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Title: The impact of digging on the evolution of the rodent mandible

Running Title: Rodent mandible morphology

Authors: Andrew F. McIntosh¹, Philip G. Cox²

Affiliations:

¹Department of Science, School of Arts and Sciences, Abraham Baldwin Agricultural College,
Tifton, GA 31793, USA

²Department of Archaeology and Hull York Medical School, University of York, Heslington,
York, YO10 5DD, UK

Abstract

There are two main (but not mutually exclusive) methods by which subterranean rodents construct burrows: chisel-tooth digging, where large incisors are used to dig through soil; and scratch digging, where forelimbs and claws are used to dig instead of incisors. A previous study by the authors showed that upper incisors of chisel-tooth diggers were better adapted to dig but the overall cranial morphology within the rodent sample was not significantly different. This study analyzed the lower incisors and mandibles of the specimens used in the previous study to show the impact of chisel-tooth digging on the rodent mandible. We compared lower incisors and mandibular shape of chisel-tooth digging rodents with non-chisel-tooth digging rodents to see if there were morphological differences between the two groups. The shape of incisors was quantified using incisor radius of curvature and second moment of area. Mandibular shape was quantified using landmark based geometric morphometrics. We found that lower incisor shape was strongly influenced by digging group using a Generalized Phylogenetic ANCOVA (analysis of covariance).

A phylogenetic Procrustes ANOVA (analysis of variance) showed that mandibular shape of chisel-tooth digging rodents was also significantly different from non-chisel-tooth digging rodents. The phylogenetic signal of incisor radius of curvature was weak, whereas that of incisor second moment of area and mandibular shape was significant. This is despite the analyses revealing significant differences in the shape of both mandibles and incisors between digging groups. In conclusion, we showed that although the mandible and incisor of rodents is influenced by function, there is also a degree of phylogenetic affinity that shapes the rodent mandibular apparatus.

Keywords

Rodent mandibular morphology; geometric morphometrics; phylogenetic comparative methods

Research Highlights

Our results show that the shape of the rodent mandible and its accompanying incisor is adapted for digging in chisel-tooth digging rodents. However, evolutionary integration of the incisor and mandible is weak within the rodent sample. We can infer from this that the rodent incisor and mandible have evolved separately, perhaps in a modular process.

1. INTRODUCTION

Subterranean rodents spend most of their lives underground and as such frequently show specialized morphological adaptations for burrowing (for review see Stein, 2000). There are two main methods of burrow construction within subterranean rodents: chisel-tooth digging, where rodents use their incisors to excavate soil; and scratch digging, where rodents use their claws and enlarged forelimbs to dig (Hildebrand, 1985; Lessa & Thaeler, 1989). These are not necessarily mutually exclusive activities with some rodent species using both incisors and claws in a complementary fashion. Rodent incisors are labially covered with hard enamel and so rodents that have adapted their digging apparatus to use incisors can potentially exploit harder soils. In contrast, scratch digging tends to be restricted to softer soils as rodent claws are made of keratin, which may experience excessive wear and cracks in harder soils (Lessa & Thaeler, 1989).

A number of craniodental traits associated with chisel-tooth digging rodents have been documented. These include more procumbent incisors, wider crania, shorter rostra and larger temporal fossae, compared to non-tooth digging rodents (Landry, 1957; Agrawal, 1967; Lessa, 1990; Samuels & Van Valkenburgh, 2009; Gomes Rodrigues et al., 2016). These traits are thought to facilitate wider gapes and larger bite forces, both of which are essential for chisel-tooth digging (McIntosh & Cox, 2016a).

Incisor procumbency in subterranean rodents has been extensively researched due to its interspecific variability and correlation with chisel-tooth digging (e.g. Landry, 1957; Lessa and Thaeler, 1989; Vassallo, 1998; Korth & Rybczynski, 2003; Mora et al., 2003; Becerra et al., 2013; Echeverría et al., 2017). Incisor procumbency describes how far forward, and at what angle, the

incisor protrudes from the mouth. Procumbency is predominantly controlled by the radius of curvature (RoC) of the incisor (for further discussion see Landry, 1957). McIntosh & Cox (2016b) showed that the upper incisor of chisel-tooth digging rodents have a larger RoC for their size compared to non-tooth diggers. This enlargement of incisor radius of curvature, coupled with a reduction in rostral length (McIntosh & Cox, 2016a), explains why the root of the upper incisor of chisel-tooth diggers is positioned further back into the skull, a trait seen especially in bathyergids (Ellerman, 1940; Stein, 2000). A larger radius of curvature provides space for a greater extent of periodontal ligament between the tooth and alveolus and thus serves to dissipate the high forces generated at the incisor tip during digging (Becerra et al., 2012). It may also increase upper incisor procumbency for a more favorable angle of attack when excavating vertical burrow walls (Landry, 1957; Lessa, 1990).

Lower incisor procumbency, unlike that of the upper incisors, does not correlate with digging method within subterranean rodents (Landry, 1957; Stein, 2000), which could lead to the assumption that chisel-tooth digging rodents dig with their more procumbent upper incisors. However, a recent *in vivo* kinematic study of the chisel-tooth digging mole-rat *Fukomys micklemi* showed that, in fact, both the upper and lower incisors are used in concert with both jaw adductor and head depressor muscles (Van Wassenbergh et al., 2017). Assuming all chisel-tooth diggers use both their upper and lower incisors to dig, we can hypothesise that the lower incisors of chisel-tooth diggers have adapted to dig in a similar manner to the upper incisors. This study will measure two traits of the lower incisor to test this hypothesis: radius of curvature and second moment of area (following McIntosh & Cox, 2016b).

