

# Contemporary and sub-fossil house mice (*Mus musculus* Linnaeus, 1758) (Rodentia: Muridae) from Iran

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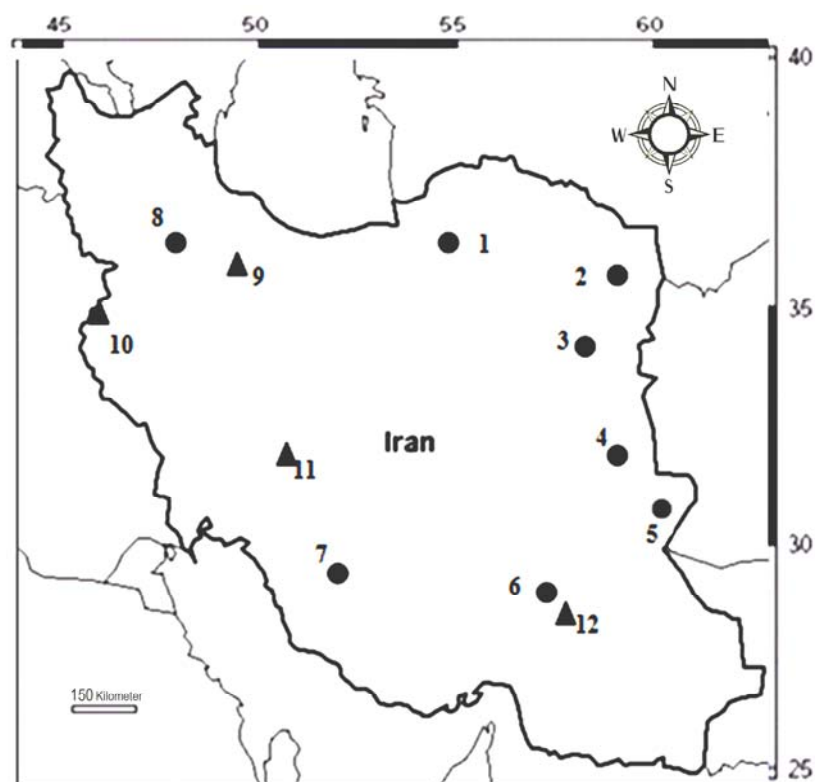
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The house mouse (*Mus musculus*) is a polytypic species, with its geographic distribution largely the result of human migrations during the Late Glacial and Holocene. This study used geometric morphometry (GM) on upper and lower molars to discriminate among the three subspecies of the house mouse present in Iran. Moreover cranial remains of this species were uncovered on several archaeological sites of the Iranian Plateau. The GM results were applied to sub-fossil lower molars from Isfahan (Qaleh Bozi), Kordestan (Kani Mikail), Qazvin (Zagheh), and Jiroft (Konar Sandal). The present-day Iranian populations that have been previously separated into three subspecies (*M. musculus musculus*, *M. musculus domesticus*, and *pro-castaneus* type) by molecular studies can also be distinguished by dental geometric morphometrics. These subspecies are distinguished by variation in the shape of the first and second upper molar (M1/, M2/) and the first lower molar (M/1). Comparison of these results to the Late Glacial/Early Holocene material from the above mentioned fossil bearing localities shows marked similarity with the extant house mouse of the southeastern Iranian Plateau, the *pro-castaneus* type.

