



Dental Shape Variation and Phylogenetic Signal in the Rattini Tribe Species of Mainland Southeast Asia

A. Hulme-Beaman, Julien Claude, Y. Chaval, A. Evin, S. Morand, J. Vigne,
K. Dobney, T. Cucchi

► To cite this version:

A. Hulme-Beaman, Julien Claude, Y. Chaval, A. Evin, S. Morand, et al.. Dental Shape Variation and Phylogenetic Signal in the Rattini Tribe Species of Mainland Southeast Asia. *Journal of Mammalian Evolution*, 2019, 26 (3), pp.435-446. 10.1007/s10914-017-9423-8 . hal-01920127

HAL Id: hal-01920127

<https://hal.umontpellier.fr/hal-01920127>

Submitted on 26 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License



Dental Shape Variation and Phylogenetic Signal in the Rattini Tribe Species of Mainland Southeast Asia

A. Hulme-Beaman^{1,2,3,4} · J. Claude⁵ · Y. Chaval⁶ · A. Evin⁵ · S. Morand⁷ · J. D. Vigne² · K. Dobney^{1,3,8} · T. Cucchi^{1,2}

Published online: 19 January 2018

© The Author(s) 2018. This article is an open access publication

Abstract

The Rattini tribe comprises some of the most speciose 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

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10914-017-9423-8>) contains supplementary material, which is available to authorized users.

✉ A. Hulme-Beaman
ardernhb@gmail.com

¹ Department of Archaeology, University of Aberdeen, St. Mary's Building, Elphinstone Road, Aberdeen, UK

² CNRS-Muséum National d'Histoire Naturelle, UMR 7209, Archéozoologie, Archéobotanique Sociétés, Pratiques et Environnement, 55 rue Buffon, 75005 Paris, France

³ Department of Archaeology, Classics and Egyptology, University of Liverpool, 12-14 Abercromby Square, Liverpool L69 7WZ, UK

⁴ Research Centre in Evolutionary Anthropology and Palaeoecology, School of Natural Sciences and Psychology, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, UK

⁵ Institut des Sciences de l'Evolution, Université de Montpellier, UMR CNRS, UM, EPHE, IRD, 2 Place Eugène Bataillon, CC065, 34095 Montpellier, Cedex 5, France

⁶ CEFS (Comportement et Ecologie de la Faune Sauvage - Wildlife, Behaviour and Ecology Research Unit), U.R. I.N.R.A. 0035, Centre Inra Occitanie-Toulouse, Chemin de Borde Rouge, Auzeville, BP 52627 31326 Castanet-Tolosan Cedex, France

⁷ CNRS-CIRAD ASTRE, Faculty of Veterinary Technology, Kasetsart University, Bangkok, Thailand

