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Research Article

Morphological evolution of the skull in closely related bandicoot rats: a comparative study using geometric morphometrics

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Abstract

We addressed the effects of phylogeny, ecology, and allometry on shape variation in ventral cranium, mandible and maxillary tooth-row in all five extant bandicoot rats. These rats are classified into two genera (*Bandicota* and *Nesokia*) and occupy different ecological niches along fossorial to aquatic gradient. The analysed structures are controlled by different gene loci, have diverse developmental patterns and different functional roles what induced us to hypothesize that they respond differently to the interplay between phylogenetic constrains and selective pressures. This was indeed the case in our results. Ventral cranial shape contained an apparent phylogenetic structuring at various levels of taxonomic hierarchy of bandicoot rats and therefore accurately replicated the taxonomic hierarchy within the group. Molar crowns provided a taxonomic grouping that was less straightforward in comparison with the skull. Possibly this was due to a stasis, which could persist in molar shape as a long time pattern, while the residual variation was correlated to a diet. The phylogenetic structuring was diluted in the mandible, probably by adaptive trends for the ecological niche. Unsurprisingly, an ecological gradient from a fossorial to aquatic ecotype explained 19.1% of mandibular shape variation. The major differences between ecotypes were on mandibular landmarks associated with insertion of major muscles that move the mandible during chisel-tooth drilling in fossorial *B. bengalensis* and *N. indica*. Among the three structures, the mandible was also the most affected by allometry, with size accounting for 14.0% of shape variability. *Nesokia* and *Bandicota* are by far the youngest murine taxa still attributed to a generic level. Small genetic differences however sharply contrast with unique shape features, evident in craniodental structures of these rats. This is particularly relevant for the endangered *N. bunnii* which is only known from a small range in Iraq. Morphological uniqueness emphasizes its "value" in conservation policies more accurately than genetic metrics, making it more "visible" in a bunch of pest rats.

Introduction

Bandicoot rats are murine (Murinae) rodents conventionally classified into two genera, *Nesokia* and *Bandicota*. These genera are believed to be in a sister position (Fabre et al., 2012; Patnaik, 2014) and some authors questioned whether they deserve taxonomic ranking as distinct genera (Musser and Carleton, 2005). Although the group is small, taxonomic uncertainties are common. Morphologically the most distinct of the three species of *Bandicota* is *B. bengalensis* which is classified into a subgenus *Gunomys* as its sole member while the remaining species *B. indica* and *B. savilei* are retained in the nominotypical subgenus *Bandicota* (Musser and Brothers, 1994). This arrangement was challenged in a multigenic phylogenetic reconstruction which yielded a sister position of *B. bandicota* and *B. indica*, placing *B. savilei* as a basal group in the genus (Fabre et al., 2012). *Nesokia*, as presently understood (Musser and Carleton, 2005), contains two species, a widespread and common *N. indica* and a rare and enigmatic *N. bunnii*. The latter was unknown until 1981 when described as a member of a new genus *Erythronesokia* (Khajuria, 1981). Whereas *N. indica* and all three species of *Bandicota* reach very high local population densities in many parts of their ranges and are controlled as major agricultural and urban pests (Aplin et al., 2003), *N. bunnii* is restricted to a small marshy area in lower Mesopotamia. It is known from only five individuals collected in the 1970s (Khajuria, 1981; Al-Robaae and Felten, 1990) and is classified in the IUCN Red List as Endangered species (Stuart, 2008). The main argument for down ranking *Erythronesokia* to a synonym of *Nesokia* stems from an analysis of skull shape which used ratios of linear cranial measurements with length of skull as denominator (Al-Robaae and Felten, 1990). Such ratios involve undesirable statistical properties which may pose bias in a comparison of morphological shape. The results by Al-Robaae and Felten (1990) have not been challenged ever since and no new evidence has been published on *Erythronesokia* over the last quarter of a century.

Clear discontinuities in external and craniodental morphology separate species of bandicoot rats (Niethammer, 1977; Khajuria, 1981; Musser and Brothers, 1994). Different species practice different modes of life and exploit contrasting ecological niches along the fossorial–aquatic continuum. *Nesokia indica* and *B. bengalensis*, which "appear to occupy the same ecological niche" (Aplin et al., 2003), are more fossorial than their congeners and, indeed, the remaining murines in southern Asia (Ellerman, 1961; Marshall, 1977). Of the remaining three bandicoot rats, *N. bunnii* lives deep within swamps and builds reed platforms above water level (Al-Robaae and Felten, 1990) hence it seems to be the species most dependent on the aquatic environment. *Bandicota indica* and *B. savilei* are more catholic in habitat requirements than *N. bunnii* but still depend heavily on water bodies. E.g. *B. indica* occupy "all parts of the human landscape", however as an excellent swimmer it is normally associated with marshes, ponds and river-

