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A geometric morphometric analysis of the shape of the first upper molar in mice of the genus *Mus* (Muridae, Rodentia)

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Mus; geometric morphometrics; thin-plate spline; phylogeny; phenotypic evolution.

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Abstract

Phenotypic variation in the shape of the first upper molar among 595 mice, representing nine extant and three extinct taxa of the genus *Mus*, was studied with thin-plate spline analysis. The reliability of classification of individual specimens into known groups based on their molars varied from 75 to 100%, depending on group and method used. Including 13 sliding semilandmarks to the analysis improved the detection of different kinds of size and shape variation as well as visualization of shape differences between studied groups. Correlation between phylogenetic and morphometric distances suggested about 80% contribution of phylogenetic inertia to the molar shape variation; moreover, the importance of localized versus global shape changes was similar in the detection of phylogenetic signals. Finally, shape changes along individual evolutionary lineages were revealed, suggesting a few cases of reversals, convergence and/or retention of ancestral shape. The evolution of mouse molars has thus been driven by random effects of drift together with stabilizing selection and convergence.

Introduction

Given their durability, molars are often used by palaeontologists and also by neontologists for addressing various evolutionary problems, including systematic and evolutionary relationships among mammal species or even populations. For small rodent species, individual teeth are usually the only complete structures present in palaeontological record. Traditionally, teeth have been evaluated qualitatively or simple dental measurements have been taken (mostly the molar row length or the distance between the incisor and the third molar). Recently, traditional morphometrics has been supplemented with methods of geometric morphometrics (Bookstein, 1991; Rohlf & Marcus, 1993), which have proven to be powerful tools for identifying and quantifying molar shape differences at interspecific and intraspecific levels (van Dam, 1996; Pavlinov, 1999; Renaud *et al*., 1999, 2005; Polly, 2003; Janžekovič & Kryštufek, 2004; Renaud & Michaux, 2004; Kryštufek & Janžekovič, 2005; Renaud, 2005).

Even though it may be argued that it is not worthwhile to estimate evolutionary relationships from single simple structures such as molars (Rohlf, Loy & Corti, 1996), it would be interesting to assess the extent to which molar shape variation in a group of rodent taxa reflects true phylogeny. Theoretically, we can expect at least six evolutionary scenarios with different outcomes (Fig. 1). A phylogenetic signal (often termed 'phylogenetic inertia') is present in morphometric data if closely related taxa are more similar to each other than are less related ones. There are several possible approaches to assessing the strength of the phylogenetic inertia in morphometric data; for instance, we can compare a phenogram constructed from empirical data with a true phylogeny using any of tree-comparison statistics (possibly using a parametric bootstrap procedure as suggested by Cole, Lele & Richtsmeier, 2002) or, alternatively, we can simply estimate congruence between a matrix of phylogenetic distances and a matrix of one of various kinds of morphometric distances (Monteiro & Abe, 1999). Clearly, whichever of these approaches we adopt we must assume that the phylogenetic relationships among the taxa under study are a priori estimated and that this estimate has been made without error (Cole *et al*., 2002). In addition, to avoid circularity, phylogenetic relationships should be estimated from data other than the morphometric data being tested (e.g. from molecular sequences).

In this study, morphometric variation in the shape of the first upper molars (M^1) in a group of both extant and extinct taxa of the genus *Mus* was analysed. Mice are characterized by rather uniform morphology and this applies, with some exceptions (Marshall, 1977; Marshall & Sage, 1981; Orsini *et al*., 1983; Kryštufek & Macholán, 1998), also to their molars. On the other hand, except for a few unresolved polytomies (e.g. the relationship among subgenera or among three Asian species *Mus caroli*, *Mus cervicolor* and *Mus cookii*; see Lundrigan, Jansa & Tucker, 2002; Tucker,

