

This is a repository copy of *The Impact of Digging on Craniodental Morphology and Integration*.

White Rose Research Online URL for this paper:
<http://eprints.whiterose.ac.uk/103865/>

Version: Accepted Version

Article:

McIntosh, Andrew and Cox, Philip Graham orcid.org/0000-0001-9782-2358 (2016) The Impact of Digging on Craniodental Morphology and Integration. *Journal of evolutionary biology*. pp. 2383-2394. ISSN 1420-9101

<https://doi.org/10.1111/jeb.12962>

Reuse

Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

1 **Title: The impact of digging on craniodental morphology and integration**

2

3 **Running head: Rodent craniodental morphology**

4

5 **Authors:**

6 Andrew F. McIntosh¹

7 Philip G. Cox²

8

9 **Institutional addresses:**

10 ¹Centre for Anatomical and Human Sciences, Hull York Medical School, University of Hull,
11 Hull, UK

12 ²Centre for Anatomical and Human Sciences, Hull York Medical School, University of York,
13 York, UK

14 ³Department of Archaeology, University of York, York, UK

15

16 **Corresponding author:**

17 Philip G. Cox

18 Email address: philip.cox@hyms.ac.uk

19 Telephone: +44 1904 321744

20

21

22

23

24

25

26 **ABSTRACT**

27 The relationship between the form and function of the skull has been the subject of a great
28 deal of research, much of which has concentrated on the impact of feeding on skull shape.
29 However, there are a number of other behaviours that can influence craniodental morphology.
30 Previous work has shown that subterranean rodents that use their incisors to dig (chisel-tooth
31 digging) have a constrained cranial shape which is probably driven by a necessity to create
32 high bite forces at wide gapes. Chisel-tooth digging rodents also have an upper incisor root
33 that is displaced further back into the cranium compared with other rodents. This study
34 quantified cranial shape and upper incisors of a phylogenetically diverse sample of rodents to
35 determine if chisel-tooth digging rodents differ in craniodental morphology. The study
36 showed that the crania of chisel-tooth digging rodents shared a similar place in morphospace,
37 but a strong phylogenetic signal within the sample meant that this grouping was non-
38 significant. It was also found that the curvature of the upper incisor in chisel-tooth diggers
39 was significantly larger than in other rodents. Interestingly, most subterranean rodents in the
40 sample (both chisel-tooth and scratch diggers) had upper incisors that were better able to
41 resist bending than those of terrestrial rodents, presumably due to their similar diets of tough
42 plant materials. Finally, the incisor variables and cranial shape were not found to covary
43 consistently in this sample, highlighting the complex relationship between a species'
44 evolutionary history and functional morphology.

45

46 **Keywords: Subterranean rodents; cranial evolution; geometric morphometrics;**

47 **phylogenetic comparative methods**

48

49

50

51 **INTRODUCTION**

52 The relationship between the form and function of the skull and teeth in vertebrates is highly
53 complex and has been the subject of much study over the years (e.g. Moss & Young, 1960;
54 Herring, 1993; Weishampel, 1993; Preuschoft & Witzel, 2004). A great deal of research has
55 focused on the process of feeding, and has sought to determine how the forces imposed by
56 mastication shape the cranium and mandible (e.g. Maynard Smith & Savage, 1959; Turnbull,
57 1970; Herring & Teng, 2000; Cox, 2008). However, there are a number of other factors that
58 can also influence the form of the skull, such as the relative size of the brain, the size and
59 orientation of the eyes, the environment in which an animal lives, and any non-masticatory
60 behaviours performed by the skull. Examples of such behaviours include: head-butting in
61 goats (Farke, 2008), dam construction in beavers (Cox & Baverstock, 2016), and digging
62 with the teeth in subterranean rodents (Samuels & Van Valkenburgh, 2009).

63

64 Subterranean rodents are a specialized group of rodents that live almost exclusively
65 underground, and as such experience very different selection pressures to terrestrial rodents.
66 For instance, burrowing underground requires 360-3400 times the energy of moving a similar
67 distance above ground (Vleck, 1979; Jarvis & Bennett, 1991). This extent of energy
68 expenditure has required the evolution of efficient methods of soil excavation. The majority
69 of subterranean rodents show one of two types of digging method: chisel-tooth digging, using
70 the incisors, and scratch digging, which uses only the forelimbs (Hildebrand, 1985). The
71 ability to use incisors for digging has allowed chisel-tooth digging rodents the freedom to
72 exploit harder soils (Lessa & Thaeler, 1989; Lessa, 1990).

73

74 Chisel-tooth digging evolved independently a number of times, including at least once in
75 each of the six extant families of subterranean rodents (Stein, 2000). It has been noted that

76 chisel-tooth digging rodents tend to converge in cranial shape, showing larger temporal
77 fossae; wider and taller crania; enlarged zygomatic arches; longer rostra; more procumbent
78 incisors and incisors that are more resistant to bending stresses (Landry, 1957; Agrawal,
79 1967; Samuels & Van Valkenburgh, 2009; Becerra et al., 2012, 2013, 2014; McIntosh &
80 Cox, 2016; Gomes Rodrigues et al., 2016). It is thought that many of these morphological
81 features improve bite force and gape in subterranean rodents that use their incisors to dig in
82 hard soils (Becerra et al., 2013; McIntosh & Cox, 2016; Gomes Rodrigues et al., 2016).

83

84 An extensively studied modification in chisel-tooth digging rodents is the increase in upper
85 incisor procumbency, that is, the angle of anterior projection of the upper incisors (Landry,
86 1957; Agrawal, 1967; Lessa & Thaeler, 1989; Lessa & Patton, 1989; Vassallo, 1998; van der
87 Merwe & Botha, 1998; Mora et al., 2003; McIntosh and Cox, 2016). Vassallo (1998)
88 hypothesised that this increased procumbency allows for a more favourable angle of attack
89 for breaking hard soils. Incisor procumbency in rodents is governed by overall curvature of
90 the incisor and its placement within the rostrum (Landry, 1957; Akersten, 1981). Landry
91 (1957) pointed out that to keep the incisor in its functional plane, the only way the
92 procumbency can change without altering incisor morphology (incisor curvature) is by
93 raising or lowering the posterior end of the incisor. However, rostral space in rodents is
94 nearly completely occupied by the incisor (Fig. 1), and so this type of movement would not
95 be possible (Landry, 1957).

96

97 The rodent cranium is a complexly integrated structure (Hallgrímsson et al., 2009) and
98 understanding how different structures covary within the cranium could potentially explain
99 morphological diversity in some clades and constraints in others. Covariation of incisor
100 morphology and cranial shape has never been studied in rodents, and as the incisor takes up

101 such a large space within the craniofacial structure of rodents, it is potentially an underlying
102 factor in chisel-tooth digging cranial convergence.

103

104 In order to address this issue, we will quantify incisor morphology in a diverse number of
105 rodents from both terrestrial and subterranean habitats to show how chisel-tooth digging
106 influences incisor morphology. Secondly, we will quantify cranial shape and attempt to verify
107 the findings of Samuels & Van Valkenburgh (2009) using phylogenetic comparative methods
108 (Felsenstein, 1985; Rohlf, 2001). Finally, we will assess how incisor morphology and cranial
109 shape covary and determine the extent of morphological integration between the upper
110 incisor and cranium in rodents.

111

112 **MATERIALS AND METHODS**

113 This study analysed 65 adult crania from a diverse group of rodents representing 20 genera
114 and 11 families: Bathyergidae, Caviidae, Cricetidae, Ctenomyidae, Dipodidae,
115 Erethizontidae, Geomyidae, Muridae, Octodontidae, Sciuridae and Spalacidae (Table 1). The
116 study focuses on the impact of chisel-tooth digging on craniodental morphology. However,
117 other factors such as diet, habitat and phylogeny have been shown to influence cranial and
118 incisor morphology in rodents (Samuels, 2009; Croft et al., 2011; Hautier et al., 2012). In
119 order to account for these potential additional influences, the sample contains
120 phylogenetically distant subterranean and terrestrial rodents with different ecologies (Table
121 1). The specimens were scanned on an X-Tek Metris microCT scanner at the University of
122 Hull (Medical and Biological Engineering Research Group). The resulting scans had
123 isometric voxels with dimensions ranging between 0.01 mm and 0.07 mm.

