

Digital dissection of the masticatory muscles of the naked mole-rat, *Heterocephalus glaber* (Mammalia, Rodentia)

The naked mole-rat, *Heterocephalus glaber*, of the family Bathyergidae is a subterranean rodent that feeds on underground roots and tubers and digs extensive tunnel systems with its incisors. It is a highly unusual mammal with regard to its social structure, longevity, pain insensitivity and cancer resistance, all of which have made it the subject of a great deal of research in recent years. Yet, much of the basic anatomy of this species remains undocumented. In this paper, we describe the morphology of the jaw-closing musculature of the naked mole-rat, as revealed by contrast-enhanced micro-computed tomography. This technique uses an iodine stain to enable the imaging of soft tissues with microCT. The iodine-enhanced scans were used to create 3D reconstructions of the naked mole-rat masticatory muscles from which muscle masses were calculated. The jaw-closing musculature of *Heterocephalus glaber* is relatively very large compared to other rodents and is dominated by the superficial masseter, the deep masseter and the temporalis. The temporalis in particular is large for a rodent, covering the entirety of the braincase and much of the rear part of the orbit. The morphology of the masseter complex described here differs from two other published descriptions of bathyergid masticatory muscles, but is more similar to the arrangement seen in other rodent families. The zygomaticomandibularis (ZM) muscle does not protrude through the infraorbital foramen on to the rostrum and thus the naked mole-rat should be considered protrogomorphous rather than hystricomorphous, and the morphology is consistent with secondarily lost hystricomorphy as has been previously suggested for Bathyergidae. Overall, the morphology of the masticatory musculature indicates a species with a high bite force and a wide gape – both important adaptations for a life dominated by digging with the incisors.

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6 Introduction

7 The naked mole-rat, *Heterocephalus glaber* (Rüppell, 1842), is found in the hot, dry regions of
8 the Horn of Africa (Honeycutt *et al.* 1991), and is probably the most well-known member of the
9 Bathyergidae – a highly specialised group of subterranean rodents known as the African mole-
10 rats or blesmols. Like all bathyergids except *Bathyergus* (which mainly uses its foreclaws), the
11 naked mole-rat digs with its incisors and is able to close its lip folds behind the incisors to
12 prevent soil from entering the mouth during tunnelling (Tucker, 1981). Naked mole-rats feed
13 exclusively on underground roots and tubers, and their so-called ‘chisel-toothed’ digging through
14 hard, compact soil in the search for widely dispersed food resources is likely to have influenced
15 the musculature of the jaw. The naked mole-rat is the only species within the genus
16 *Heterocephalus*, and phylogenetic analyses indicate that it is the most basal extant species of the
17 family (Allard & Honeycutt, 1992), with an estimated divergence from the other bathyergid
18 genera between 40 and 48 million years ago (Faulkes *et al.*, 2004), or 33-35 million years ago,
19 depending on the fossil calibration of the molecular clock (Faulkes *et al.*, 2011; Ingram *et al.*,
20 2004). On this basis, some authors place the naked mole-rat in its own subfamily, the
21 Heterocephalinae, distinct from the remaining genera in the Bathyerginae (e.g., Wilson & Reeder,
22 2005). The naked mole-rat first came to prominence scientifically when it was shown that its
23 complex social structure is in fact a rare example of eusociality in vertebrates (Jarvis, 1981,
24 1991). That is, naked mole-rats have a caste system analogous to that of ants and termites, with a
25 breeding female, or queen, at the top of the hierarchy, and smaller, non-breeding workers at the
26 bottom. Since then, naked mole-rats have been discovered to possess many other unusual
27 qualities that have placed them in the forefront of research in a number of fields. For example,
28 naked mole-rats appear to be insensitive to acid-induced pain (Smith *et al.*, 2011), extraordinarily
29 resistant to cancer (Seluanov *et al.*, 2009), and extremely long-lived for a small mammal
30 (Buffenstein, 2005).

31 According to recent estimates (Faulkes & Bennett, 2013), the Bathyergidae comprises thirty or
32 more species, in six genera, and is inferred to have originated in the Eocene of Africa (Huchon &
33 Douzery, 2001) although the earliest known fossils are Miocene in age (Faulkes *et al.*, 2004;
34 Mein & Pickford, 2008). On the basis of lower jaw and inner ear morphology, the bathyergids
35 were considered members of the Hystricognathi by Tullberg (1899). Subsequent works tended to
36 preserve this relationship, with the Bathyergidae frequently being closely allied to two other

37 families of Old World hystricognaths, the Thryonomyidae and Petromuridae (Landry, 1957;
38 Wood, 1974). However, a degree of doubt remained over the placement of bathyergids owing to
39 the unusual morphology of their masticatory muscles. Almost all living rodents can be classified
40 as sciuriform (squirrel-like), myomorph (mouse-like) or hystricomorph (porcupine-like) based
41 on the morphology of the masseter muscle (Brandt, 1855; Wood, 1965). Most members of the
42 Hystricognathi are defined as being hystricomorph, owing to the possession of an enlarged
43 infraorbital foramen, through which a substantial portion of the zygomaticomandibularis (ZM)
44 muscle (or medial masseter) extends to take an origin on the rostrum. However, in most
45 Bathyergidae, the infraorbital foramen is small, simply transmitting the infraorbital artery and the
46 infraorbital branch of the maxillary nerve (Maier & Schrenk, 1987). Thus, no part of the
47 masticatory musculature attaches to the rostrum. This morphology is termed protrogomorphous,
48 and is thought to be the ancestral condition for rodents also seen in many Eocene fossil taxa
49 (Wood, 1965) and the extant mountain beaver, *Aplodontia rufa* (Druzinsky, 2010), although
50 claims of hystricomorphy in *Aplodontia* have also been made (Eastman, 1982). It should be
51 noted, however, that a moderate enlargement of the infraorbital foramen is seen in two recently-
52 split extant genera of blesmols, *Cryptomys* (Boller, 1970; Morlock, 1983) and *Fukomys* (Van
53 Daele, *et al.*, 2009), as well as in fossil genera from the Miocene of East Africa, (Lavocat, 1973,
54 1974). In *Cryptomys* and *Fukomys* this enlargement is accompanied by a very limited extension
55 of the ZM through the foramen on to the rostrum (Boller, 1970; Van Daele *et al.*, 2009).