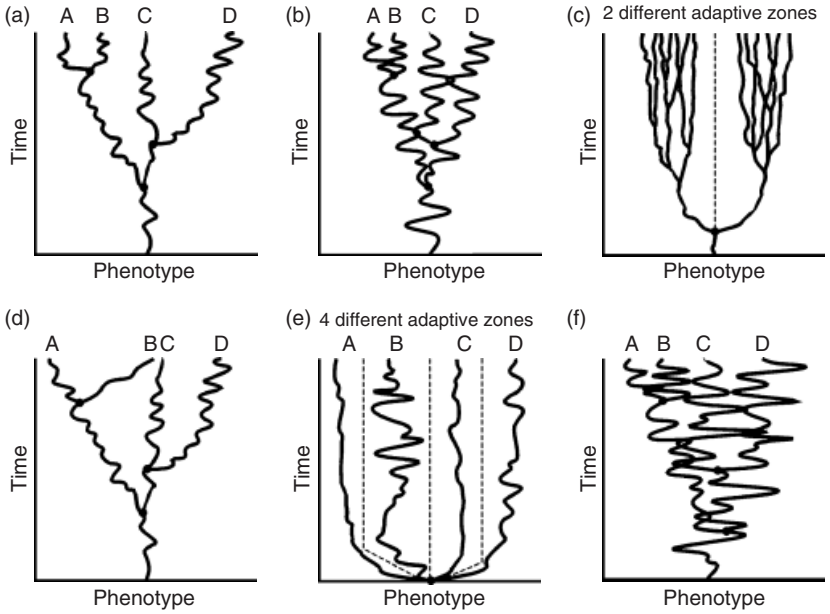

Figure 1 (a) Evolution of a phenotypic trait under random drift: the observed differences in the trait between taxa are proportional to the time elapsed since they shared a common ancestor (cf. the distance between A and B vs. C and D). (b) Evolution under the Ornstein–Uhlenbeck model (Felsenstein, 1988; Martins, 1994) where populations move at random on their adaptive peaks under the influence of drift whereas weak stabilizing selection acts as a restraining force returning the populations back towards the optima. (c) A phylogenetic signal produced by strong stabilizing selection: if two species enter different adaptive zones, selection within each zone pulls the species towards different adaptive peaks causing substantial phenotype divergence between the two lineages; in the same time, the selection regimes do not allow the species to cross the border between the zones, and even though subsequent speciation events occur little phenotype divergence appears among new species within each zone. Therefore, the phylogenetic signal is strong at a high taxonomic level while being obscured at lower levels. (d) An example of a phylogenetic signal produced by strong directional selection causing convergence of the trait in taxa B and C. (e) A 'star radiation' scenario where taxa speciate very rapidly because of entering different adaptive zones and thus any phylogenetic signal is obscured. (f) An example of evolutionary lability of a trait resulting either from a high degree of genetic variation within lineages or from rapid fluctuations of environmental conditions with populations tracking fast-moving adaptive peaks through the morphological space. From Martins (1994) and Cole *et al.* (2002).

Sandstedt & Lundrigan, 2005), the *Mus* phylogeny is known to have a reasonably low level of ambiguity (e.g. Boursot *et al.*, 1993; Lundrigan *et al.*, 2002; Suzuki *et al.*, 2004) and can be used for comparative purposes. This allows us to address various questions, namely: How reliable is the classification of individual specimens into known groups based on their molars? How strong is a phylogenetic signal in the data studied? What shape changes have mouse molars undergone along individual evolutionary lineages? Molar outlines were analysed in 24 samples of mice representing nine extant species or subspecies of *Mus* (subgenera *Mus* and *Nannomys*) and three extinct taxa of different age (*Mus auctor*, 6.5 Myr; *Progonomys debruijni*, 9.5 Myr; *Antemus chinjiensis*, 13.5 Myr; see Jacobs, 1978, for dating) using landmark-based methods of geometric morphometrics. These techniques are based on a priori defined points that are (at least geometrically) homologous from specimen to specimen. This approach allows partitioning size from shape as well as affine (uniform) from non-affine (non-uniform) shape changes among objects and provides graphic tools for visualization of these changes (Bookstein, 1991).

Materials and methods

Material investigated

The right first upper molars from 586 mice of the genus *Mus* from various areas of Europe, Asia and Africa were analysed. Population samples were pooled into 24 geographical groups, for convenience referred to as 'populations' throughout the paper (population codes and sample sizes are given in parentheses):

Mus spretus: 1. Morocco (*spr*MA, 30); 2. Tunisia (*spr*TN, 20); 3. Spain (*spr*E, 25); 4. France (*spr*F, 21); *Mus macedonicus*: 5. (former Yugoslavian) Macedonia (*mac*MK, 25); 6. Thrace, Greece (*mac*GR, 30); 7. Anatolia, Turkey (*mac*TR1, 26); 8. Cilicia, S Turkey (*mac*TR2, 26); 9. Syria (*mac*SYR, 31); *Mus spicilegus*: 10. Austria (*spi*A, 20); 11. Ukraine and Moldavia (*spi*UKR, 36); 12. Serbia, Yugoslavia (*spi*SCG, 9); 13. *Mus spicilegus adriaticus*, Montenegro, Albania, west Greece (*adriaticus*, 27); *Mus musculus musculus*: 14. Czech Republic (*mus*CZ, 21); 15. Slovakia (*mus*SK, 20); 16. Russia (*mus*RUS, 19); *Mus musculus domesticus*: 17. Germany (*dom*D, 23); 18. Switzerland (*dom*CH, 29); 19. Greece (*dom*GR, 25); *M. caroli*: 20. Vietnam (*car*VN, 30); 21. Thailand (*car*THA, 22); *M. cervicolor*: 22. Thailand (*cervicolor*, 21); *Mus terricolor* (= *Mus dunni*): 23. India, Pakistan, Nepal (*terricolor*, 16); *Mus* (*Nannomys*) *minutoides*: 24. Cameroon, Chad, Gabon, Guinea (*minutoides*, 34).

Three extinct species considered to be ancestors of extant *Mus* were added to the material: 25. *Mus auctor* (3), 26. *Progonomys debruijni* (4) and 27. *Antemus chinjiensis* (2), all three from Pakistan Siwaliks (Jacobs, 1978). The total sample size thus increased to 595 specimens. Codes of the animals, sample localities and the size of each sample are available upon request from the author.