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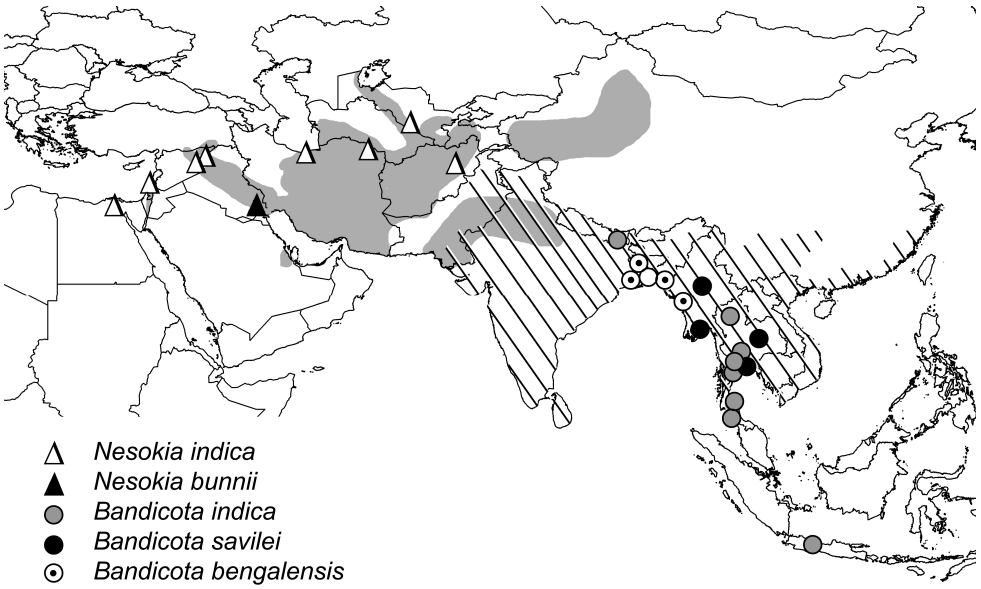

Figure 1 – Location of the samples used in this study. Note differences in biogeographic patterns between the Oriental *Bandicota* (striped) and the primarily Palaearctic *Nesokia* (shaded grey).

side habitats (Aplin et al., 2003). The ecological niche of *B. savilei* is puzzling. This small bandicoot rat shows similar habitat preferences as the larger *B. indica* except for being "relatively intolerant of prolonged inundation" (Aplin et al., 2003).

Morphological contrasts between species and genera were meticulously documented in taxonomic studies (Ellerman, 1941; Al-Robaae and Felten, 1990; Musser and Brothers, 1994) but were not quantified and viewed in a phylogenetic and ecological framework so far. In this study we address morphometric relationships among all five bandicoot rats using three morphological structures, the ventral cranium, the mandible and the upper molars. The three structures are controlled by different gene loci, have diverse developmental patterns and different functional roles (cf. Caumul and Polly, 2005, and references therein). Variations in size and shape were analysed using the landmark based methods of geometric morphometrics. Geometric methods are utilized because they provide both quantitative variables appropriate for multivariate statistical methods as well as depictions of morphological shape variation within a sample, and thus facilitate linkages between raw data and biological patterns (Adams et al., 2013).

By measuring variance in size and shape of the ventral cranium, the mandible, and the occlusal surface of the upper molar row in all the extant bandicoot rats, we set out to address several main issues. (1) First, we quantified the craniodental makeup and contrasted species on this ground. (2) Next, we investigated whether some of the structures studied produce phenetic trees of compatible typology to the accepted taxonomic hierarchy (Musser and Carleton, 2005) and to partial phylogenetic trees provided by Michaux et al. (2007) and Fabre et al. (2012). (3) Alternatively we explored putative adaptive plasticity in craniodental structures which would dilute the phylogenetic structuring by associating species with their ecological niche.

Methods

Sample

We studied museum vouchers of bandicoot rats, belonging to three species within the genus *Bandicota* (*B. bengalensis*, *B. indica* and *B. savilei*) and two species within the genus *Nesokia* (*N. bunnii* and *N. indica*). Specimens are deposited in the following collections (acronyms are parenthesized): Senckenberg Nature Museum and Research Institute, Frankfurt am Main, Germany (SMF), Siberian Zoological Museum, Russian Academy of Sciences, Novosibirsk, Russia (SZM), and Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany (ZFMK). Only adult specimens with fully erupted permanent dentition were selected for present analysis which involved crania, mandibles and upper molar rows of 98 specimens. Individuals with heavily worn molar crowns were excluded from the dental study. A list of sample sizes is given in Tab. 1 and a map of geographic positions in Fig. 1. The complete list of specimens studied is to be found in Appendix S1.

Data collection

Crania, mandibles and molars were photographed with a Monica Minolta DG-7D digital camera under constant conditions. Images of crania were taken in ventral view with the palate parallel to the photographic plane, images of left mandibles in labial view, and images of upper right molars in occlusal view. Twenty two-dimensional landmarks were digitized on the right side of the ventral cranium, 16 on the mandible, and 18 on the occlusal surface of the molar row (Fig. 2) using TpsDig software (Rohlf, 2013, 2015).

For each structure, landmark coordinates of all specimens were aligned using Generalized Procrustes Analysis (GPA) (Rohlf and Slice, 1990; Dryden and Mardia, 1998). GPA standardized size and removed the differences in landmark configurations due to position and orientation. Size information was preserved as centroid size (CS), calculated as the square root of the sum of squared distances between each landmark and the centroid of the landmark configuration (Bookstein, 1991), and shape information as Procrustes coordinates.

