

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 ‘chisel-toothed’ digging through hard,
14 compact soil in the search for widely dispersed food resources is likely to have influenced the
15 musculature of the jaw. The naked mole-rat is the only species within the genus *Heterocephalus*,
16 and phylogenetic analyses indicate that it is the most basal extant species of the family (Allard &
17 Honeycutt, 1992), with an estimated divergence from the other bathyergid genera between 40 and
18 48 million years ago (Faulkes *et al.*, 2004), or 33-35 million years ago, depending on the fossil
19 calibration of the molecular clock (Faulkes *et al.*, 2011; Ingram *et al.*, 2004). On this basis, some
20 authors place the naked mole-rat in its own subfamily, the Heterocephalinae, distinct from the
21 remaining genera in the Bathyerginae (e.g., Wilson & Reeder, 2005). The naked mole-rat first
22 came to prominence scientifically when it was shown that its complex social structure is in fact a
23 rare example of eusociality in vertebrates (Jarvis, 1981, 1991). That is, naked mole-rats have a
24 caste system analogous to that of ants and termites, with a breeding female, or queen, at the top of
25 the hierarchy, and smaller, non-breeding workers at the bottom. Since then, naked mole-rats have
26 been discovered to possess many other unusual qualities that have placed them in the forefront of
27 research in a number of fields. For example, naked mole-rats appear to be insensitive to acid-
28 induced pain (Smith *et al.*, 2011), extraordinarily resistant to cancer (Seluanov *et al.*, 2009), and
29 extremely long-lived for a small mammal (Buffenstein, 2005).

30 According to recent estimates (Faulkes & Bennett, 2013), the Bathyergidae comprises thirty or
31 more species, in six genera, and is inferred to have originated in the Eocene of Africa (Huchon &
32 Douzery, 2001) although the earliest known fossils are Miocene in age (Faulkes *et al.*, 2004;
33 Mein & Pickford, 2008). On the basis of lower jaw and inner ear morphology, the bathyergids
34 were considered members of the Hystricognathi by Tullberg (1899). Subsequent works tended to
35 preserve this relationship, with the Bathyergidae frequently being closely allied to two other
36 families of Old World hystricognaths, the Thryonomyidae and Petromuridae (Landry, 1957;

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

55 Given the variable morphology of the ZM and infraorbital foramen seen amongst extant and
56 fossil genera, bathyergid protrogomorphy was the subject of some debate for a number of years,
57 particularly with regard to its evolutionary history: Does the bathyergid condition represent
58 retention of the primitive condition or is the morphology secondarily derived? Tullberg (1899)
59 and Wood (1965, 1985) believed that bathyergids show the ancestral condition, and that
60 *Cryptomys* demonstrates nascent hystricomorphy. On the other hand, Landry (1957), Lavocat
61 (1974) and Luckett & Hartenberger (1985) were of the opinion that bathyergids evolved from a
62 hystricomorph ancestor, and that their current morphology represents a reversal to the primitive
63 condition. Maier & Schrenk (1987) added support to the latter view by showing that small
64 bundles of fibres of the ZM muscle protrude through the infraorbital foramen early in
65 development in two genera of blesmols, *Bathyergus* and *Georychus*, but subsequently retreat
66 from the rostrum, and are absent at birth. More recently, molecular analyses have supported a
67 close evolutionary association of the Bathyergidae, Thryonomyidae (cane-rats) and Petromuridae
68 (dassie rat), within the Hystricognathi (Blanga-Kanfi *et al.*, 2009; Fabre *et al.*, 2012). Given the

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

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

89 One reason underlying the lack of published literature on the masticatory musculature of the
90 naked mole-rat may be the small size of this species, which renders traditional dissection more
91 difficult than for larger species. However, its small size also makes the naked mole-rat an ideal
92 candidate for digital dissection via contrast-enhanced micro-computed tomography (microCT)
93 and virtual reconstruction. This technique is based on the work of Metscher (2009) and Jeffery *et*
94 *al.* (2011) and involves the staining of biological specimens with iodine potassium iodide (I₂KI)
95 to enable the visualisation of soft tissues with microCT. The musculature of several rodent
96 species has already been successfully imaged using this technique (Cox & Jeffery, 2011; Hautier
97 *et al.*, 2012; Baverstock *et al.*, 2013) as well as that of other vertebrates (Tsai & Holliday, 2011;
98 Düring *et al.*, 2013; George & Holliday, 2013; Gignac & Kley, 2014; Lautenschlager *et al.*,