56 Given the variable morphology of the ZM and infraorbital foramen seen amongst extant and
57 fossil genera, bathyergid protrogomorphy was the subject of some debate for a number of years,
58 particularly with regard to its evolutionary history: Does the bathyergid condition represent
59 retention of the primitive condition or is the morphology secondarily derived? Tullberg (1899)
60 and Wood (1965, 1985) believed that bathyergids show the ancestral condition, and that
61 *Cryptomys* demonstrates nascent hystricomorphy. On the other hand, Landry (1957), Lavocat
62 (1974) and Lockett & Hartenberger (1985) were of the opinion that bathyergids evolved from a
63 hystricomorph ancestor, and that their current morphology represents a reversal to the primitive
64 condition. Maier & Schrenk (1987) added support to the latter view by showing that small
65 bundles of fibres of the ZM muscle protrude through the infraorbital foramen early in
66 development in two genera of blesmols, *Bathyergus* and *Georchus*, but subsequently retreat
67 from the rostrum, and are absent at birth. More recently, molecular analyses have supported a
68 close evolutionary association of the Bathyergidae, Thryonomyidae (cane-rats) and Petromuridae

69 (dassie rat), within the Hystricognathi (Blanga-Kanfi *et al.*, 2009; Fabre *et al.*, 2012). Given the
70 clear hystricomorphy of *Thryonomys* and *Petromus* (Woods, 1972), under this phylogenetic
71 arrangement it would be more parsimonious to assume that blesmols evolved from a
72 hystricomorph ancestor, than that they retained the protrogomorph condition, and hence that
73 hystricomorphy evolved at least three times independently within the hystricognath radiation (in
74 Hystricidae; in the ancestor of Thryonomyidae+Petromuridae; and in the Caviomorpha), as well
75 as once in the Ctenodactyloidea, the sister-clade to Hystricognathi.

76 Despite the controversy surrounding mole-rat muscular morphology, there are very few detailed
77 descriptions of the masticatory musculature of bathyergids. In his monumental study of rodent
78 anatomy, Tullberg (1899) included three bathyergid species: *Georychus capensis*, *Georychus*
79 *coecutiens* (now synonymised with *Cryptomys hottentotus* according to Wilson & Reeder [2005])
80 and *Bathyergus maritimus* (now *B. suillus*). As mentioned above, the jaw-closing muscles of
81 *Cryptomys hottentotus* were also described by Boller (1970). Morlok (1983) examined all five
82 genera of bathyergids recognised at the time (*Bathyergus*, *Cryptomys*, *Georychus*, *Heliophobius*
83 and *Heterocephalus*), but only provided detailed descriptions of the musculature of *Cryptomys*.
84 More recently, the jaw-closing muscles of the genus *Fukomys* (formerly part of *Cryptomys* [Kock
85 *et al.*, 2006]) were briefly described by Van Daele *et al.* (2009). The Bathyergidae were excluded
86 from the comparative study of New and Old World hystricomorphs of Woods (1972) owing to
87 their perceived protrogomorphy. This study aims to fill at least part of this gap in the comparative
88 literature by examining the masticatory musculature of one particularly notable absentee, the
89 naked mole-rat.

90 One reason underlying the lack of published literature on the masticatory musculature of the
91 naked mole-rat may be the small size of this species, which renders traditional dissection more
92 difficult than for larger species. However, its small size also makes the naked mole-rat an ideal
93 candidate for digital dissection via contrast-enhanced micro-computed tomography (microCT)
94 and virtual reconstruction. This technique is based on the work of Metscher (2009) and Jeffery *et*
95 *al.* (2011) and involves the staining of biological specimens with iodine potassium iodide (I₂KI)
96 to enable the visualisation of soft tissues with microCT. The musculature of several rodent
97 species has already been successfully imaged using this technique (Cox & Jeffery, 2011; Hautier
98 *et al.*, 2012; Baverstock *et al.*, 2013) as well as that of other vertebrates (Tsai & Holliday, 2011;

99 Düring *et al.*, 2013; George & Holliday, 2013; Gignac & Kley, 2014; Lautenschlager *et al.*,
100 2014). The aim of this study is to provide a description of the jaw-closing musculature of *H.*
101 *glaber*. Given the demands made on the masticatory system by the chisel-tooth digging by which
102 it excavates tunnels, knowledge of the masticatory muscles is of prime importance for
103 understanding the biology of the naked mole-rat. The descriptions provided here will enable
104 comparisons to be made between the naked mole-rat and the other genera of bathyergids already
105 described in the literature, as well as with other members of the Hystricognathi, both fossorial
106 and non-fossorial.

107 **Materials and Methods**

108 *Sample and scanning*

109 Three naked mole-rat individuals were obtained from collections held at Queen Mary University
110 of London. The specimens were all non-breeding workers and had been preserved in 95% ethanol
111 for several years. In order to visualise the bony morphology, the specimens were imaged using
112 microCT at the Department of Engineering, University of Hull. The scans were performed at 100
113 kV and 37 μ A, with a copper filter averaging two frames per projection. Isometric voxel sizes
114 ranged between 0.015 and 0.02 mm. Following the initial scanning, the specimens were
115 immersed in a 5% solution of I₂KI dissolved in phosphate-buffered formal saline for a period of
116 two weeks. The stained specimens were then microCT scanned, again at the Department of
117 Engineering, University of Hull. The scan was performed at 80 kV and 60 μ A without a filter,
118 with 4000 projections averaging 2 frames per projection, and using a beryllium target. Voxels
119 were isometric and ranged between 0.022 and 0.024 mm in size.

120 *Digital reconstruction*

121 Only one of the contrast-enhanced microCT scans (corresponding to specimen ‘Hetero3’) was
122 sufficiently well-resolved to enable virtual reconstruction of the masticatory muscles. This scan
123 was imported as a stacked TIFF into Avizo 8.0 (FEI Visualization Sciences Group, Burlington,
124 MA, USA), and the masticatory muscles of the right side of the head were reconstructed.
125 Although the muscles were clearly visible as individual components, the contrast difference
126 between muscle and bone was not sufficiently different to enable automatic thresholding of the
127 muscles. Thus manual segmentation was employed to produce the 3D muscle reconstructions.
128 The cranium and mandible were also reconstructed to facilitate the visualisation of attachment
129 areas. However, the bony components were reconstructed from the initial unstained scans to

130 allow automatic thresholding. Bone and muscle reconstructions were then brought together and
131 aligned in Avizo 8.0 to produce high resolution figures and movies. Downsampled surface files of
132 the skull, mandible and muscles were combined using Adobe 3D Reviewer (Adobe Systems Inc.,
133 San Jose, CA, USA) to produce a 3D interactive PDF (Supplementary File S1) following the
134 method outlined by Lautenschlager (2014). The reconstructed surface file, along with the
135 microCT scan, was deposited in Hydra, the University of Hull data repository, under the
136 accession number 8475 (<https://hydra.hull.ac.uk/resources/hull:8475>). The remaining two scans,
137 whilst not of sufficient quality for digital reconstruction, were at least detailed enough to provide
138 comparisons to Hetero3, and were of help in determining muscle morphology.