**Key words:** House mouse, sub-fossil, upper and lower molar, subspecies, Iran

## INTRODUCTION

The house mouse, *Mus musculus*, a worldwide complex species, is the most recent phylogenetic offshoot of the genus *Mus* (Boursot et al., 1993). According to genetic studies, the Indian subcontinent is considered to be the origin of the species (Bonhomme et al., 1994; Boursot et al., 1996; Darvish et al., 2006; Din et al., 1996). A well-preserved mouse skull was recovered from a pedogenically modified two million year old mudstone layer east of Chandigarh, India and was designated *M. linnaeusi* (Patnaik et al., 1996). Comparison of the fossil skull with those of the extant species of the subgenus *Mus* reveals its close relationship to the house mouse lineage (Patnaik et al., 1996). Subsequent to establishment of *M. musculus* in northern India, radiation in several directions led to well-defined peripheral subspecies (Bonhomme et al., 1994). Currently, this species includes three geographically and genetically distinct subspecies: *M. musculus musculus*, *M. m. domesticus*, and *M. m. castaneus*, which are sometimes elevated to species level (Boursot et al., 1996; Darvish, 2004; Darvish et al., 2006; Orth et al., 1996; Rajabi-Maham, 2007; Vanlerberghe et al., 1986). The nominotypical subspecies, *M. m. musculus*, occurs in Eastern Europe and throughout northern Asia, *M. m. domesticus* is distributed in Western Europe and has colonized much of the world as a commensal with humans, and *M. m. castaneus* extends from Central Asia throughout southeastern Asia; it has spread to Taiwan and eastward through the Moluccas to New Guinea and also to the Mariana Isles (Wilson and Reeder, 2005). The systematic status of the intermediate populations of



**FIGURE 1.** Geographic distribution of *Mus musculus* specimens in Iran (● Modern: 1 Gonbad; 2 Mashhad; 3 Gonabad; 4 Birjand; 5 Zabol; 6 Kerman; 7 Shiraz; 8 Zanjan. ▲ sub-fossil: 9 Qazvin; 10 Kani Mikaiil; 11 Isfahan; 12 Jiroft).

the house mouse in Eastern Iran, Afghanistan, Pakistan, and northern India is long debated among scholars and remains unresolved (Darvish, 2008; Darvish et al., 2006). Based on the existence of introgression through narrow and permanent hybrid zones in Europe, some researchers describe these populations as semi-species or distinct 'good' species (Bonhomme et al., 1989; Marshall and Sage, 1981; Orsini et al., 1983; Sage et al., 1993). Others emphasize the polytypic nature of *M. musculus* and consider these populations to be subspecies (Din et al., 1996).

According to recent reports, both molecular and morphological evidence is useful for resolving the status of *M. musculus* (Darvish, 2008). Newly developed geometric morphometric methods on biological shapes (Leandro et al., 2002) have been effective in interspecific distinction of closely related species and in distinguishing the commensal form from other species of *Mus* (Cucchi, 2005). The aim of this study was to apply outline-based geometric morphometry to analyze molars of *Mus musculus* that have been collected from different regions of Iran, and to determine the antiquity of each subspecies from sub-fossil dental remains.

#### **MATERIALS AND METHODS**

The right upper and lower molars from 92 specimens of *Mus musculus* belonging to populations from eight localities in Iran were analyzed (Table 1, Fig. 1). The studied specimens belong to three subspecies: *M. musculus musculus*, *M. m. domesticus* and *pro-castaneus* type (Darvish et al. 2006; Orth et al. 1996; Rajabi-Maham, 2007; Vanlerberg et al. 1986). In addition, seven sub-fossil specimens (Late Glacial/ Early Holocene) were collected from four localities on the Iranian Plateau. The site of Qaleh Bozi near Isfahan (Biglari et al. 2009) is dated to the Middle Palaeolithic C. 30000 BC, the Kani Mikaiil cave in Kurdistan has a sequence going from C. 20000 BC to mid Holocene (Roustaei

et al., 2002a and b), Hashemi et al., 2006), Zagheh in the Qazvin Plain is a Late Neolithic site (Mashkour et al., 1999 and Molla-Salehi et al., 2008) and finally Konar Sandal near Jiroft is a Bronze Age site (Mashkour and Hashemi 2008) (Table 2, Fig. 1). The microvertebrate samples were recovered within a specific wet or dry sieving program directed by M. Mashkour within the archaeological excavations for a systematic chronostratigraphic collection of paleoenvironmental data. It should be stressed here that although the sub-fossil samples were uncovered in these archaeological sites, this is not a guarantee for their age. In fact, the accumulation process of microvertebrate remains in anthropogenic sites is a complex process which needs thorough taphonomic studies and ideally a direct absolute dating on the examined samples. For the case of the *Mus* samples studied here, it could be safely admitted that the Zagheh sample comes from layers that are all from the 7<sup>th</sup>/6th millennium BC. The Qaleh Bozi Rock shelters are still active sites for cliff nesting raptors according to the important number of modern pellets visible in the cave. Although the sample comes from the sedimentation under the cave floor, it is not yet possible to allocate a precise date to this sample. The same remarks can be addressed to the Kani Mikail sample. As for the Konar Sandal sample, it was recovered from the sediments under the surface, but again the precise date for this sample is for the moment unclear. These samples are currently stored in the osteological collections of the Rodentology Research Department at Ferdowsi University of Mashhad.

