicus*, *M. spicilegus* and *M. musculus* using the outline method. The aim of this study is to determine whether variation in the shape of the molars distinguishes these species.

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Materials & Methods

Specimens analyzed in this study came from Iran (house mouse *Mus musculus*) and Europe (*Mus macedonicus*, *Mus spicilegus*). Specimens from Iran were collected from Mashhad (36º17´N, 59º35´E, N=11), Gonbad (37º15´N, 55º10´E, N=14), Gonabad (34º21´N, 58º42´E, N=20), Zabol (31º2´N, 61º29´E, N=6), Birjand (32º52´N, 59º12´E, N=20), Kerman (30º16´N, 57º4´E, N=6), Shiraz (29º36´N, 52º31´E, N=8) and Zanjan (36º40´N, 48º29´E, N=7). All samples are deposited in the Rodent Collection of the Zoology Museum of Ferdowsi University, Mashhad, Iran (ZMFUM).

Digital images of the right upper and lower molars of 122 mice were captured using a JVC™ digital camera connected to an Olympus BH-2 stereomicroscope with objective lens 1 and magnification 2.5 x. Images were then organized with tpsUtil version 1.21 (Rohlf 2003) software and recorded using a series of points along the outline of the first and second upper molars (M1/, M2/) and the first lower molar (M/1) with tpsDig v2.12 software. Points were recorded in a sequence beginning at a particular point. Following best practice, we started at an identifiable point: the number of points was 150 points per outline. The program GMTP version 2 (Geometric Morphometric tools package) (Taravati 2009) was used to convert the data from tps format into a format readable for EFAwin (Isaev 1995). The data were then opened and analysed in EFAwin using Fourier decomposition to describe the points using harmonics. The first 15 harmonics were used here as an adequate description of the original data. GMTP then converted the output file of EFAwin to PAST format.

Variation in molar shape among species was assessed by Canonical Discriminant Function Analysis (CDA) based on the harmonic coefficients, using SPSS v15. CDA constructs new axes (one fewer than the number of groups) that maximise the separation of the groups (the between- to the within-group variance), subject to these axes being uncorrelated with one another.

Results

Fig. 1 shows the results of the CDA for the harmonic coefficients from the first upper molar, displaying the first and second axes, along both of which there is highly significant discrimination among the taxa. The first axis separates *M. spicilegus* from the two other species, and the second axis was effective in separating *M. macedonicus* from *M. musculus* (Fig. 1).

The CDA scatterplot for the second upper molar (Fig. 2) showed that each species occupied different areas of the graph. *M. musculus* was separated along the first axis from the two other species, and the second axis distinguished *M. macedonicus* and *M. spicilegus* from each other (Fig. 2).

The CDA for the first lower molar (Fig. 3) once more demonstrated that *M. musculus*, *M. macedonicus* and *M. spicilegus* can be separated from each other using molar shape variation.