124

125 Surface reconstructions of crania and incisors were created from the microCT scans in Avizo
126 8.0 (FEI, Hillsboro, Oregon). Two measures determining incisor shape (Landry, 1957) were
127 calculated: incisor length (straight line distance from apex to tip: a in Fig. S1); and incisor
128 radius of curvature (RoC), which was derived using Heron's formula (Fig. S1) from a circle
129 fitted to three points along the dorsal midline of the incisor surface (at the apex, tip and most
130 dorsal point of the curve). Following Lin et al. (2010), this method assumed that incisor
131 growth is circular, although technically rodent incisors grow helically (Herzberg & Schour,
132 1941). The relationship between incisor length and RoC was assessed using ordinary least
133 squares (OLS) regression, conducted in R (R Foundation for Statistical Computing, Vienna),
134 to determine the overall shape of the incisor. If the gradient of the regression line fitted
135 through the origin is 0.5, then the length is double the radius of curvature and thus the incisor
136 is a semicircle.

137

138 Rodent incisors are long relative to their diameter (i.e. take up a large proportion of a circle)
139 and so can be affected by bending stresses (Bacigalupe et al., 2002). Second moment of area
140 (SMA) is a geometric measurement that defines the resistance to bending of a cross-section
141 of an object and is a good indicator of structural strength (Alexander, 1983), a potentially
142 important property for incisors used to dig through hard soils. The SMA of each incisor at its
143 midpoint cross-section was calculated using the BoneJ plugin (Doubé et al., 2010) in ImageJ
144 (Schneider et al., 2012).

145

146 The sample included a large range of body masses (Paralomys can be as small as 12 g,
147 whereas Bathyergus can grow up to 2 kg; Nowak, 1999) and past studies on rodent incisors
148 have shown that variables such as SMA correlate strongly with size (e.g. Verzi et al., 2010).
149 Thus, condylobasal cranial length was included as a covariate to account for scaling. Incisor

150 morphology variables and cranial length were logged in all analyses due to size differences
151 and the necessity to linearize variables to carry out statistical procedures.

152

153 As closely related species tend to be more similar to each other than to more distantly related
154 species they cannot be considered as completely independent units (Felsenstein, 1985;
155 Garland et al., 2005), a prerequisite for standard statistical tests. Phylogenetic Generalized
156 Least Squares (PGLS; Grafen, 1989; Martins & Hansen, 1997) was performed to show if any
157 relationship between size and incisor morphology existed after phylogenetic information was
158 incorporated into the analysis.

159

160 It is possible to test how much phylogenetic signal is present in the data, a statistical
161 procedure that quantifies the expected covariation of species traits under a selected
162 evolutionary model (e.g. Brownian motion) on a phylogeny (for review see Blomberg &
163 Garland, 2002). For the univariate analyses in this study, Pagel's λ (Pagel, 1999) was used to
164 estimate the phylogenetic signalling in the data. Pagel's λ is a scaling parameter that
165 measures the correlation of traits relative to expected correlation under a Brownian motion
166 model of evolution. Normally, λ ranges from zero (no phylogenetic signal and data is
167 equivalent to a "star" phylogeny) to one (data consistent with selected phylogenetic tree
168 under a Brownian motion model of evolution) or beyond (the evolutionary process is more
169 orderly than Brownian motion). Pagel's λ and PGLS regressions in this study are quantified
170 simultaneously using the method proposed by Revell (2010). Phylogenetic ANCOVA models
171 using PGLS (to test for differences in incisor RoC relative to cranial length between chisel-
172 tooth diggers and non-tooth diggers [including non-fossorial rodents]) were fitted to genus
173 means of the sample using the nlme (Pinheiro et al., 2016) and ape (Paradis et al., 2004)

174 packages in R. The phylogeny used in all analyses was modified from Fabre et al. (2012),
175 with branch lengths in millions of years.

176

177 The cranial shape of each specimen was quantified using 3D landmark coordinates (Geomys
178 was not included in this part of the study due to extensive damage of the cranium). 29
179 homologous landmarks were recorded from each cranial reconstruction using Avizo (Fig. 2
180 and Table S1). From this landmark data, variation in the shape of the crania was analysed
181 with geometric morphometrics (for review see O'Higgins, 2000). The landmark co-ordinates
182 were subjected to the Procrustes method of generalized least squares (GLS) superimposition
183 (Rohlf & Slice, 1990). A principal component analysis (PCA) of genus-averaged Procrustes
184 coordinates revealed the greatest axes of shape variation within the sample. The variance
185 encompassed by the first ten axes is given in Table S2. Surface warps of the extreme ends of
186 the principal components axes were also included to visualise the shape variation within the
187 data. As Pagel's λ cannot be estimated accurately for multivariate data (Adams, 2014a),
188 phylogenetic signal in the data was quantified by calculating the K statistic (Blomberg et al.,
189 2003), generalized to accept multivariate shape data (Adams, 2014a). Although the K statistic
190 and λ statistic are derived differently (K is a scaled ratio of variance and λ is a scaling metric)
191 their outcomes are normally similar i.e. <1 implies data has less phylogenetic signal than
192 expected under Brownian motion and >1 has more phylogenetic signal than expected under
193 Brownian motion.

194

195 A phylogenetic principal components analysis (pPCA) was also performed on the Procrustes
196 coordinates. This analysis centres the data on the ancestral root of a phylogeny
197 ("phylogenetic mean") and extracts principal components from the variance covariance
198 matrix informed by phylogenetic propinquity, so that the major axes represent the major

199 features of shape variation in the evolutionary process (Revell, 2009; Polly et al., 2013; but
200 see Uyeda et al., 2015). To calculate surface warps associated with extremes of pPC axes, an
201 average surface calculated from Procrustes coordinates is warped to an ancestral state
202 reconstruction at the root of the phylogeny (Yang et al., 1995). Appropriately scaled
203 eigenvectors from the corresponding pPC were then used to show the shape differences along
204 the pPC axes. The rationale for including both PCA and pPCA analyses is that PCA gives
205 information about the distribution of the taxa in shape space, whereas the pPCA reveals the
206 important morphological variation in the evolution of this group of rodents.

207

208 To test for differences between skull shapes of chisel-tooth diggers and non-tooth diggers, we
209 used Procrustes ANOVA (analysis of variance) as well as phylogenetically informed
210 Procrustes ANOVA in a phylogenetic framework under a Brownian motion model of
211 evolution (Adams, 2014b). In this procedure, sum of squares (SS) is measured based on the
212 SS of Procrustes distances among specimens (see Goodall, 1991), which is equivalent to a
213 distance-based ANOVA design (Anderson, 2001). GLS superimposition, phylogenetic signal
214 testing, principal components analysis, ANOVA and surface warps were processed using the
215 geomorph package in R (Adams & Otárola-Castillo, 2013), and pPCA was performed using
216 the phytools package in R (Revell, 2012).

217

218 Morphological integration and covariation of biological forms has been extensively studied
219 using geometric morphometrics and partial least squares (PLS) (e.g. Rohlf & Corti, 2000;
220 Bookstein et al., 2003; Bastir et al., 2005; Hautier et al., 2012; Klingenberg, 2014). PLS
221 quantifies the maximum amount of covariation between two sets of variables, using a
222 correlation or covariance (for geometric morphometric studies) matrix of traits (Rohlf &
223 Corti, 2000). This sets it apart from regression analysis which determines whether variation

224 in the independent variable(s) can predict variation in the dependent variable. In this study,
225 one set of variables contained the Procrustes coordinates of cranial shape. The second set of
226 variables was the incisor measurements, incisor RoC and SMA. Generalized Procrustes
227 analysis removes variation due to isometric scaling from the cranial shape variables, but
228 retains allometric shape variation (Drake, 2011). Multivariate regressions of Procrustes co-
229 ordinates on log-transformed centroid size were performed to assess the effects of allometry
230 on cranial shape variation. Size was removed from the incisor variables when measuring
231 covariation between cranium and incisors. Cranial length was used as the independent
232 variable to regress against incisor variables. Regression was carried out in the PGLS
233 framework to obtain the allometric regression vector (Revell, 2009), and residuals for taxon
234 averages were calculated from this vector. The residuals were then used in PLS analyses with
235 cranial shape variables to measure covariation. Multivariate regressions of cranial shape on
236 size were performed in the geomorph package in R (Adams & Otárola-Castillo, 2013).
237 Regressions of incisor variables on cranial length were performed in the phytools package in
238 R (Revell, 2012).

