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Abstract

The Rattini tribe comprises some of the most specious genera in the mammalian kingdom. Many of these species are also highly morphologically conserved. As a result, identifying Rattini tribe animals, particularly those of the *Rattus* genus, to species level is extremely difficult. Problems with identification of conservative morphologies, particularly of the skeleton, have led to difficulties in understanding the fossil remains and as a result the systematics of this group. Here, we apply geometric morphometrics to the first lower molar of 14 species of the Rattini tribe. We find that the morphological data present a strong phylogenetic signal. However, within *Rattus,* this signal is rather complex and possibly hints at rapid evolutionary shape and size changes. In modern species, it is possible to identify specimens to species level with a good degree of confidence. We find that using both size and shape together affords further confidence with identification. However, we caution against the over-reliance on size in environments with unknown species composition and climate, particularly in archaeological contexts. This approach should prove to be a useful tool for identifying fossil and sub-fossil remains, particularly where biomolecular markers are absent in circumstances of poor preservation.

Keywords Rattini tribe · geometric morphometrics · dental morphology · phylogenetic signal · identification · Southeast Asia

Introduction

Despite having undergone a number of extensive adaptive radiations and occupying numerous ecological niches (Fabre et al. 2012), members of the Rattini tribe, particularly the

Rattus genus*,* are extremely morphologically conserved at the genus level (Taylor et al. 1982; Robins et al. 2007; Pagès et al. 2010; Rowe et al. 2011). As a result, they can be difficult to identify to species level (Chaimanee and Jaeger 2001; but see Pergams et al. 2015) and generally require the use of

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biomolecular markers (e.g., Robins et al. 2007; Chaval et al. 2016). This, in turn, has led to difficulties in establishing and understanding the taxonomy, systematics, and distribution of many of these species before the use of molecular analyses (Pagès et al. 2010). Species of the Rattini tribe also represent some of the most invasive organisms in the world (Aplin et al. 2011), a likely consequence of their highly plastic behavior. As such, assessing the shape variability of skeletal and dental elements regularly found in fossil and sub-fossil deposits is important for tracking the evolution and rapid divergence of these species. Fundamental questions remain unanswered — e.g., whether there is sufficient shape variability among modern species of the Rattini tribe to distinguish between species and if so, does it reflect a phylogenetic signal that will be informative for fossil remains? Here, we address these questions by examining the morphology of one of the most regularly found and best-preserved elements in the fossil record, the first lower molar, of the major Rattini tribe species of Mainland Southeast Asia using geometric morphometrics.

The Rattini tribe (as described by Pagès et al. 2010) comprises a total of 35 genera and 167 species. It includes a range of specialist and generalist species, including some of the most successful and destructive commensal and agricultural pest species known today, such as *Rattus norvegicus* (the brown rat) and *R. rattus* (the black rat) (Myers et al. 2000; Lecompte et al. 2008; Pagès et al. 2010; Morand et al. 2013; Capizzi et al. 2014; Kosoy et al. 2015). The identification of Rattini tribe species is particularly important in the study of fossil and sub-fossil records. Identification of rodents to species levels informs research about the species composition of local rodent communities and how these have changed through time, particularly in the case of changes due to human activity (Weissbrod et al. 2017), but also in environmental changes (Cuenca-Bescós et al. 2009; Stoetzel et al. 2011). Identification to species level is also central to the understanding of the rate and route of species translocations and invasions (Prendergast et al. 2017). However, this is often hindered by poor DNA preservation conditions of hot and humid environments, particularly in regions such as Southeast Asia. As Southeast Asia holds the greatest diversity of Rattini tribe species, it is thought to represent the geographic origin of the tribe (Pagès et al. 2010; Fabre et al. 2012). Numerous fossil deposits are also found in Mainland Southeast Asia along with many specimens thought to belong to this tribe, further indicating Southeast Asia as a likely origin for this group (Chaimanee and Jaeger 2001; Pearch et al. 2013). However with problems of DNA preservation in such regions and with older specimens, identification is limited to morphological examination.

Teeth are among the few elements that can be diagnostic to species level and survive well in both the archaeological and paleontological context, with first mandibular molars often being found in large numbers (e.g. Cucchi et al. 2013;

Pearch et al. 2013; Weissbrod et al. 2017). As such, they have traditionally formed the primary taxonomic criterion for rodent systematics (Musser 1972, 1981, 1986; Musser and Newcomb 1983) and identification of specimens from fossil and sub-fossil deposits is often based on dental remains (Cucchi et al. 2013; Pearch et al. 2013; Weissbrod et al. 2017). However, the often disconnected nature of the fossil record, and the morphological conservative nature of these species (i.e., they all look very similar, with little change through time), has resulted in difficulties in understanding Rattini tribe systematics (Wood 1965; Hautier et al. 2009; Fabre et al. 2012). Species within the various tribes of Rodentia have been continuously re-classified, so much so that many species within the Rattini tribe have been assigned to the *Rattus* genus at one time or another (Mares 1975; Lecompte et al. 2008; Rowe et al. 2008; Pagès et al. 2011). Advances in biomolecular approaches and recent phylogenetic analyses are providing much greater insight into the systematics of this tribe (Pagès et al. 2010). There have also been significant advances in the quantification of shape of skeletal elements, particularly in the form of landmark based shape analysis, and others included in the field of geometric morphometrics (Adams et al. 2004), but these have yet to be applied systematically to members of the Rattini tribe to investigate their extant taxonomy and their evolution in the fossil record.