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

106 **Materials and Methods**

107 *Sample and scanning*

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

119 *Digital reconstruction*

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

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

138 *Analysis*

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

153 The arrangement of jaw-closing muscles in the naked mole-rat revealed by contrast-enhanced
154 microCT was compared to previously published descriptions of masticatory muscles in other
155 bathyergids: Tullberg (1899); Boller (1970); Morlock (1983) and Van Daele *et al.* (2009). In
156 addition, to understand how naked mole-rat masticatory muscles are similar to or differ from
157 rodents more generally, the results here were compared to published descriptions of other rodents
158 including sciuriforms, myomorphs and hystricomorphs, as well as the only living
159 protrogomorph, *Aplodontia rufa*. The literature consulted was as follows: Müller, 1933
160 (*Hydrochoerus*); Greene, 1935 (*Rattus*); Schumacher & Rehmer, 1962 (*Cavia*, *Rattus*); Wood,

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

176 **Results**

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

181 *Superficial masseter*

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

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

205 *Deep masseter*

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

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

221 Hermanson, 1985; Hautier *et al.*, 2012) and *Pedetes capensis* (Offermans & De Vree, 1989). The
222 muscle originates in the jugal fossa on the ventrolateral surface of the zygomatic arch and runs
223 horizontally and posteriorly to the condylar process of the mandible. Despite careful analysis of
224 the microCT scans, no such muscle could be discerned in *Heterocephalus*, or has it been
225 described in other bathyergids (Tullberg, 1899; Boller, 1970; Morlok, 1983; Van Daele *et al.*,
226 2009).

227 *Zygomaticomandibularis*

228 The ZM is the deepest of the three muscle layers running from the zygomatic arch to the
229 mandible. In the naked mole-rat, as in *Fukomys* (Van Daele *et al.*, 2009), it is clearly separable
230 from the deep masseter that lies immediately lateral to it. The iodine-enhanced microCT images
231 in this study show three distinct portions of the ZM (Fig. 5): (1) a posterior ZM originating from
232 the glenoid fossa and running antero-ventrally to insert on the middle of the lateral mandibular
233 ramus; (2) an anterior ZM with an origin spanning the jugo-squamosal suture on the medial
234 surface of the zygomatic arch with an insertion on the ventral part of the coronoid process; and
235 (3) an infraorbital ZM that originates from the anteriormost part of the orbit where the zygomatic
236 arch meets the skull and the small infraorbital foramen pierces the maxilla and inserts at the base
237 of the coronoid process lateral to the distal molar. No part of the infraorbital ZM was seen to pass
238 through the infraorbital foramen, but this muscle was so named as it appears to be homologous
239 with the infraorbital ZM in other rodents (e.g., Weijs, 1973; Olivares *et al.*, 2004; Satoh & Iwaku,
240 2006, 2009; Cox & Jeffery, 2011).

241 Our description of the ZM largely matches that of Tullberg (1899) and Morlock (1983), differing
242 only in nomenclature. Tullberg (1899) clearly identifies three portions of the medial masseter
243 (equivalent to the ZM here), but assigns the rostral two portions to the '*Portio anterior*
244 *masseteris medialis*' rather than splitting them into infraorbital and anterior sections. Morlock
245 (1983) identifies the three parts as (caudal to rostral): (1) posterior ZM, (2) anterior ZM, and (3)
246 '*maxillo-mandibularis*'. There are, however, substantial differences in the muscle arrangement
247 described by Boller (1970) in *Cryptomys*. The middle part, here identified as the anterior ZM,
248 was determined by Boller (1970) to be a ventral extension of the temporalis muscle (*pars*
249 *zygomatica*) on to the lateral surface of the mandible. This was not thought to be the case in the
250 naked mole-rat specimen scanned for this study as, in common with Morlock (1983) no clear
251 connection between this muscle and the temporalis could be seen.

252 *Temporalis*

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

258 *Medial pterygoid*

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

268 *Lateral pterygoid*

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

274 **Discussion**

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

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

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

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

338 The iodine-enhanced microCT scans show very clearly that the zygomaticomandibularis muscle
339 does not pass through the infraorbital foramen and on to the rostrum in *Heterocephalus glaber*.
340 Thus, the naked mole-rat displays the protrogomorphous morphology (Wood, 1965), as do most
341 other genera in the Bathyergidae (Tullberg, 1899), although there is a very small extension of the

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

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

374 soft tissues, especially muscle. Although a great deal of tissue shrinkage is evident in the scans
375 presented here, this is mostly an artefact of the lengthy fixation time; such extreme shrinkage is
376 highly unlikely in fresher specimens. It is hoped that iodine-enhanced microCT will become part
377 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.