239

240 As in the methods above, any inter-generic analysis must also account for the non-
241 independence of the data. Incorporating phylogeny whilst quantifying morphological
242 integration at the inter-generic level shows how morphological covariation has evolved along
243 a tree (Klingenberg & Marugán-Lobón, 2013). Phylogenetic PLS is calculated by
244 incorporating the evolutionary covariance matrix from PGLS to calculate PLS scores (Adams
245 & Felice, 2014). The strength of association between cranial and incisor variables is
246 quantified using the RV coefficient (Klingenberg, 2009). RV coefficient ranges from 0
247 (variables are independent) to 1 (variables are dependent). All statistical tests of covariation
248 and association were carried out using phylogenetic PLS. However, non-phylogenetic PLS

249 was also carried out in order to visualise morphological variation along the PLS axes, using
250 surface warps. Phylogenetic and non-phylogenetic PLS analyses, accompanying surface
251 warps and RV coefficient calculations were implemented in the geomorph package in R
252 (Adams & Otárola-Castillo, 2013).

253

254 **RESULTS**

255 The relationship between upper incisor length and upper incisor RoC in each genus is
256 displayed in Fig. 3A. Incisor shape can be changed by RoC, or by the proportion of the circle
257 taken up by the incisor (represented by incisor length). OLS model fitted to origin (Fig. 3A)
258 shows a positive relationship between the two variables). The gradient of the line was nearly
259 half (0.52), which means that every upper incisor analysed in the sample was approximately
260 semicircular in shape. As shape change was found to be so limited, all further analyses
261 concentrated on incisor size, as represented by radius of curvature.

262

263 Fig. 3B shows the relationship between cranial length and upper incisor RoC. Firstly, to test
264 for homogeneity between slopes (a prerequisite for ANCOVA models), an ANCOVA was
265 conducted which included the interaction term between log cranial length (covariate) and
266 digging method (categorical-variable). This was not significant ($P>0.05$) showing that an
267 ANCOVA model is suitable to apply to our data. Generalized phylogenetic ANCOVA
268 revealed that chisel-tooth digging rodents have a significantly larger upper incisor RoC
269 ($P<0.01$) than other rodents. Phylogenetic signal in this data, measured simultaneously with
270 PGLS model using λ , was 0.60, which is significantly different from the null hypothesis of a
271 star phylogeny ($P<0.01$).

272

273 Fig. 3C shows the relationship between cranial length and SMA of the upper incisors.
274 Interaction between log cranial length (covariate) and digging method (categorical variable)
275 was significant ($P < 0.01$). This meant an ANCOVA could not be applied to the data as the
276 slopes were not similar between groups. PGLS was applied to the data instead and the
277 residuals were examined to show the relationship between the data (Fig. 3D). The residuals of
278 the PGLS of cranial length and upper incisor SMA show that not only do the chisel-tooth
279 digging rodents have a relatively larger upper incisor SMA, but so do the other subterranean
280 rodents compared to the terrestrial taxa (see Table 1 for groupings). Phylogenetic signal in
281 this PGLS analysis was 0.51 ($P < 0.01$).

282

283 Fig. 4 represents some of the variation in cranial shape using PCA (Fig. 4A) and pPCA (Fig.
284 4B), with both method showing a very similar pattern of shape variation. However,
285 phylogenetic signal in the data is significant ($K_{\text{mult}} = 0.49$, $P < 0.01$). It can be seen that most of
286 the chisel-tooth digging rodents group in the same part of the subspace (towards negative end
287 of PC1). The only chisel-tooth digging rodent that departs from the group is *Spalacopus*
288 (which lies positively on PC1 with respect to the rest of the chisel-tooth digging group). The
289 non-tooth digging rodents do not group tightly and are spread over different parts of the
290 subspace. A Procrustes ANOVA indicates that chisel-tooth and non-tooth digging groups can
291 be distinguished in morphospace ($F = 3.57$, $P < 0.01$). However, a Procrustes ANOVA
292 incorporating the phylogeny leads to non-significance between groups ($F = 2.25$, $P > 0.05$),
293 unsurprising given the significant amount of phylogenetic signal in the data. Shape variation
294 across the two PC axes is represented by the warps on the extremes of the pPC axes. Positive
295 pPC1 scores are associated with a longer skull with a narrow, straight-sided rostrum. More
296 negative pPC1 scores are associated with a shorter skull and a more tapered rostrum. In
297 general, pPC2 separates chisel-tooth digging taxa from the other genera (although *Spalacopus*

298 is again separated from the other chisel-tooth diggers). Cranial morphology varies along this
299 axis from curved crania with short rostra (negative pPC2, chisel-tooth diggers) to flatter
300 crania with more elongated rostra (positive pPC2, non-tooth diggers). Multivariate
301 regressions of Procrustes co-ordinates on centroid size were not significant, indicating that
302 allometry did not have an important effect in either the original or the phylogenetically
303 corrected analyses.

304

305 The surface warps associated with the non-phylogenetic PLS (Fig. 5) indicate that, in this
306 sample, elongated, narrow crania and wide rostra are associated with relatively small incisors
307 with low SMA (negative PLS1) – these are largely non-tooth digging genera. Shorter, wider
308 crania with narrow rostra are associated with relatively large incisors with high SMA. These
309 are found at the positive end of cranial and incisor PLS1 and are mostly chisel-tooth digging
310 rodents. It is particularly notable that, in this sample, bathyergids and spalacids appear to be
311 covarying in a similar manner. However, using a phylogenetic PLS analysis, the covariation
312 between cranial and incisor morphology was not found to be significant.

313

314 **DISCUSSION**

315 This study has shown that there is a clear correlation between size (RoC) of the upper incisor
316 and digging method in rodents (Fig. 3B), although there is also a moderate phylogenetic
317 signal in the data ($\lambda = 0.60$). Despite the seemingly complicated relationship between
318 phylogeny and ecology in the evolution of incisor RoC, it is clear that chisel-tooth digging
319 rodents have acquired a larger incisor RoC for their size compared to rodents that do not use
320 their incisors to dig. Landry (1957) assessed upper incisor RoC in a phylogenetically diverse
321 group of rodents and concluded that a large upper incisor RoC (and arc length) is required to
322 improve upper incisor procumbency. McIntosh & Cox (2016) showed that, within the

323 Bathyergidae, chisel-tooth digging species have a craniomandibular morphology that
324 facilitates a wide gape. A wide gape coupled with more procumbent incisors gives a more
325 effective angle of attack (Mora et al., 2003) and enables the incisor tip to be in contact with
326 the soil throughout a complete gape motion, hence removing a larger amount of soil relative
327 to a rodent with less procumbent incisors.

328

329 Increasing procumbency via an increase in the RoC of the upper incisor requires the root of
330 the incisor to be further displaced into the pterygoid region of the skull. The cranium is a
331 complex structure which plays host to the brain and other sensitive sensory structures, and as
332 the cranium is highly integrated (Cheverud, 1982; Hallgrímsson et al., 2007; Klingenberg &
333 Marugán-Lobón, 2013), any cranial morphological change could have an effect on these
334 systems. Alternatively, increasing incisor procumbency could be achieved by moving the
335 incisor root forward whilst keeping the RoC constant, and decreasing the arc length of the
336 incisor (see Landry, 1957, for further discussion). This would mean the root of the incisor
337 would not be required to expand further back into the skull. Another strategy could be to
338 increase the length of the rostrum to incorporate the larger incisor, as seen in species of
339 *Ctenomys* (Mora et al., 2003), but this would result in a loss of mechanical efficiency of the
340 major masticatory muscles (McIntosh & Cox, 2016). Long incisors originating further back
341 in the skull, as seen in chisel-tooth diggers, may well be an adaptation for the use of incisors
342 for digging in hard soils. An elongated incisor within the rostrum gives a larger surface area
343 in contact with the skull that can then dissipate the larger forces generated at the tip during
344 chisel-tooth digging (Landry, 1957; Becerra et al., 2012).

345

346 SMA, an indicator of bending strength, did not correlate in the same way as incisor RoC.
347 Firstly, studying the residuals of the analysis (Fig. 3D), it is clear that this variable does not

348 show differences between chisel-tooth digging rodents and non-tooth digging rodents.
349 Instead, it seems that the difference lies between the subterranean and terrestrial rodents. The
350 subterranean rodents have a larger incisor SMA for their size compared with the terrestrial
351 rodents. For example, one of the (relatively) largest incisor SMAs was measured in *Geomys*,
352 a subterranean, scratch digging rodent. Subterranean rodent diets are mostly made up of
353 geophytes and other subterranean plants, which tend to be hard and fibrous materials (see
354 Busch et al., 2000). Therefore, it appears that subterranean rodents have adapted to resist the
355 increased pressure at the incisor tip due to their hard food diet by making the incisor more
356 resistant to bending. Incisor morphology has been shown to strongly correlate with diet in
357 caviomorph rodents (Croft et al., 2011). We propose that subterranean rodent incisors are
358 resistant to bending due to their hard food diets, but chisel-tooth digging rodents also have an
359 adaptation to deal with the additional forces exhibited during incisor digging in hard soils by
360 lengthening their incisors to dissipate these forces.