Measurement error and sensitivity analysis

The measurement error due to digitizing was assessed by using Procrustes analysis of variance (Procrustes ANOVA) (Klingenberg and McIntyre, 1998). Images of 30 specimens were digitized two times for each skeletal element. The mean squares for individual variation ex-

Table 1 – Summary of the genera and species examined in this study with sample sizes for crania, mandibles and molars. The numbers of known male and female specimens are parenthesized (M + F). Their sum does not match the total because only some of museum vouchers have been sexed.

Genus	Species	Cranium	Mandible	Molar
Bandicota	*bengalensis*	23 (10 + 10)	29 (12 + 15)	20 (10 + 8)
	indica	30 (10 + 12)	37 (5 + 14)	32 (11 + 14)
	savilei	17 (7 + 9)	17 (5 + 9)	15 (6 + 8)
Nesokia	*bunnii*	3 (0 + 0)	3 (0 + 0)	3 (0 + 0)
	indica	18 (0 + 1)	12 (0 + 0)	19 (0 + 0)
Total		91 (27 + 32)	98 (22 + 38)	89 (27 + 30)

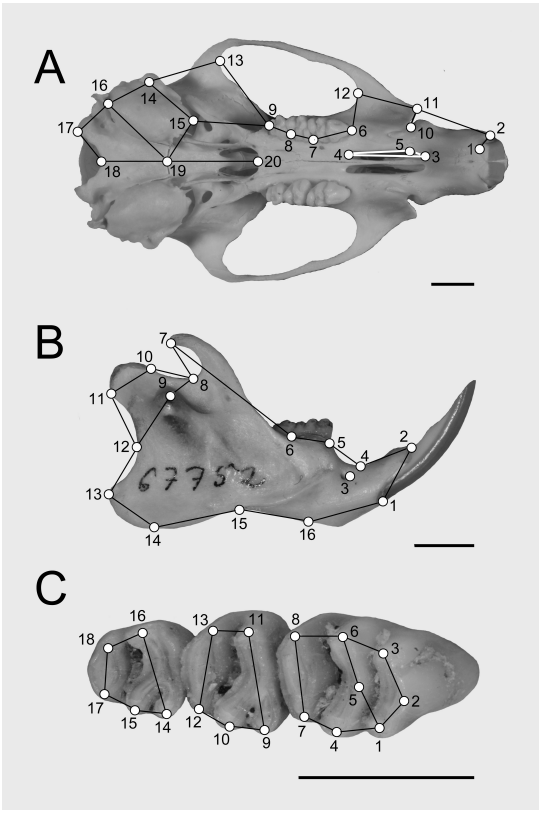

Figure 2 – The position of landmarks used to characterize the shape of (A) ventral cranium, (B) labial side of the mandible, and (C) occlusal surface of the upper molars (labial side is at the top). Wireframe used for "stylized" drawings is superimposed on the configuration of landmarks. Scale bars equal to 5 mm.

ceeded the measurement error component by more than 100-fold for CS and 11-fold for shape, which means landmark precision was adequate and a digitizing error was low. Sampling error was assessed using the sensitivity analysis (Cardini et al., 2015), in which the largest sample (i.e. *Bandicota indica* n⩾30) was randomly split into progressively smaller subsamples (in 1/2, 1/3, 1/5 and 1/10 of the original sample size) to estimate how reducing sample size affects the calculation of the CS sample mean and variance and shape variance (V1, sum of the variances of all shape coordinates, according to Cardini et al., 2015). Comparison of the means and variances of the subsamples to the original values of *B. indica* and to the range of variation of the same parameters of the other *Bandicota* and *Nesokia* species showed that a sample size of 10 specimens gives a reasonable degree of accuracy for the CS and 15 specimens for the shape (in all three structures, V1 varied between ±10 and 15% relative to "true" value).