A previous study on craniodental morphology in subterranean rodents showed that the cranial shape of chisel-tooth diggers grouped tightly in morphospace (Samuels & Van Valkenburgh, 2009). This study did not consider the phylogenetic relatedness of the species in question. A similar study by the authors of this paper confirmed the original study's finding. However, when the analysis was performed in a phylogenetic context, the grouping of chisel-tooth diggers was non-significant (McIntosh & Cox, 2016b). Interspecific analyses such as these must take phylogenetic affinity into account due to the non-independence of the data points (Felsenstein, 1985). From our previous study, we tentatively concluded that, when phylogeny is considered, the overall geometry of the cranium within rodents is not impacted by choice of digging method (although a wider sample will need to be tested to give more confidence in this interpretation). However, there is no doubt that changes within the cranium can improve chisel-tooth digging performance (see above references for cranial characteristics in chisel-tooth digging rodents). The cranium within vertebrates houses the brain and other sensory organs and as such is likely to be more evolutionarily conservative relative to the mandible (e.g. Linde-Medina et al., 2016). The impact of digging on the evolution of the mandible however has not been tested. The mandible is a single bone that primarily functions to facilitate mastication and thus possesses attachment sites for the jaw closing muscles. Therefore, it is thought that the primary influence on the shape of the mandible is the power and motion of jaw movement, provided by muscles of mastication. Chisel-tooth diggers have relatively large masticatory muscles and bite force for their size (Van Daele et al., 2009; Cox & Faulkes, 2014). We hypothesize that these large muscle attachments, and the need to generate large bite forces, will significantly influence the shape of the mandible in chisel-tooth digging rodents.

In addition to the above analyses, we will also investigate the relationship between incisor shape and mandibular morphology. The rodent lower incisor fills a large proportion of the internal space in the mandibular bone. Indeed, in some cases the incisor root extends as far as the mandibular condyle (Stein, 2000). Thus, we hypothesise that lower incisor morphology will affect how the rodent mandible is shaped. Along with testing for differences in lower incisor and mandible morphology, we will test for covariation between incisor and mandibular morphology to assess the level of evolutionary integration of these structures, which may influence their overall shape.

2. MATERIALS AND METHODS

This study analysed 54 adult hemi-mandibles from a diverse group of rodents representing 20 genera and 10 families: Bathyergidae, Caviidae, Cricetidae, Dipodidae, Erethizontidae, Geomyidae, Muridae, Octodontidae, Sciuridae and Spalacidae (Table 1). These mandibles are from the same specimens that were used in a previous study on craniodental morphology (McIntosh & Cox, 2016b). The specimens were scanned on an X-Tek Metris micro-CT scanner at the University of Hull (Medical and Biological Engineering Research Group). The resulting scans had isometric voxels with dimensions ranging between 0.01 and 0.07 mm. All image data (original microCT-scans or surface reconstructions derived from them) are available from www.morphosource.org. DOI numbers are given alongside specimen and scanning details in supplementary datafile S1.

Mandible reconstructions and lower incisor segmentations were created from micro-CT scans using Avizo 8.0 (FEI, Hillsboro, OR). Radius of curvature was derived using Heron's formula from a circle fitted to three points along the dorsal midline of the incisor surface (at the apex, tip

and most dorsal point of the curve) following the method used by McIntosh & Cox (2016b). Second moment of area (SMA), a geometric measurement that indicates resistance to bending of a cross section of the lower incisor, was measured using the BoneJ plugin (Doube et al., 2010) for ImageJ (Schneider et al., 2012). Second moment of area is a good indicator of structural strength (Alexander, 1983) and so is likely to correlate with mechanically demanding activities such as chisel-tooth digging.

A large range of body masses was represented within the study (*Phyllotis* can be as small as 12 g, whereas *Bathyergus* can grow up to 2 kg; Nowak, 1999) and so cranial length was included in all regression analyses to account for size. Incisor morphology variables and cranial length were logged in all analyses due to size differences and to linearize variables for statistical procedures.

All bivariate statistical analyses used the phylogenetic generalized linear model (PGLM) to account for phylogenetic dependence within our sample (Felsenstein, 1989; Grafen, 1989). Phylogenetic ANCOVA models using PGLM were fitted to genus means of the sample using the nlme (Pinheiro et al., 2016) and ape (Paradis et al., 2004) packages in R. The ANCOVAs were used to test the differences in incisor RoC and SMA whilst controlling for size, between chisel-tooth diggers and non-tooth diggers (including non-fossorial rodents). The phylogeny used in all analyses was modified from Fabre et al. (2012), with branch lengths in millions of years (Figure. 1).

Phylogenetic signal quantifies the expected covariation of species traits under Brownian motion in a phylogeny relative to the observed traits (for review see Blomberg & Garland, 2002). This

allows us to quantify the strength of phylogenetic constraints on the morphology. Pagel's λ (Pagel, 1999) was used to estimate the phylogenetic signal in the data. λ is a scaling parameter that measures the correlation of traits relative to expected correlation under a Brownian motion model of evolution. Normally, λ ranges from zero (no phylogenetic signal and data are equivalent to a 'star' phylogeny) to one (data consistent with selected phylogenetic tree under a Brownian motion model of evolution) or beyond (the evolutionary process is more orderly than Brownian motion). λ and PGLM regressions in this study are quantified simultaneously using the method proposed by Revell (2010).