139 *Analysis*

140 Muscle volumes were calculated by Avizo 8.0 and converted to masses assuming muscle density
141 of 1.0564 g/cm³ (Murphy & Beardsley, 1974). It was clear from the microCT images that the
142 specimen had suffered extensive soft tissue shrinkage. This is likely to be a result of the iodine
143 staining (Vickerton *et al.*, 2013) as well as the lengthy preservation time. Therefore the absolute
144 mass of each masticatory muscle should be approached with some caution. Vickerton *et al.*
145 (2013) provided estimates of percentage shrinkage for various concentrations of I2KI and
146 staining durations, but calculation of degree of muscle shrinkage is not possible in this study as it
147 is unknown exactly how long the specimens had been in ethanol before staining. However, the
148 muscle attachment sites, their relative positions and their relationship to other anatomical
149 structures were not affected by shrinkage. Moreover, the preservation and staining techniques
150 affect all muscles equally, so the topology and relative proportions of the muscles can be analysed
151 with confidence. Condylbasal cranial length (the midline distance along the cranial base from
152 the anterior extremity of the premaxillae to the margin of the foramen magnum) of the specimen
153 was measured to be 18.9 mm.

154 The arrangement of jaw-closing muscles in the naked mole-rat revealed by contrast-enhanced
155 microCT was compared to previously published descriptions of masticatory muscles in other
156 bathyergids: Tullberg (1899); Boller (1970); Morlock (1983) and Van Daele *et al.* (2009). In
157 addition, to understand how naked mole-rat masticatory muscles are similar to or differ from
158 rodents more generally, the results here were compared to published descriptions of other rodents
159 including sciuriforms, myomorphs and hystricomorphs, as well as the only living
160 protrogomorph, *Aplodontia rufa*. The literature consulted was as follows: Müller, 1933

161 (*Hydrochoerus*); Greene, 1935 (*Rattus*); Schumacher & Rehmer, 1962 (*Cavia*, *Rattus*); Wood,
162 1965 (*Marmota*, *Myocastor*, *Ondatra*); Turnbull, 1970 (*Sciurus*, *Rattus*, *Hystrix*); Woods, 1972
163 (*Proechimys*, *Echimys*, *Isothrix*, *Mesomys*, *Myocastor*, *Octodon*, *Ctenomys*, *Erethizon*, *Cavia*,
164 *Chinchilla*, *Dasyprocta*, *Thryonomys*, *Petromus*); Weijs, 1973 (*Rattus*); Woods & Howland, 1979
165 (*Capromys*, *Geocapromys*, *Plagiodontia*, *Myocastor*); Woods & Hermanson, 1985 (*Capromys*,
166 *Geocapromys*, *Plagiodontia*, *Myocastor*, *Echimys*, *Octodon*, *Erethizon*, *Coendou*, *Dasyprocta*,
167 *Atherurus*, *Thryonomys*, *Petromus*); Offermans & De Vree, 1989 (*Pedetes*); Ball & Roth, 1995
168 (*Sciurus*, *Microsciurus*, *Sciurillus*, *Tamiasciurus*, *Tamias*, *Glaucomys*); Thorington & Darrow,
169 1996 (*Aplodontia*, *Paraxerus*, *Funisciurus*, *Myosciurus*, *Heliosciurus*, *Protoxerus*, *Funambulus*,
170 *Calliosciurus*, *Tamiops*, *Xerus*, *Atlantoxerus*, *Ratufa*); Olivares *et al.*, 2004 (*Aconaemys*,
171 *Octomys*, *Tympanoctomys*, *Spalacopus*, *Octodon*, *Octodontomys*); Satoh & Iwaku, 2004
172 (*Cricetulus*, *Mesocricetus*, *Phodopus*, *Tscherkia*); Satoh & Iwaku, 2006 (*Onychomys*); Satoh &
173 Iwaku, 2009 (*Neotoma*, *Peromyscus*); Druzinsky, 2010 (*Aplodontia*, *Cynomys*, *Tamias*,
174 *Marmota*, *Ratufa*, *Sciurus*, *Thomomys*); Hautier & Saksiri, 2009 (*Laonastes*); Hautier, 2010
175 (*Ctenodactylus*); Cox & Jeffery, 2011 (*Cavia*, *Rattus*, *Sciurus*); Baverstock *et al.*, 2013 (*Mus*);
176 Becerra *et al.*, 2014 (*Chinchilla*, *Ctenomys*, *Octodon*).

177 Results

178 The absolute masses and relative proportions of the jaw-closing muscles are given in Table 1. The
179 muscles of mastication are described below and shown in Figs. 1-5. A 3D interactive
180 reconstruction is provided in the supplementary PDF (Supplementary File S1) and a rotating
181 reconstruction is given in the supplementary movie (Supplementary File S2).

182 *Superficial masseter*

183 The superficial masseter is a moderately large muscle in the naked mole-rat, forming just under a
184 quarter of the masticatory muscle mass (Table 1). It takes a small tendinous origin from the
185 ventral surface of the anterior zygomatic arch where it meets the skull. From its origin, the
186 superficial masseter runs postero-ventrally along the anterior border of the deep masseter, and
187 inserts along the ventral margin of the mandible. It also wraps around the mandibular margin and
188 extends over the medial mandibular surface. This section of the superficial masseter, known as
189 the *pars reflexa* (Turnbull, 1970), pushes backwards, around the insertion of the medial
190 pterygoid, almost reaching the posteriormost tip of the medial surface of the angle (Figs. 2 and 3).

191 The microCT images of the naked mole-rat do not indicate that the superficial masseter is
192 separated into smaller divisions. In most other descriptions of hystricomorph rodents, the
193 superficial masseter is either divided into a *pars horizontalis* and a *pars reflexa* (e.g., Turnbull,
194 1970), or into a main section and a small *pars anterior* that originates from the mesial edge of the
195 tendon (e.g., Woods, 1972; Woods & Hermanson, 1985; Hautier & Saksiri, 2009; Hautier, 2010).
196 No *pars anterior* was identified in the naked mole-rat, but an extensive *pars reflexa* was clearly
197 visible. Two previous descriptions of bathyergid musculature have reported a superficial masseter
198 morphology not found in any other rodent species. Boller (1970) described a large superficial
199 masseter in *Cryptomys* that completely covered the deep masseter in lateral view. He divided this
200 muscle into three parts, termed M2, M1a and M1b, the latter being essentially synonymous with
201 the superficial masseter described here. This configuration was also reported for *Fukomys* by Van
202 Daele *et al.* (2009), although the authors note that the separation between the superficial masseter
203 and deep masseter is very difficult. In this study, no part M1a or M2 could be identified, with all
204 musculature dorsal to the superficial masseter being assigned to the deep masseter – a view
205 shared by Morlock (1983).