⁸ Department of Archaeology, Simon Fraser University, Burnaby, BC, Canada

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

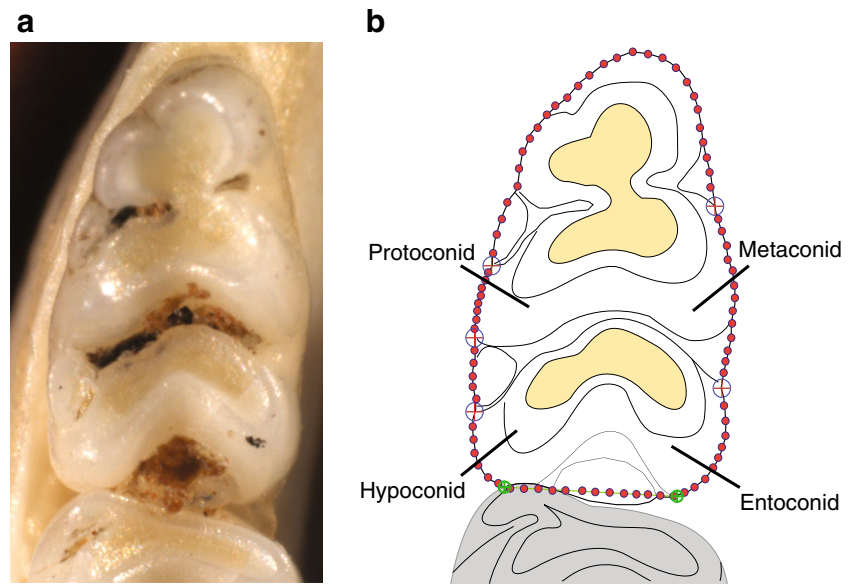
Sliding semi-landmarks (SSLMs) (Bookstein 1997) were then used to describe the five curves between these landmarks along the external outline of the tooth, which is unaffected by occlusal wear until the most advanced wear stages. Starting from landmark 1, we used 20, 30, 10, 10, and 30 SSLMs for each 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						
<i>Bandicota indica</i>	13	15		7	76.55	46.15	100	0.0524	0.0341
<i>Bandicota savilei</i>	15	13		4	82.36	46.15	100	0.0437	0.0313
<i>Berylmys berdmorei</i>	14	11		7	74.14	46.15	100	0.0251	0.0277
<i>Berylmys bowersi</i>	11	15		4	73.17	38.46	100	0.0346	0.0267
<i>Leopoldamys edwardsi</i>	8	15	1	4	97.63	76.92	100	0.0199	0.022
<i>Maxomys surifer</i>	15	13		4	91.98	69.23	100	0.0217	0.0255
<i>Niviventer fulvescens</i>	20	14		4	90.55	76.92	100	0.0601	0.0252
<i>Rattus argentiventer</i>	18	16		3	78.54	46.15	100	0.0179	0.0204
<i>Rattus exulans</i>	14	15		9	76.09	38.46	100	0.0289	0.0254
<i>Rattus sakeratensis</i>	14	15		6	61.17	23.08	92.31	0.0139	0.0241
<i>Rattus nitidus</i>	9	4		2	88.65	69.23	100	0.0227	0.0198
<i>Rattus norvegicus</i>	15	13		2	78.25	46.15	100	0.0276	0.0241
<i>Rattus rattus</i>			29	1	83.08	46.15	100	0.0186	0.0198
<i>Rattus tanezumi</i>	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 jackknifing 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. Loss 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.