361

362 It is clear from examining both PCA and pPCA plots (Figs. 4A and B, respectively) that
363 cranial shape has significantly converged in chisel-tooth diggers. The tight grouping of
364 chisel-tooth digging rodents shows homoplasy (Polly et al., 2013), as phylogenetically distant
365 spalacids and bathyergids group closely together, even after phylogenetic information has
366 been incorporated in the pPCA plot. The exception to this trend is *Spalacopus* which,
367 although a chisel-tooth digging rodent with a relatively large incisor RoC (Fig. 3B), does not
368 have a similar cranial shape to other chisel-tooth diggers. The cranium of *Spalacopus* is more
369 rounded with a wider rostrum than that of the bathyergid and spalacid chisel-tooth diggers.
370 This could be due to the arrangement of the incisors in the cranium of *Spalacopus*, which are
371 located in alveolar sheaths that are lateral to the cheek teeth and thus avoid the internal
372 cranial space (Lessa, 1990). Other chisel-tooth diggers do not have this lateralization of the

373 alveolar sheath and incorporate the incisor alveolus into internal cranial spaces, potentially
374 constraining cranial shape. However, only one specimen of *Spalacopus* was available for this
375 study, and further research on a greater number of specimens is needed to address this issue
376 thoroughly.

377

378 Convergence of cranial shape with digging methods has already been shown in rodents
379 (Samuels & Van Valkenburgh, 2009) and was also found in this study (Procrustes ANOVA,
380 $P < 0.01$). However, Samuels & Van Valkenburgh (2009) did not take into account
381 phylogenetic similarity between species. In this sample, the phylogenetic signal of cranial
382 shape was significant ($K = 0.49$, $P < 0.01$). This was a surprising result given the amount of
383 convergence of chisel-tooth digging crania shown in the morphospace in both principal
384 component methods (Figs. 4A and B) and the fact that chisel-tooth digging has arisen
385 independently at least three times in the sample used (Fig. 3D). However, when phylogeny of
386 the data is included in the analysis, chisel-tooth digging crania are not dissimilar to non-tooth
387 digging crania (phylogenetically informed Procrustes ANOVA, $P > 0.05$). This result is likely
388 driven by the phylogenetic distribution of chisel-tooth diggers within the sample. Eight of the
389 nine chisel-tooth digging genera belong to one of two families, the Bathyergidae and the
390 Spalacidae). The remaining genus, *Spalacopus*, does not converge on the same cranial
391 morphology of the other chisel-tooth diggers. Phylogenetic comparative methods reduce the
392 weighting of taxa that are more closely related relative to taxa that are phylogenetically more
393 distant. So, despite the large phylogenetic distance between bathyergids and spalacids (two
394 families whose last common ancestor may have been in the Cretaceous; Fabre et al., 2012),
395 the sample here may not have been wide enough to pick up on any biological signal.

396

397 The multivariate regression of Procrustes co-ordinates on log centroid size did not find a
398 significant allometric component to the variation of the cranial shape in the sample. This was
399 true whether or not phylogenetic information was incorporated into the analysis. This result is
400 in direct contradiction to Gomes Rodrigues et al. (2016) who found a high significance
401 ($P < 0.001$) in the regression of cranial shape component on log centroid size. However, it
402 should be noted that the sample of Gomes Rodrigues et al. (2016) was taken exclusively from
403 the Ctenohystrica, whereas the sample here covers all parts of the rodent family tree.
404 Alternatively, the difference between our result and that of Gomes Rodrigues et al. (2016)
405 may simply be a lack of statistical power as a consequence of the relatively low sample size
406 in our analysis.

407

408 When phylogenetic information was incorporated into the PLS analysis, the covariation
409 between cranial and incisor morphology was not found to be significant. This result indicates
410 that, from an evolutionary perspective, cranial and incisor covariation is not consistent, even
411 though from a strictly morphological perspective, there appears to be some association
412 between incisor and cranial form (Fig. 5). The fact that chisel-tooth digging spalacids and
413 bathyergids group fairly tightly in the non-phylogenetic PLS plot (Fig. 5) suggests that, at
414 least in these two families, tooth digging may be driving the convergent evolution of both
415 large, wide incisors and short, wide crania, perhaps to resist the high forces generated by
416 tunnelling. However, the lack of significant association in the phylogenetic PLS analysis
417 suggests that the incisor and cranium do not covary over evolutionary time across all rodents,
418 and may in fact be separate modules (although, as above, this may be a result of low
419 statistical power owing to the relatively limited sample size). Such modularity has been
420 proposed to provide evolutionary flexibility in the face of different functional pressures
421 (Wagner & Altenberg, 1996; Kirschner & Gerhart, 1998), which may explain how chisel-

422 tooth digging has been able to evolve independently in several families of subterranean
423 rodents.

424

425 In summary, this study has shown that digging method is associated with skull morphology in
426 rodents. Chisel-tooth digging clearly imposes a set of functional demands that have led to the
427 convergent evolution of particular cranial and incisor morphologies. However, covariation
428 between the incisor and cranium is not consistent throughout the rodents, suggesting that
429 these two elements may not be tightly integrated, and may in fact be able to respond flexibly
430 to different selection pressures. Overall, the results indicated a complex interplay between
431 phylogeny and function driving the evolution of skull and tooth shape in rodents.

432

433 **DATA ACCESSIBILITY**

434 All numerical data deposited at http://figshare.com/authors/Philip_Cox/6179885

435

436 **ACKNOWLEDGEMENTS**

437 The authors would like to extend their thanks to our colleagues that supplied us with the
438 specimens used in this study: Dr Rob Asher and Mr Mathew Lowe (University Museum of
439 Zoology, Cambridge); Professor Christiane Denys and Dr Violaine Nicolas (Museum
440 National d'Histoire Naturelle, Paris); Dr Radim Šumbera (University of South Bohemia); Mr
441 Tony Parker (World Museum Liverpool); Professor Dominique Adriaens (Ghent University);
442 and Professor Nigel Bennett (University of Pretoria). We are grateful to Professor Michael
443 Fagan and Mrs Sue Taft (University of Hull) for microCT scanning specimens; and to Dr
444 Pierre-Henri Fabre (University of Montpellier) for providing the rodent phylogeny. We thank
445 Dr Lionel Hautier (University of Montpellier), Dr Helder Gomes Rodrigues (Museum
446 National d'Histoire Naturelle, Paris), Dr Nick Milne (University of Western Australia) and

447 Professor Paul O'Higgins, Dr Sam Cobb, Dr Laura Fitton and Professor John Currey
448 (University of York) for many stimulating and helpful conversations about this research. We
449 also thank Professor Chris Klingenberg, Dr Vida Jojić, and another anonymous reviewer for
450 the many insightful comments that have improved this manuscript. Andrew McIntosh was
451 funded by a University of Hull studentship.

452

453 **REFERENCES**

- 454 Adams, D.C. 2014a. A generalized K statistic for estimating phylogenetic signal from shape
455 and other high-dimensional multivariate data. *Syst. Biol.* **63**: 685-697.
- 456 Adams, D.C. 2014b. A method for assessing phylogenetic least squares models for shape and
457 other high-dimensional multivariate data. *Evolution* **68**: 2675-2688.
- 458 Adams, D.C. & Felice, R.N. 2014. Assessing trait covariation and morphological integration
459 on phylogenies using evolutionary covariance matrices. *PLoS ONE* **9**: e94335.
- 460 Adams, D.C. & Otárola-Castillo, E. 2013. Geomorph: an R package for the collection and
461 analysis of geometric morphometric shape data. *Methods Ecol. Evol.* **4**: 393-399.
- 462 Agrawal, V. 1967. Skull adaptations in fossorial rodents. *Mammalia* **31**: 300-312.
- 463 Akersten, W.A. 1981. A graphic method for describing the lateral profile of isolated rodent
464 incisors. *J. Vert. Paleo.* **1**: 231-234.
- 465 Alexander, R.M. 1983. *Animal mechanics*. Blackwell Scientific Publication: Oxford, UK.
- 466 Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance.
467 *Austral. Ecol.* **26**: 32-46.
- 468 Bacigalupe, L.D., Iriarte-Díaz, J. & Bozinovic, F. 2002. Functional morphology and
469 geographic variation in the digging apparatus of cururos (Octodontidae: *Spalacopus cyanus*).
470 *J. Mammal.* **83**: 145-152.