Size and shape analyses

The GPA and all subsequent analyses were performed for each structure (cranium, mandible and molars) separately. Sexual dimorphism and interspecific differences in size and shape were tested using uni- and multivariate analysis of variance (t-test, ANOVA and MANOVA) for the effect of species, sex, and their interaction. The number of shape variables used in MANOVA was reduced to the first few principal components (PCs). The number of PCs was chosen by measuring the correlation between the matrix of Procrustes shape distances in the full shape space and pair-wise Euclidean distances in the reduced shape space (for 5, 10, and 15 PCs; cf. Cardini et al., 2010). The first 15 PCs explained 93.7%, 93.0% and 91.4% of total variance in the cranium, mandible and molars, respectively, and all had a correlation with Procrustes distances of 0.999, which represented a good summary of the total variation in shape. Sexual dimorphism could be analysed only in *Bandicota*. For pairwise comparisons of all five species in size, a Tukey post-hoc honest significant difference (HSD) test for unequal sample sizes was performed. Because in geometric morphometric analyses parametric tests may be strongly affected by small samples (Cardini and Elton, 2007), they were performed or repeated without *N. bunnii* (N=3). Size differences were visualized with box and whiskers graphs of species centroid sizes. A principal components analysis (PCA) was used to summarize and explore patterns of variation among specimens in the shape space. To visualize shape features associated with the most variation, scatter plots of the first two principal components (PCs) were drawn. Shape changes along the first two PCs and species' mean shapes were presented by wire-frame graphs based on the thin plate spline algorithm (Bookstein, 1991). To assess the statistical significance of pair wise comparisons of shape between species, a nonparametric permutation test of Procrustes distances between species mean shapes was conducted. Procrustes distances (Bookstein, 1991) are computed directly in the shape space and best summarize differences among mean shapes. To control results for increased likelihood of type I error from multiple comparisons, we used a sequential Bonferroni correction. A matrix of Procrustes distances among the species mean shapes was used to produce a phenetic tree employing the UPGMA (unweighted pair-group method using an arithmetic average) cluster analysis. We also estimated the effect of size (interspecific allometry) and ecology on the overall shape variation by multivariate regressions of shape variables (Procrustes coordinates) onto size (CS) and ecological categories, respectively. The three ecotypes (cf. Introduction) used as covariates in regression were: fossorial (*N. indica*, *B. bengalensis*), aquatic (*N. bunnii*) and generalized (*B. indica*, *B. savilei*). The statistical significance of regressions was estimated by a permutation test with 10,000 iterations against the null hypothesis of complete independence between the dependent (shape) and independent variable (size, ecological categories).

Analyses were performed using the MorphoJ software (Klingenberg, 2011) and IBM SPSS Statistics (2012).

Results

Sexual dimorphism

Information on sex was available only for specimens of *Bandicota*; hence only this genus was considered in the analysis of secondary sexual dimorphism. Sexual size dimorphism was not statistically significant in any skeletal element (cranium: $F_{1, 52}$=0.93, p=0.3382; mandible: $F_{1, 54}$=1.58, p=0.2143; molars: $F_{1, 51}$=0.00, p=0.9855). Also all sex×species interactions for size were insignificant (p>0.2). In shape, differences between males and females were significant only in the cranium (Wilks' λ=0.526, $F_{15, 38}$=2.28, p=0.0201; mandible: Wilks' λ=0.620, $F_{15, 40}$=1.63, p=0.1088; molars: Wilks' λ=0.755, $F_{15, 37}$=0.80, p=0.6702). The sex×species interaction was significant only in the mandible (Wilks' λ=0.364, $F_{30, 80}$=1.75, p=0.0253). Tests performed for each species separately did not show or confirm sexual dimorphism either in size or shape of the skull. Sexes were therefore pooled in all subsequent analyses.

Size variation

Size variation among the five species was significant in all skeletal elements (cranium: $F_{4, 86}$=155.28, p<0.0001; mandible: $F_{4, 93}$=239.26, p<0.0001; molars: $F_{4, 84}$=58.40, p<0.0001). Graph visualisations and pair wise comparisons showed that *B. indica* and *N. bunnii* were largest in all aspects (Fig. 3) and that the differences between them were non-significant (p>0.160). On the other side of the range, *B. bengalensis* was smallest in molar and mandibular morphology and of comparable cranial size to *N. indica* (p=0.627). The middle- and similar-sized *N. indica* and *B. savilei* differed only in cranial size (p= 0.024). All other pair wise comparisons were highly significant (p<0.001). Results of tests repeated without a small sample of *N. bunnii* did not differ considerably.

Shape variation

Highly significant differences were observed among the analysed species (without *N. bunnii* N=3) in the shape of all three skull structures (cranium: Wilks' λ=0.0013, $F_{45, 208}$=39.14, p<0.0001; mand-

ible: Wilks' λ=0.0148, $F_{45, 229}$=15.964, p<0.0001; molars: Wilks' λ=0.0529, $F_{45, 202}$=7.61, p< 0.0001). Pair-wise comparisons of the species using permutations of Procrustes distances and PCA graphs visualizing shape relationships retrieved different patterns of shape variation in the three skeletal elements.

Cranium – A PCA scatter plot revealed a clear distinction between *Bandicota* and *Nesokia* species in the shape of the ventral cranium (Fig. 4A). Specimens of the two genera were visibly separated along the first PC axis (explaining 45.7% of the total variance). Wireframe graphs displaying shape changes associated with the PC1 show that specimens of *Bandicota* are characterized by a relatively larger incisive foramen, shorter upper molar tooth row, and narrower zygomatic plate. In general, the *Bandicota* skulls have a relatively longer and narrower temporal region and rostrum, and less expanded zygomatic arches. Species were well separated also along the second PC axis (14.9% of variance) and the pattern corresponded to cranial size differences (Fig. 3A). Therefore the smaller the species, the higher the PC2 scores tended to be. Wireframe graphs show that specimens with positive PC2 scores and smaller skulls have broader crania with shorter and wider rostral regions. Procrustes distances among all species pairs were highly significant (p<0.0005) ranging from 0.028 (*B. indica* vs. *B. savilei*) to 0.076 (*N. indica* vs. *B. bengalensis*). As expected, in the UPGMA phenogram, species of the same genus grouped together (Fig. 4B). Average shapes gave insight into species specific morphometric characteristics, amongst which the most impressive was the difference in the length of incisive foramen between *B. bengalensis* (longest) and *N. indica* (shortest). The relation of shape and size along PC2 was reflected in a highly significant (p<0.0001) interspecific allometric effect; size accounted for 11.9% of the overall cranial shape variation. A relatively small proportion (11.2%) of shape variation was explained by ecological categorisation (p<0.0001). All analyses performed on the cranium were repeated also without the LM4 (posterior part of the incisive foramen), which was very variable along PC1. Even after the removal of LM4 the grouping of specimens in the PCA scatterplot changed only slightly and stayed the same in the UPGMA phenogram.