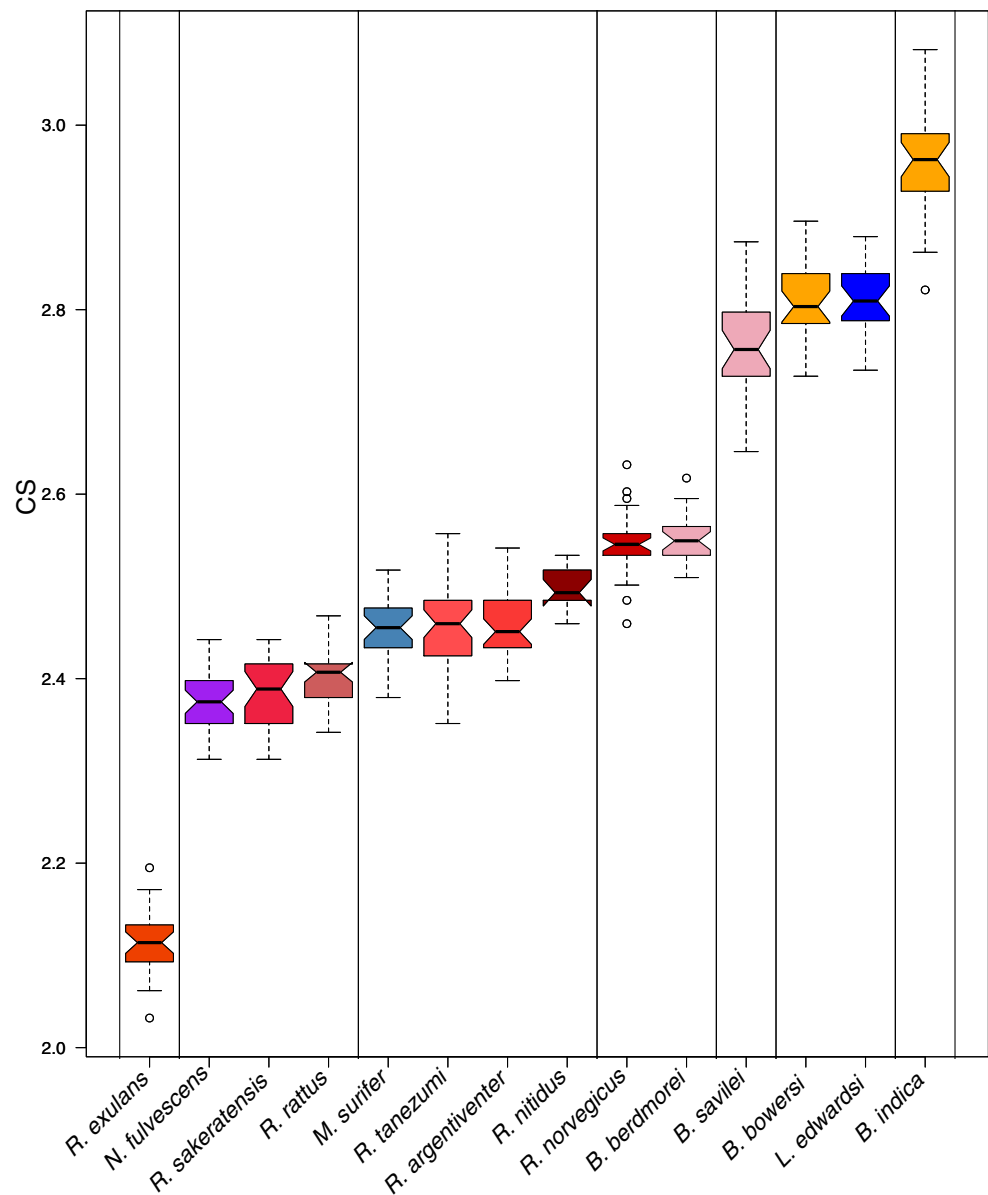
Results

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	<i>p</i> 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	19	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 ($K_{mult} = 0.809$, $p < 0.001$) 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 ($K_{mult} = 0.739$, $p = 0.101$) 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 ($K_{mult} = 1.007$, $p = 0.0195$ and $K_{mult} = 1.02$, $p = 0.0235$, 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