206 *Deep masseter*

207 The deep masseter is very similar in size to the superficial, forming 25% of the total muscle mass
208 (Table 1). It lies immediately dorsal to the superficial masseter, originating along the length of the
209 ventral border of the zygomatic arch and inserting on the masseteric ridge on the ventral part of
210 the mandibular ramus (Fig. 4). As mentioned above, the deep masseter is not covered by part M2
211 of the superficial masseter as described by Boller (1970) and Van Daele *et al.* (2009), but is
212 clearly visible in lateral view (as noted by Morlock, 1983). Thus, the naked mole-rat has a
213 morphology similar to that seen in many hystricomorph taxa, such as *Proechimys* (Woods, 1972),
214 *Myocastor* (Woods & Howlands, 1979) and *Plagiodontia* (Woods & Hermanson, 1985). In some
215 rodents, particularly sciuriforms and myomorphs, the deep masseter has often been split into
216 anterior and posterior portions (e.g., Greene, 1935; Weijs, 1973; Ball & Roth, 1995; Thorington
217 & Darrow, 1996; Cox & Jeffery, 2011), but, in common with many descriptions of
218 hystricomorphous rodents (e.g., Müller, 1933; Offermans & De Vree, 1989; Woods, 1972; Woods
219 & Hermanson, 1985), no division of the deep masseter was found in *Heterocephalus glaber*.

220 A further division of the deep masseter, known simply as the ‘posterior masseter’ has been
221 described in many hystricognaths (Woods, 1972; Woods & Howland, 1979; Woods &

222 Hermanson, 1985; Hautier *et al.*, 2012) and *Pedetes capensis* (Offermans & De Vree, 1989). The
223 muscle origins in the jugal fossa on the ventrolateral surface of the zygomatic arch and runs
224 horizontally and posteriorly to the condylar process of the mandible. Despite careful analysis of
225 the microCT scans, no such muscle could be discerned in *Heterocephalus*, and nor has it been
226 described in other bathyergids (Tullberg, 1899; Boller, 1970; Morlok, 1983; Van Daele *et al.*,
227 2009).

228 *Zygomaticomandibularis*

229 The ZM is the deepest of the three muscle layers running from the zygomatic arch to the
230 mandible. In the naked mole-rat, as in *Fukomys* (Van Daele *et al.*, 2009) it is clearly separable
231 from the deep masseter that lies immediately lateral to it. The iodine-enhanced microCT images
232 in this study show three distinct portions of the ZM (Fig. 5): a posterior ZM originating from the
233 glenoid fossa and running antero-ventrally to insert on the middle of the lateral mandibular
234 ramus; an anterior ZM with an origin spanning the jugo-squamosal suture on the medial surface
235 of the zygomatic arch, and an insertion on the ventral part of the coronoid process; and an
236 infraorbital ZM that originates from the anteriormost part of the orbit where the zygomatic arch
237 meets the skull and the small infraorbital foramen pierces the maxilla, and inserts at the base of
238 the coronoid process lateral to the distal molar. No part of the infraorbital ZM was seen to pass
239 through the infraorbital foramen, but this muscle was so named as it appears to be homologous
240 with the infraorbital ZM in other rodents (e.g., Weijs, 1973; Olivares *et al.*, 2004; Satoh & Iwaku,
241 2006, 2009; Cox & Jeffery, 2011).

242 The above description of the ZM largely matches that of Tullberg (1899) and Morlock (1983),
243 differing only in nomenclature. Tullberg (1899) clearly identifies three portions of the medial
244 masseter (equivalent to the ZM here), but assigns the rostral two portions to the '*Portio anterior*
245 *masseteris medialis*' rather than splitting them into infraorbital and anterior sections. Morlock
246 (1983) identifies the three parts as (caudal to rostral): posterior ZM, anterior ZM and '*maxillo-*
247 *mandibularis*'. There are, however, substantial differences in the muscle arrangement described
248 by Boller (1970) in *Cryptomys*. The middle part, here identified as the anterior ZM, was
249 determined by Boller (1970) to be a ventral extension of the temporalis muscle (*pars zygomatica*)
250 on to the lateral surface of the mandible. This was not thought to be the case in the naked mole-
251 rat specimen scanned for this study as, in common with Morlock (1983) no clear connection
252 between this muscle and the temporalis could be seen.

253 *Temporalis*

254 The temporalis is the largest jaw-closing muscle in *Heterocephalus glaber*, forming around 32%
255 of the masticatory muscle mass (Table 1). Its origin covers the entire parietal bone and much of
256 the frontal and squamosal as well, extending from the dorsal midline down to the zygomatic
257 process of the squamosal, and from the occipital bone across the braincase well into the orbit
258 (Fig. 5). The insertion is on the tip and widely across the medial surface of the coronoid process.

259 *Medial pterygoid*

260 The medial pterygoid is fairly small component of the masticatory complex in the naked mole-
261 rat, comprising just 6% of the total jaw-closing muscle mass (Table 1). It is split into two
262 branches at its origin – a small branch that attaches to the lateral surface of the pterygoid plate,
263 and a much larger branch that originates deep within the pterygoid fossa. The fossa opens into the
264 braincase in *Heterocephalus glaber* as it does in all bathyergids and all hystricognaths except
265 *Hydrochoerus* (Woods, 1972). The two branches of the medial pterygoid unite and run caudally,
266 ventrally and laterally to take a long, narrow insertion on the medial surface of the angle of the
267 mandible (Fig. 3). As mentioned above the insertion of the medial pterygoid is almost completely
268 surrounded by the *pars reflexa* of the superficial masseter (Figs. 2 and 3).

269 *Lateral pterygoid*

270 The lateral pterygoid originates from the lateral pterygoid plate and part of the alisphenoid bone,
271 dorsal to the smaller branch of the medial pterygoid. From its origin it extends postero-laterally,
272 in an almost horizontal plane, to the medial surface of the mandibular condyle (Fig. 3). The
273 insertion is immediately dorsal to the *pars reflexa* of the superficial masseter. The muscle is very
274 small, forming around 2% of the masticatory musculature (Table 1).

275 **Discussion**

276 The technique of iodine-enhanced microCT scanning (Metscher, 2009; Jeffery *et al.*, 2011) was
277 used to visualise the jaw-closing musculature of the naked mole-rat. Although the specimen
278 studied had been preserved for several years in ethanol and undergone a substantial amount of
279 muscle shrinkage, the iodine potassium iodide staining was very successful in revealing the
280 different layers and sections of the masticatory muscles.