The morphology of a hemi-mandible from each specimen was quantified using 3D-landmark coordinates. Left hemi-mandibles made up most of the sample. A small number of right hemi-mandibles were also quantified (due to damage on the left) and reflected before any initial shape analysis. The *Ctenomys* specimen was not included in this part of the study due to extensive damage of the whole mandible. Each landmark represented homologous anatomical points between specimens. Mandibular surfaces were reconstructed from microCT-scans and 14 landmarks were recorded from each surface using Avizo (Figure. 2 and Table A1). From this landmark data, variation in the shape of the mandible was analysed with geometric morphometrics (for review see O'Higgins, 2000). The landmark co-ordinates were subjected to the Procrustes method of generalized least squares (GLS) superimposition (Rohlf & Slice, 1990). This process involves translating, scaling and rotating the coordinates to minimize the differences between each specimen. A principal component analysis (PCA) of genus-averaged Procrustes coordinates shows the largest shape variation between genera. Surface warps of the extreme ends of the principal components axes were also included to visualise the shape variation within the data.

185
186 As Pagel's λ cannot be estimated accurately for multivariate data (Adams, 2014a), phylogenetic
187 signal in the data was quantified by calculating the κ statistic (Blomberg et al., 2003), generalized
188 to accept multivariate shape data (Adams, 2014a). Although the κ statistic and λ statistic are
189 derived differently (κ is a scaled ratio of variance and λ is a scaling metric) their outcomes are
190 normally similar, that is <1 implies data have less phylogenetic signal than expected under
191 Brownian motion and >1 implies data have more phylogenetic signal than expected under
192 Brownian motion.

193
194 A phylogenetic Procrustes ANOVA under a Brownian motion model of evolution (Adams, 2014b)
195 was performed on Procrustes coordinates to test for differences between mandible shapes of chisel-
196 tooth diggers and non-tooth diggers. Procrustes sum of squares (SS) is measured based on the SS
197 of Procrustes distances among specimens (see Goodall, 1991), which is equivalent to a distance-
198 based ANOVA design (Anderson, 2001). GLS superimposition, phylogenetic signal testing,
199 principal components analysis, ANOVAs and surface warps were processed using the geomorph
200 package in R (Adams & Otárola-Castillo, 2013).

201
202 To measure evolutionary covariation between lower incisor and mandibular morphology, a
203 phylogenetic partial least square analysis (pPLS) was performed (Adams & Felice, 2014)
204 following the method in McIntosh & Cox, 2016b. Mandibular morphology in this analysis is
205 represented by Procrustes coordinates. As the Procrustes procedure removes isometric scaling but
206 retains allometric effects (Drake, 2011), we performed a multivariate regression of Procrustes
207 coordinates on log-transformed mandibular centroid size in a phylogenetic context (Adams,

2014b). Size was also removed from incisor variables using cranial length as a size surrogate following Revell, 2009. These size corrected variables were used to perform the pPLS in the geomorph package.

3. RESULTS

The relationship between rodent lower incisor RoC and digging method is represented by Figure 3. A generalized phylogenetic ANCOVA revealed that chisel-tooth digging rodents have a significantly larger lower incisor RoC ($P < 0.01$) compared to the rest of the sample, after accounting for size and phylogenetic affinity. A phylogenetic signal for this analysis was significant, with a λ value less than zero ($P < 0.01$). Chisel-tooth digging rodents also have a significantly larger lower incisor SMA ($P < 0.01$), which is represented by Figure 4. Phylogenetic signal was also present and significant in this analysis, with a λ value of 1.06 ($P < 0.01$). The SMA analysis also showed that *Bathyergus* and *Geomys* (both scratch digging subterranean rodents) overlap with chisel-tooth digging rodents. In fact, *Bathyergus* has the largest SMA of all the rodents, after controlling for size.

Mandibular shape variation in morphospace is represented by Figure 5. The eigenvalues and factor loadings for the first 10 principal components are given in Table A2 and Table A3. A phylogenetically informed Procrustes ANOVA of the mandibular Procrustes coordinates showed that chisel-tooth digging rodents differed significantly from the other rodent mandibles ($F = 7.630$; $R^2 = 0.310$; $P = 0.016$). However, the mandibular shape coordinates also revealed a significant phylogenetic signal, with a κ value of 0.58 ($P < 0.01$). Variations of shape associated with PC1 are changes in the mandibular body, the angular process and diastema length. Negative scores on PC1

correlate with taller mandibular bodies, less posteriorly extended angular processes and shorter diastemata. Positive PC1 scores are associated with shorter mandibular bodies, longer diastemata and more posteriorly extended angular processes. Although PC1 represents the most variation of the sample, it seems to represent a more phylogenetic structure within the sample (discussed further below), whereas PC2 represents most of the variation that accounts for the difference between the two rodent groups. As shown by the warps at the extreme ends of PC2 (Figure 5), the main difference in mandibular shape appears to be associated with the mandibular coronoid process and condyle. On the negative end of PC2, where the majority of non-tooth digging rodents are situated, the coronoid process is reduced in height relative to the condyle. On the positive end of PC2, where the chisel-tooth diggers are positioned, the coronoid process is increased in height relative to the condyle.

Multivariate regression of mandible shape on log centroid size was non-significant ($F = 1.769$; $P = 0.108$). Therefore, it was not necessary to account for allometric shape changes in the analyses. Also, there was no covariation found between size corrected incisor variables and mandibular shape variables, as the phylogenetically informed partial least squares analysis was non-significant ($R = 0.602$; $P = 0.345$).

4. DISCUSSION

The results of this study show significant differences in the lower incisor morphology of chisel-tooth digging and non-tooth digging rodents. Figure 3 shows that the RoC of lower incisors is relatively larger in chisel-tooth digging rodents. Our previous study showed a similar relationship between RoC of the upper incisors of chisel-tooth diggers and digging method (McIntosh & Cox,

2016b). As mentioned in the introduction, incisors with a larger surface area will benefit from a greater extent of periodontal ligament to dissipate excessive forces during tooth digging (Moxham & Berkovitz, 1995; van Driel et al., 2000; Becerra et al., 2012). Chisel-tooth diggers have clearly evolved enlarged upper and lower incisors. This result adds to the evidence that chisel-tooth digging rodents use both their upper and lower incisors, operated by their enlarged masticatory, neck and back muscles, to dig (Van Wassenburgh et al., 2017).