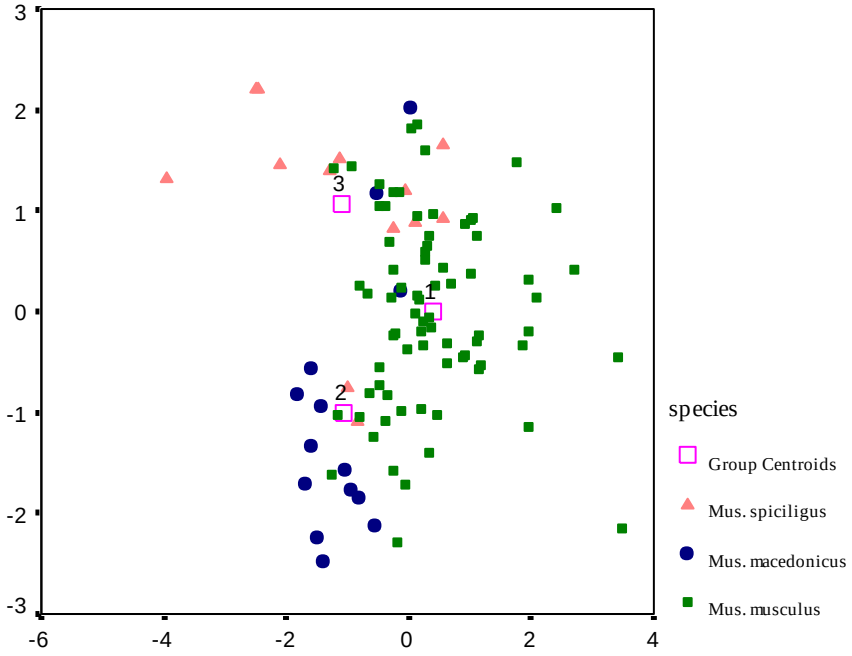

Figure 1: Scatter plot of the two axes of the Canonical Discriminant Function Analysis of the outline data for the first upper molar. Together they account for 67% of the discrimination (Wilks $\lambda = 0.231$, $p<0.001$), and the second axis alone for 32% (Wilks $\lambda = 0.579$, $p<0.001$).

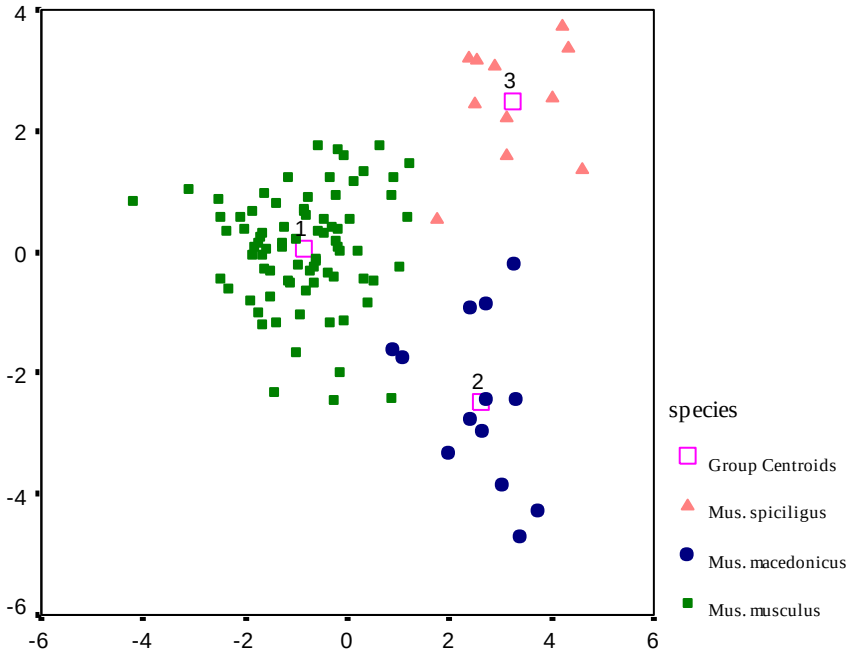

Figure 2: Scatter plot of the two axes of the Canonical Discriminant Function Analysis of the outline data for the second upper molar. Together they account for 62% of the discrimination (Wilks $\lambda = 0.113$, $p<0.001$), and the second axis alone for 36% (Wilks $\lambda = 0.405$, $p<0.001$).