281 The results show that the masticatory system of the naked mole-rat is very large. Although the
282 absolute muscle masses are of limited use, given the extensive muscle shrinkage that has taken
283 place, it should be noted that the total muscle masticatory mass (0.242 g) is 75% of that reported
284 for the rat (Cox & Jeffery, 2011), despite the naked mole-rat skull being under half the length of
285 the rat skull, and the average body mass of non-breeding adult naked mole-rats in captivity (30-
286 50 g; Jarvis & Sherman, 2002) being between 14 and 23% of the body mass estimated for the rat
287 specimen used by Cox & Jeffery (2011). Thus, even before muscle shrinkage has been accounted
288 for, the naked mole-rat clearly has very large jaw-closing muscles compared to other rodents. In
289 fact, Jarvis & Sherman (2002) report that the jaw muscles constitute around a quarter of the entire
290 muscle mass of the naked mole-rat.

291 The masticatory complex is dominated by three muscles: the superficial masseter, the deep
292 masseter and the temporalis. The superficial and deep masseters together form almost 50% of the
293 masticatory musculature. Gorniak (1977) and Byrd (1981) suggested that these muscles have an
294 important role in the closing and power strokes of biting at both the incisors and molars. Thus,
295 the large masseter in the naked mole-rat is likely to deliver a high bite force. In addition, the
296 strong horizontal component of pull in the superficial masseter is likely to make this muscle the
297 main protractor of the lower jaw, as proposed by Hiiemae (1971). The microCT images revealed
298 a large portion of the superficial masseter that wraps around the ventral margin of the mandible
299 and inserts on the medial surface of the ramus, known as the *pars reflexa*. Various functions have
300 been suggested for this part of the muscle, including fine control of jaw opening (Weijs &
301 Dantuma, 1975) and increase in the resting length of the muscle to facilitate wider gapes (Sato
302 & Iwaku, 2004). The lack of posterior masseter in the naked mole-rat is somewhat unusual, given
303 its presence in most other hystricognaths (Woods, 1972; Woods & Howland, 1979; Woods &
304 Hermanson, 1985). However, it is perhaps not surprising as its presence seems to be correlated
305 with hystricomorphy (a posterior masseter is found in *Pedetes* as well; Offermans & De Vree,
306 1989) and it has not been reported in other bathyergids (Tullberg, 1899; Boller, 1970; Morlok,
307 1983; Van Daele *et al.*, 2009). Offermans & De Vree (1993) found that the function of the
308 posterior masseter was largely propalinal movement of the mandible, so it may be that such
309 motion of the jaws is not important for mole-rats. Indeed, observations indicate that jaw
310 movements are in fact mainly oblique in bathyergids (Gomes Rodrigues, pers. comm.).

311 The temporalis muscle in the naked mole-rat extends across the entire temporal region of the
312 cranium and also pushes into the orbit. As a proportion of total jaw adductor muscle mass (32%),
313 it is very large compared to other species in the Hystricognathi e.g., *Cavia* (11%: Cox & Jeffery,
314 2011), *Hydrochoerus* (5%: Müller, 1933) and *Hystrix* (17%: Turnbull, 1970). However, it should
315 be noted that temporalis muscles of similar relative size have been recorded in *Aplodontia rufa*
316 (34%) and many sciuriforms (25-30%) by Druzinsky (2010), and may in fact be the primitive
317 condition for rodents. The highly reduced size of the eye in the naked mole-rat may have partly
318 facilitated the increase in size of the temporalis, enabling its anterior expansion into the relatively
319 unoccupied orbit (Lavocat, 1973). It has also been suggested that a large temporalis may result
320 from the widening and flattening of the skull seen in fossorial species (Druzinsky, 2010). Such a
321 large temporalis is likely to be acting as a powerful elevator of the jaw (Hiimeae, 1971),
322 producing substantial forces at the teeth. The ventralmost fibres of temporalis running along the
323 zygomatic process of the squamosal have a largely horizontal direction of pull, and thus are likely
324 to act as a strong retractor of the jaw. Overall, it appears that the two largest masticatory muscles
325 of the naked mole-rat may provide it with the potential to generate high bite forces and to
326 produce a wide gape. Both these are highly useful characteristics in a subterranean species that
327 digs extensive tunnels in search of widely dispersed food resources with its incisors, as the naked
328 mole-rat does (Stein, 2000). Brett (1991) radio tracked individuals in a colony of 87 naked mole-
329 rats and found that 3.6-4.5 tonnes of soil were excavated in a single year - the equivalent of 2.3-
330 2.9 km of new tunnels. These are impressive statistics for a small rodent of average 34 g body
331 mass. Such endeavours impose high costs with respect to tooth wear, yet incisor growth is not
332 dissimilar to that of other rodents (Berkovitz & Faulkes, 2001), and perhaps offset as a limiting
333 factor by the social behaviour of the naked mole-rat, where digging activity is distributed among
334 a large workforce with strong jaws. It is most likely that digging through hard soil is the main
335 constraint on jaw anatomy and musculature, as the underground roots and tubers on which naked
336 mole-rats feed are not woody or particularly tough to chew. For example, one of the most
337 common geophytes eaten by naked mole-rats in Kenya (*Pyrenacantha*) has a succulent flesh with
338 just a thin epidermis (< 1 mm thick), and this is typical of the other food plant species they
339 consume (Brett, 1991).

340 The iodine-enhanced microCT scans show very clearly that the zygomaticomandibularis muscle
341 does not pass through the infraorbital foramen and on to the rostrum in *Heterocephalus glaber*.
342 Thus, the naked mole-rat displays the protrogomorphous morphology (Wood, 1965), as do most