Mandible – Contrary to the cranium, mandibular shape did not group species according to taxonomic affiliation. In the PCA scatter plot (Fig. 5A), the PC1 (27.3% of variance) well distinguished between the aquatic (*N. bunnii*) and generalised species (*B. indica* and *B. savilei*) on one hand, and the two fossorial (*B. bengalensis* and *N. indica*) on the other. Major mandibular features delimiting the two groups are the shapes of the angular, coronoid and alveolar processes. The first group having positive PC1 scores had a longer and narrower angular process and a slightly more slender condylar process but relatively short alveolar process, whereas the second group had a short and blunt angular process, a more robust condylar process, and a prominent alveolar process, which was shifted posteriorly. No obvious grouping was seen along PC2 (12.3% of variance), except for *N. bunnii* having only negative PC2 scores. Shape variation along PC2 was characterized mostly by modifications of the mandibular ramus, in particular the angular and alveolar process, as well as a slight horizontal bending of the alveolar region. The full range of variability along PC2 was observed in all species (except for *N. bunnii* which could be due to the small sample size), therefore this relatively large portion of mandibular shape variation is a general intraspecific feature of the two genera and could be explained by relatively high mandibular plasticity. Shape differences were significant among all species pairs (p<0.003). Procrustes distances among mean mandibular shapes of the five species ranged from 0.023 (*B. indica* vs. *B. savilei*) to 0.065 (*N. bunnii* vs. *B. bengalensis*). In the UPGMA phenogram, the most similar *B. indica* and *B. savilei* grouped with the large aquatic *N. bunnii*, whereas *N. indica* grouped with *B. bengalensis* (Fig. 5B). Average species shapes revealed the massiveness of the *N. bunnii* mandible compared to all other species, which are generalized or fossorial. The relation of shape and size was highly significant (p<0.0001). Size accounted for 14.0% of the interspecific mandibular shape variation. As evident from the PCA graph, a relatively large portion of shape variation was ecology-related; more exactly, an ecological gradient from fossorial to aquatic ecotype explained 19.1% of shape variation; this association was highly significant (p<0.0001).

Molars – The PCA graph (Fig. 6A) revealed a pattern of shape variation in molars that was more complex than in the other two skeletal elements. First, no straightforward grouping was observed. Secondly, intraspecific variability was more pronounced. The first PC explained

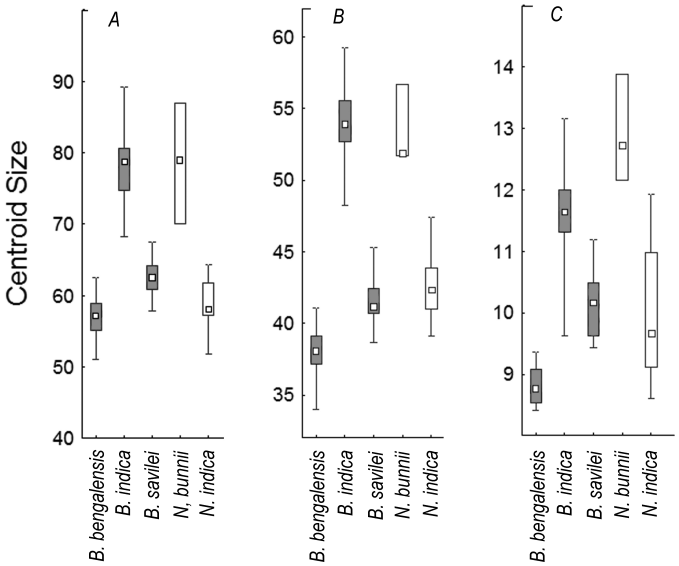

Figure 3 – Plot of centroid size medians, interquartile range (25th–75th percentile), maximal and minimal values for the *Bandicota* (boxes shaded) and *Nesokia* species for (A) the ventral cranium, (B) the mandible data set and (C) the molar data set.