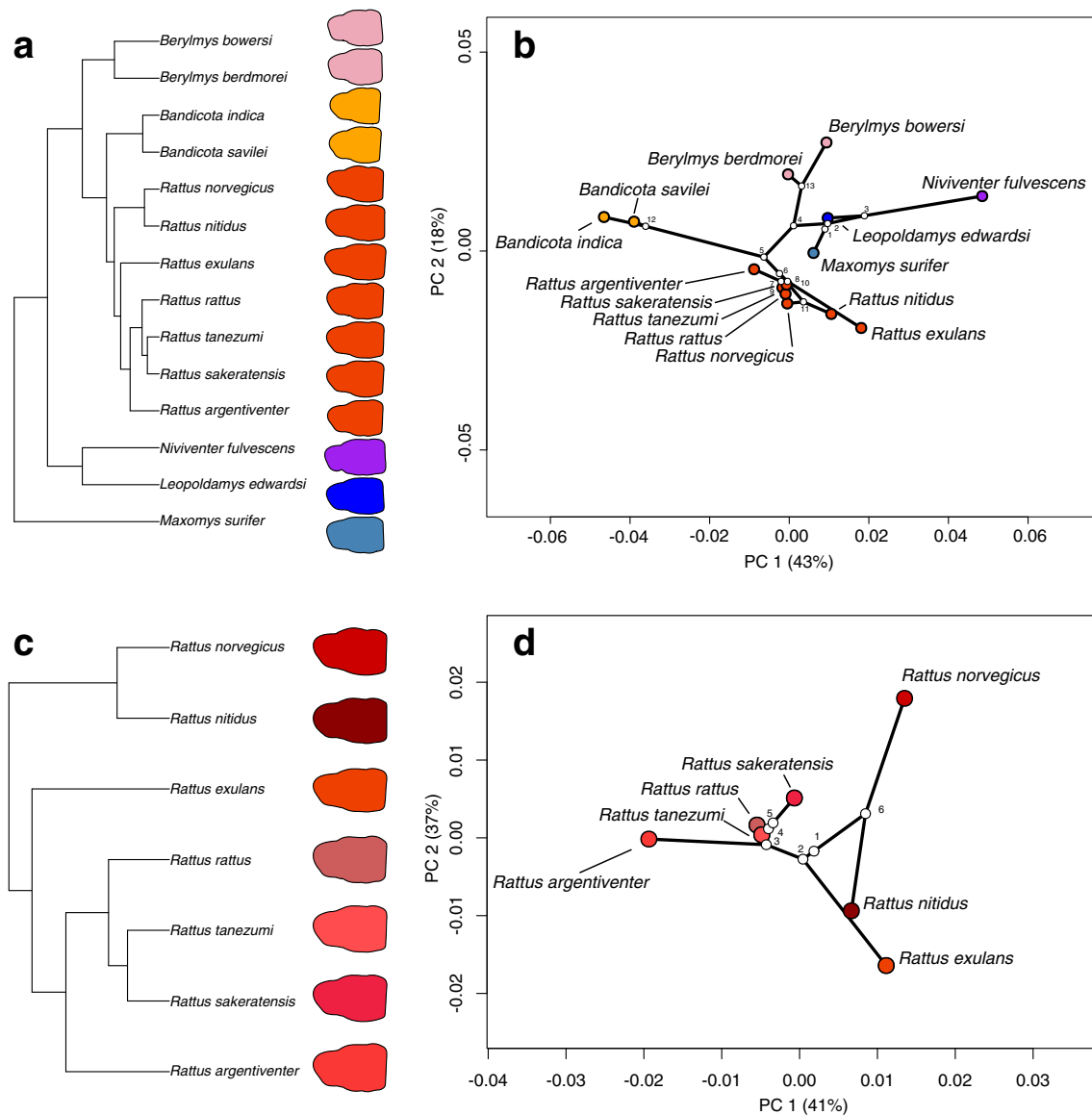


Fig. 3 Phylogenies mapped to morphospace: **a**, phylogeny of all species including multiple genera and the mean shape of each species with a different color for each genus; **b**, phylogeny of all species mapped to morphospace with colors corresponding to **a**; **c**, phylogeny of only

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 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.

Acknowledgements We would like to thank Pierre-Henri Fabre for providing the phylogeny for this study. The collection of specimens used was funded by the French ANR Biodiversity, grant ANR 07 BDIV 012 CERoPath project (www.ceropath.org), and by the French ANR CP&ES, grant ANR 11 CPEL 002 BiodivHealthSEA project (www.biodivhealthsea.org). We also thank Madoudou Garba and Gauthier Dobigny (CBGP-IRD) for providing additional specimens. We greatly thank all local communities and their leaders for permission and invaluable help during field trapping. Special thanks to the CERoPath and BiodivHealthSEA teams and the drivers for their invaluable help during fieldwork. We would also like to thank Maeve McMahon for help with manuscript editing and preparation.

Compliance with Ethical Standards All applicable international, national, and institutional guidelines for the care and use of animals were followed. Rodent species included in the study are neither on the CITES list, nor the Red List (IUCN). Animals were treated in accordance with the guidelines of the American Society of Mammalogists, and within the European Union legislation guidelines (Directive 86/609/EEC). Each trapping campaign was validated by the national, regional, and local health authorities. Approval notices for trapping and investigation of rodents were provided by the Ministry of Health Council of Medical Sciences, National Ethics Committee for Health Research (NHCHR) Lao PDR, number 51/NECHR, and by the Ethical Committee of Mahidol University, Bangkok, Thailand, number 0517.1116/661.

Voucher specimens are deposited at Institut des Sciences de l'Évolution. Reference collection tissues are hosted by the Faculty of Veterinary Technology, Kasetsart University, Bangkok, Thailand (curators: Anamika Kamchanabanthoeng / S. Morand).