For the outline method, digital images were captured using a JVC digital camera connected to an Olympus BH-2 stereomicroscope. Tpsdig V. 2.12 was used for digitizing points around the outline of molars (M1/, M2/, M/1) at 150 points per outline. Points were recorded in a sequence starting at a particular point, and best practice was to start at an identifiable point. The coordinates of these points, after converting to EFAWin (Isaev, 1995) format (EFAWin software was used for Elliptic Fourier analysis) using GMTTP V 2.00 (Taravati, 2009), are entered into EFAWin software and number of harmonics to be computed. The criterion for the number of harmonics is whether there is a sufficiently good fit of the outlines (in this study we used 15 harmonics). The EFAWin output file was converted to PAST format by GMTTP and finally harmonic coefficients used for assessment of variation in the shape of the molars by Canonical Discriminant Function Analysis (CDA) and cluster analyses, using SPSS V. 15 and PAST V. 1.94. The multivariate analysis of variance (MANOVA, Neff and Marcus, 1980) was conducted to identify differences between populations, and CDA was performed to classify samples into groups and determine separation among groups. Cluster analysis was carried out to evaluate the degree of similarity or no similarity among subspecies. For cluster analysis, group centroids were obtained from the canonical discriminant analysis and then, using the average Euclidean distance between groups, a dendrogram was drawn.

## RESULTS

### GEOMETRIC MORPHOMETRICS

Results of MANOVA showed significant differences among subspecies ( $P < 0.01$ ). Canonical discriminant function analysis was performed for maximum separation between subspecies. The results of CDA for M1/ is shown in Figure 2. In this analysis, the first and second statistically significant functions ( $P < 0.05$ ) explain 67% and 32% of the total variance, respectively. *Mus musculus domesticus* was separated from two other subspecies along the second component while *M. m. musculus* and *M. m. castaneus* showed separation along the first component.

The MANOVA analysis of the harmonic coefficients showed significant differences among subspecies ( $P < 0.007$ ). Figure 3 shows a scatterplot resulting from CDA for harmonic coefficients of M2/ in the space of the first and second components. The first and second statistically significant function ( $P < 0.05$ ) explained 70% and 29% of the total variance, respectively. The resulting scatterplot showed a separation between *M. m. domesticus* and the two other subspecies, which were slightly separated along the second function (Fig. 3).

**TABLE 1.** Geographic origin of the *Mus musculus* samples.

Locality	Number	Latitude/Longitude	Map code	subspecies
Gonbad	14	37° 15' N 55° 10' E	1	<i>M. m. musculus</i>
Mashhad	11	36° 17' N 59° 35' E	2	<i>M. m. musculus</i>
Gonabad	20	34° 21' N 58° 42' E	3	<i>M. m. musculus</i>
Birjand	20	32° 52' N 59° 12' E	4	<i>M. m. castaneus</i>
Zabol	6	31° 2' N 61° 29' E	5	<i>M. m. castaneus</i>
Kerman	6	30° 16' N 57° 4' E	6	<i>M. m. castaneus</i>
Shiraz	8	29° 36' N 52° 31' E	7	<i>M. m. castaneus</i>
Zanjan	7	36° 40' N 48° 29' E	8	<i>M. m. domesticus</i>

**TABLE 2.** Geographic origin of the sub-fossil samples.

Locality	Number	Map code
Qazvin (Zagheh)	4	9
Kurdistan(Kani Mikaiil)	1	10
Isfahan(Qaleh Bozi)	1	11
Jiroft(Konar Sandal)	1	12

MANOVA of the harmonic coefficients for the M/1 indicated significant differences ( $P < 0.003$ ). The CDA scatterplot of harmonic coefficients for the first lower molar showed separation among subspecies (Fig. 4). The first two functions explained 98% of the total variance that is suitable amount for study of molar shape differences between subspecies. *Mus musculus domesticus* was separated along the first component from the two other subspecies, and the second component discriminated *M. m. musculus* from *M. m. castaneus* (Fig. 4).