The SMA of lower incisors was also found to be significantly larger in chisel-tooth diggers (Figure 4). This significance was not found in the previous study looking at the upper incisors of the same sample (McIntosh & Cox, 2016b). The previous study found that upper incisor SMA was associated with the fossorial rodents in the sample and not just chisel-tooth diggers i.e. both scratch and chisel-tooth diggers. Although an ANCOVA could not be implemented in the previous study due to a significant interaction between slopes of the digging groups, in our current study, there is no interaction between slopes and therefore the lower incisors of chisel-tooth diggers are more resistant to bending (i.e., have a larger SMA). This likely indicates higher bite forces, as the SMA of lower incisors is strongly correlated with bite force (Freeman & Leman, 2008), which may represent an adaptation to digging. However, it should be noted that the increase in SMA, and hence bite force, could also be a dietary adaptation to enable the gnawing of hard food items. For instance, it can be seen from Figure 4 that many of the non-tooth digging genera that fall within the range of the chisel-tooth diggers are able to generate high bite forces (Freeman & Lemen, 2008) in order to incorporate hard food items, such as geophytes (*Bathyergus*, *Ctenomys*, *Geomys*) or nuts (*Sciurus*), into their diet (Wilson et al., 2016).

The analyses of lower incisor RoC and SMA produced different phylogenetic signal values. Pagel's λ calculated from the incisor RoC analysis was less than zero. A negative phylogenetic signal can arise when closely related taxa are more different in a given trait than a randomly chosen pair of taxa (Diniz-Filho et al., 2012). This negative signal can be explained by the fact that chisel-tooth digging has evolved independently at least 4 times along the phylogeny in our sample (within the bathyergids, geomyids [*Thomomys*], spalacids and octodontids [*Spalacopus*]; see Figure 1). As incisor RoC is significantly larger in chisel-tooth diggers, this trait may have evolved from recent divergences within the phylogeny.

In contrast, incisor SMA had a high phylogenetic signal. A high value of phylogenetic signal is normally associated with traits that have evolved early along the phylogenetic tree (see Revell et al., 2008 for review). As such it could be inferred that the rodents in our sample had their incisor SMA fixed early in their evolutionary timescale. However, this is probably not the case given that chisel-tooth digging has evolved independently along the phylogeny at least 4 times in our sample (see above). Many of the non-tooth digging rodents in our sample also had high incisor SMA values (see Figure 4), possibly related to diet as mentioned above. This could explain why the phylogenetic signal was high compared with the incisor RoC, which did not have the same overlap between tooth digging and non-tooth digging rodents (see Figure 3). Although calculating the phylogenetic signal is useful to show if there is phylogenetic affinity within the sample, concentrating on the strength of the signal may be misleading. Indeed, calculation of phylogenetic signal using fewer than 20 data points may inflate type II errors (Münkemüller et al., 2012). There are many variables that need to be considered when inferring character evolution, such as sample size, accuracy of phylogenetic tree, model selection of evolutionary process and rate. These

variables are outside the scope of this study. However, expanding our data set to answer other questions on the evolutionary history of rodents will be a fruitful avenue of study.

The phylogenetic ANOVA shows significant differences in mandible shape (Figure 5) between tooth diggers and non-tooth diggers that were not seen in the cranium (McIntosh & Cox, 2016b). This result confirms our original hypothesis that the shape of the mandible has been strongly influenced by chisel-tooth digging. In the mandibular morphospace (Figure 5), PC2 separates the two groups and represents large variation in the morphology of the coronoid process. Chisel-tooth diggers have enlarged coronoid processes, which provide a greater surface area for insertion of the temporalis muscles. These enlarged muscle attachments on the mandibles of chisel-tooth diggers along with larger SMAs and ROCs of the lower incisors provide strong evidence that the mandible and its incisor has been adapted to dig in hard soils that would require a higher bite force. It should be noted, however, that not all morphological variation in the mandible can be related to digging behaviour. Shape changes along PC1 are largely related to differences in the robustness of the mandible (e.g., mandibular body, angular process, and diastema), which might be expected to correlate with digging method, but in fact do not separate chisel-tooth diggers from other rodents. Rather, it seems that PC1 reveals phylogenetic signal within the sample (also shown by the significant Blomberg's κ value), with squirrel- and mouse-related taxa associated with negative PC1 scores and the Ctenohystrica (guinea pig-related rodents) associated with positive PC1 scores (Figure 5). This phylogenetic division of taxa across the morphospace is perhaps not surprising as it follows Tullberg's (1899) classification of rodents into Sciurognatha and Hystricognatha based on the morphology of the mandible, although, more recently, it has been shown that such a binary division masks a much greater range of variation in mandibular morphology within rodents

(Hautier et al., 2011). Nonetheless, these results demonstrate how the rodent mandible is shaped by both function and ancestral history and are a reminder that phylogeny must always be considered in functional morphological studies containing an inter-specific sample.