Here, we use geometric morphometrics to explore phenotypic variation of the first lower molar of Southeast Asian species within the Rattini tribe. We select this element because of its prevalence in archaeological sites and fossil deposits. We test the hypothesis that there is a taxonomic and phylogenetic signal present in the first lower molar morphology of the seven major Mainland Southeast Asian *Rattus* species and seven of their immediate relatives from the following genera: *Maxomys*, *Niviventer*, *Leopoldamys*, *Berylmys*, *Sundamys*, and *Bandicota*. We aim to develop a protocol for identification of isolated lower first mandibular molars from sub-fossil and fossil material where poor DNA preservation prohibits genetic analyses.

Materials

We focus on species from Mainland Southeast Asia because of the presence there of both *Rattus* and close relatives (Pagès et al. 2010) and the availability of specimens previously identified by genetic markers. Museum specimens are often shown to be misidentified or representing cryptic variation or species (Robins et al. 2007; Pagès et al. 2010). To avoid these problems, we focused on comparative specimens that were consistently identified by a single research group (Ceropath project, http://www.ceropath.org/). Although extremely important to future understanding of the diversification of the genus

Rattus, the taxonomic status and systematics of Sahulian *Rattus* from New Guinea and Australia remain little known, despite recent biomolecular investigation (Rowe et al. 2008, 2011; Robins et al. 2014). As such, it has been excluded from our analyses.

The first lower molar of 395 subadult and adult specimens were examined in this study. They represented 13 of the 30 known Rattini species (from ten genera) currently present in Mainland Southeast Asia, along with one closely related species from outside Mainland Southeast Asia (*R. rattus*) (see Table 1). All specimens were photographed in the occlusal view for geometric morphometric analyses. None of the specimens were at advanced tooth wear stages. The ratio of sexes was near equal for each species. The specimens were previously trapped in the wild, vouchered, and identified by genotyping as part of the CEROPATH project (00121 0505, 07 BDIV 012).

Methods

To quantify the form of the first lower molar, we recorded five fixed landmarks on the external outline of the tooth in the occlusal view (Fig. 1) from photos using tpsDig 2.12 (Rohlf 2008):

1. The most anterior point of the entoconid on the outline of the tooth
2. The most anterior point of the metaconid on the outline of the tooth
3. The most anterior point of the protoconid on the outline of the tooth
4. The most posterior point of the protoconid on the outline of the tooth
5. The most anterior point of the hypoconid on the outline of the tooth

Sliding semi-landmarks (SSLMs) (Bookstein 1997) were then used to describe the five curves between these landmarks along the external outline of the tooth, which is unaffected by occlusal wear until the most advanced wear stages. Starting from landmark 1, we used 20, 30, 10, 10, and 30 SSLMs for each curve, respectively. The posterior end of the tooth was described using an artificial line in all cases. This was taken from the maximum posterior point of the hypoconid to the maximum posterior point of the entoconid to minimize variation introduced by the presence or absence of the second molar. This line is highly reproducible based on the use of the maximum posterior points. We use this approach because in fossil deposits, unlike in modern collections, the first molar can regularly be found in isolation or with the neighboring second molar missing. As a result, using an artificial line reduces variance introduced by presence/absence of the neighboring tooth, allowing this methodology to be used on fossil material where presence or absence might be influenced by the preservation conditions of the site.

Table 1 The number of specimens examined in this paper (Female/ Male/ Unknown) and the approximate number of regions specimens come from, with the Mean, Min and Max correct cross validation results from 1000 iterations of resampling to equal sample size a for each species individually

Species	Sample size			Regions	Mean %	Min %	Max %	Distance to mean	Variance
	F	M	U						
Bandicota indica	13	15		7	76.55	46.15	100	0.0524	0.0341
Bandicota savilei	15	13		4	82.36	46.15	100	0.0437	0.0313
Berylmys berdmorei	14	11		7	74.14	46.15	100	0.0251	0.0277
Berylmys bowersi	11	15		4	73.17	38.46	100	0.0346	0.0267
Leopoldamys edwardsi	8	15	1	4	97.63	76.92	100	0.0199	0.022
Maxomys surifer	15	13		4	91.98	69.23	100	0.0217	0.0255
Niviventer fulvescens	20	14		4	90.55	76.92	100	0.0601	0.0252
Rattus argentiventer	18	16		3	78.54	46.15	100	0.0179	0.0204
Rattus exulans	14	15		9	76.09	38.46	100	0.0289	0.0254
Rattus sakeratensis	14	15		6	61.17	23.08	92.31	0.0139	0.0241
Rattus nitidus	9	4		2	88.65	69.23	100	0.0227	0.0198
Rattus norvegicus	15	13		2	78.25	46.15	100	0.0276	0.0241
Rattus rattus			29	1	83.08	46.15	100	0.0186	0.0198
Rattus tanezumi	20	20		12	31.37	0	76.92	0.0100	0.0264

The Procrustes distance of each species to the total mean and the variance of each individual species is also presented