Canonical discriminant analysis of the first and second upper molars and first lower molar differentiated the three studied subspecies. The second upper and first lower molar conclusively distinguished *M. musculus domesticus* from the two another subspecies (Figs 2, 3 and 4). Results of CDA of the first lower molar of the sub-fossil specimens showed proximity to the *pro-castaneus* type (Fig. 5).

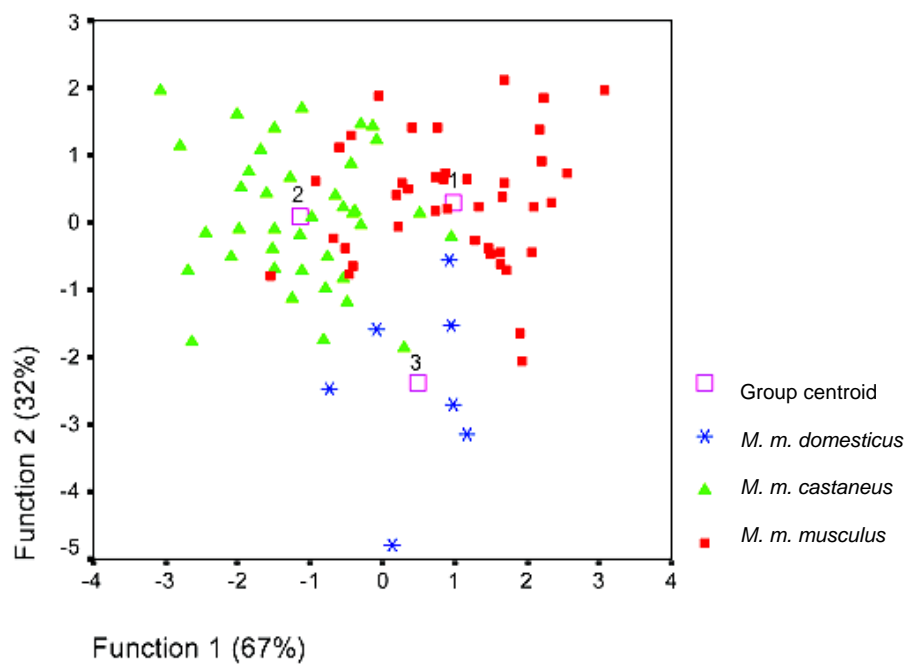


FIGURE 2. Scatter plot of CDA for the first upper molar in three sub-species of *M. musculus*.

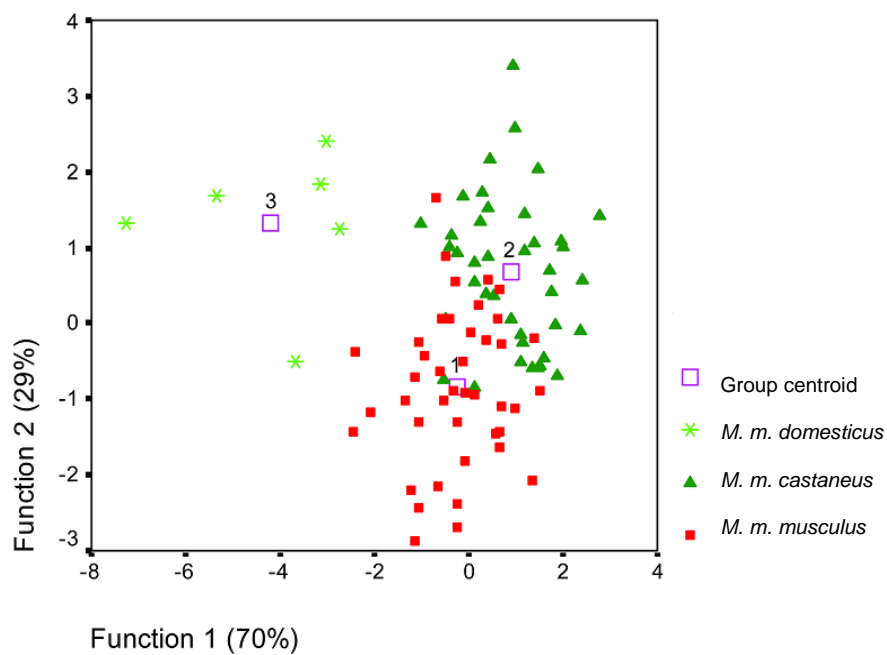


FIGURE 3. Scatterplot of CDA for the second upper molar of *Mus m. domesticus*, *M. m. musculus*, and *M. m. castaneus*.