The specimens under study are deposited in Université Montpellier II, France (J.-C. Auffray's collection: *M. spretus* from Tunisia and Morocco, part of *M. spretus* from Spain and France); National Museum of Natural History,

Smithsonian Institution, Washington DC, USA (part of *M. spretus* from Spain, part of *M. macedonicus* from Turkey, *M. m. musculus* from Russia, all *M. caroli*, *M. cervicolor*, *M. terricolor*); Slovenian Museum of Natural History, Ljubljana, Slovenia (*M. macedonicus* from Macedonia, part of *M. macedonicus* from Turkey, *M. spicilegus* from Serbia and Montenegro); Charles University, Prague, Czech Republic (V. Vohralík's collection: *M. macedonicus* from Greece and Syria, part of *M. macedonicus* from Turkey, *M. spicilegus* from Greece, *M. m. domesticus* from Greece); Naturhistorisches Museum Wien, Vienna, Austria (*M. spicilegus* from Austria); I. I. Schmalhausen Institute of Zoology, Kiev, Ukraine (S. V. Mezhzherin's collection: *M. spicilegus* from Ukraine and Moldavia); Institute of Vertebrate Biology, Brno, Czech Republic (part of *M. spretus* from France, *M. m. musculus* from the Czech Republic and Slovakia); Zoologische Staatssammlung München, Munich, Germany (part of *M. m. domesticus* from Germany); Université Lausanne, Switzerland (*M. m. domesticus* from Switzerland); Museum of Vertebrate Zoology, University of California, Berkeley, USA (part of *M. m. domesticus* from Germany); and Muséum National d'Histoire Naturelle, Paris, France [all *M.* (*N.*) *minutoides*].

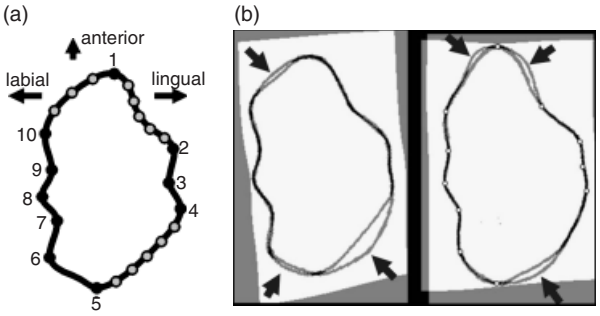

Figure 2 (a) Positions of 10 landmarks (black dots) along the outline of the right first upper molar and approximate locations of 13 sliding semilandmarks (grey dots). See text for details. (b) Examples of molar outlines showing shape differences that are not correlated with the position of the landmarks. An average (reference) configuration was computed from each pair of landmark configurations (there are two pairs of outlines shown in the figure) and then the images of both molars were 'unwarped', that is averaged pixel by pixel so that each landmark coincided with its position in the reference. The grey parts of contours correspond to areas that vary between the specimens while not being captured by the position of any landmark (arrows). The unwarping procedure was performed using the tpsSuper program (Rohlf, 2004*a*).

Data acquisition

Skulls were oriented so that the occlusal surface was horizontal. Each outline was redrawn with a drawing attachment and scanned. M^1 images of extinct *M. auctor*, *P. debruijni* and *A. chinjiensis* were redrawn from Jacobs (1978). The *x*- and *y*-coordinates of 10 landmarks were digitized using tpsDig2 (Rohlf, 2005*a*). These landmarks are points of maximum curvature of the outline associated with cusps and valleys between these cusps (Fig. 2a) and so can be regarded as type 2 landmarks *sensu* Bookstein (1991). They represent points of action of biomechanical forces corresponding with growing and differentiation of dental epithelium around enamel knots during development (Jernvall, 1995). In order to minimize problems with capturing shape differences in parts of objects without landmarks (see Fig. 2b), a series of 13 sliding semilandmarks (Bookstein, 1997) was digitized along each outline (Fig. 2a). Unlike true landmarks, these points were allowed to move along a curve so as to minimize the amount of shape change between each of the specimens and the average of all specimens. For this purpose, the curve was approximated by the direction of a chord drawn between the adjacent points and the points were positioned, iteratively, so as to minimize the bending energy (see below). All configurations of landmarks and semilandmarks were superimposed using the Procrustes generalized least-squares procedure (Rohlf & Slice, 1990) implemented in the tpsRelw program (Rohlf, 2005*b*).

Analyses

Variation in molar shape among species was assessed by discriminant function analysis (DFA) and canonical variate analysis (CVA) based on the matrix of partial warps (see below). The matrix of Mahalanobis distances was used for the construction of UPGMA (Sneath & Sokal, 1973) and neighbor-joining (N-J; Saitou & Nei, 1987) trees.

In order to summarize variation among the configurations, thin-plate spline relative warp analysis (TPSRW; Rohlf, 1993) was carried out on the landmark data using tpsRelw (Rohlf, 2004*a*). First, eigenvectors, called principal warps, were extracted from the bending energy matrix and each specimen was then projected onto them to yield the partial warps. The bending energy is a function of both the amount of transformation in shape and the degree of localization of this transformation (i.e. the closer the two points between which the shape change takes place, the higher the bending energy). The matrix of partial warp scores was used for the multivariate analyses described above as well as for extraction of relative warps; the latter allowed summarizing and visualizing variation among the outlines. The partial warps were first scaled with $\alpha = 0$, giving the same weight to all partial warps; then, values of $\alpha = -1$ and 1, giving greater weight to partial warps at smaller and larger spatial scale, respectively, were also applied.