343 other genera in the Bathyergidae (Tullberg, 1899), although there is a very small extension of the
344 ZM through the infraorbital foramen in *Cryptomys* (Boller, 1970; Morlock, 1983) and *Fukomys*
345 (Van Daele *et al.*, 2009). In contrast, all other families in the Hystricognathi are hystricomorph
346 (i.e., they possess a large infraorbital portion of the ZM that passes through an enlarged
347 infraorbital foramen and takes a wide origin on the rostrum). However, the 3D reconstructions
348 demonstrate that, although the naked mole-rat is technically protrogomorphous, it is very
349 different in morphology to the other extant protrogomorph, *Aplodontia rufa*. In the mountain
350 beaver, the origin of the ZM is restricted to the medial surface of the zygomatic arch and the
351 internal surface of the maxillary root of the zygoma (Druzinsky, 2010). It does not have the wide
352 attachment at the front of the orbit seen in *Heterocephalus glaber*. The morphology of the ZM in
353 the naked mole-rat very much resembles the hystricomorphous condition without the extension
354 on to the rostrum. This morphology concurs with the assumption of secondarily lost
355 hystricomorphy, based on the position of the Bathyergidae within the rodent phylogeny (Fabre *et*
356 *al.*, 2012), and the presence of hystricomorphy in fossil genera (Lavocat, 1973) and ontogeny
357 (Maier & Schrenk, 1987). It appears that most of the blesmols, *Heterocephalus* included, have
358 undergone a shortening of the rostrum (Landry, 1957), which may account for the retreat of the
359 ZM from the snout. Such a rostral shortening is also seen in other fossorial hystricognaths e.g.,
360 *Ctenomys* (Vassallo & Verzi, 2001) which also has a relatively reduced infraorbital part of the
361 ZM compared to the semi-fossorial *Octodon* and the terrestrial *Chinchilla* (Bercerra *et al.*, 2014).
362 It should be noted that shortening of the rostrum would shorten the out-lever of the masticatory
363 system, thus contributing to high bite forces. The loss of the infraorbital ZM may also have been
364 an adaptation (alongside the enlarged *pars reflexa* of the superficial masseter, mentioned above)
365 to increasing gape for incisor digging. Overall, the condition of the muscles in *Heterocephalus* is
366 also a reminder that rodent masticatory muscles are in fact a suite of continuous characters, and
367 that imposing discrete descriptive terms (protrogomorph, hystricomorph) on them may result in
368 erroneous interpretations of evolutionary history and relationships.

369 In conclusion, the naked mole-rat has evolved an enlarged set of masticatory muscles,
370 particularly the superficial masseter and temporalis. These large muscles may contribute,
371 alongside rostral shortening, to a presumably high bite force and wide gape necessary for digging
372 with the incisor teeth. The overall morphology is protrogomorphous, but is consistent with
373 evolution from a hystricomorphous ancestor, with the infraorbital portion of the
374 zygomaticomandibularis having been lost possibly through rostral shortening. The contrast-

375 enhanced microCT technique has been shown to be a highly effective tool for the visualisation of
376 soft tissues, especially muscle. Although a great deal of tissue shrinkage is evident in the scans
377 presented here, this is mostly an artefact of the lengthy fixation time; such extreme shrinkage is
378 highly unlikely in fresher specimens. It is hoped that iodine-enhanced microCT will become part
379 of the standard toolkit of anatomical investigation in the future.

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Figure 1

Masticatory muscles of *Heterocephalus glaber*

Right lateral view of a 3D reconstruction of the cranium, mandible and masticatory muscles of *Heterocephalus glaber*. Abbreviations: iozm, infraorbital zygomaticomandibularis; dm, deep masseter, sm superficial masseter; t, temporalis. Scale bar = 5mm.

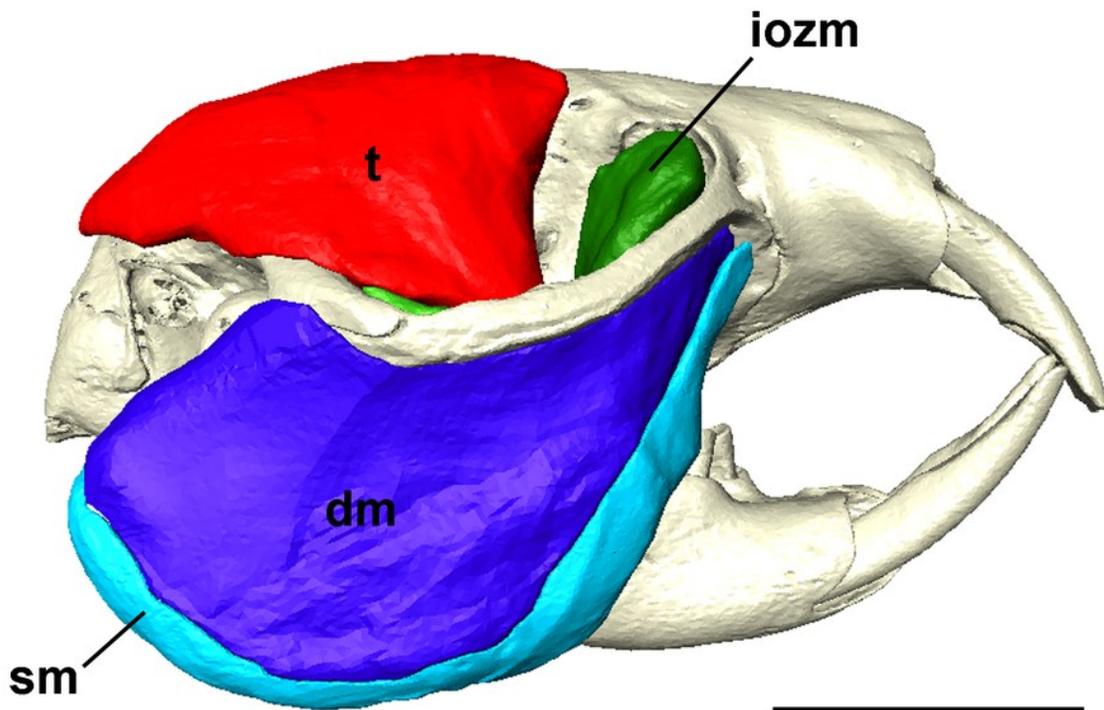


Figure 2

Coronal microCT slice of *Heterocephalus glaber*

Coronal microCT slice through the head of *Heterocephalus glaber* stained with iodine potassium iodide. Abbreviations: dm, deep masseter (dark blue); lp, lateral pterygoid (yellow); mp, medial pterygoid (orange); pr, *pars reflexa* of the superficial masseter (light blue); sm, superficial masseter (light blue); t, temporalis (red). White line on 3D reconstruction shows position of slice. Scale bar = 5mm.