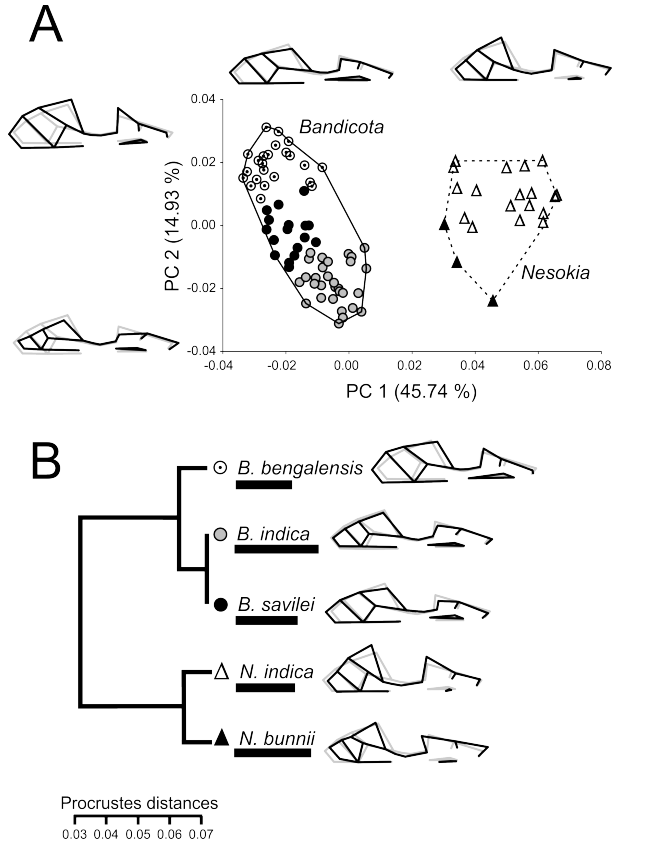

Figure 4 – Scatter plot of the first two PCs (percentage of variance in parentheses) for the ventral cranium (A) with wire frame graphs showing shape changes along the two PC axes for the unit of 0.1 in the negative and positive direction (black) compared to the mean of each PC (grey). The UPGMA phenogram of the overall shape variation for the *Bandicota* and *Nesokia* species for the ventral cranium (B) with wire frame graphs (magnified three times) illustrating differences between means of each individual group (black) and the mean of all groups (grey). Centroid size is visualized for each species using horizontal bars. Symbols correspond to sampling sites shown in Fig. 1.

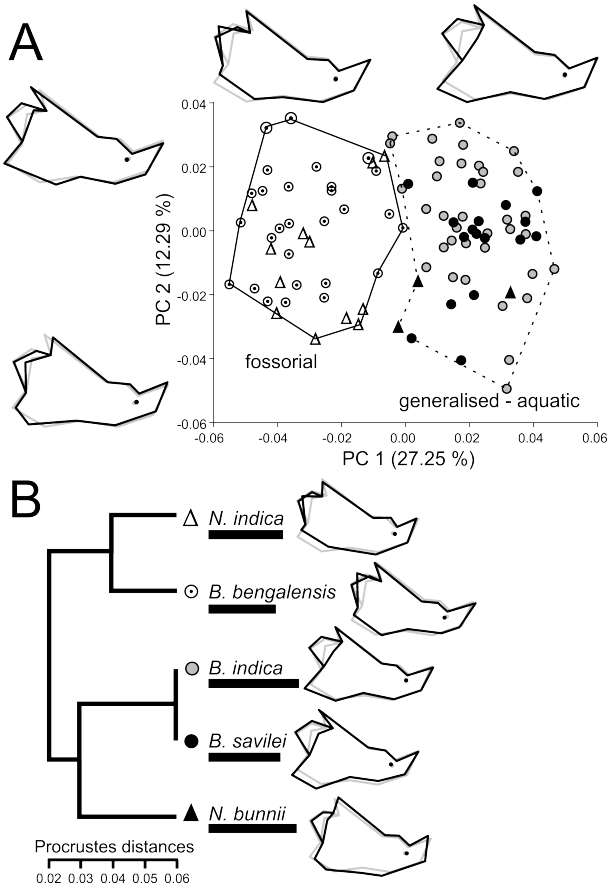

Figure 5 – Scatter plot of the first two PCs (percentage of variance in parentheses) for the mandible (A) with wire frame graphs showing shape changes along the two PC axes for the unit of 0.1 in the negative and positive direction (black) compared to the mean of each PC (grey). The UPGMA phenogram of the overall shape variation for the *Bandicota* and *Nesokia* species for the mandible (B) with wire frame graphs (magnified three times) illustrating differences between means of each individual group (black) and the mean of all groups (grey). Centroid size is visualized for each species using horizontal bars. Symbols correspond to sampling sites shown in Fig. 1.

a relatively large portion of the total variance (35.3%), but specimens of most, especially *Bandicota*, species were scattered along the whole range (approx. -0.10 to 0.09). The *Nesokia* species deviated from this general distribution by having mostly positive PC1 scores, which were associated with a broader transverse occlusal surface in all three molars. Shape changes along PC2 (13.8% of variance) were characterized by inward curving of the molar tooth row for positive PC2 scores and outward curving for negative scores. No obvious pattern of specimen distribution could be observed along PC2, except that *B. savilei* was the most variable with mainly negative scores, while *N. indica* (from the four larger samples) had the narrowest range. Opposite to the cranium and mandible, the scatter of specimens from both *Nesokia* species completely overlapped along the two PCs, showing their common molar shape features, but also overlapped with all three *Bandicota* species, displaying the great variability and similarity of molars of the two genera. As expected, not all species pairs were significantly different in molar shape. The highest *p*-value was observed within the genus *Nesokia* (*N. bunnii* vs. *N. indica*; p=0.2433; Procrustes distance = 0.045). After the Bonferroni correction, pairwise tests of *B. bengalensis* vs. *B. indica* (p=0.0157) and vs. *N. bunnii* (p=0.0107) were non-significant. Other pairs had p-values lower than 0.005. The *B. bengalensis* vs. *B. indica* species pair displayed also the shortest Procrustes distance (0.031), as reflected in the UPGMA phenogram (Fig. 6B). The longest Procrustes distance was calculated between *N. bunnii* and *B. savilei* (0.098). Despite a lower support, the UPGMA clustering of the species into the two genera was similar to that of the cranium. Average species shapes showed big differences in the pattern of the occlusal surface, *B. savilei* having the narrowest transverse occlusal surface and both *Nesokia* species the broadest. *Nesokia bunnii* had the shortest surface in the longitudinal direction. Allometry and ecological categories had little influence on shape, explaining only 2.6% (p=0.0376) and 5.1% (p=0.0005) of the total variation, respectively.