A contribution of evolutionary history to the morphological variation of the mouse first upper molar was estimated as the coefficient of determination (R^2) from association between the matrix of phylogenetic distances (expressed in units of time; see Supplementary Material Appendix S1) and matrices of morphometric (Mahalanobis) distances (Monteiro & Abe, 1999). The matrix association was tested with the Mantel test (Mantel, 1967) with 5000 permutations.

Finally, shape changes along phylogenetic lineages were evaluated with the tpsTree program (Rohlf, 2004*b*): first, a user-defined N-J tree was inferred from molecular data and

then the shape of internal nodes [hypothetical taxonomic units (HTUs)] was estimated using squared change parsimony (Maddison, 1991; McArdle & Rodrigo, 1994). For inferring the tree, 1140-bp cytochrome *b* sequences were retrieved from GenBank for the taxa under study (Lundrigan *et al*., 2002; Suzuki *et al*., 2004; Veyrunes *et al*., 2005) and aligned with ClustalX (Thompson *et al*., 1997). Felsenstein 1984 (F84) distances (Kishino & Hasegawa, 1989) were then computed and the N-J tree was constructed using the PHYLIP package (Felsenstein, 2004). Shape changes along particular phylogenetic trajectories were visualized as follows. First, mean shapes for all extant species and their HTUs were computed from all aligned landmark configurations. Then, using the tpsSplin program (Rohlf, 2004*a*), the shape change along a lineage was quantified through a thin-plate spline transformation and visualized as a deformation of a reference (i.e. one of the ancestral configurations) on to the configuration of one of the descendants.

Where not stated otherwise, the following programs were used for the multivariate anlyses: Statistica (StatSoft, Inc., 2004) for DFA, NTSYS-pc (Rohlf, 1997) for CVA, and PHYLIP for inferring the UPGMA and N-J trees.

Results

The pattern of morphological variation described by the first two relative warps is shown in Fig. 3. The shape changes along the first relative warp are expressed by overall contraction (typical for *M. spicilegus*) or elongation (typical for the Asian species and African *M. minutoides*) of the molar along the anterior–posterior axis, as well as by the relative position of landmarks 2–5 and 10. Similar shape changes distinguished the populations along the second relative warp, with *M. terricolor*, *M. minutoides* and *M. spicilegus* from Austria and Serbia (*spi*A and *spi*SCG, respectively) at one extreme and *Antemus*, *M. macedonicus* from Greece (*mac*GR) and *M. caroli* from Thailand (*car*THA) at the other. In the middle of the graph there is a group of west-Palearctic taxa (except *M. spicilegus*). The uniform shape changes were defined by the anterior/posterior transition of the lingual side of the molar (landmarks 2–4) relative to the labial part (landmarks 6–10).

MANOVA of partial warps (including a uniform component) revealed a highly significant morphological differentiation among populations (Wilk's $\lambda = 0.00049$; $P \ll 0.001$). However, DFA showed notable differences between species in correct classification: as shown in Table 1, all the extinct species were correctly classified in 100% whereas some of the extant taxa performed rather poorly (*terricolor*: 75.0%; *cervicolor*: 76.2%), the only exception being *M. spicilegus* with 92.4% of correct assignments. As expected, the percentage increased when centroid size was added to the analysis: when the extinct taxa were removed, the average percentage of correct assignments was 82.5% whereas this reached 87.9% when centroid size was added.

The results of canonical analysis are shown in Fig. 4a. The first canonical axis, explaining 27.7% of among-group variation relative to the within-group variance–covariance

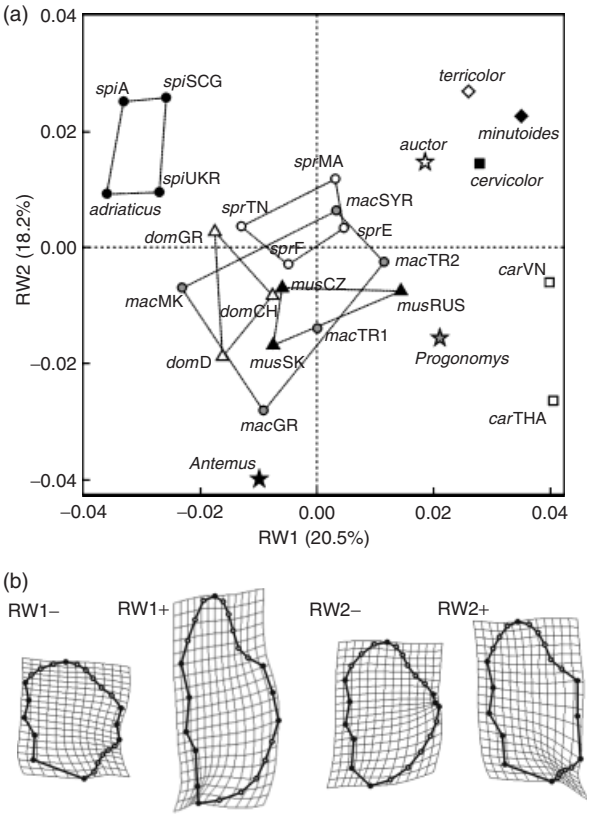

Figure 3 (a) Projection of the first two relative warps; open circles: *Mus spretus*; grey circles: *Mus macedonicus*; black circles: *Mus spicilegus*; black triangles: *Mus musculus musculus*; open triangles: *Mus musculus domesticus*; open squares: *Mus caroli* (see Materials and methods for details). (b) Thin-plate spline deformation grids showing positive and negative displacements along the first and second relative warp axes (deformations arbitrarily magnified).

matrix, contrasted a group of all *M. spicilegus* populations (including *M. s. adriaticus*) with Asian species (*M. caroli*, *M. cervicolor*, *M. terricolor*), *A. chinjiensis*, *M. auctor* and African *M. minutoides*. In between, there is a group of taxa with rather 'average' M^1 shape. The second axis (17.6% of variation) separated taxa within the west-Palearctic group. The third canonical variate (12.9%) is more difficult to interpret.