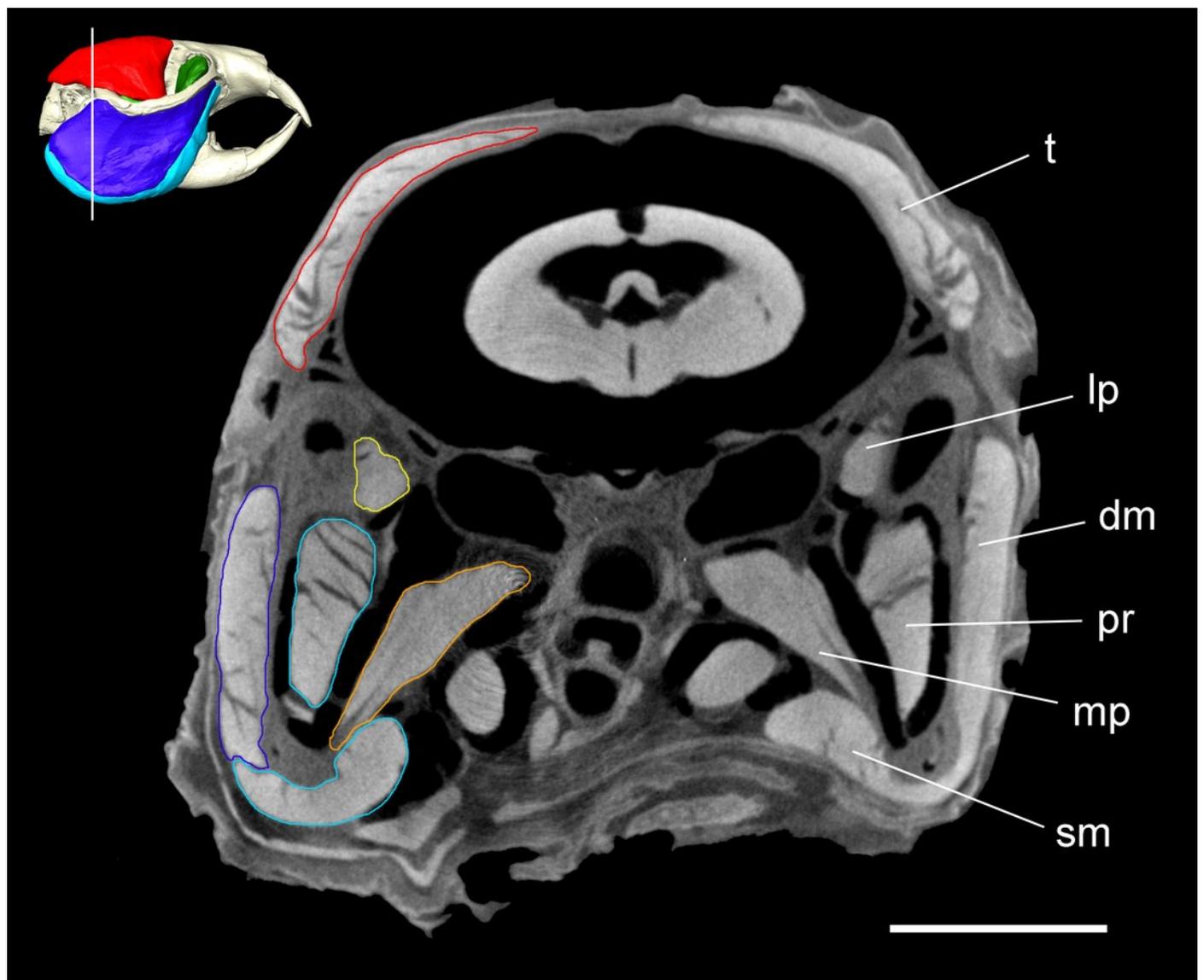


Figure 3

Superficial masseter and pterygoid muscles of *Heterocephalus glaber*

Right lateral view of a 3D reconstruction of the cranium, mandible, superficial masseter and pterygoid muscles of *Heterocephalus glaber*. Cranium and mandible transparent for visualisation of muscles attaching to medial mandibular surface. Abbreviations: lp, lateral pterygoid; mp, medial pterygoid; pr, *pars reflexa* of the superficial masseter; sm, superficial masseter. Scale bar = 5mm.

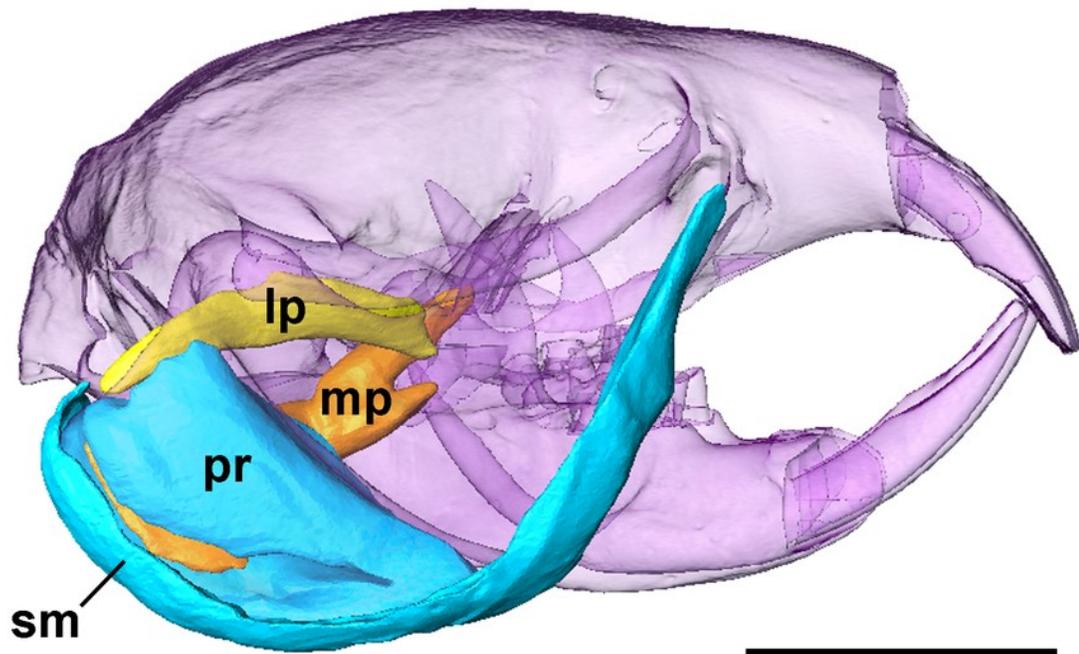


Figure 4

Transverse microCT slice of *Heterocephalus glaber*

Transverse microCT slice through the head of *Heterocephalus glaber* stained with iodine potassium iodide. Abbreviations: azm, anterior zygomaticomandibularis (dark green); dm, deep masseter (dark blue); mp, medial pterygoid (orange); pr, *pars reflexa* of the superficial masseter (light blue); pzm, posterior zygomaticomandibularis (light green); sm(t), tendon of superficial masseter (light blue). White line on 3D reconstruction shows position of slice.

Scale bar = 5mm.