Despite incisor and mandible morphology showing significant differences between chisel-tooth and non-tooth digging rodents, there was no covariation found between the lower incisor and mandible. Incorporating the phylogeny into a PLS analysis shows how the rodent incisor and mandible has evolved along a tree (Klingenberg and Marugán-Lobón, 2013). This result shows that the evolution of these two structures is not consistent within rodents. In our previous study, we found that the crania and upper incisors of the same specimens were also not covarying through time (McIntosh & Cox, 2016b). Our results provide evidence towards the hypothesis that rodent incisors and cranio-mandibular morphology are not evolving in the same direction and are in fact separate modules (for review see Klingenberg, 2014), which is complementary to previous work showing weak levels of integration across the rodent mandible overall (Zelditch et al., 2008). This evolutionary independence has been proposed to allow structures to rapidly evolve in response to environmental pressures (Wagner & Altenberg, 1996; Kirschner & Gerhart, 1998) and is perhaps why chisel-tooth digging has been able to evolve independently in several rodent families.

In conclusion, this study found that the mandible and lower incisor show significant morphological differences in chisel-tooth digging rodents, probably to increase bite force and gape (McIntosh & Cox, 2016a). This is in contrast to chisel-tooth digging crania of the same specimens, which were not found to be significantly different from non-tooth digging rodents (McIntosh & Cox, 2016b). This study has therefore provided strong evidence that the mandible is more adaptable towards

selective pressures compared with the cranium. As a single bone that primarily is used for muscle attachment, any changes within the mandible are unlikely to affect other systems within the craniomandibular apparatus. The cranium however contains multiple bones and houses the brain and other sensory organs, along with the attachment areas of muscles of mastication, and is therefore more evolutionary conservative compared with the more labile mandible.

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Conflict of Interest

The authors have no conflict of interest to declare.

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Tables

Table 1 List of genera analysed including number of specimens (N) and digging mode.

Subterranean rodent genera are in bold. Terrestrial rodents are not assigned a digging mode.

Family	Genus number	Genus	N	Primary Digging Mode
Bathyergidae	4	<i>Bathyergus</i>	10	Scratch
	3	<i>Cryptomys</i>	1	Chisel-tooth
	6	<i>Fukomys</i>	2	Chisel-tooth
	5	<i>Georychus</i>	2	Chisel-tooth
	2	<i>Heliophobius</i>	8	Chisel-tooth
	7	<i>Heterocephalus</i>	3	Chisel-tooth
Caviidae	9	<i>Cavia</i>	2	
Cricetidae	15	<i>Phyllotis</i>	1	
Ctenomyidae	12	<i>Ctenomys</i>	1	Scratch*

Dipodidae	20	<i>Dipus</i>	1	
Erethizontidae	8	<i>Erethizon</i>	1	
Geomyidae	13	<i>Geomys</i>	1	Scratch
	14	<i>Thomomys</i>	3	Chisel-tooth
Muridae	16	<i>Rattus</i>	2	
Octodontidae	11	<i>Octodon</i>	1	Scratch
	10	<i>Spalacopus</i>	1	Chisel-tooth
Spalacidae	18	<i>Cannomys</i>	1	Chisel-tooth
	17	<i>Rhizomys</i>	3	Chisel-tooth
	19	<i>Tachyoryctes</i>	3	Chisel-tooth
Sciuridae	1	<i>Sciurus</i>	7	

*The genus *Ctenomys* contains both scratch digging and chisel-tooth digging species. The specimen used in this analysis was an individual of the species *Ctenomys opimus* which is known to be a scratch digger (Eisenberg & Redford, 1992).

Figure Legends

Figure 1. Phylogeny of rodent genera used in this study, modified from Fabre et al. 2012.

Numbers correspond to Table 1 and Figures 3-5. Chisel-tooth digging genera are shown in blue, non-chisel-tooth digging genera are shown in red.

Figure 2. Landmark configuration represented on *Bathyergus suillus* in lateral (top) and medial (bottom) view (see Table A1 for corresponding landmark numbers and descriptions).

Figure 3. Phylogenetic ANCOVA representing the relationship between cranial length and lower incisor RoC for chisel-tooth digging genera (diamond points and dashed regression line) and non-tooth-digging genera (circular points and full regression line). Genus numbers given in Figure 1 and Table 1.

Figure 4. Phylogenetic ANCOVA representing the relationship between cranial length and lower incisor SMA for chisel-tooth digging genera (diamond points and dashed regression line) and non-tooth-digging genera (circular points and full regression line). Genus numbers given in Figure 1 and Table 1.

Figure 5. Principal components analysis (PCA) with associated virtual deformations representing mandibular shape variation at the extreme ends of PC1 and PC2. Genus numbers given in Figure 1 and Table 1. Chisel-tooth digging genera are shown as diamond points, non-chisel-tooth digging genera are shown as circular points. Note that no landmarks were placed on the incisors, so the form of the incisor in the warped surfaces are extrapolations based on mandibular shape alone and should be treated with caution.

Appendices

Table A1 Anatomical description of landmark points displayed on Figure 2

#	Landmark description
1	Dorsalmost point on incisal alveolar margin
2	Ventralmost point on incisor alveolar margin
3	Anteriormost point on dorsal symphysis
4	Ventralmost point on dorsal margin of diastema
5	Anteriormost point on alveolar margin of cheek teeth
6	Posteriormost point on alveolar margin of cheek teeth
7	Tip of coronoid process
8	Posteriormost point on condyle
9	Anteriormost point on curve between condyle and angle
10	Posterior tip of angular process
11	Anteriormost point of masseteric fossa
12	Anteriormost point on condyle
13	Ventralmost point on curve between condyle and coronoid
14	Inferiormost point on ventral mandibular margin

557 Table A2 Eigenvalues on first 10 principal component axes

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Principal Component Axis	Eigenvalues
1	37.58
2	17.65
3	11.35
4	7.20
5	6.79
6	4.96
7	3.81
8	2.88
9	2.01
10	1.71

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560 Table A3 Factor loadings on first 10 principal component axes