The distinct position of *M. spicilegus* among the west-Palearctic group of mice molar was also displayed by the UPGMA dendrogram (not shown). The N-J tree placed all but one population into specific and/or subspecific clades (Fig. 4b), the only exception being *M. macedonicus* from Macedonia (*mac*MK). However, the tree reflects true phylogeny only roughly. The Mantel test revealed significant correlation between phylogenetic and morphological distances ($r = 0.895$, $t = 3.382$, $p[\text{rand } Z > \text{obs. } Z] = 0.0004$), suggesting an ~80% contribution of phylogenetic history to the shape differences. Figure 5 shows a positive but nonlinear relationship between the two matrices. Using

Table 1 Classification matrix derived from discriminant function analysis based on partial warp scores

Species	% correct	1	2	3	4	5	6	7	8	9	10	11	12	Total
spretus	84.4	81	6	3	1	3	1	1	0	0	0	0	0	96
macedonicus	87.0	7	120	2	3	4	1	1	0	0	0	0	0	138
spicilegus	92.4	2	3	85	1	1	0	0	0	0	0	0	0	92
musculus	76.3	3	6	0	45	2	3	0	0	0	0	0	0	59
domesticus	80.5	7	4	2	2	62	0	0	0	0	0	0	0	77
caroli	82.7	2	4	0	1	0	43	0	0	1	0	1	0	52
cervicolor	76.2	3	1	0	0	0	1	16	0	0	0	0	0	21
terricolor	75.0	0	0	0	0	0	0	0	12	4	0	0	0	16
minutoides	88.2	0	0	0	1	0	0	1	3	30	0	0	0	34
auctor	100.0	0	0	0	0	0	0	0	0	0	3	0	0	3
Progonomys	100.0	0	0	0	0	0	0	0	0	0	0	4	0	4
Antemus	100.0	0	0	0	0	0	0	0	0	0	0	0	2	2
Mean/total	84.7	105	144	92	53	72	49	19	15	35	3	5	2	594

Rows are predicted groups and columns are actual groups.

different scaling factors of $\alpha = -1$ and $+1$ had a negligible impact on the results.

Shape transformations along the mouse phylogeny (upper right) are shown as thin-plate splines in Fig. 6. In the upper left part of the figure, the estimated shape of a hypothetical ancestor of all the extant taxa studied (*HTU*1) is shown together with the thin-plate spline depicting the shape transformation from *P. debruijni* to this ancestor. With some caution due to the very limited sample size of *Progonomys* molars investigated, we can see a trend towards elongation of the anterior cusp and deepening valleys between cusps on the labial side of the molar, and an anterior transition of landmark 8. A similar pattern was revealed by thin-plate spline deformation of *A. chinjiensis* on to *HTU*1 (not shown). In the middle row, there are transformations from *HTU*1 to African and Asian species that are outgroups of the west-Palearctic clade as well as to a hypothetical ancestor of this clade. We can note that similar trends in M^1 morphology characterized the evolution of two species of 'pygmy' mice, *M.* (*N.*) *minutoides* and *M. terricolor*, the most noticeable of them being considerable elongation of the anterior cusp. More interestingly, evolutionary trajectories in the M^1 outline of the two Asian relatives, *M. caroli* and *M. cervicolor*, look largely like mirroring each other: the most apparent shape changes appear to be the anterior transition of landmark 10 and the labial transition of landmark 5 in the former species, whereas the opposite changes were characteristic for the latter. Along the lineage leading to the west-Palearctic group, the molar became relatively shortened as shown by the projection of *HTU*1 on to *HTU*5. In *M. spicilegus*, this trend was further strengthened and accompanied by the anterolabial shift of landmark 10 and deepening the valleys between cusps so that the molar has an apparently 'warped' occlusal surface. On the other hand, *M. spretus* and *M. macedonicus* have more or less retained the ancestral shape of the molar.

The molecular phylogeny from Fig. 6 was then superimposed on the three-dimensional ordination plot from principal components analysis of a matrix of average landmark configuration for the nine extant taxa and their estimated HTUs in order to visualize evolutionary trajectories through shape space (Fig. 7). The average configurations of *Antemus*, *Progonomys* and *M. auctor* were also added and their presumed phylogenetic relationships (Jacobs, 1978) indicated (dotted arrows). We can see a transition along the first principal axis from *Antemus* through *Progonomys* and *M. auctor* to a hypothetical ancestor of the extant *Mus* taxa (*HTU*1) and further to African *M.* (*N.*) *minutoides*. After the branching off of *M. terricolor*, the M^1 shape of this species converged, to an extent, to the shape similar to *M. minutoides*. Unlike the Asian species, phenotypic divergence within the west-Palearctic group is relatively low, except for *M. spicilegus* accumulating a high number of autapomorphies. The lowest divergence was revealed in *M. spretus*, largely retaining shape estimated as ancestral for the group; we can also note partial convergence between *M. m. musculus* and *M. macedonicus*.