Fig. 1 **a**, *Rattus exulans* lower
first molar in occlusal view; **b**, a
schematic of the same tooth with
landmarks (large blue circles) and
sliding semi-landmarks (small red
circles). The boundaries of the
cusps and the stylids (small flat or
saddle-like surface joining cusps)
are difficult to precisely identify,
but have been illustrated in the
schematic for clarity
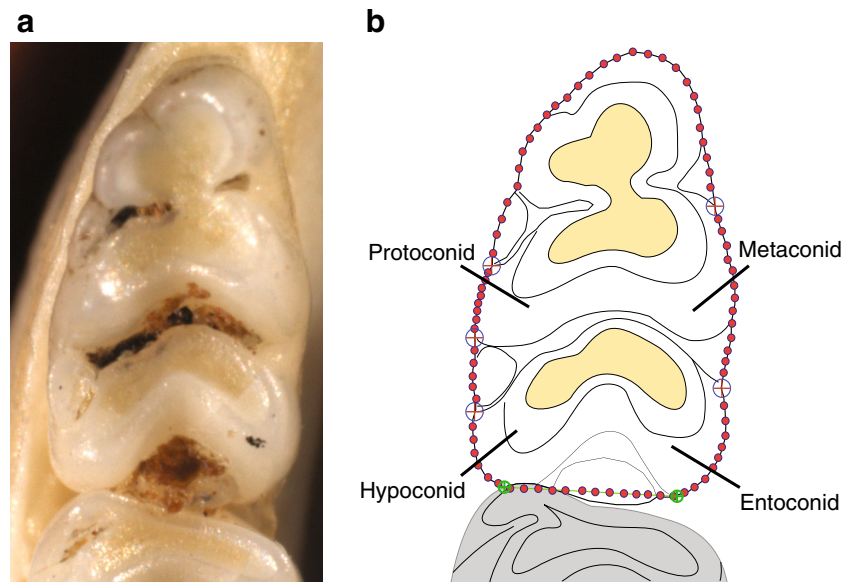
Variance introduced by digitizing error was calculated from a subset of ten randomly selected specimens from different species (3 *R. exulans*, 2 *R. tanezumi*, 2 *Niviventer fulvescens*, 1 *Maxomys surifer,* 1 *Bandicota indica,* and 1 *Bandicota savilei*) digitized five times each. Shape differences between individuals were tested with a Procrustes ANOVA (Claude 2008).

Landmark and SSLM configurations digitized from photographs were aligned using a generalized Procrustes analysis to remove factors of size, position, and orientation from the form coordinates. SSLMs were slid during this stage using the minimum Procrustes distance method in TpsRelw 1.41 (Rohlf 2005). Centroid size (CS) was calculated at this stage for examining between species differences. All statistics were carried out in R. A Tukey honest significant difference (Tukey HSD, Tukey 1949) test was carried out on the CS of all species using the Vegan package (Oksanen et al. 2017). Sexual dimorphism of molar size was also tested using an ANOVA on CS for each species. The end results were then corrected for multiple comparisons using the Bonferroni adjustment.

A principal component analysis was applied to the aligned shape coordinates and the resulting principal components were considered shape variables for standard statistical analyses in R (R Core Team 2016). Two strategies of dimensionality reduction were employed: the number of principal components that represented ~95% of variance (in this case 13 principal components representing 94.64% variance); and the number of principal components that achieved highest discrimination between species using a resampling approach to account for unbalanced sample sizes (in this case 18 principal components representing 96.71% variance) (Evin et al. 2013 — MevolCVP R functions). Results from statistical analyses using both dimensionality reduction strategies are reported.

As there was likely to be size differences between species (e.g., *R. exulans* being very small and *Bandicota* spp. being very large), we also considered the possibility of discriminating species on form (size + shape). We performed a principal component analysis on a new matrix of CS in the first column and the Procrustes coordinates in the following columns (Mitteroecker et al. 2004). In the case of form variables, principal component 1 represents ~99% of variance due to the huge size variation of the dataset. Dimensionality reduction for form was only carried out to the level that achieved the highest discrimination (after Evin et al. 2013), which resulted in 18 principal components.

Sexual dimorphism in shape was tested using a MANOVA for each species individually and the results were then corrected for multiple comparisons using the Bonferroni adjustment. Between species differences in shape and form were tested using a MANOVA on the total dataset and then also pairwise between species. The pairwise MANOVA results were corrected for multiple comparisons using the Bonferroni adjustment. If the MANOVA results were significant, the ability to identify between species was assessed by carrying out a linear discriminant analysis on principal components, coupled with a leave-one-out correct cross validation analysis. Groups were resampled to equal sample size iteratively 1000 times and a stepwise correct cross validation percentage was generated following Evin et al. (2013). The correct cross validation percentage was generated using a jack-knifing approach whereby each specimen was considered an unknown and was then identified to one of the reference groups, the percentage of correctly identified specimens was then considered the correct cross validation percentage. The maximum, minimum, and mean correct cross validation percentages of the resampling approach (1000 iterations) to the linear discriminant analysis were reported for each of the two

dimensionality reduction approaches (95% variance and maximum correct cross-validation) as the approximate ability to identify between species (after Evin et al. 2013). These percentages are reported versus the null percentage, which is the chance of a specimen being correctly identified accidentally (e.g., with two groups there is a 50% chance of a specimen being correctly identified by accident). The cross validation results from an iterative resampling to equal sample size (1000 times) were examined to assess the rate of identification for each individual species and the rate of misidentification of species with low correct cross validation percentages. This made it possible to assess which species were regularly misidentified as other species.