471 Bastir, M., Rosas, A. & Sheets, H.D. 2005. The morphological integration of the Hominoid
472 skull: a partial least squares and PC analysis with implications for European middle
473 Pleistocene mandibular variation. In: *Modern Morphometrics in Physical Anthropology* (D.
474 Slice, ed.), pp. 265-284. Kluwer Acad, New York, NY.

475 Becerra, F., Casinos, A. & Vassallo, A.I. 2013. Biting performance and skull biomechanics of
476 a chisel tooth digging rodent (*Ctenomys tuconax*; Caviomorpha; Octodontoidea). *J. Exp.*
477 *Zool.* **319A**: 74-85.

478 Becerra, F., Echeverría, A.I., Casinos, A. & Vassallo, A.I. 2014. Another one bites the dust:
479 bite force and ecology in three caviomorph rodents (Rodentia, Hystricognathi). *J. Exp. Zool.*
480 **321A**: 220-232.

481 Becerra, F., Vassallo, A.I., Echeverría, A.I. & Casinos, A. 2012. Scaling and adaptations of
482 incisors and cheek teeth in caviomorph rodents (Rodentia, Hystricognathi). *J. Morphol.*
483 **273A**: 1150-1162.

484 Blomberg, S.P. & Garland, T. 2002. Tempo and mode in evolution: phylogenetic inertia,
485 adaptation and comparative methods. *J. Evol. Biol.* **15**: 899-910.

486 Blomberg, S.P., Garland, T. & Ives, A.R. 2003. Testing for phylogenetic signal in
487 comparative data: behavioral traits are more labile. *Evolution* **57**: 717-745.

488 Bookstein, F.L., Gunz, P., Mitteröcker, P., Prossinger, H., Schæfer, K. & Seidler, H. 2003.
489 Cranial integration in *Homo*: singular warps analysis of the midsagittal plane in ontogeny and
490 evolution. *J. Hum. Evol.* **44**: 167-187.

491 Busch, C., Antinuchi, C.D., Del Valle, J.C., Kittlein, M., Malizia, A.I., Vassallo, A.I. et al.
492 2000. Population ecology of subterranean rodents. In: *Life Underground: The Biology of*
493 *Subterranean Rodents* (E.A. Lacey, J.L. Patton & G.M. Cameron eds), pp. 183-226.
494 University of Chicago Press, Chicago, IL.

495 Cardini, A. & Elton, S. 2008. Does the skull carry a phylogenetic signal? Evolution and
496 modularity in the guenons. *Biol. J. Linn. Soc.* **93**: 813-834.

497 Cheverud, J.M. 1982. Phenotypic, genetic, and environmental morphological integration in
498 the cranium. *Evolution* **36**: 499-516.

499 Cox, P.G. 2008. A quantitative analysis of the Eutherian orbit: correlations with masticatory
500 apparatus. *Biol. Rev.* **83**: 35-69.

501 Cox, P.G. & Baverstock H. 2016. Masticatory muscle anatomy and feeding efficiency in the
502 American beaver, *Castor canadensis* (Rodentia, Castoridae). *J. Mamm. Evol.* **23**: 191-200.

503 Croft, D.A., Niemi, K. & Franco, A. 2011. Incisor morphology reflects diet in caviomorph
504 rodents. *J. Mammal.* **92**: 871-879.

505 Doube, M., Kłosowski, M.M., Arganda-Carreras, I., Cordelières, F.P., Dougherty, R.P.,
506 Jackson, J.S. et al. 2010. BoneJ: Free and extensible bone image analysis in ImageJ. *Bone* **47**:
507 1076-1079.

508 Drake, A.G. 2011. Dispelling dog dogma: an investigation of heterochrony in dogs using 3D
509 geometric morphometric analysis of skull shape. *Evol Dev* **13**: 204-213.

510 Fabre, P.-H., Hautier, L., Dimitrov, D. & Douzery, E. 2012. A glimpse on the pattern of
511 rodent diversification: a phylogenetic approach. *BMC Evol. Biol.* **12**: 88.

512 Farke, A.A. 2008. Frontal sinuses and head-butting in goats: a finite element analysis. *J. Exp.*
513 *Biol.* **211**: 3085-3094.

514 Felsenstein, J. 1985. Phylogenies and the comparative method. *Am. Nat.* **125**: 1-15.

515 Gomes Rodrigues, H., Šumbera, R. & Hautier, L. 2016. Life in burrows channelled the
516 morphological evolution of the skull in rodents: the case of African mole-rats (Bathyergidae,
517 Rodentia). *J. Mamm. Evol.* **23**: 175-189.

518 Goodall, C.R. 1991. Procrustes methods in the statistical analysis of shape. *J. R. Stat. Soc.*
519 *Ser. B* **53**: 285-339.

520 Grafen, A. 1989. The phylogenetic regression. *Phil. Trans. R. Soc. Lond. B* **326**: 119-157.

521 Hallgrímsson, B., Jamniczky, H., Young, N.M., Rolian, C., Parsons, T.E., Boughner, J.C. et
522 al. 2009. Deciphering the palimpsest: studying the relationship between morphological
523 integration and phenotypic covariation. *Evol. Biol.* **36**: 355-376.

524 Hallgrímsson, B., Lieberman, D.E., Young, N.M., Parsons, T. & Wat, S. 2007. Evolution of
525 covariance in the mammalian skull. *Novartis Foundation Symposium Vol. 284*. John Wiley,
526 New York, NY.

527 Hautier, L., Lebrun, R. & Cox, P.G. 2012. Patterns of covariation in the masticatory
528 apparatus of hystricognathous rodents: Implications for evolution and diversification. *J.*
529 *Morph.* **273**: 1319-1337.

530 Herring, S.W. 1993. Formation of the vertebrate face: epigenetic and functional influences.
531 *Am. Zool.* **33**: 472-483.

532 Herring, S.W. & Teng, S. 2000. Strain in the braincase and its sutures during function. *Am. J.*
533 *Phys. Anthropol.* **112**: 575-593.

534 Herzberg, F. & Schour, I. 1941. The pattern of appositional growth in the incisor of the rat.
535 *Anat. Rec.* **80**: 497-506.

536 Hildebrand, M. 1985. Digging of quadrupeds. In: *Functional Vertebrate Morphology* (M.
537 Hildebrand, D.M. Bramble, K.F. Liem & D.B. Wake, eds), pp. 89-109. The Belknap Press,
538 London.

539 Jarvis, J. & Bennett, N. 1991. Ecology and behavior of the family Bathyergidae. In: *The*
540 *Biology of the Naked Mole-rat* (P.W. Sherman, J.U.M. Jarvis & R.D. Alexander, eds), pp. 66-
541 96. Princeton University Press, Princeton, NJ.

542 Kendall, D.G. 1984. Shape manifolds, procrustean metrics, and complex projective spaces.
543 *Bull. Lond. Math. Soc.* **16**: 81-121.

544 Kirschner, M. & Gerhardt, J. 1998. Evolvability. *Proc. Nat. Acad. Sci. USA* **95**: 8420-8427.

545 Klingenberg, C.P. 2009. Morphometric integration and modularity in configurations of
546 landmarks: tools for evaluating a priori hypotheses. *Evol. Dev.* **11**: 405-421.

547 Klingenberg, C.P. 2014. Studying morphological integration and modularity at multiple
548 levels: concepts and analysis. *Phil. Trans. R. Soc. Lond. B* **369**: 20130249.

549 Klingenberg, C.P. & Marugán-Lobón, J. 2013. Evolutionary covariation in geometric
550 morphometric data: analyzing integration, modularity, and allometry in a phylogenetic
551 context. *Syst. Biol.* **62**: 591-610.

552 Landry, S.O. 1957. Factors affecting the procumbency of rodent upper incisors. *J. Mammal.*
553 **38**: 223-234.

554 Lessa, E.P. 1990. Morphological evolution of subterranean mammals: integrating structural,
555 functional, and ecological perspectives. In: *Evolution of Subterranean Mammals at the*
556 *Organismal and Molecular Levels* (E. Nevo & O.A. Reig, eds.), pp. 211-230. Wiley-Liss,
557 New York, NY.

558 Lessa, E.P. & Patton, J.L. 1989. Structural constraints, recurrent shapes, and allometry in
559 pocket gophers (genus *Thomomys*). *Biol. J. Linn. Soc.* **36**: 349-363.

560 Lessa, E.P. & Thaeler, C.S. 1989. A reassessment of morphological specializations for
561 digging in pocket gophers. *J. Mammal.* **70**: 689-700.