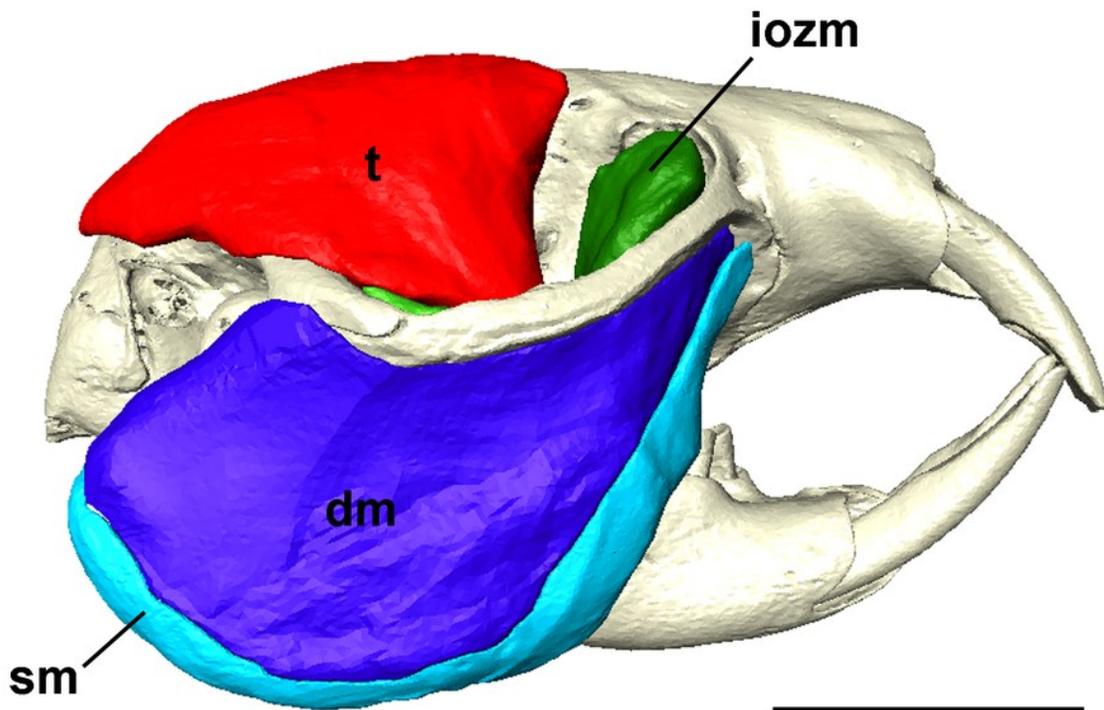


Figure 2

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.