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

- Adams DC (2014) A generalized K statistic for estimating phylogenetic signal from shape and other high-dimensional multivariate data. *Syst Biol* 63:685–697
- Adams DC, Otárola-Castillo E (2013) Geomorph: an R package for the collection and analysis of geometric morphometric shape data. *Methods Ecol Evol* 4:393–399
- Adams DC, Rohlf FJ, Slice D (2004) Geometric morphometrics: ten years of progress following the “revolution.” *Hystrix Ital J Zool* 71:5–16
- Aplin KP, Brown PR, Jacob J, Krebs CJ, Singleton GR (2003) Field methods for rodent studies in Asia and the Indo-Pacific: review of the major pest species. ACIAR Monograph Series No 100 Australian Centre for International Agricultural Research, Canberra, pp 121–223
- Aplin KP, Suzuki H, Chinen AA, Chesser RT, ten Have J, Donnellan SC, Austin J, Frost A, Gonzalez JP, Herbreteau V, Catzeffis F, Soubrier J, Fang Y-P, Robins J, Matisoo-Smith E, Bastos ADS, Maryanto I, Sinaga MH, Denys C, Van Den Bussche RA, Conroy C, Rowe K, Cooper A (2011) Multiple geographic origins of commensalism and complex dispersal history of black rats. *PLoS One* 6:e26357
- Armitage PL (1994) Unwelcome companions: ancient rats reviewed. *Antiquity* 68:231–240
- Armitage PL, West B, Steedman K (1984) New evidence of black rat in Roman London. *Lond Archaeol* 4:375–383
- Bastos AD, Nair D, Taylor PJ, Brettschneider H, Kirsten F, Mostert E, von Maltitz E, Lamb JM, van Hooft P, Belmain SR, Contrafatto G, Downs S, Chimimba CT (2011) Genetic monitoring detects an overlooked cryptic species and reveals the diversity and distribution of three invasive *Rattus* congeners in South Africa. *BMC Genet* 12:26
- Blomberg SP, Garland TJ, Ives AR (2003) Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* 57: 717–745
- Bookstein FL (1997) Landmark methods for forms without landmarks: morphometrics of group differences in outline shape. *Med Image Anal* 1:225–243
- Butler MA, King AA (2004) Phylogenetic comparative analysis: a modeling approach for adaptive evolution. *Am Nat* 164:683–695
- Capizzi D, Bertolino S, Mortelliti A (2014) Rating the rat: global patterns and research priorities in impacts and management of rodent pests. *Mammal Rev* 44:148–162
- Chaimanee Y, Jaeger J (2001) Evolution of *Rattus* (Mammalia, Rodentia) during the Plio-Pleistocene in Thailand. *Hist Biol* 15:181–191
- Chaval Y, Waengsothorn S, Morand S, Cosson JF, Claude J (2016) A new taxonomic toolkit for identification of two sympatric species of *Bandicota* (Rodentia: Muridae) from Mainland Southeast Asia. *Mammalia* 80:425–439
- Chingangbam DS, Laishram JM, Suzuki H (2015) Molecular phylogenetic characterization of common murine rodents from Manipur, northeast India. *Genes Genet Syst* 90:21–30
- Claude J (2008) *Morphometrics with R*. Springer, New York
- Claude J (2013) Log-Shape ratios, Procrustes superimposition, elliptic Fourier analysis: three worked examples in *R. hystrix*. *Ital Mammal* 24:94–102
- Conroy CJ, Rowe KC, Rowe KMC, Kamath PL, Aplin KP, Hui L, James DK, Moritz C, Patton JL (2013) Cryptic genetic diversity in *Rattus* of the San Francisco Bay region, California. *Biol Invasions* 15:741–758
- Cucchi T, Barnett R, Martinková N, Renaud S, Renvoisé E, Evin A, Sheridan A, Mainland I, Wickham-Jones C, Tougaard C, Quéré JP, Pascal M, Heckel G, O'Higgins P, Searle JB, Dobney KM (2014) The changing pace of insular life: 5000 years of