562 Lin, G., Xie, J., Ci, H., Tang, L., Su, J. & Zhang, T. 2010. Morphological adaptations of
563 incisors in the subterranean Gansu zokor, *Myospalax cansus* (Rodentia, Spalacidae). *Folia*
564 *Zool.* **59**: 295-300.

565 Martins, E.P. & Hansen, T.F. 1997. Phylogenies and the comparative method: a general
566 approach to incorporating phylogenetic information into the analysis of interspecific data.
567 *Am. Nat.* **149**: 646-667.

568 Maynard Smith, J. & Savage, R.J.G. 1959. The mechanics of mammalian jaws. *Sch. Sci. Rev.*
569 **40**: 289-301.

570 McIntosh, A.F. & Cox, P.G. 2016. Functional implications of craniomandibular morphology
571 in African mole-rats (Rodentia: Bathyergidae). *Biol. J. Linn. Soc.* **117**: 447-462.

572 Mora, M., Olivares, A.I. & Vassallo, A.I. 2003. Size, shape and structural versatility of the
573 skull of the subterranean rodent *Ctenomys* (Rodentia, Caviomorpha): functional and
574 morphological analysis. *Biol. J. Linn. Soc.* **78**: 85-96.

575 Moss, M.L. & Young R.W. 1960. A functional approach to craniology. *Am. J. Phys.*
576 *Anthropol.* **18**: 281-292.

577 Nowak, R.M. 1999. *Walker's Mammals of the World*. Johns Hopkins University Press,
578 Baltimore, MD.

579 O'Higgins, P. 2000. The study of morphological variation in the hominid fossil record:
580 biology, landmarks and geometry. *J. Anat.* **197**: 103-120.

581 Pagel, M. 1999. Inferring the historical patterns of biological evolution. *Nature* **401**: 877-884.

582 Paradis, E., Claude, J. & Strimmer, K. 2004. APE: analyses of phylogenetics and evolution in
583 R language. *Bioinformatics* **20**: 289-290.

584 Pinheiro, J., Bates, D., Debroy, S., Sarkar, D. & R Core Team (2016). *nlme: Linear and*
585 *Nonlinear Mixed Effects Models (R package version 3.1-111.)*.

586 Polly, P.D, Lawing, A.M., Fabre, A.-C. & Goswami, A. 2013. Phylogenetic principal
587 components analysis and geometric morphometrics. *Hystrix* **24**: 33-41.

588 Preuschoft, H. & Witzel, U. 2004. Functional structure of the skull in Hominoidea. *Folia*
589 *Primatol.* **75**: 219-252.

590 Revell, L.J. 2009. Size-correction and principal components for interspecific comparative
591 studies. *Evolution* **63**: 3258-3268.

592 Revell, L.J. 2010. Phylogenetic signal and linear regression on species data. *Methods Ecol.*
593 *Evol.* **1**: 319-329.

594 Revell, L.J. 2012. phytools: an R package for phylogenetic comparative biology (and other
595 things). *Methods Ecol. Evol.* **3**: 217-223.

596 Rohlf, F.J. 2001. Comparative methods for the analysis of continuous variables: geometric
597 interpretations. *Evolution* **55**: 2143-2160.

598 Rohlf, F.J. & Corti, M. 2000. Use of two-block partial least-squares to study covariation in
599 shape. *Syst. Biol.* **49**: 740-753.

600 Rohlf, F.J. & Slice, D. 1990. Extensions of the Procrustes method for the optimal
601 superimposition of landmarks. *Syst. Zool.* **39**: 40-59.

602 Samuels, J.X. 2009. Cranial morphology and dietary habits of rodents. *Zool. J. Linn. Soc.*
603 **156**: 864-888.

604 Samuels, J.X. & Van Valkenburgh, B. 2009. Craniodental adaptations for digging in extinct
605 burrowing beavers. *J. Vert. Paleo.* **29**: 254-268.

606 Schneider, C.A., Rasband, W.S. & Eliceiri, K.W. 2012. NIH Image to ImageJ: 25 years of
607 image analysis. *Nature Methods* **9**: 671-675.

608 Stein, B.R. 2000. Morphology of subterranean rodents. In: *Life Underground: The Biology of*
609 *Subterranean Rodents* (E.A. Lacey, J.L. Patton & G.N. Cameron, eds.), pp. 19-61. University
610 of Chicago Press, Chicago, IL.

611 Turnbull, W.D. 1970. Mammalian masticatory apparatus. *Fieldiana (Geol.)* **18**: 147-356.

612 Uyeda, J.C., Caetano, D.S. & Pennell, M.W. 2015. Comparative analysis of principal
613 components can be misleading. *Syst. Biol.* **64**: 677-689

614 Van der Merwe, M. & Botha, A.J. 1998. Incisors as digging tools in molerats (Bathyergidae).
615 *S. Afr. J. Zool.* **33**: 230-235.

616 Vassallo, A.I. 1998. Functional morphology, comparative behaviour, and adaptation in two
617 sympatric subterranean rodents genus *Ctenomys* (Caviomorpha: Octodontidae). *J. Zool.* **244**:
618 415-427.

619 Verzi, D.H., Álvarez, A., Olivares, A.I., Morgan, C.C. & Vassallo, A.I. 2010. Ontogenetic
620 trajectories of key morphofunctional cranial traits in South American subterranean ctenomyid
621 rodents. *J. Mammal.* **91**: 1508-1516.

622 Vleck, D. 1979. The energy cost of burrowing by the pocket gopher *Thomomys bottae*.
623 *Physiol. Zool.* **52**: 122-136.

624 Wagner, G.P. & Altenberg, L. 1996. Complex adaptations and the evolution of evolvability.
625 *Evolution* **50**: 967-976.

626 Weishampal, D.B. 1993. Beams and machines: Modeling approaches to the analysis of skull
627 form and function. In: *The Skull. Vo1. 3: Functional and Evolutionary Mechanisms* (J.
628 Hanken and B.K. Hall, eds), pp. 303-344. University of Chicago Press, Chicago, IL.

629 Yang, Z., Kumar, S. & Nei, M. 1995. A new method of inference of ancestral nucleotide and
630 amino acid sequences. *Genetics* **141**: 1641-1650.

631

632

633

634

635

636

637

638

639

640

641

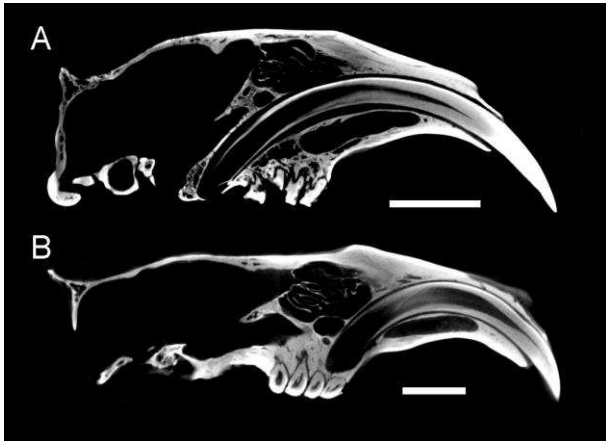
642

643

644 **Table 1.** List of genera analysed including specimen number, diet and mode of digging.
 645 Abbreviations for dietary categories: O, omnivore; GH, generalist herbivore; SH, specialist
 646 herbivore. Dietary categories follow method of Samuels (2009). **Subterranean rodent**
 647 **genera are in bold.**

Family	Genus #	Genus	N	Diet	Digging mode
Bathyergidae	1	Bathyergus	10	SH	Scratch
	2	Cryptomys	1	SH	Chisel-tooth
	3	Fukomys	9	SH	Chisel-tooth
	4	Georychus	3	SH	Chisel-tooth
	5	Heliophobius	10	SH	Chisel-tooth
	6	Heterocephalus	5	SH	Chisel-tooth
Caviidae	7	Cavia	2	SH	
Cricetidae	8	Paralomys	1	GH	
Ctenomyidae	9	Ctenomys	1	SH	Scratch
Dipodidae	10	Dipus	1	GH	
Erethizontidae	11	Erethizon	1	SH	
Geomyidae	12	Geomys	1	SH	Scratch
	13	Thomomys	1	SH	Scratch
Muridae	14	Rattus	2	O	
Octodontidae	15	Octodon	1	GH	
	16	Spalacopus	1	SH	Chisel-tooth
Spalacidae	17	Cannomys	1	SH	Chisel-tooth
	18	Rhizomys	3	SH	Chisel-tooth
	19	Tachyoryctes	4	SH	Chisel-tooth
Sciuridae	20	Sciurus	7	O	

648 **FIGURES**



649

650 **Fig. 1.** Parasagittal slice of CT scan in two subterranean rodents: A, chisel-tooth digging
651 *Georychus capensis*; and B, scratch digging *Bathyergus suillus*. Notice the posterior
652 displacement of the incisor root in *Georychus capensis* compared with *Bathyergus suillus*.