Discussion

Unlike microtines, mice of the genus *Mus* are characterized by rather uniform molar shapes. Nevertheless, in some groups of fossil (van Dam, 1996; Renaud *et al.*, 1999, 2005; Renaud & Michaux, 2004) and extant murines (Janžekovič & Kryštufek, 2004; Kryštufek & Janžekovič, 2005), geometric morphometric methods have been quite successful in describing morphological variation and discrimination between the taxa investigated. Among the nine recent species and subspecies analysed in this study, the highest accuracy of DFA classification was revealed in *M. spicilegus* (~92%). This result corresponds with that of Marshall & Sage (1981), who described M^1 of this species (reported as *Mus hortulanus* in their paper) as distinct from others in the forward position of the anterolabial cusp (labial anterocone *sensu* Jacobs, 1978). This is confirmed by the anterior displacement of landmark 10 (cf. Figs 4 and 7). However,

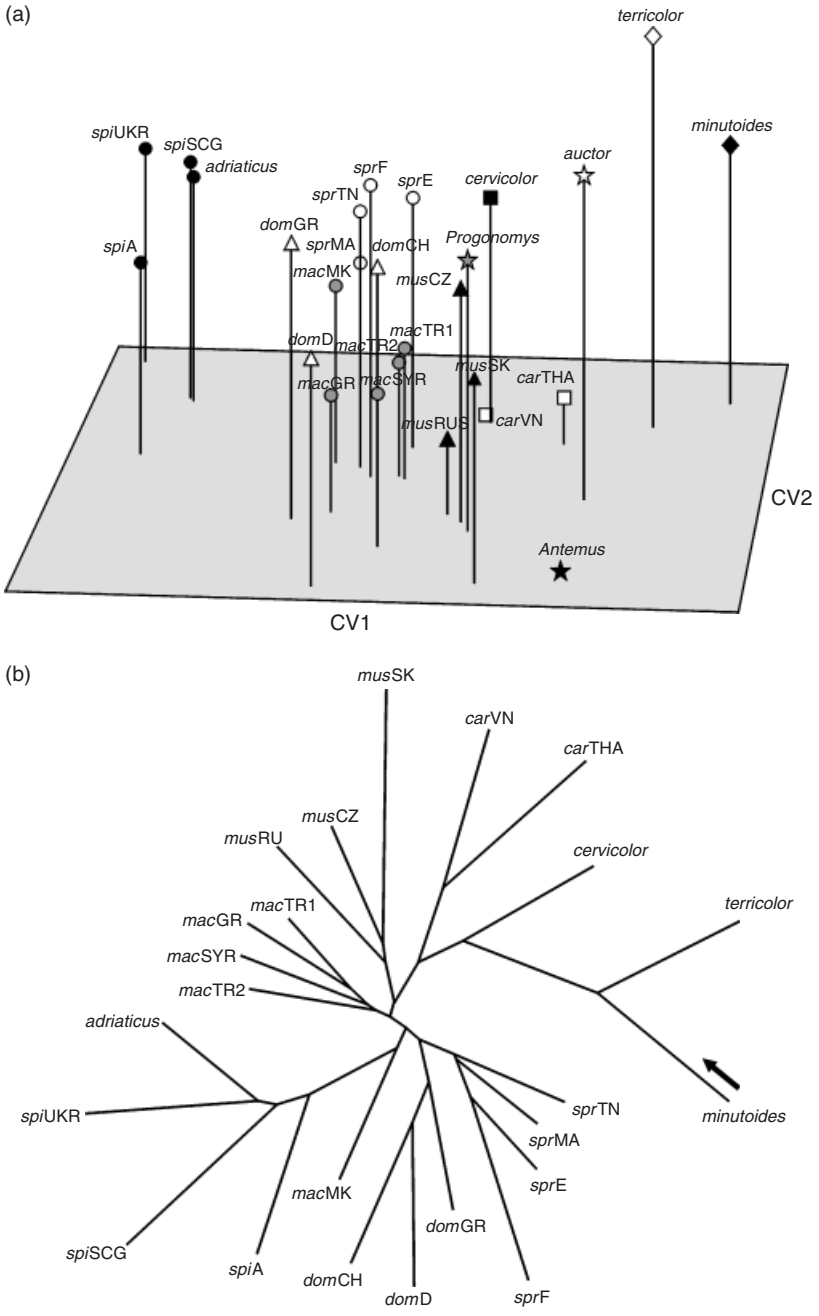

Figure 4 (a) Three-dimensional plot of the first three canonical variates based on partial warp scores and explaining 58.3% of the total shape variation. (b) Unrooted neighbor-joining tree inferred from Mahalanobis distances between the populations. The arrow indicates the position of the root estimated from molecular data (Boursot *et al.*, 1993). See Materials and methods, and Fig. 3 for details.

rigorous quantification of shape differences by thin-plate spline also allowed the detection of other characteristic features of *M. spicilegus* M^1: overall compression of the molar along the antero-posterior axis and protruding labial and lingual cusps which, together with deepening valleys between these cusps, give the molar typical 'warped' appearance.