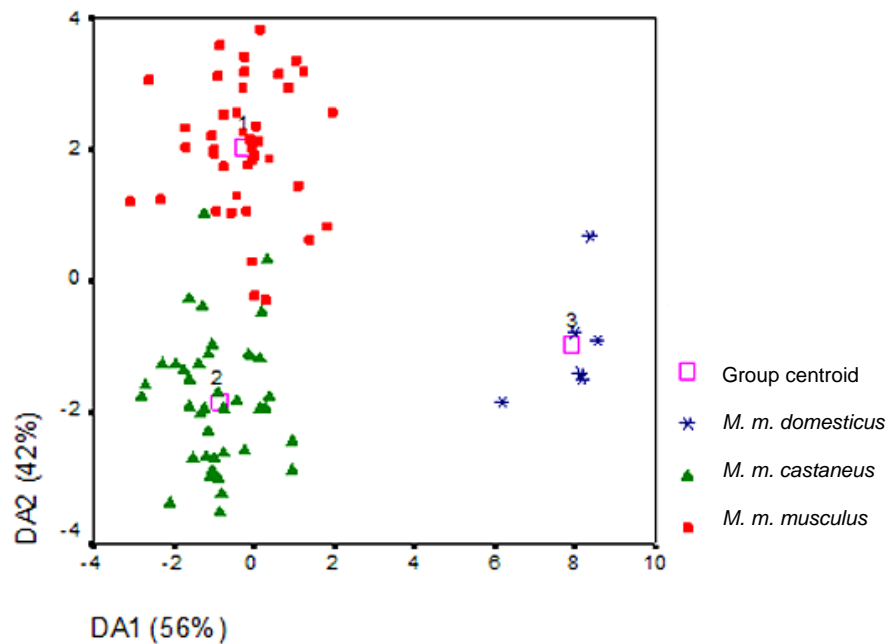


FIGURE 4. Scatterplot of CDA for the first lower molar of *Mus m. domesticus*, *M. m. musculus*, and *M. m. castaneus*.

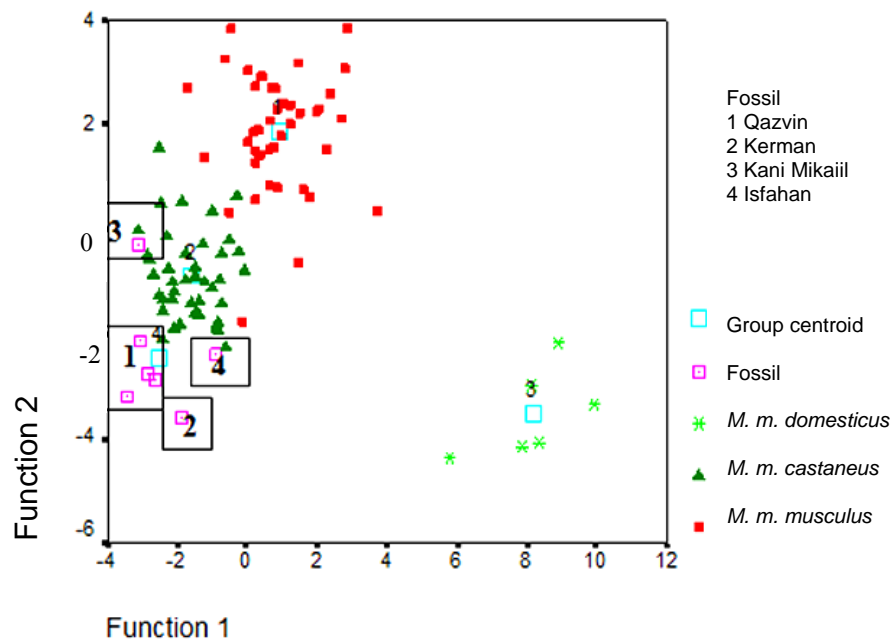
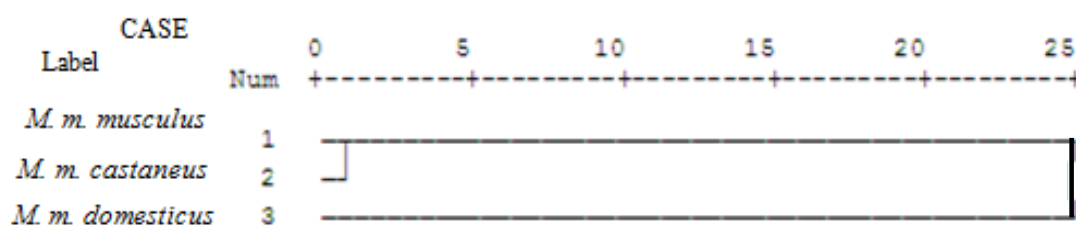