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
1	-0.020	0.005	0.254	-0.031	0.120	0.174	0.048	0.263	-0.077	0.126
2	0.016	0.141	0.002	-0.026	-0.183	0.083	0.051	0.142	-0.022	-0.154
3	-0.029	0.178	0.207	0.097	-0.165	0.008	0.023	0.047	0.090	0.095
4	0.059	0.157	0.122	0.128	-0.008	0.180	0.014	0.183	-0.035	-0.136
5	0.044	0.100	-0.055	0.064	-0.095	-0.036	0.050	0.093	-0.036	-0.099
6	0.057	0.056	0.125	0.261	0.113	0.037	-0.075	0.338	0.077	-0.114
7	-0.029	-0.085	0.162	0.099	0.195	-0.070	-0.432	-0.133	-0.079	0.008
8	-0.034	-0.009	0.061	-0.052	-0.115	-0.096	-0.061	-0.025	-0.119	0.037
9	0.033	0.111	0.121	0.034	-0.092	-0.002	-0.127	-0.235	-0.054	0.063
10	-0.011	-0.051	-0.009	0.117	-0.104	-0.155	-0.007	-0.155	-0.037	-0.157
11	-0.036	-0.042	0.011	-0.015	-0.044	-0.098	0.040	0.032	0.045	-0.083
12	0.069	0.008	-0.042	0.012	-0.039	-0.133	0.003	-0.149	-0.070	0.144
13	0.060	-0.090	-0.037	0.143	-0.037	-0.169	0.001	-0.074	-0.090	-0.144
14	-0.010	-0.069	-0.011	-0.082	0.009	-0.009	-0.088	-0.185	0.069	-0.022

15	0.086	-0.045	-0.049	-0.053	-0.072	-0.012	0.119	-0.375	-0.138	-0.044
16	0.121	0.227	-0.465	-0.108	-0.244	-0.116	-0.149	0.092	0.004	0.298
17	0.012	-0.086	-0.014	-0.033	0.186	0.039	-0.009	0.119	-0.179	0.274
18	0.165	-0.274	-0.063	-0.245	0.007	0.023	0.042	0.330	-0.347	0.030
19	0.485	-0.228	0.196	-0.026	-0.217	-0.312	0.018	-0.026	0.300	0.117
20	-0.005	0.139	0.000	-0.028	-0.036	0.098	0.115	-0.079	0.070	-0.073
21	-0.165	0.320	-0.382	0.057	0.290	-0.202	0.096	-0.050	-0.088	0.078
22	0.041	0.213	0.019	0.079	0.140	0.248	0.280	-0.104	-0.248	-0.094
23	-0.020	-0.066	0.007	0.079	0.112	-0.141	-0.052	0.070	-0.145	0.218
24	-0.073	-0.130	0.102	0.137	-0.105	-0.050	0.251	0.075	0.051	-0.117
25	-0.217	-0.292	0.077	-0.131	-0.080	0.237	-0.176	-0.126	-0.216	-0.206
26	0.015	-0.159	0.001	0.105	0.068	-0.035	0.147	-0.216	-0.155	-0.045
27	0.061	0.011	-0.158	-0.086	0.188	0.334	-0.342	-0.010	0.303	-0.230
28	-0.461	-0.105	-0.121	0.285	0.035	-0.317	-0.020	0.171	0.160	-0.137
29	0.028	0.140	-0.042	-0.151	-0.438	0.114	-0.004	0.112	-0.046	-0.202
30	-0.310	0.193	0.324	0.091	-0.270	0.129	-0.168	-0.146	0.028	0.399
31	-0.346	0.161	0.187	-0.678	0.102	-0.227	0.156	0.010	0.128	-0.032
32	-0.025	-0.069	0.099	0.147	0.157	0.115	0.075	-0.103	0.187	0.101
33	0.183	0.006	0.031	-0.189	0.226	-0.026	0.156	-0.189	0.305	-0.195
34	0.208	0.271	0.057	0.047	0.134	0.073	0.160	0.046	-0.004	-0.007
35	0.040	0.097	-0.038	0.027	0.038	-0.072	-0.191	0.165	0.320	-0.042
36	-0.055	-0.180	0.009	0.113	-0.025	-0.098	0.348	0.121	0.003	0.029
37	0.258	0.133	-0.076	0.039	0.107	0.023	-0.119	-0.084	-0.122	0.071
38	-0.035	0.026	-0.057	0.031	0.077	0.082	-0.009	-0.126	-0.028	-0.062
39	-0.068	-0.093	-0.214	-0.018	-0.138	-0.069	-0.222	0.032	-0.137	-0.262
40	-0.147	-0.316	-0.367	0.039	-0.142	0.429	0.226	-0.063	0.315	0.293
41	0.010	-0.143	0.035	-0.067	0.264	-0.044	-0.064	0.003	0.038	0.152
42	0.046	-0.159	-0.012	-0.210	0.082	0.060	-0.104	0.211	-0.023	0.122

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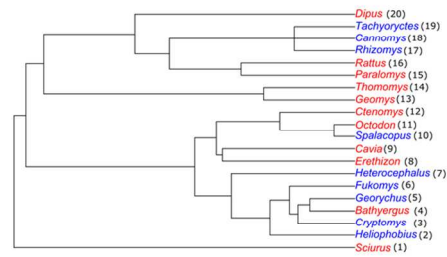


Figure 1 Phylogeny of rodent genera used in this study. Modified from Fabre et al. 2012. Numbers correspond to Figures 3-5

338x190mm (96 x 96 DPI)