653 Scale bars = 10 mm.

654

655

656

657

658

659

660

661

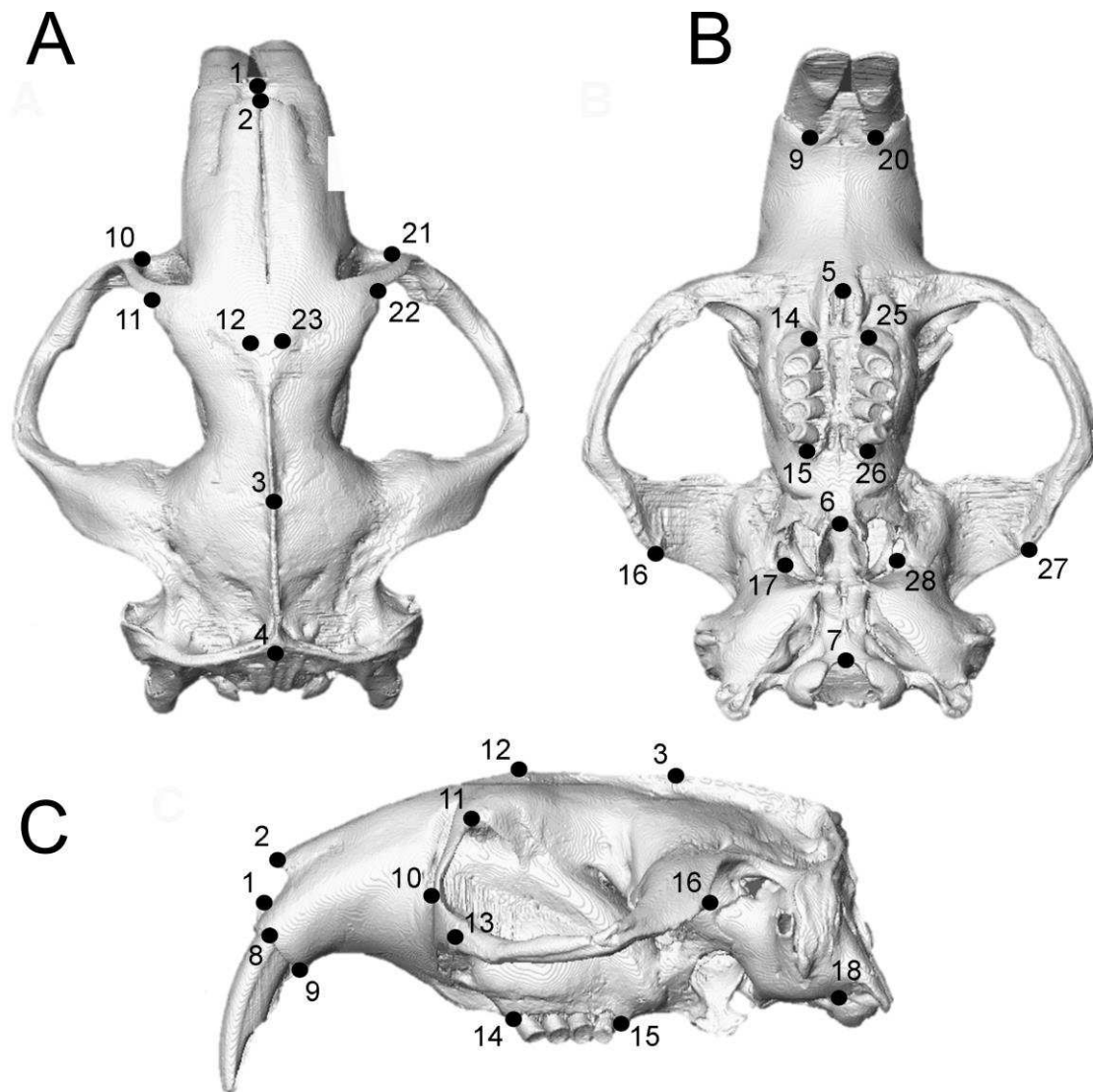
662

663

664

665

666



667

668 **Fig. 2.** Landmark configuration represented on *Fukomys mechowii*. A, dorsal view. B, ventral
 669 view. C, lateral view. See Table S1 for corresponding landmark numbers and descriptions.

670

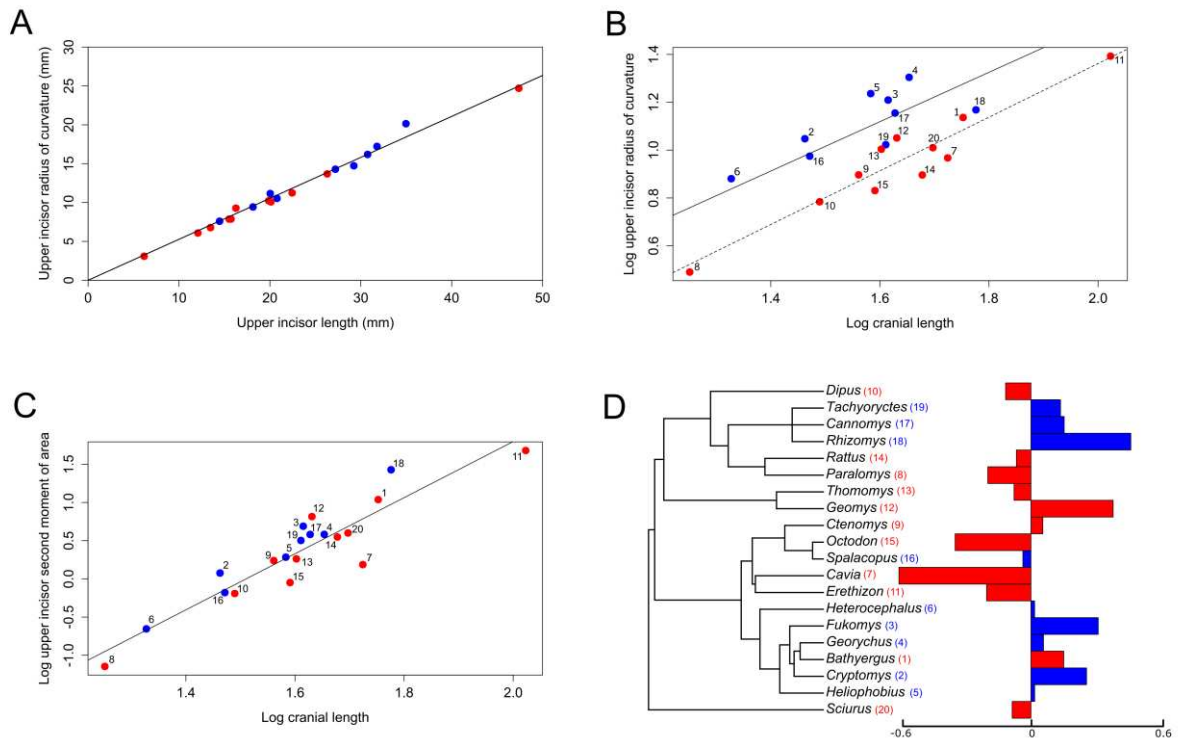
671

672

673

674

675



676

677 **Fig. 3.** A, OLS model fitted through origin showing the relationship between upper incisor
 678 length and upper incisor RoC. B, Phylogenetic ANCOVA representing the relationship
 679 between cranial length, upper incisor RoC and digging method. C, PGLS representing the
 680 relationship between cranial length and upper incisor second moment of area. D, Phylogeny
 681 of data with accompanying SMA residual values from PGLS of cranial length and upper
 682 incisor SMA. Chisel-tooth digging genera are in blue. Non-tooth digging genera are in red
 683 (including non-fossorial species). Corresponding genus numbers are given in Table 1.