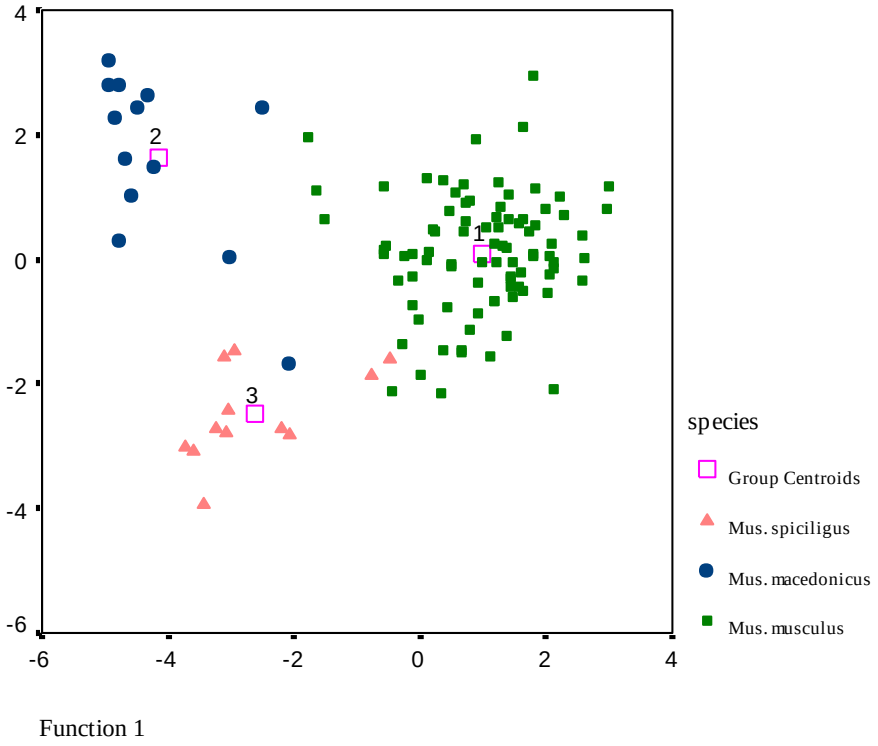

Figure 3: Scatter plot of the two axes of the Canonical Discriminant Function Analysis of the outline data for the first lower molar. Together they account for 77% of the discrimination (Wilks λ = 0.110, $p<0.001$), and the second axis alone for 22% (Wilks $\lambda = 0.500$, $p<0.001$).

Discussion

Discriminating between close species of mice is difficult (Cucchi 2005), but analyzing shape using outlines can be highly effective for interspecific distinctions. In this study we demonstrated that the outline of the molars can be a powerful tool for distinguishing the commensal (*M. musculus*) from the wild species (*M. spicilegus* and *M. macedonicus*), and that this method, as with classical morphometry and morphology, would be an appropriate method for distinguishing among species. House and wild mice can be clearly distinguished based on the shapes of their first and second upper and first lower molars. Therefore, we can conclude that there are significant differences in the molar shapes among *M. musculus*, *M. macedonicus* and *M. spicilegus*. Using the outline method, Cucchi *et al.* (2002) separated commensal house mice from *M. macedonicus* using the first lower molar. Among the molars, the first upper molar showed overlap between species (see Fig. 1) probably because variation in the shape of first upper molar is less than that of the second upper and first lower molar.

Several aspects of molar shape are important in the separation of species from each other. For example, tubercle E (tE) on the anterior lobe of the first lower molar (Orsini 1982) is more developed in *M. macedonicus* and *M. spicilegus* than in *M. musculus* (cf. Fig. 4). In the house mouse the tubercles on the anterior lobe of the first lower molar are more trilobic compared to wild mice. Orsini (1982) used tubercle E on the anterior lobe of the first lower molars to separate the commensal sub-species (*M. m. domesticus*) from wild species (*M. spretus* and *M. macedonicus*). We suggest that this morphological criterion with variation in the shape of the molar is involved in the separation of the three species studied.

This study focused mainly on the morphology of the teeth. Following this, it is necessary to study geographical and genetic variation of these three species. We could not estimate the effect of geographic and ecological factors on the shape of the molars, because the specimens

of *M. macedonicus* and *M. spicilegus* come from the animal house. However, environmental factors play a major role in dental morphology.

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الملخص العربي

التحاليل الهندسية والمظهرية لضروس ثلاثة أنواع من تحت جنس ***Mus* (Rodentia, Muridae)** باستخدام طريقة الخطوط العريضة

شابانی م.1 - غاسیمزادیا ف.1 - دارفیش ج.2

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هناك تقنيات جديدة نسبياً لقياس الاختلافات الهندسية والمظهرية ويتم تطبيقها فى المجالات البيولوجية وخصوصاً التى تعتمد على الاختلافات فى الشكل. تم فى هذه الدراسة بيان الاختلافات فى شكل الضروس العلوية والسفلية لعدد 122 فأر ينتمون لنوع واحد من إيران (*Mus musculus*)، ونوعين من أوروبا (*Mus macedonicus, Mus spiciligus*)، باستخدام تقنية الخطوط العريضة. أوضحت النتائج أن هناك اختلافات فى شكل الضروس فى عينات الدراسة وبخاصة فى التفاصيل الخاصة بالشكل. من بين الضروس، كان الضرس الثانى العلوى والأول السفلى لهما تأثير أكثر فى فصل الأنواع عن بعضها البعض. ولذا يمكننا استخلاص أن استخدام تقنية الخطوط العريضة للضروس تمدنا بطريقة مفيدة فى تمييز الأنواع.