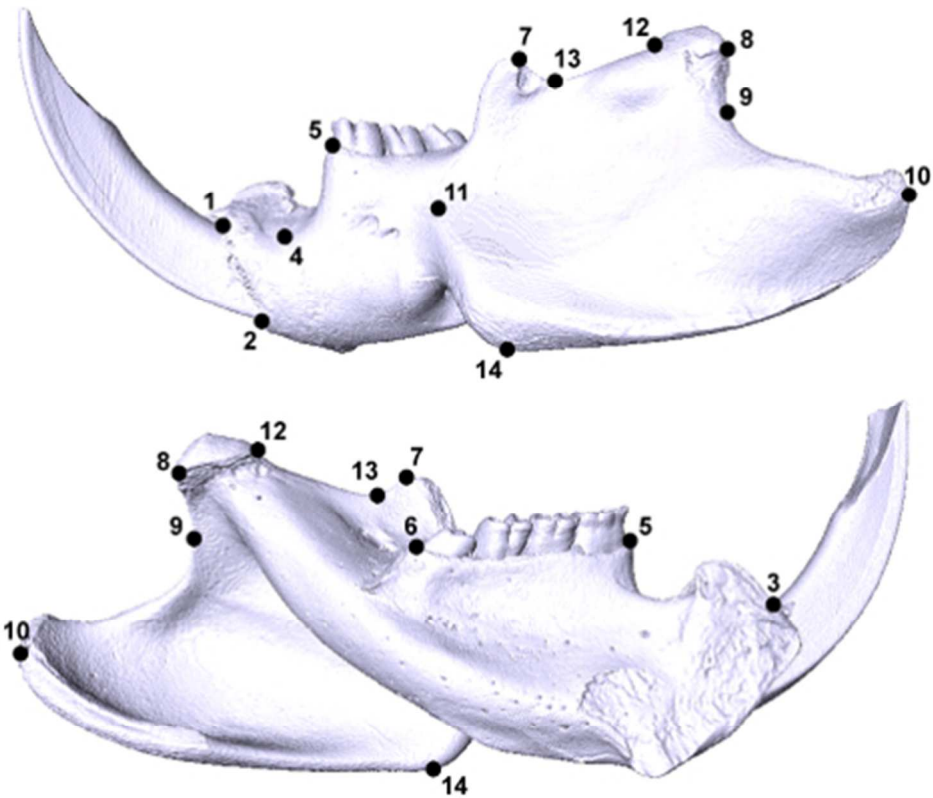


Figure 2 landmark configuration represented on *Bathyergus suillus* in lateral (top) and medial (bottom) view (see Table A1 for corresponding landmark numbers and descriptions)

79x71mm (150 x 150 DPI)

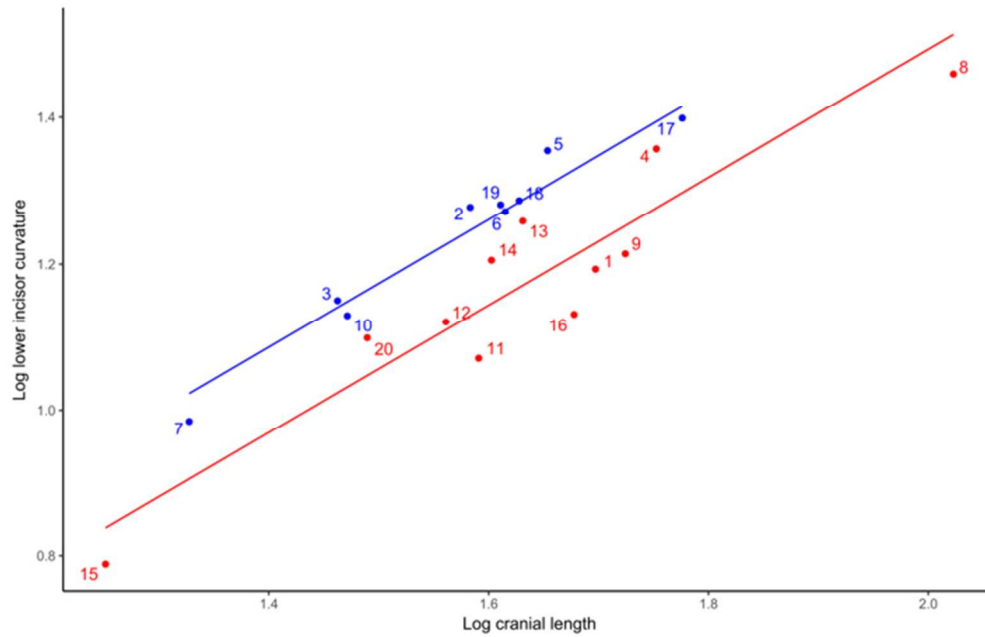


Figure 3 Phylogenetic ANCOVA representing the relationship between cranial length, lower incisor RoC and digging method

121x78mm (150 x 150 DPI)