- microevolution in the Orkney vole (*Microtus arvalis orcadensis*). *Evolution* 68:2804–2820
- Cucchi T, Kovács ZE, Berthon R, Orth A, Bonhomme F, Evin A, Siaharsvie R, Darvish J, Bakhshaliyev V, Marro C (2013) On the trail of Neolithic mice and men towards Transcaucasia: zooarchaeological clues from Nakhchivan (Azerbaijan). *Biol J Linn Soc* 1084:917–928
- Cuenca-Bescós G, Straus LG, González Morales MR, García Pimienta JC (2009) The reconstruction of past environments through small mammals: from the Mousterian to the Bronze Age in El Mirón Cave (Cantabria Spain). *J Archaeol Sci* 36:947–955
- Dayan T, Simberloff D (1994) Character displacement, sexual dimorphism, and morphological variation among British and Irish mustelids. *Ecology* 75:1063–1073
- Dayan T, Simberloff D (1998) Size patterns among competitors: ecological character displacement and character release in mammals with special reference to island populations. *Mammal Rev* 28:99–124
- Dayan T, Simberloff D, Tchernov E, Yom-Tov Y (1989a) Inter- and intraspecific character displacement in mustelids. *Ecology* 70:1526–1539
- Dayan T, Simberloff D, Tchernov E, Yom-tov Y (1992) Canine carnassials: character displacement in the wolves, jackals and foxes of Israel. *Biol J Linn Soc* 45:315–331
- Dayan T, Tchernov E, Yom-tov Y, Simberloff D (1989b) Ecological character displacement in Saharo-Arabian *Vulpes*: outfoxing Bergmann's rule. *Oikos* 55:263–272
- Evin A, Cucchi T, Cardini A, Strand Vidarsdottir U, Larson G, Dobney K (2013) The long and winding road: identifying pig domestication through molar size and shape. *J Archaeol Sci* 40:735–743
- Fabre P, Hautier L, Dimitrov D, Douzery EJP (2012) A glimpse on the pattern of rodent diversification: a phylogenetic approach. *BMC Evol Biol* 12:1–19
- Felsenstein J (1973) Maximum-likelihood estimation of evolutionary trees from continuous characters. *Am J Hum Genet* 25:471–492
- Harmon LJ, Glor RE (2010) Poor statistical performance of the Mantel test in phylogenetic comparative analyses. *Evolution* 64:2173–2178
- Harmon LJ, Losos JB, Davies TJ, Gillespie RG, Gittleman JL, Jennings WB, Kozak KH, Mcpeek MA, Moreno-roark F, Near TJ, Purvis A, Ricklefs RE, Schluter D, Schulte II JA, Seehausen O, Sidlauskas BL, Torres-carvajal O, Weir JT, Mooers AØ (2010) Early bursts of body size and shape evolution are rare in comparative data. *Evolution* 64:2385–2396
- Harmon LJ, Weir JT, Brock CD, Glor RE, Challenger W (2008) GEIGER : investigating evolutionary radiations. *Bioinformatics* 24:129–131
- Hautier L, Fabre P-H, Michaux J (2009) Mandible shape and dwarfism in squirrels (Mammalia Rodentia): interaction of allometry and adaptation. *Naturwissenschaften* 96:725–730
- Klingenberg CP, Gidaszewski NA (2010) Testing and quantifying phylogenetic signals and homoplasy in morphometric data. *Syst Biol* 59:245–261
- Kosoy M, Khlyap L, Cosson J-F, Morand S (2015) Aboriginal and invasive rats of genus *Rattus* as hosts of infectious agents. *Vector-Borne Zoonotic Dis* 15:3–12
- Lack JB, Greene DU, Conroy CJ, Hamilton MJ, Braun JK, Mares MA, Van Den Bussche RA (2012) Invasion facilitates hybridization with introgression in the *Rattus rattus* species complex. *Mol Ecol* 21:3545–3561
- Lecompte E, Aplin KP, Denys C, Catzefflis F, Chades M, Chevret P (2008) Phylogeny and biogeography of African Murinae based on mitochondrial and nuclear gene sequences with a new tribal classification of the subfamily. *BMC Evol Biol* 8:199
- Mares MA (1975) South American mammal zoogeography: evidence from convergent evolution in desert rodents. *Proc Natl Acad Sci USA* 72:1702–1706