Species shape variance was calculated as the trace of the Procrustes variance-covariance matrix among specimens within a species. The variance of species was then correlated with the mean percentage correct cross validation of each species in the iterative resampling test, to see if species with larger variance were also harder to identify in this dataset. Variance scores were also compared against sample sizes and the size of the regions samples were collected from to assess if this was a contributing influence and possible bias on different levels of variance in this study (see Supplementary Information). To further discount geographical bias, we compared the variance of the closest geographic specimens versus the variance of those over a greater geographic area (see Supplementary Information for results and functions written in R for this analyses). The Procrustes distances between the mean of the total dataset and the mean shapes of each of the species was also calculated to identify which species were closest to an intermediate shape.

The shape data were then compared to divergence data generated from genetic data of the same species to assess phylogenetic signal using a multivariate generalized K-statistic ($Kmult$, Adams 2014). The phylogenetic tree published in Fabre et al. (2012), which included DNA (both mtDNA and nuclear) sequences from these specimens, was used and pruned to remove tips representing taxa that are not present in this morphological dataset (Blomberg et al. 2003, Adams 2014). The pruning was done using the ape package (Paradis et al. 2004). The test developed originally by Blomberg et al. (2003) tests the phylogenetic signal according to what we may expect under a Brownian model of evolution and performs better than a Mantel test approach (Harmon and Glor 2010). This analysis was carried out for all the species in the dataset and then also carried out on species of the genus *Rattus*, as this was the only genus with more than two species present in the dataset. Lose of phylogenetic signal in the *Rattus* shape data was then assessed using a jack-knifing approach (i.e., iteratively removing species one by one and assessing the change in phylogenetic signal reported at each iteration). The phylogenetic signal was then visualized by mapping the phylogeny onto the first two principal

components using a squared change parsimony procedure for predicting ancestors (Klingenberg and Gidaszewski 2010). This mapping of the phylogeny onto the morphospace was done using the geomorph package (Adams and Otárola-Castillo 2013).

Size data were assessed for a phylogenetic signal using a similar procedure to that described in Meloro and Raia (2010) and Piras et al. (2012). This compares a single continuous variable (in this case CS) to phylogenetic data under a number of different models of evolution (Brownian [Felsenstein 1973], Ornstein-Uhlenbeck [Butler and King 2004], accelerating-decelerating [Harmon et al. 2010], Pagel's lambda [Pagel 2000], and a white noise model [following Piras et al. 2012]). This was carried out using the Geiger package (Harmon et al. 2008). The model with the lowest Akaike Information Criterion (AIC) was chosen as the best fit. We also ran a test of significance for phylogenetic signal in size data using the K-statistic (Blomberg et al. 2003) with functions from the package phytools (Revell 2012). As for shape, phylogenetic signal was tested in both the complete dataset and a subset of data representing only the *Rattus* genus.

All data generated and analyzed during this study are included in this published article and its supplementary information files.
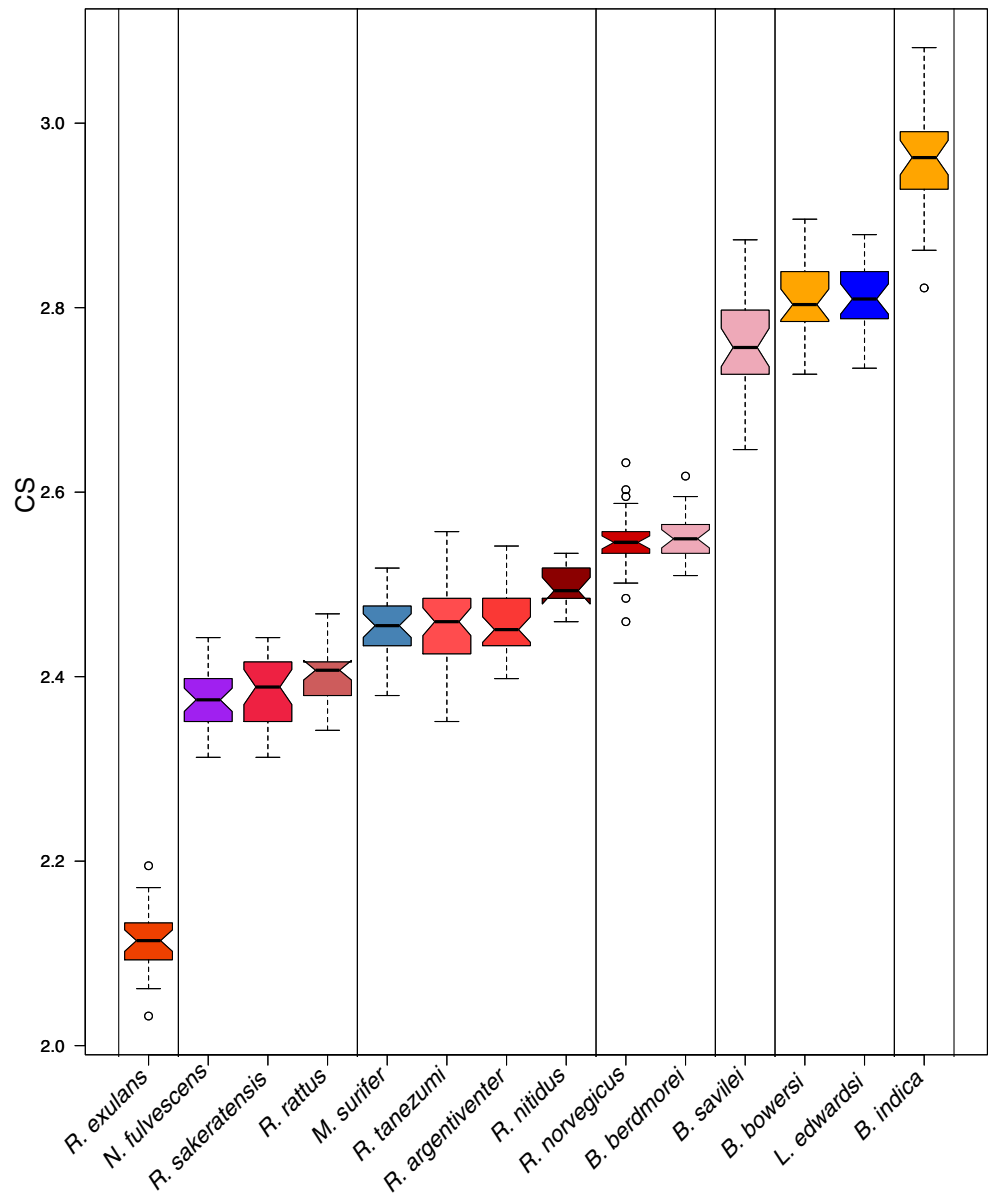
Results