684

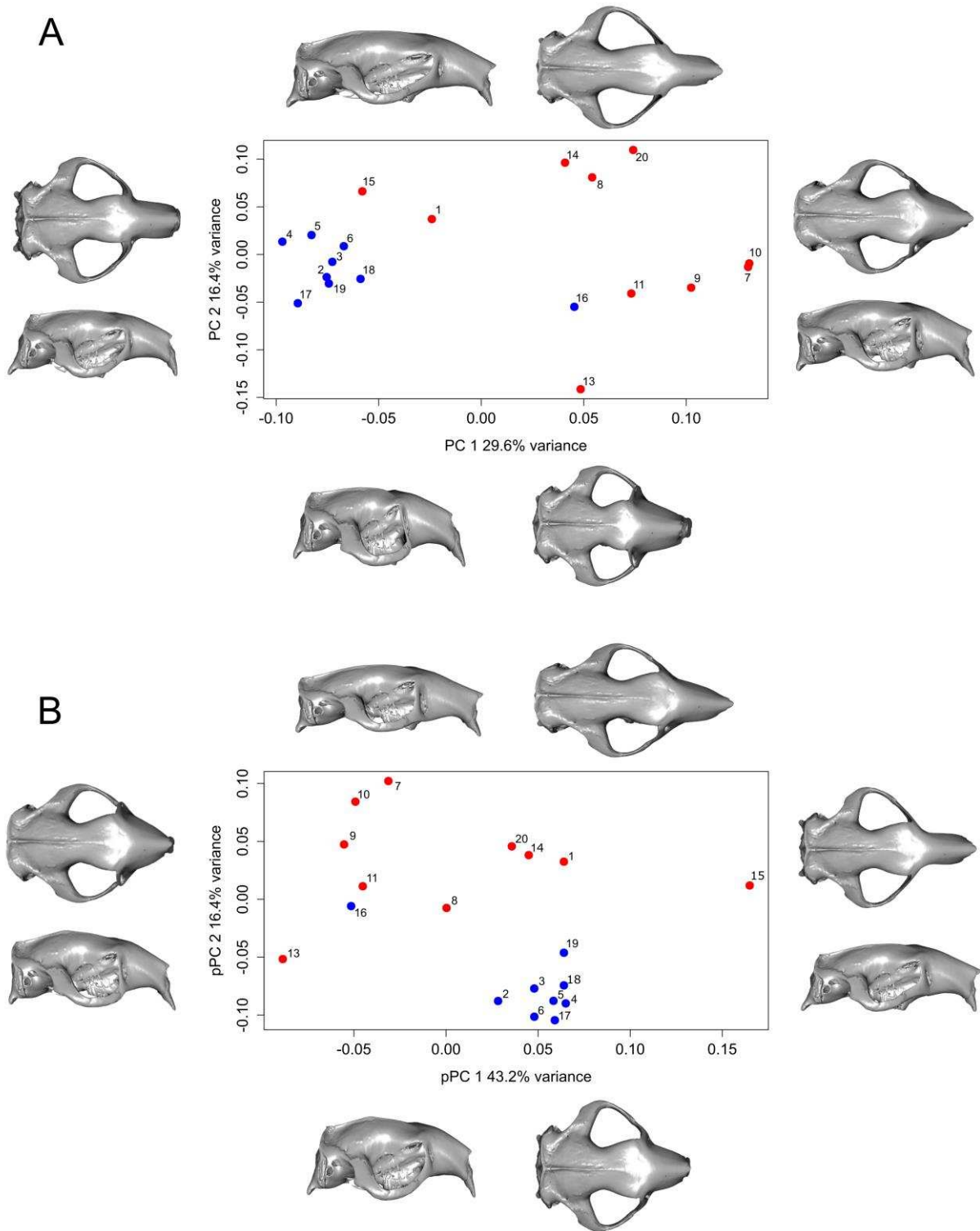
685

686

687

688

689



690

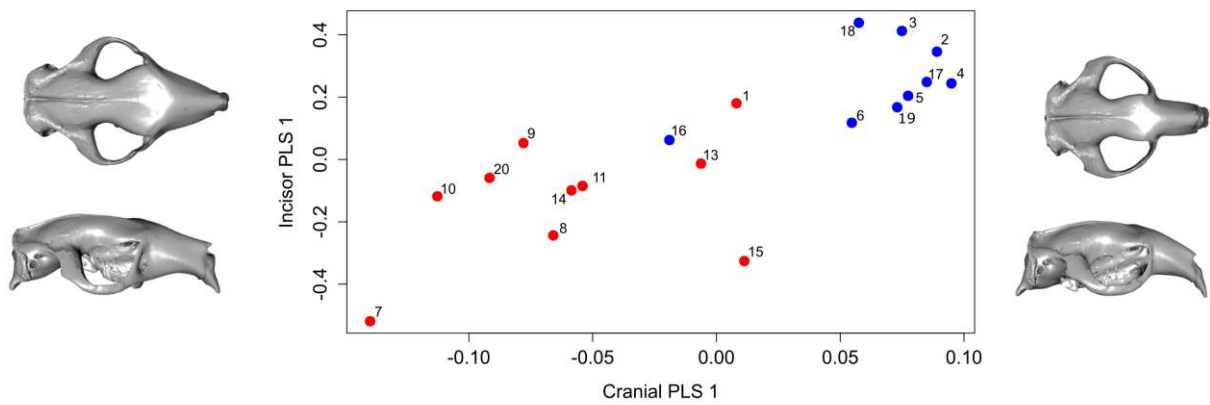
691 **Fig. 4.** A, Principal components analysis with associated virtual deformations representing
 692 shape variation at the extreme ends of PC1 and PC2. B, Phylogenetic principal components
 693 analysis with associated virtual deformations representing non-phylogenetic shape variation
 694 at the extreme ends of pPC1 and pPC2 axes. Chisel-tooth digging genera are in blue. Non-

695 tooth digging genera are in red (including non-fossorial species). Corresponding genus
696 numbers are given in Table 1.

697

698

699



700

701 **Fig. 5.** Partial least squares analysis showing cranial shape and incisor covariation across
702 PLS1 (accounts for 93.7% squared total covariance). Virtual deformations of the cranium
703 shown at the extreme ends of cranial PLS1. Chisel-tooth digging genera are in blue. Non-
704 tooth digging genera are in red (including non-fossorial species). Corresponding genus
705 numbers are given in Table 1.

706

707

708

709

710

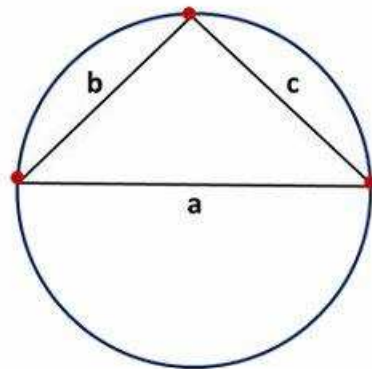
711

712

713

714 SUPPORTING INFORMATION

715



$$\text{RoC} = \frac{abc}{\sqrt{(2a^2b^2 + 2b^2c^2 + 2c^2a^2 - a^4 - b^4 - c^4)}}$$

716

717 **Figure S1.** Measuring incisor RoC. The length of the incisor (L) is measured using the base

718 of the triangle (a). Red points represent the 3 landmarks placed on the surface of the incisor.

719

720

721

722

723

724

725

726

727

728

729

730

731

732 **Table S1.** Cranial landmarks used in geometric morphometric analysis.

Number	Landmark definition
1	Midpoint of ventral margin of nasal opening
2	Antermost point on internasal suture
3	Bregma
4	Posteriormost point on dorsal midline
5	Midpoint between anterior extremities of incisive foramina
6	Posteriormost midline point on palatine
7	Midline point on ventral margin of foramen magnum
8 & 19	Dorsalmost point on incisal alveolar margin
9 & 20	Posteriormost point on incisal alveolar margin
10 & 21	Lateralmost point on margin of infraorbital foramen
11 & 22	Dorsalmost point on orbital margin
12 & 23	Posteriormost point of naso-frontal suture
13 & 24	Antermost point of maxillo-jugal suture
14 & 25	Anterior extremity of cheek tooth row
15 & 26	Posterior extremity of cheek tooth row
16 & 27	Posterior tip of zygomatic arch
17 & 28	Posteriormost point of foramen ovale
18 & 29	Lateralmost point of hypoglossal foramen

733
 734 Landmarks 1-7 recorded from midline, landmarks 8-18 recorded on left side of skull,
 735 landmarks 19-29 recorded on right side of skull.

736
 737

738 **Table S2.** Variance represented by first 10 principal components of non-phylogenetically
 739 informed and phylogenetically informed analyses.

740

Principal component	Non-phylogenetic		Phylogenetic	
	Percentage variance	Cumulative variance	Percentage variance	Cumulative variance
1	29.51	29.51	43.20	43.20
2	16.47	45.98	16.35	59.55
3	11.59	57.56	9.05	68.61
4	10.04	67.60	6.08	74.68
5	7.34	74.94	4.57	79.25
6	5.54	80.48	3.58	82.83
7	4.41	84.90	3.09	85.92
8	3.75	88.64	2.61	88.53
9	2.60	91.24	2.50	91.04
10	2.23	93.47	1.99	93.03

741