Discrimination of the remaining species/subspecies varied from group to group (~75–88%), yet both UPGMA and N-J trees placed a vast majority of populations into distinct specific/subspecific clades (see Figs 3 and 6). It should be noted that the performance of TPS increased by using a set of sliding semilandmarks, which enabled capturing shape changes in areas without landmarks as illustrated by image unwarping shown in Fig. 1b (the results of TPS without semilandmarks are not shown here).

In spite of the relative success of clustering and N-J methods in inferring specific/subspecific clades, morphological uniformity of mouse molars and the presence of homoplasy in the data caused the phylogeny of the genus to be reflected only roughly by M^1 outlines. This result is not surprising because one can hardly expect 'true' phylogenetic

relationships to be inferred from a single and simple structure such as the first upper molar. Moreover, morphometric data are phenetic and as such they represent mixtures of homologous and homoplasious similarities (Sneath & Sokal, 1973; Felsenstein, 1982; see also criticisms by Pimentel & Riggins, 1987; Felsenstein, 1988, 2002; Bookstein, 1994). Notwithstanding, the phylogenetic signal in the data under study appeared to be quite strong because up to 80% of molar shape variation was explained by phylogenetic relationships among the taxa. As various values of alpha from −1 to +1 had no apparent effects on the results, we can conclude that the importance of localized versus global shape changes was similar in the detection of phylogenetic signals.

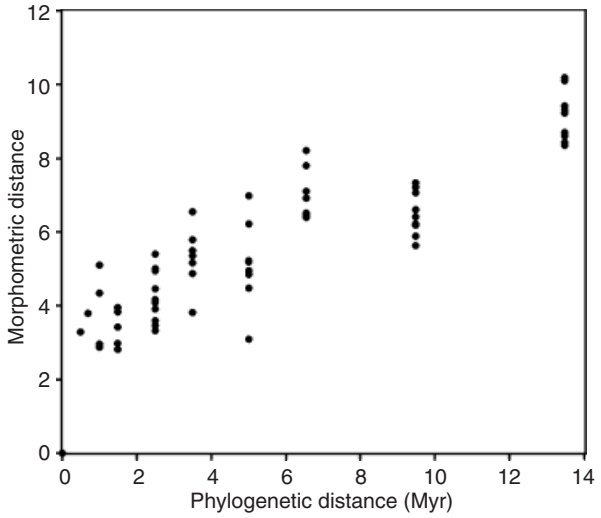

Figure 5 Morphometric (Mahalanobis) distances based on partial warp scores plotted against phylogenetic distances expressed in units of time (Myr; see Supplementary Material Appendix S1). Note that the relationship between the distances is not linear; however, the correlation between the matrices is highly significant in both cases.

Quantification of shape changes through thin-plate splines along the mouse phylogeny, estimated from cytochrome *b* sequences, allowed tracking evolutionary trajectories in morphometric space and comparison with published qualitative descriptions. According to Jacobs (1978; see also Michaux, 1971), among the primitive dental characters of the Murinae was the presence of all major cusps on M^1 excluding posterostyle (postero-lingual cusp, between landmarks 4 and 5 in Fig. 2) and the relatively posterior position of anterostyle (antero-lingual cusp, landmark 2). *Progonomys debruijni* was characterized as being 'related to later members of (the *Progonomys-Mus*) lineage in having the anterior portion of M^1 expanded' (Jacobs, 1978, p. 81). However, comparison of average *Antemus* and *Progonomys* molar shapes (drawn from Jacobs, 1978) through thin-plate spline deformation showed an opposite trend, indicated by the slightly *posterior* displacement of landmark 1; in addition, the anterostyle (landmark 2) expanded anteriorly with compression of the postero-labial