In the measurement error test, shape differences between replicates were non-significant (Df 4, residual Df 45, Pillai = 0.263, approx. $F = 0.274$, $p > 0.99$), whereas shape differences among specimens were highly significant (Df 9, residual Df 40, Pillai = 8.64, approx. $F = 94.2$, $p < 0.001$). Shape measurement error was calculated to represent ~2% of the among individual variance from different species. Sexual dimorphism was tested in shape and size, and no comparisons were found to be significant ($p > 0.1$), as a result sex was not considered in any further analyses. Sample size and sampling region size were not found to have a significant influence on variance (see Supplementary Information).

Seven CS groups were identified using the Tukey HSD test (Fig. 2). Size could not be used to confidently identify species except for the largest species (*Bandicota indica*) and the smallest species (*R. exulans*) within the studied sample. 98.47% of *Bandicota indica* specimens were larger than the range of any other species, while all *R. exulans* specimens were smaller than the range of any other species.

MANOVA results of shape variables indicated there are highly significant differences in molar morphology among the species examined (Table 2). All, but one, pairwise species comparisons indicated species shape differences were highly significant (all $p < 0.001$). The one pairwise species comparison that did not yield significant results was that of

Fig. 2 CS boxplot by species. The CS of the samples are presented here and the size groupings identified from Tukey HSD are indicated by the boundary lines plotted between groups on the boxplot

R. tanezumi versus *R. nitidus*, which produced varying significant ($p < 0.001$) and non-significant ($p > 0.1$) results depending on the dimensionality reduction used (principal components 1–2 $p < 0.01$; 1–3:8 $p > 0.1$; 1–9 and greater $p < 0.001$). Species were identified using shape alone to a good degree of confidence (Table 2). However, no species

Table 2 MANOVA and linear discriminant analysis results of between species analyses using Shape and then Form with two different dimensionality reduction approaches in each case: the first dimensionality reduction in both cases is to 95% of variance; the second dimensionality reduction in both cases is to maximum discrimination

Variables	PCs	Df	Pillai	Approx. F	Num df	Den df	p value	Min CVP	Mean CVP	Max CVP	Null CVP
Shape	13	13	4.13	13.65	169	4953	<0.001	69.82	76.54	82.84	7.43
	18		4.85	12.43	234	4888		73.37	79.53	84.62	
Form (CS + shape)	13		4.45	15.23	169	4953		82.97	86.74	91.76	
	19		5.14	13.65	234	4888		84.06	88.7	94.51	
CS	–	–	–	–	–	–	–	31.67	43.45	52.75	

PCs refers to the number of dimensions used for the below results

was identified with 100% confidence using shape variables alone. *Rattus tanezumi* had the lowest level of correct identification at 31.37% (versus a null percentage of 7.43% chance of random correct identification); its mean shape was also the closest to the overall mean shape of all species (Table 1). Iterative resampling to equal sample size showed that on average *R. tanezumi* was misidentified as *R. argentiventer* ~19% of the time and was also misidentified as *R. exulans, R. rattus, R. sakeratensis* (sensu Aplin et al. 2011), and *R. nitidus,* between 5 and 15% of instances for each (also see Supplementary Information Table 1). There was no significant correlation between variance scores and percent correctly identified.