- Meiri S, Cooper N, Purvis A (2008) The island rule: made to be broken? *Proc Biol Sci* 275:141–148. <https://doi.org/10.1098/rspb.2007.1056>
- Meiri S, Dayan T, Aviv T (2003) On the validity of Bergmann's rule. *J Biogeogr* 30:331–351
- Meiri S, Raia P, Phillimore AB (2011) Slaying dragons: limited evidence for unusual body size evolution on islands. *J Biogeogr* 38:89–100
- Meloro C, Raia P (2010) Cats and dogs down the tree: the tempo and mode of evolution in the lower carnassial of fossil and living Carnivora. *Evol Biol* 37:177–186
- Millien V, Damuth J (2004) Climate change and size evolution in an island rodent species: new perspectives on the island rule. *Evolution* 58:1353–1360
- Millien V, Lyons SK, Olson L, Smith FA, Wilson AB, Yom-Tov Y (2006) Ecotypic variation in the context of global climate change: revisiting the rules. *Ecol Lett* 9:853–869
- Mitteroecker P, Gunz P, Bernhard M, Schaefer K, Bookstein FL (2004) Comparison of cranial ontogenetic trajectories among great apes and humans. *J Hum Evol* 46:679–697
- Morand S, Bordes F, Chen H-W, Claude J, Cosson J-F, Galan M, Czirjak GA, Greenwood AD, Latinne A, Michaux J, Ribas A (2013) Global parasite and *Rattus* rodent invasions: the consequences for rodent-borne diseases. *Integr Zool* 10:409–423
- Morand S, Bordes F, Blasdell K, Pilosof S, Cornu J-F, Chaisiri K, Chaval Y, Cosson J-F, Claude J, Feyfant T, Herbreteau V, Dupuy S, Tran A (2015) Assessing the distribution of disease-bearing rodents in human-modified tropical landscapes. *J Applied Ecol* 52:784–794
- Musser GG (1972) Identities of taxa associated with *Rattus rattus* (Rodentia, Muridae) of Sumba Island, Indonesia. *J Mammal* 53:861–865
- Musser GG (1981) The giant rats of Flores and its relatives east of Borneo and Bali. *Bull Am Mus Nat Hist* 169:67–176
- Musser GG (1986) Sundaic *Rattus*: definitions of *Rattus baluensis* and *Rattus korinchi*. *Am Mus Novitates* 2862:1–24
- Musser GG, Newcomb C (1983) Malaysian murids and the giant rat of Sumatra. *Bull Am Mus Nat Hist* 174:327–598
- Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GA, Kent J (2000) Biodiversity hotspots for conservation priorities. *Nature* 403:853–858
- Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H (2017) vegan: Community ecology package. R package version 2.4-5. <https://CRAN.R-project.org/package=vegan>
- Pagel M (2000) Inferring the historical patterns of biological evolution. *Nature* 401:877–884
- Pagès M, Chaval Y, Herbreteau V, Waengsothorn S, Cosson J-F, Hugot J-P, Morand S, Michaux J (2010) Revisiting the taxonomy of the Rattini tribe: a phylogeny-based delimitation of species boundaries. *BMC Evol Biol* 10:184
- Pagès M, Corbet G, Orth A, Volobouev V, Michaux J, Catzefflis F (2011) Morphological chromosomal and genic differences between sympatric *Rattus rattus* and *Rattus satarae* in South India. *J Mammal* 92:659–670
- Paradis E, Claude J, Strimmer K (2004) APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* 20:289–290
- Pearch MJ, Bumrungsri S, Schwenninger J, Ward DJ, Harrison DL (2013) A review of the Cainozoic small mammal fauna of Thailand with new records (Chiroptera ; Scandentia ; Eulipotyphla) from the late Pleistocene. *Cainozoic Res* 10:59–98
- Pergams ORW, Byrn D, Lee KLY, Jackson R (2015) Rapid morphological change in black rats (*Rattus rattus*) after an island introduction. *PeerJ* 3:e812
- Pimm SL (1989) Theories of predicting success and impact of introduced species. In: Drake JA, Mooney HA, di Castri F, Groves RH, Kruger FJ, Rejmánek M, Williamson M (eds) *Biological Invasions: A Global Perspective*. John Wiley & Sons, New York, pp 351–367