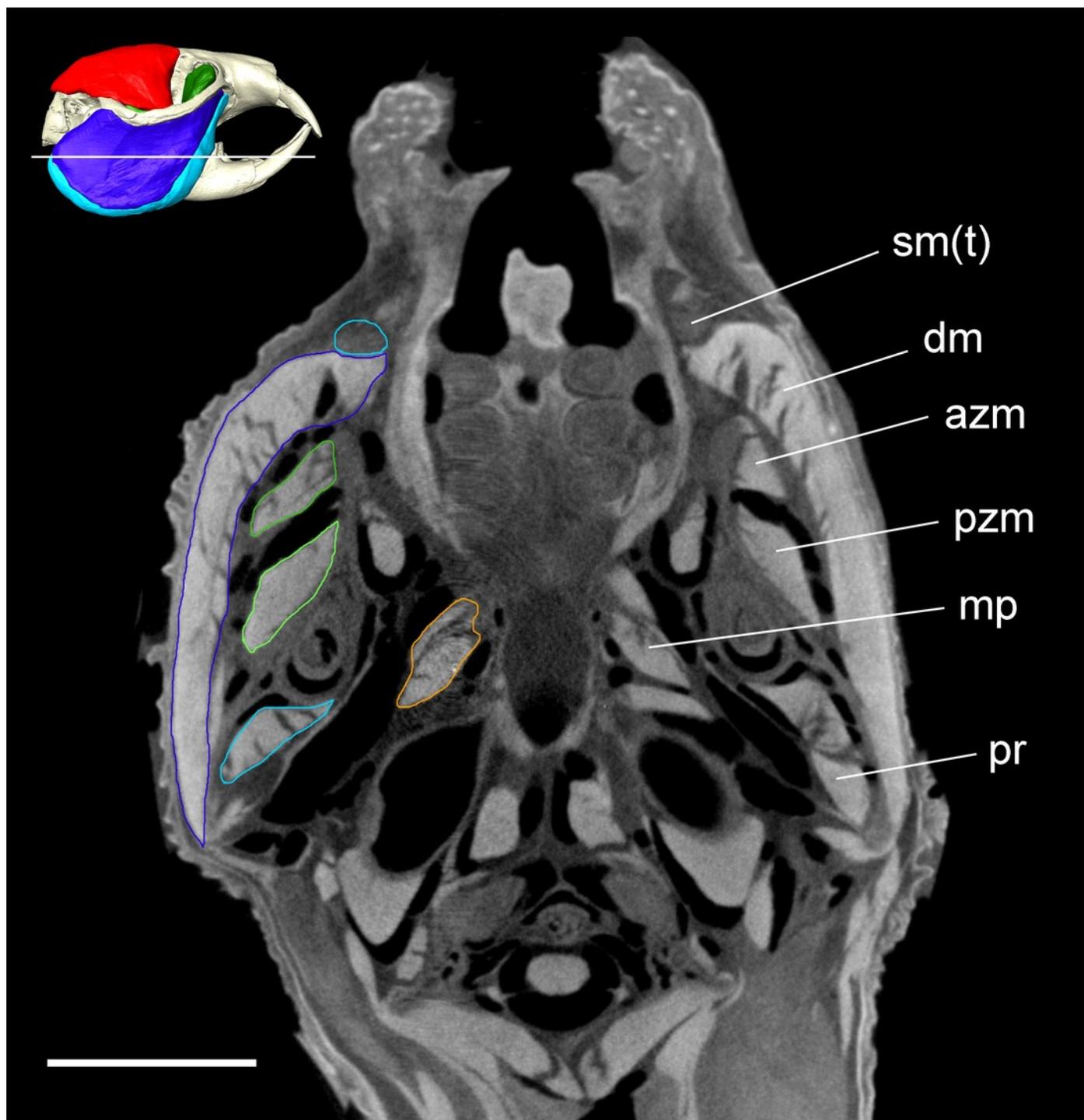


Figure 5

Temporalis and zygomaticomandibularis muscles of *Heterocephalus glaber*

Right lateral view of a 3D reconstruction of the cranium, mandible, temporalis and zygomaticomandibularis muscles of *Heterocephalus glaber*. Abbreviations: azm, anterior zygomaticomandibularis; iozm, infraorbital zygomaticomandibularis; pzm, posterior zygomaticomandibularis; t, temporalis. Scale bar = 5mm.

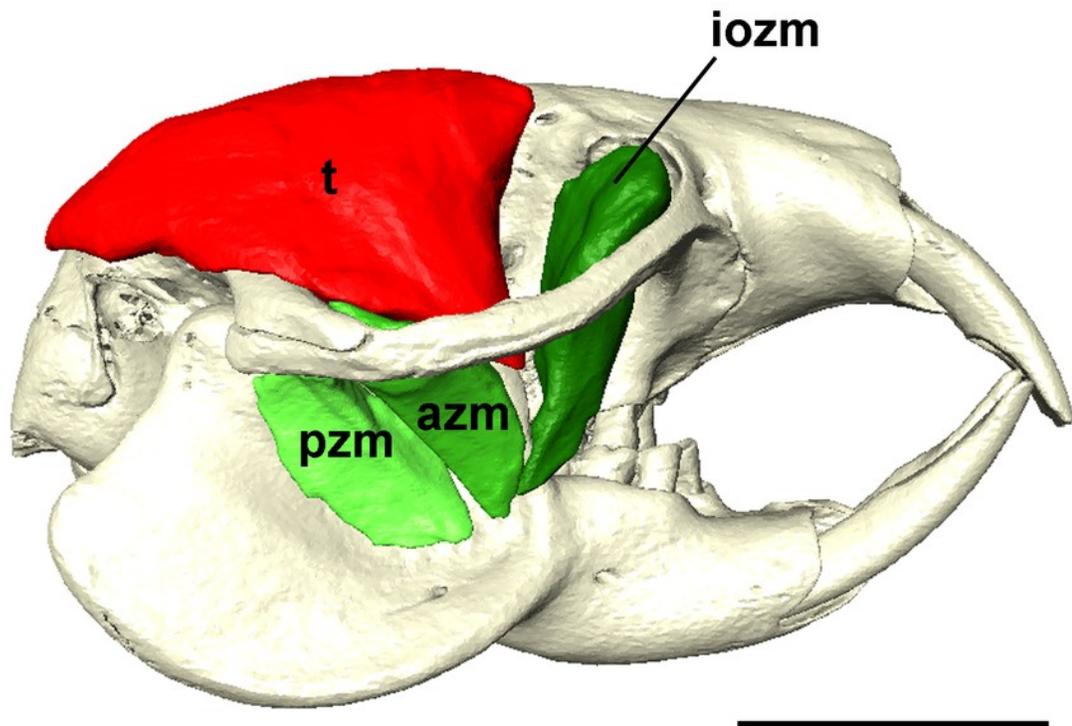


Table 1 (on next page)

Masses and relative proportions of masticatory muscles of *Heterocephalus glaber*

Muscle	Mass (g)	Percentage
Superficial masseter	0.057	23.4
Deep masseter	0.062	25.5
Anterior ZM	0.007	2.9
Posterior ZM	0.006	2.6
Infraorbital ZM	0.005	5.4
Temporalis	0.078	32.2
Medial pterygoid	0.015	6.1
Lateral pterygoid	0.013	2.0
Total	0.242	100.0