Discussion

The present analysis adds to previous groundwork on cranial morphology of bandicoots (Ellerman, 1941; Al-Robaae and Felten, 1990; Musser and Brothers, 1994) by exploring patterns of shape variation in three craniodental structures. We addressed the effects of phylogeny, ecology, and allometry on shape variation in ventral cranium, mandible and maxillary tooth-row. Specifically, we hypothesized that different structures responded differently to the interplay between phylogenetic constrains and selective pressures. This was indeed the case in our results, where independent structures contained different amount of phylogenetic structuring (cf. Meloro et al., 2011). While UPGMA trees constructed from the metrics of ventral cranial shape and shape of molars replicated the taxonomic hierarchy of bandicoot rats, the mandible retrieved very different morphometric relationships among species.

The close match between shapes of the ventral cranium with the current taxonomic division of bandicoot rats is not entirely unexpected. Species, genera and subgenera in this group of rodents result from morphological analyses (for a review see Musser and Brothers, 1994) and identification keys list dissimilarities, which clearly emerged also in our results. E.g. *Nesokia* and *Bandicota* are distinguished by relative length of incisive foramina, and *B. bandicota* is differentiated from its congeners by larger bullae and relatively wider braincase (e.g., Ellerman, 1941, 1947, 1961). To obtain confidence into the phylogenetic value of our results, we compared phenetic trees with partial molecular

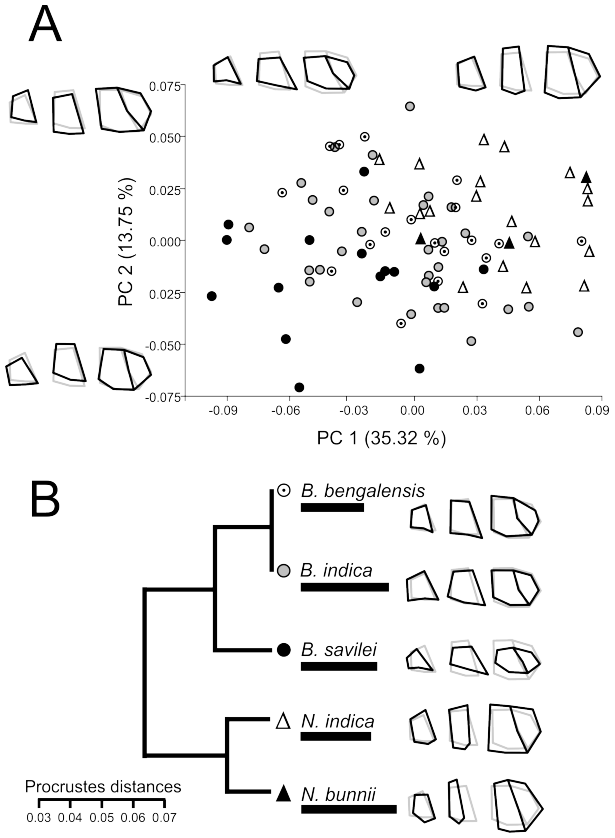

Figure 6 – Scatter plot of the first two PCs (percentage of variance in parentheses) for the molar tooth row (A) with wire frame graphs showing shape changes along the two PC axes for the unit of 0.1 in the negative and positive direction (black) compared to the mean of each PC (grey). The UPGMA phenogram of the overall shape variation for the *Bandicota* and *Nesokia* species for molar tooth row (B) with wire frame graphs (magnified three times) illustrating differences between means of each individual group (black) and the mean of all groups (grey). Centroid size is visualized for each species using horizontal bars. Symbols correspond to sampling sites shown in Fig. 1.