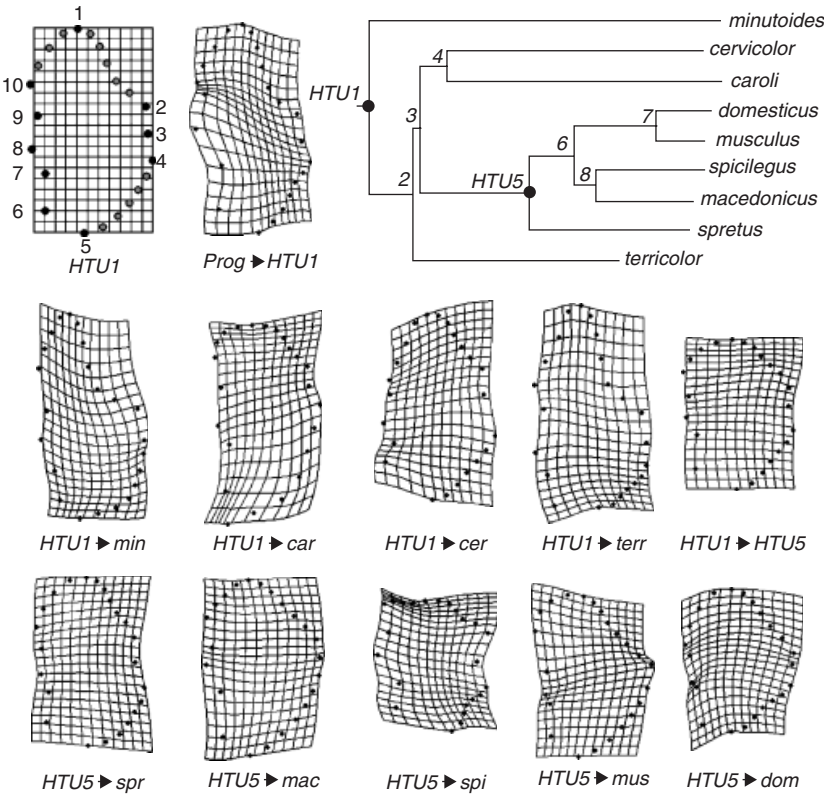

Figure 6 Molecular phylogeny of the studied taxa (upper right) with estimated configuration of landmarks and semilandmarks at the root of the tree (*HTU1*) and hypothesized shape transformation from *Progonomys* (*Prog*) to this root (upper left). Thin-plate splines in the middle and bottom row show displacements of landmarks from an ancestral form to one of its descendants. The shape transformations were arbitrarily magnified. *HTU5*, hypothetical ancestor of the west-Palearctic group; *min*, *Mus* (*Nannomys*) *minutoides*; *terr*, *Mus terricolor*; *car*, *Mus caroli*; *cer*, *Mus cervicolor*; *dom*, *Mus musculus domesticus*; *mus*, *Mus musculus musculus*; *spi*, *Mus spicilegus*; *mac*, *Mus macedonicus*; *spr*, *Mus spretus*.

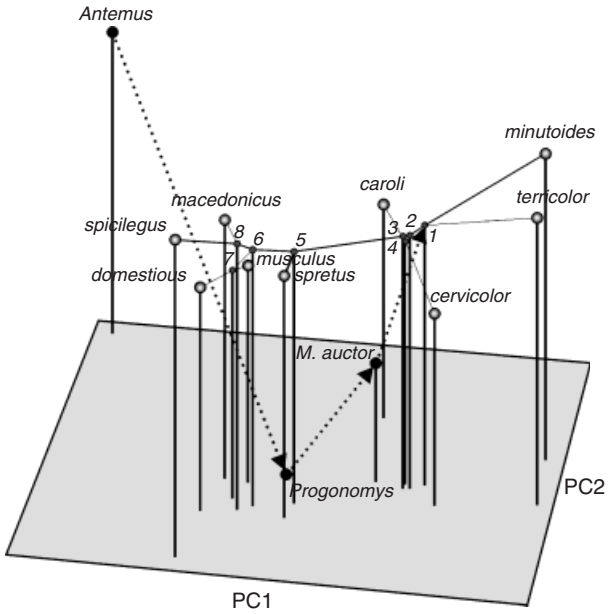

Figure 7 Projection of the studied taxa into the first three principal components based on consensus landmark configurations averaged across species/subspecies after Procrustes superimposition. The molecular phylogeny from Fig. 6 is superimposed (solid lines), together with presumed phylogenetic relationships of fossil taxa (dotted arrows), in order to show the estimated evolutionary trajectory in shape space. The internal nodes are numbered as in Fig. 6.

part of the molar as depicted by transition of landmarks 6 and 8 towards each other (not shown). Elongation of the anterior portion of the molar (precingulum) is a characteristic trend for descendants of *Progonomys*: *M. auctor*, a hypothetical ancestor of all recent members of the genus *Mus* (*HTU*1), and especially for two 'pygmy' mice, *M.* (*N.*) *minutoides* and *M. terricolor*, giving them a typical slender appearance. This trend was subsequently reversed in the west-Palearctic clade (*M. spretus*, *M. macedonicus*, *M. spicilegus*, *M. musculus*) where the anterior portion was shrunk and the molars have become more compact. This relative compression is most apparent in *M. spicilegus*, whereas *M. spretus* and *M. macedonicus* largely retained the ancestral state.

The results presented in this study thus suggest that the evolution of the shape of the first upper molar within the genus *Mus* is to a large extent characterized by random divergence of lineages through drift (reflected by the contribution of phylogenetic signal to shape variation). However, the decreasing rate of morphological divergence between the taxa with the time elapsed since their last common ancestor (Fig. 5) also suggests a role of stabilizing selection (e.g. ecological factors or genetic determinism) probably acting as a restraining force returning lineages back to an adaptive optimum (cf. Fig. 1b). This process is best modelled with the Ornstein–Uhlenbeck model (Felsenstein, 1988; Martins, 1994) based on the notion of a particle moving here and there at random but tethered to a central point with an elastic band. In this case, phylogenetic correlation between species is expected to decay exponentially with time, in notable agreement with the pattern shown in Fig. 5. On the other hand, mapping shape changes onto known molecular phylogeny (Figs 6 and 7) also revealed a few cases of convergence and reversal, indicating a more complex scenario including the action of diverse evolutionary forces. However, an explanation for the striking extent of M^1 shape difference in *M. spicilegus* remains unclear.

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Supplementary material

The following material is available for this article online:

Appendix S1 The matrix of phylogenetic distances (in millions of years) used for estimation of phylogenetic inertia in the data.

This material is available as part of the online article from http://www.blackwell-synergy.com