FIGURE 5. Scatterplot of CDA for the first lower molar of the sub-species and sub-fossil samples.

#### CLUSTER ANALYSIS

Cluster analysis was performed for the molars (M1/, M2/, and M/1) with similar results. *Mus musculus musculus* and *M. musculus castaneus* formed a separate cluster from *M. musculus domesticus*. This observation was compatible with the results of canonical discriminant analysis of the second upper and first lower molar, which clearly showed *M. musculus domesticus* distinct from the other two subspecies (Fig. 6).



**FIGURE 6.** Dendrogram using average linkage (between groups) of centroids of the harmonic coefficients, illustrating the degree of similarity among sub-species.

## DISCUSSION

The Iranian Plateau is located centrally between three major subspecies of the *Mus musculus* populations: the *M. musculus musculus* in central Asia, the *M. m. domesticus* to the west, and *M. m. castaneus* to the east (Darvish et al., 2006). Ingression of these subspecies into Iran develop and create secondary contact zones which led to the study of this species and its subspecies from important different aspects such as morphological, molecular and biochemical. The present study analyzed differences in the shape of molars of *M. musculus musculus*, *M. m. domesticus*, and *M. m. castaneus* and demonstrated that the outline of the molars is useful in distinguishing subspecies of *M. musculus*.

Results showed that the three subspecies of *M. musculus* are distinct from each other and that a difference in the shape of M1/, M2/ and M/1 molars exists among subspecies. Also the results of geometric morphometry for molar shape consistently confirmed the presence of *M. m. musculus* in the north, *M. m. domesticus* in the west, and *pro-castaneus* in the southeast and central parts of Iran. These findings are in agreement with results of previous genetic and morphometric studies (Darvish, 2004, 2006).

Results of geometric morphometrics of M1/, M2/, and M/1 in CDA differentiated *M. musculus domesticus* from the two other subspecies and showed a considerable overlap between *M. musculus musculus* and *pro-castaneus*. According to genetic studies (on peripheral *M. musculus castaneus*) based on allozyme data, mtDNA markers, and Y chromosome (introns Zfy-1 and Zfy-2), *M. musculus musculus* and *M. musculus castaneus* are sister taxa (Din et al., 1996; Gündüz et al., 2000; Milishnikov et al., 2004). It is possible that the sister relationship between *M. musculus musculus* and *M. musculus castaneus* affects the morphological trait molar shape, as a result, an overlap can be seen in the outline of the molar.

Moreover, the results of CDA analysis revealed a close similarity between *pro-castaneus* and sub-fossil specimens (Fig. 5). Since the sub-fossil samples were collected from different regions of Iran, a *castaneus*-like ancestor for house mouse, widely distributed in Iran, is proposed. The centrifugal model of the origin and radiation of the house mouse (Bonhomme et al., 1994; Boursoot et al., 1993) suggests that the ancestral mouse was *castaneus*-like and originated on the Indian subcontinent and then penetrated eastern parts of Iran (Darvish, 2008). The ancestral mouse, after entering what is now Iran, most likely spread throughout the country. Fossil specimens studied belong to the ancestral mouse and confirm the centrifugal model.

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