phylogenies (Michaux et al., 2007; Fabre et al., 2012) as a priori correct reconstructions of the evolutionary history of bandicoot rats. Both data sets retrieved identical grouping at both the species and genus level. We therefore conclude that the shape of the ventral cranium reflects an apparent phylogenetic structuring. Molar crowns, which are less rich in anatomical complexity than the ventral cranium (Caumul and Polly, 2005), provided a taxonomic grouping which was less straightforward in comparison with the skull. We found molars to be on average broader in *Nesokia* than in *Bandicota*. What was not evident from our results but is documented in taxonomic papers (e.g. Musser and Brothers, 1994) is a laminate pattern of molars in *Nesokia*, while *Bandicota* retains cuspidation. Molars are diet-adapted structures in mammals. In murine rodents, broader molars, with cusps fused into laminae, associate with vegetarian diet, while narrower and tuberculate molars reflect omnivorous nourishment (Renaud et al., 2007b). Although both genera seem to be fairly catholic in their diets, *Bandicota* is more omnivorous, particularly at low population densities (Aplin et al., 2003), while *Nesokia* heavily depends on fleshy underground parts of plants (Kryštufek and Vohralík, 2005). Piras et al. (2009) conclude that shape configuration in rodent molars is best explained by persistence of stasis as a long time pattern, while the residual variation correlates to ecology. Therefore, a wide intraspecific variation in bandicoot rats may reflect environmental plasticity (e.g. Anderson et al., 2014; Piras et al., 2010), i.e. a non-heritable adaptation of local populations to a specific diet. Dietary conditions vary with a landscape context, climate, human activities and rat population densities. Considering our large geographic coverage, a small-scale phylogeographic structuring possibly further added to divergence between samples and increased the total intraspecific variability. And finally, different levels of abrasion of molar crowns increased heterogeneity within species.

The morphological analysis of mandibles did not recover the phylogeny of bandicoot rats. Caumul and Polly (2005), in their study of Palaearctic marmots, came to conclusions very similar to ours. In their opinion complex structures, like the ventral cranium, perform better in phylogenetic reconstructions because of a complex genetic background that results in a varied anatomy with several functional and developmental modules. Each module has its own semi-independent evolutionary history and provides autonomous lines of phylogenetic evidence. On the other hand, the various morphogenetic components of the mandible (see Monteiro and dos Reis, 2005) seem to be all controlled by related ecological selection.

Studies of mandible shape showed that in murine rodents the mandible is influenced by both phylogeny and ecology (e.g., Michaux et al., 2007; Renaud et al., 2007a) or in some cases mostly by phylogeny (e.g., Jojić et al., 2012). In our study, the two clusters resulting from mandibular shape were not congruent with taxonomy (grouping did not match division into genera) or with patterns of size variability (small-sized species were in both clusters). We therefore believe that the phylogenetic structuring was diluted in the mandible, probably by adaptive trends for ecological niche. The fossorial *N. indica* and *B. bengalensis* clustered together in the UPGMA tree. These rats loosen soil with the action of the mandible and dig extensive burrows using incisors and skull as a powerful shovel and drill (chisel-tooth drilling sensu Nevo, 1999). In this category of burrowing rodents the selective pressures on the mandible are strong enough to produce convergent morphotypes in distantly related species. The convergences are evident in a powerful coronoid process, prominent alveolar process and a short angular process which is bent laterally (Kryštufek and Vohralík, 2005). These osteological structures act as insertion areas of major muscles that move the mandible (Casanovas-Vilar and van Dam, 2013), i.e. a temporal muscle and two masseter muscles, the zygomatico-mandibularis (inserting on the processus alveolaris), and the superficialis (inserting on the processus angularis). The major differences between the two clusters of bandicoot rats were exactly on these mandibular landmarks. The remaining generalised (*B. indica*, *B. savilei*) and aquatic (*N. bunnii*) bandicoot rats shared mandibles of similar shape and were in the same UPGMA cluster (Fig. 5B). Their broad overlap along the first two principal components (Fig. 5A) suggests a comparable gross ecological niche. We conclude that the shape of the mandible shows signs of homoplasy in the two fossorial bandicoot rats.

Nesokia, *Bandicota* and the Ryukyu Islands endemic *Diplothrix* (classified as *Rattus* in Ellerman, 1941) evidently compose a monophyletic lineage (hereafter the *NBD* lineage) that is a sister to true *Rattus* (Lecompte et al., 2008). These genera appear in the fossil record at the Pliocene-Pleistocene boundary: *Bandicota* and *Nesokia* in Siwalik, Indostan, at about 2.5 mya and 2 mya, respectively (Patnaik, 2014), and *Diplothrix* in the Upper Pleistocene of China (Wang et al., 2010). Fossil evidence is remarkably congruent with the divergence between the *NBD* and *Rattus* retrieved by the molecular clock (2.6 mya; Lecompte et al., 2008). This estimate makes *Nesokia*, *Bandicota* and *Diplothrix* by far the youngest murine taxa still attributed to generic level. Small genetic differences however sharply contrast with unique shape features, evident in craniodental structures of *Nesokia* and *Bandicota*. To address the pace of morphological evolution in the *NBD* lineage, the inclusion of *Diplothrix*, together with various species of *Rattus* as outgroups, would be essential.

The evolutionary young and morphologically diverse *NBD* lineage is of equal interest to pest control officers as is for conservation managers. The externally unique *Diplothrix legata* and *Nesokia bunnii* are small range peripheral isolates of the *NBD* lineage, both classified in the IUCN Red list as Endangered. Morphological uniqueness emphasizes their "value" (sensu Cardini and O'Higgins, 2004) in conservation policies more accurately than genetic metrics, what makes them more "visible" in a bunch of pest rats.

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Supplemental information

Additional Supplemental Information may be found in the online version of this article:

Appendix S1 List of museum vouchers examined in this study.