The most generalist and commensal species in this dataset (namely: *R. tanezumi, R. rattus, R. exulans,* and *R. norvegicus* [Morand et al. 2015]) all varied in their distance from the total mean shape to varying degrees. *Rattus tanezumi* was the closest species to the total mean shape, whereas *R. exulans* and *R. norvegicus* were ninth and tenth out of fourteen away from the mean shape. *Rattus exulans* and *R. norvegicus* were, therefore, the furthest *Rattus* species from the mean shape. The variance scores of the generalist species were also in the middle of the range.

As CS has been demonstrated to be significantly different among many species, it can be reintroduced for a form analysis. A MANOVA on form variables indicates that form differences were highly significant (Table 1). Using form variables, *R. exulans, B. berdmorei, B. savilei,* and *L. edwardsi* were all identified with 100% correct cross validation from all other species in this dataset. *Rattus tanezumi* was again the most regularly misidentified with only 65% correct cross validation (versus 7.43% of random correct classification). Again, misidentifications of *R. tanezumi* were most often assigned to *R. argentiventer* (in 15% of instances) and then to *R. rattus, R. sakeratensis,* and *R. nitidus* (in 5–10% of instances). This was evident when examining the pairwise correct cross validation results, where analyses between *R. tanezumi* and the aforementioned species all produced lower correct cross validation scores (Table 2). In all iterations of the form analyses, *R. tanezumi* was no longer misidentified as *R. exulans.*

The K-statistic ($Kmult = 0.809$, $p < 0.001$) supports the presence of a high level of phylogenetic signal in the molar shape data. When the phylogeny is mapped onto the morphospace, it is possible to see how closely the mean shapes of the species within each genera fall together (Fig. 3a, b). However, when species of the genus *Rattus* were analyzed alone (Fig. 3c, d), the K-statistic ($Kmult = 0.739$, $p = 0.101$) revealed the phylogenetic signal within the data does not pass the 0.05 significance threshold. When the morphometric data are mapped onto the phylogeny, it appears that there is some convergence in shape between *R. nitidus* and *R. exulans* (Fig. 3b, d). The jack-knifing approach to removing species and testing phylogenetic signal revealed that

whenever *R. nitidus* and *R. norvegicus* were both present in the dataset the phylogenetic signal was lost. When either *R. nitidus* or *R. norvegicus* were removed, the phylogenetic signal is significant in the *Rattus* dataset ($Kmult = 1.007$, $p = 0.0195$ and $Kmult = 1.02$, $p = 0.0235$, respectively). This may indicate that the strong phylogenetic signal in the total dataset was not only driven by distinctive morphological differences between genera, and the phylogenetic signal within genera has contributed to this signal.

For the evolution of size, the Brownian motion model had the smallest AIC. However, the signal was not found to be significant at the 0.05 level across the entire dataset ($K = 0.6843$, $p = 0.07$). However, a much stronger phylogenetic signal in size was found within the *Rattus* genus. Again, a Brownian motion model of evolution was found to be the best fit with the lowest AIC and this was found to be significant ($K = 1.163$, $p = 0.028$).

Discussion

Identification of these Rattini tribe species is extremely difficult, as demonstrated by a number of genetic studies that have corrected field and museum identifications (Pagès et al. 2010; Chaval et al. 2016). A few species, notably *R. exulans* and *B. indica,* are more easily identified when size is incorporated as they are markedly smaller or larger than all other rats (e.g., Claude 2013; Chaval et al. 2016). However, the various groups of similarly sized species indicate that size alone is a poor indicator of species and that shape is necessary to further distinguish between species. Furthermore, as many of these species' current distribution includes islands, the possibility of insular size variation needs to be considered, particularly where these species have been introduced to islands relatively recently in geological time (Meiri et al. 2011).

Shape Versus Size

Analyses of shape variables alone provide some discrimination between species, but they also further confirm the conserved nature of the dental morphology of the species of the Rattini tribe (Rowe et al. 2011; Claude 2013).