- Piras P, Claude J, Ventura J, Cubo J, Pierre U, Paris C (2012) Ecological and functional correlates of molar shape variation in European populations of *Arvicola* (Arvicolinae Rodentia). *Zool Anzeiger* 251: 335–343
- Prendergast ME, Buckley M, Crowther A, Frantz L, Eager HM, Lebrasseur O, Hutterer R, Hulme-Beaman A, Van Neer W, Douka K, Veall M-A, Morales EMQ, Schuenemann VJ, Reiter E, Allen R, Dimopoulos EA, Helm RM, Shipton C, Mwebi O, Denys C, Horton M, Wynne-Jones S, Fleisher J, Radimilahy C, Wright H, Searle JB, Krause J, Larson G, Boivin NL (2017) Reconstructing Asian faunal introductions to eastern Africa from multi-proxy biomolecular and archaeological datasets. *PLoS One* 12(8): e0182565
- R Core Team (2016) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>
- Renaud S, Pantalacci S, Auffray JC (2011) Differential evolvability along lines of least resistance of upper and lower molars in island house mice. *PLoS One* 6(5): e18951
- Revell LJ (2012) phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol Evol* 3:217–223
- Robins JH, Hingston M, Matisoo-Smith E, Ross HA (2007) Identifying *Rattus* species using mitochondrial DNA. *Mol Ecol Notes* 7:717–729
- Robins JH, Tintinger V, Aplin KP, Hingston M, Matisoo-Smith E, Penny D, Lavery SD (2014) Phylogenetic species identification in *Rattus* highlights rapid radiation and morphological similarity of New Guinean species. *PLoS One* 9(5): e98002
- Rohlf FJ (2005) TpsRelw 1.41— Thin plate Spline Relative Warp, 1.41. ed. Ecology & Evolution, State University at Stony Brook, New York
- Rohlf FJ (2008) TpsDig 2.12— Thin plate Spline Digitization 2.12. ed. Ecology & Evolution, State University at Stony Brook, New York
- Rowe KC, Aplin KP, Baverstock PR, Moritz C (2011) Recent and rapid speciation with limited morphological disparity in the genus *Rattus*. *Syst Biol* 60:188–203
- Rowe KC, Reno ML, Richmond DM, Adkins RM, Stepan SJ (2008) Pliocene colonization and adaptive radiations in Australia and New Guinea (Sahul): multilocus systematics of the old endemic rodents (Muroidea: Murinae). *Mol Phylogenet Evol* 47:84–101
- Serrat MA, Williams RM, Farnum CE (2010) Exercise mitigates the stunting effect of cold temperature on limb elongation in mice by increasing solute delivery to the growth plate. *J Appl Physiol* 109: 1869–1879
- Shiels AB, Flores CA, Khamsing A, Krushelnicky PD, Mosher SM, Drake DR (2012) Dietary niche differentiation among three species of invasive rodents (*Rattus rattus* *R. exulans* *Mus musculus*). *Biol Invasions* 15:1037–1048
- Stoetzel E, Marion L, Nespoulet R, Abdeljalil M, Hajraoui E, Denys C (2011) Taphonomy and palaeoecology of the late Pleistocene to middle Holocene small mammal succession of El Harhoura 2 cave (Rabat-Témara Morocco). *J Hum Evol* 60:1–33
- Taylor JM, Calaby JH, Van Deusen HM (1982) A revision of the genus *Rattus* (Rodentia Muridae) in the New Guinean region. *Bull Am Mus Nat Hist* 173:179–336
- Tukey JW (1949) Comparing individual means in the analysis of variance. *Biometrics* 5:99–114
- Weissbrod L, Marshall FB, Valla FR, Khalaily H, Bar-oz G, Auffray J, Vigne J-D, Cucchi T (2017) Origins of house mice in ecological niches created by settled hunter-gatherers in the Levant 15000 y ago. *Proc Natl Acad Sci USA* 114:1–6
- Wood AE (1965) Grades and clades among rodents. *Evolution* 19:115–130
- Yom-tov Y, Yom-tov S (2004) Climatic change and body size in two species of Japanese rodents. *Biol J Linn Soc* 82:263–267