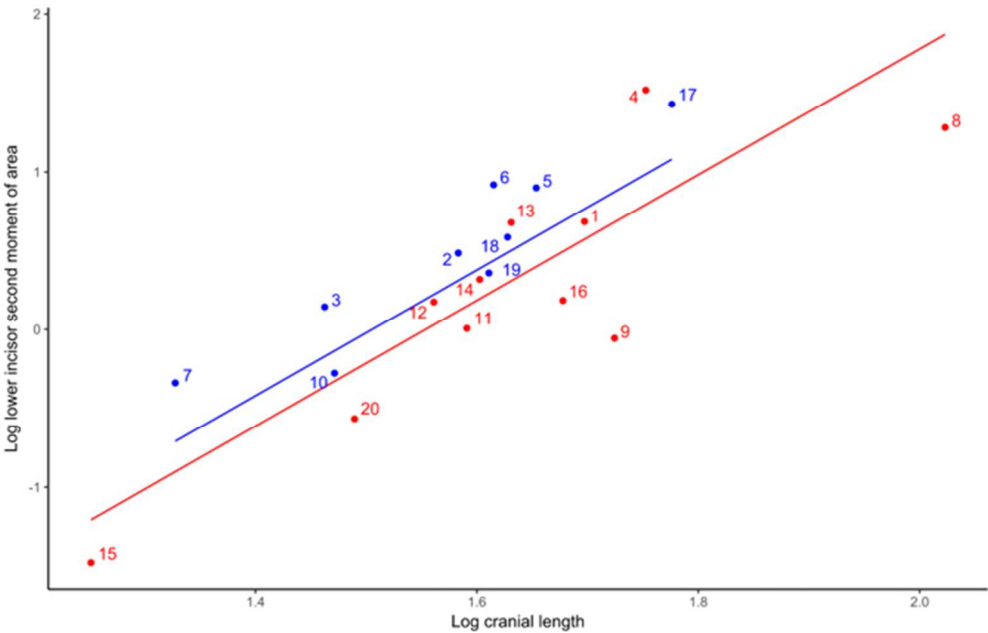


Figure 4 Phylogenetic ANCOVA representing the relationship between cranial length, lower incisor SMA and digging method

114x73mm (150 x 150 DPI)

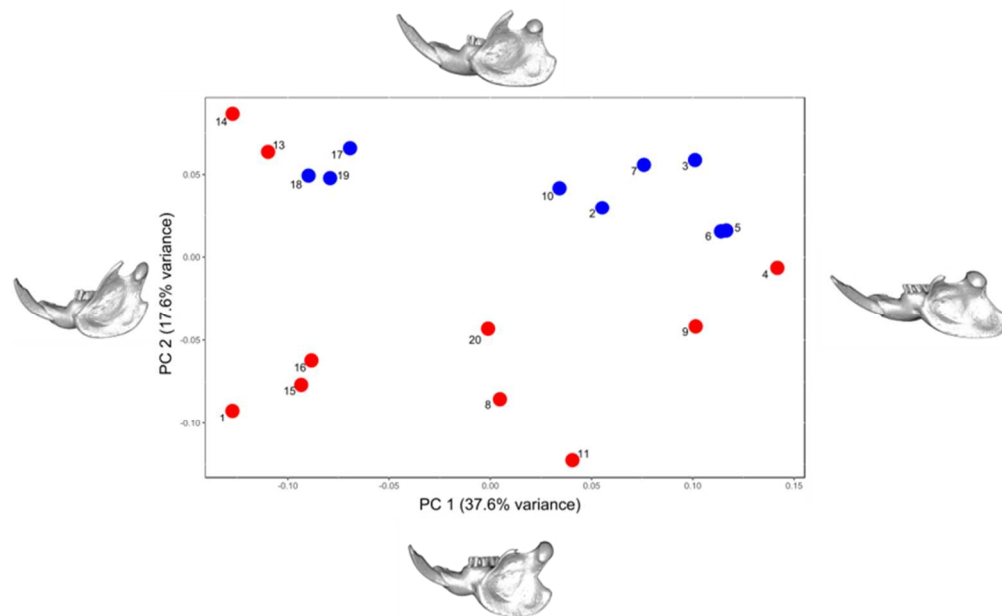
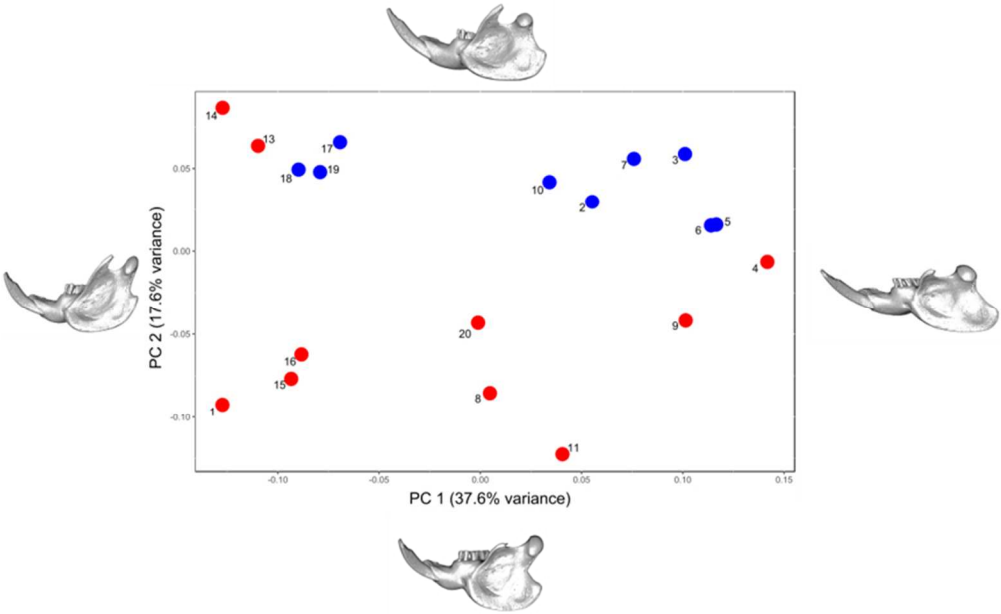


Figure 5 Principal components analysis (PCA) with associated virtual deformations representing mandibular shape variation at the extreme ends of PC1 and PC2

145x92mm (150 x 150 DPI)



Graphical Abstract Image

145x92mm (150 x 150 DPI)

Graphical Abstract Text

Our results show that the shape of the rodent mandible and its accompanying incisor is adapted for digging in chisel-tooth digging rodents. However, evolutionary integration of the incisor and mandible is weak within the rodent sample. We can infer from this that the rodent incisor and mandible have evolved separately in a modular process.

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