The form of the first lower molar (the combination of size and shape variables) is the most effective method of distinguishing between the species within our study. When preservation conditions do not allow for the use of molecular approaches for the identification of species, the geometric morphometric approach outlined here is a useful additional tool for identifying the skeletal remains of invasive taxa. Molar size must, however, be used with caution, especially for species that hold wide geographic distributions, as molar size variation can be influenced by environmental factors (Renaud et al. 2011; Cucchi et al. 2014), which is partly
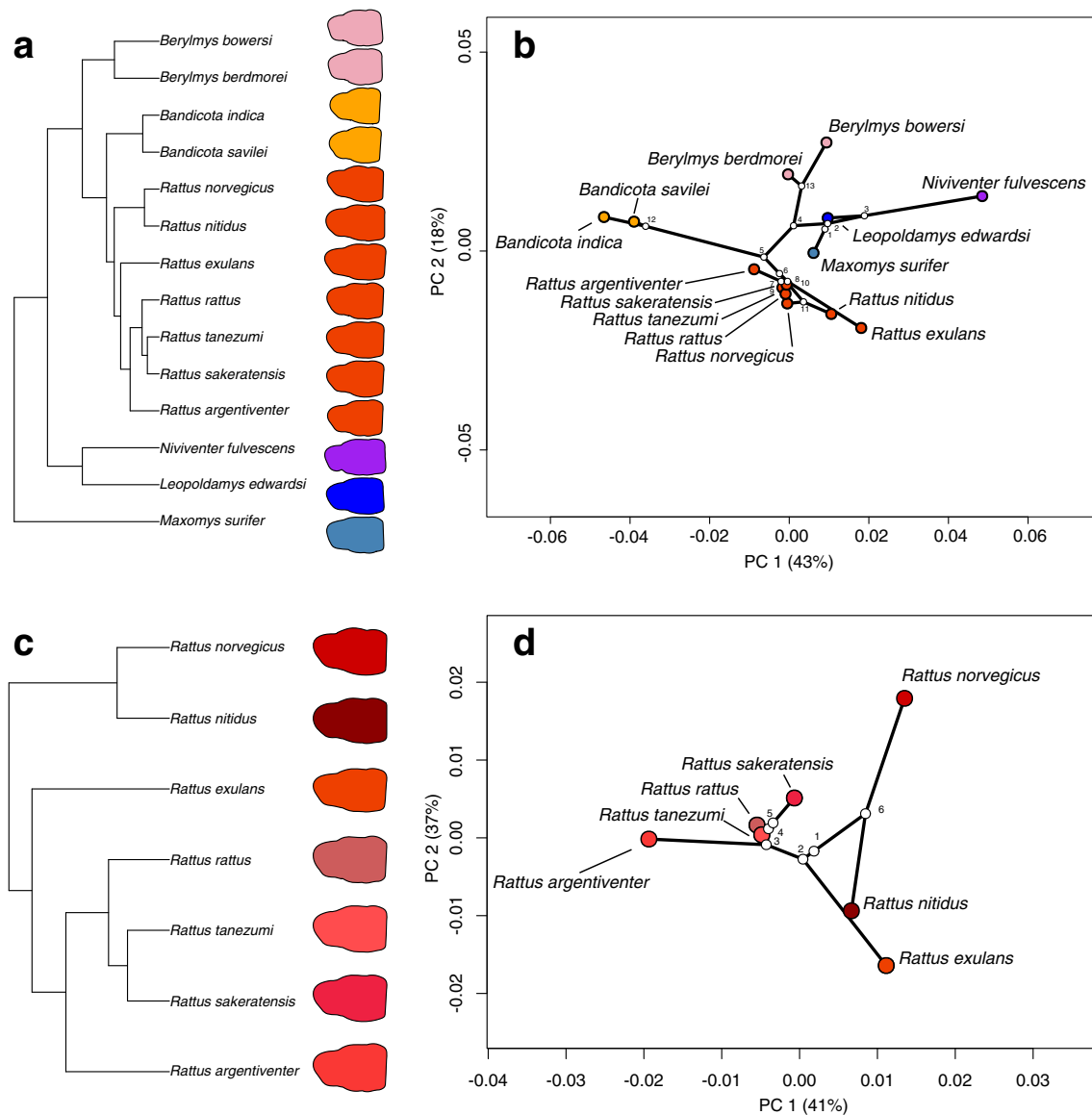
Fig. 3 Phylogenies mapped to morphospace: **a**, phylogeny of all species including multiple genera and the mean shape of each species with a different color for each genus; **b**, phylogeny of all species mapped to morphospace with colors corresponding to a; **c**, phylogeny of only *Rattus* species with mean shapes of each species and colored in red scale; **d**, phylogeny of *Rattus* species mapped to morphospace with red scale colors corresponding to c

explained by their correlation with overall body size (Meiri et al. 2003; Millien and Damuth 2004; Millien et al. 2006).

Another factor to consider when using size as a distinguishing characteristic is the effect of competition and community structure. The samples used in this study were from populations that live sympatrically with at least one, if not more, closely related species. Studies on community composition, particularly in the success rate of invasive species, increasingly demonstrate that morphologically similar sympatric species are less likely to be successful (Pimm 1989). Sympatry of related species is, therefore, expected to drive character displacement such as body size differences, as is seen in other species (Dayan and Simberloff 1998). For

example, *R. exulans* and *R. rattus* successfully niche partition when living sympatrically, but population sizes appear to be are smaller than when similar niche partitioning occurs between the morphologically more different *R. rattus* and *Mus musculus*, where population sizes remain relatively high, indicating *R. rattus* and *Mus musculus* can niche partition more successfully than *R. rattus* and *R. exulans* can (Shiels et al. 2012). There is some tentative evidence from the archaeological record of dramatic changes in size (primarily body size) for individual *Rattus* species in the absence of competition (Armitage et al. 1984; Armitage 1994). For example, in the absence of *R. norvegicus*, *R. rattus* individuals can reach much larger sizes comparable with those of *R. norvegicus*

due to lack of interspecific competition (Armitage 1994). This phenomenon is better documented in many carnivoran (and especially canid) species (Dayan et al. 1989a, 1989b, 1992; Dayan and Simberloff 1994, 1998) but is yet to be thoroughly tested in other groups such as rats. It is also possible, though, that body size and tooth size evolve independently (body size being far more plastic than tooth size, e.g., Serrat et al. 2010). Differences in rate of size evolution among skeletal elements have been observed in other rodent species (Yom-tov and Yom-tov 2004). It is, therefore, unclear as to whether size will remain a good indicator of species in other regions and climatic conditions (past and present). We found a strong phylogenetic signal for molar size in the *Rattus* genus (see next section), which could show that for this genus, molar size may not be as flexible as body size. However, this evidence does not indicate there is no plasticity in tooth size variation and while it holds an important phylogenetic signal we cannot exclude the fact that it may vary due to other factors such as competition and environment. As a result, archaeological identification of rats must use size with caution and previous identification to species level based on size should be considered carefully, especially in contexts such as islands where murid rodents usually grow larger (Meiri et al. 2008). The archaeological record provides an excellent resource for future examination of size variation and the impact of invasive species, particularly those introduced through human-mediated dispersal around the world.

The poor identification of *R. tanezumi,* and in particular the difficulty distinguishing it from *R. rattus,* in this study is unsurprising. There is on-going debate over the species status of these two species, which stems from their ability to regularly hybridize where introduced by humans (Bastos et al. 2011; Lack et al. 2012; Conroy et al. 2013). *Rattus tanezumi* does not appear to have any greater shape diversity relative to other species. As a result, we should not expect its shape to overlap substantially with other species any more than that of the other species examined here. Furthermore, *R. tanezumi* is not only misidentified as its other sister species (*R. rattus, R. argentiventer,* and *R sakeratensis*), when shape alone is analyzed it is also regularly misidentified as *R. norvegicus* and *R. exulans*. Its poor identification would then appear to be the result of its intermediate position in shape between all the *Rattus* species in these analyses.

Dental Morphologies and Phylogenetic Signal

The phylogenetic signal in shape is significant and closely related species are more similar to each other across the Rattini tribe, but this is not immediately observable within the genus *Rattus* when this group is analyzed separately. This might be due to the recent timing of divergence of closely related species, which now have highly diverged morphologies. For example, relative to other species of the *Rattus*

genus, the sister species *R. norvegicus* and *R. nitidus* are not as morphologically similar as expected considering their close genetic relationship, but distant phylogenetic relationship with other *Rattus* species (Chingangbam et al. 2015). The reason for the morphological divergence between *R. nitidus* and *R. norvegicus* is unclear. Understanding this is made difficult due to the fact the indigenous range of *R. nitidus* is not well established, because of extensive translocation likely in association with human agricultural patterns (Chingangbam et al. 2015) and because *R. nitidus* and *R. norvegicus* live sympatrically in many regions (Aplin et al. 2003). As a result, it is hard to infer all the ecological and environmental differences between these two species. Also, as we focus on Mainland Southeast Asia, we likely only have a small proportion of the morphological variability of both species, and *R. norvegicus* particularly, which has a much wider range and is thought to be native to more temperate regions of East Asia. *Rattus nitidus* molar shape is more similar to a number of *Rattus* species other than it is to its sister species *R. norvegicus*, and poor levels of correct cross validation between *R. nitidus* and *R. tanezumi* suggest there are a number of morphological similarities between these species (Supplementary Information Table 1). As a result, the lack of phylogenetic genetic signal under a Brownian motion model when *R. nitidus* is present is unsurprising. However, it might also be considered that *R. norvegicus* has rapidly diverged away from other *Rattus* species. Therefore, the reason for this shape divergence between *R. nitidus* and *R. norvegicus* is unclear and could result from different scenarios, for example: 1) it might be the case that *R. nitidus* harbors a primitive *Rattus* shape and their tooth shape did not evolve away from a typical *Rattus* shape, while the tooth shape of *R. norvegicus* underwent a greater rate of morphological evolution; or 2) the ancestor of *R. norvegicus* and *R. nitidus* diverged from the rest of *Rattus* and later the tooth shape of *R. nitidus* has converged back to that of other *Rattus* species. It is not possible to discern between these scenarios without extensive fossil evidence. It is also possible that when compared with *R. norvegicus* in Southeast Asia, *R. nitidus* occupies many more similar habitat preferences to the other *Rattus* species, as it is a serious agricultural pest like *R. tanezumi* and *R. argentiventer*, whereas *R. norvegicus* is found in more urban and village environments feeding on refuse (Aplin et al. 2003). As a result, the similarity in dental shape of most *Rattus* species could be the result of their overlapping habitat preferences. However, it can be said that it indicates that within the *Rattus* genus the molar shape diversification is rather complex, involving rapid evolutionary mechanisms. Interestingly, size was found to have a strong phylogenetic signal within the *Rattus* genus, but not at the tribe level, suggesting that ancient rather than recent evolutionary phenomena have altered the signal. The presence of a phylogenetic signal in size of *Rattus* species could reflect their

relatively short divergence period compared with the deeper divergence with the other genera in this study.

Conclusion

Members of the Rattini tribe are extremely difficult to identify; this is particularly the case when soft tissue structures and articulation are lost. Teeth are among the few elements that are diagnostic and survive well in multiple contexts (i.e., modern, archaeological, and paleontological). Using geometric morphometrics, it is possible to identify between many of these species, though some species within *Rattus* continue to present problems. There is also a strong phylogenetic signal, but this requires further testing with a greater range of species from each genus. Future studies should examine the effects of changes in community composition and niche partitioning on dental morphology and size to establish how transferable this approach is between different regions and time periods.

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