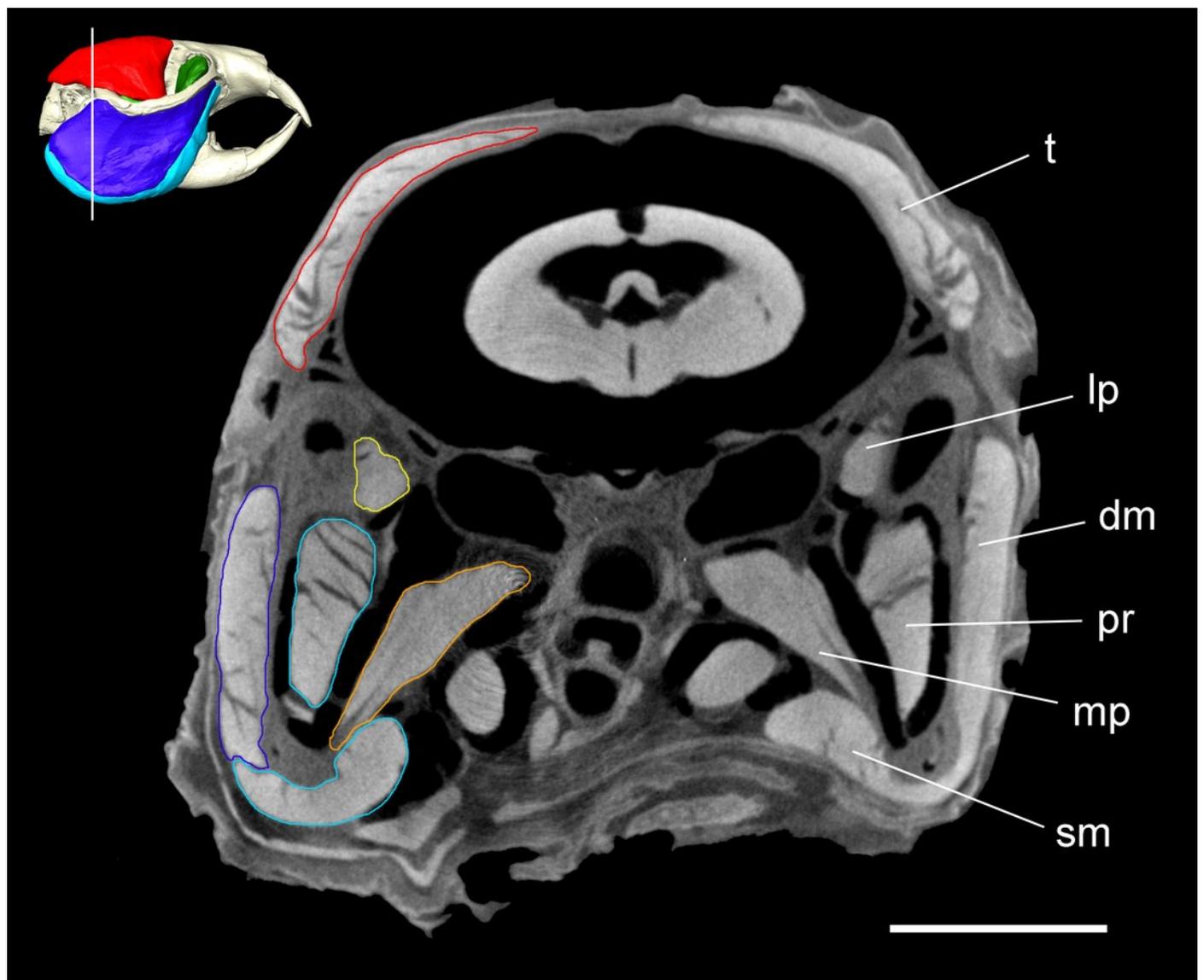


Figure 3

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.

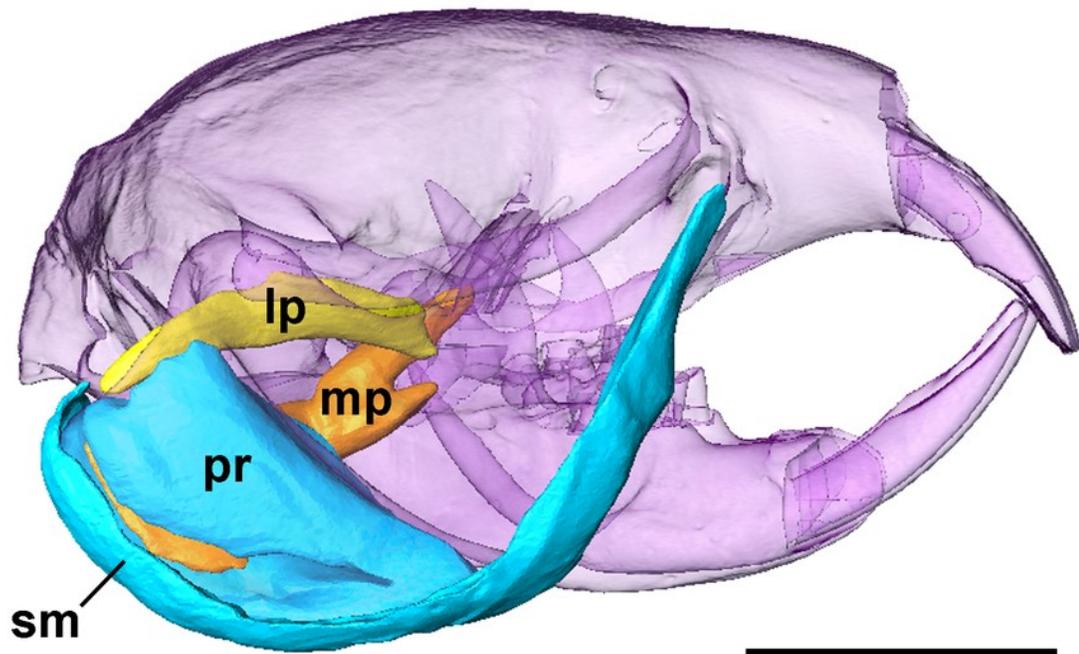


Figure 4

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.

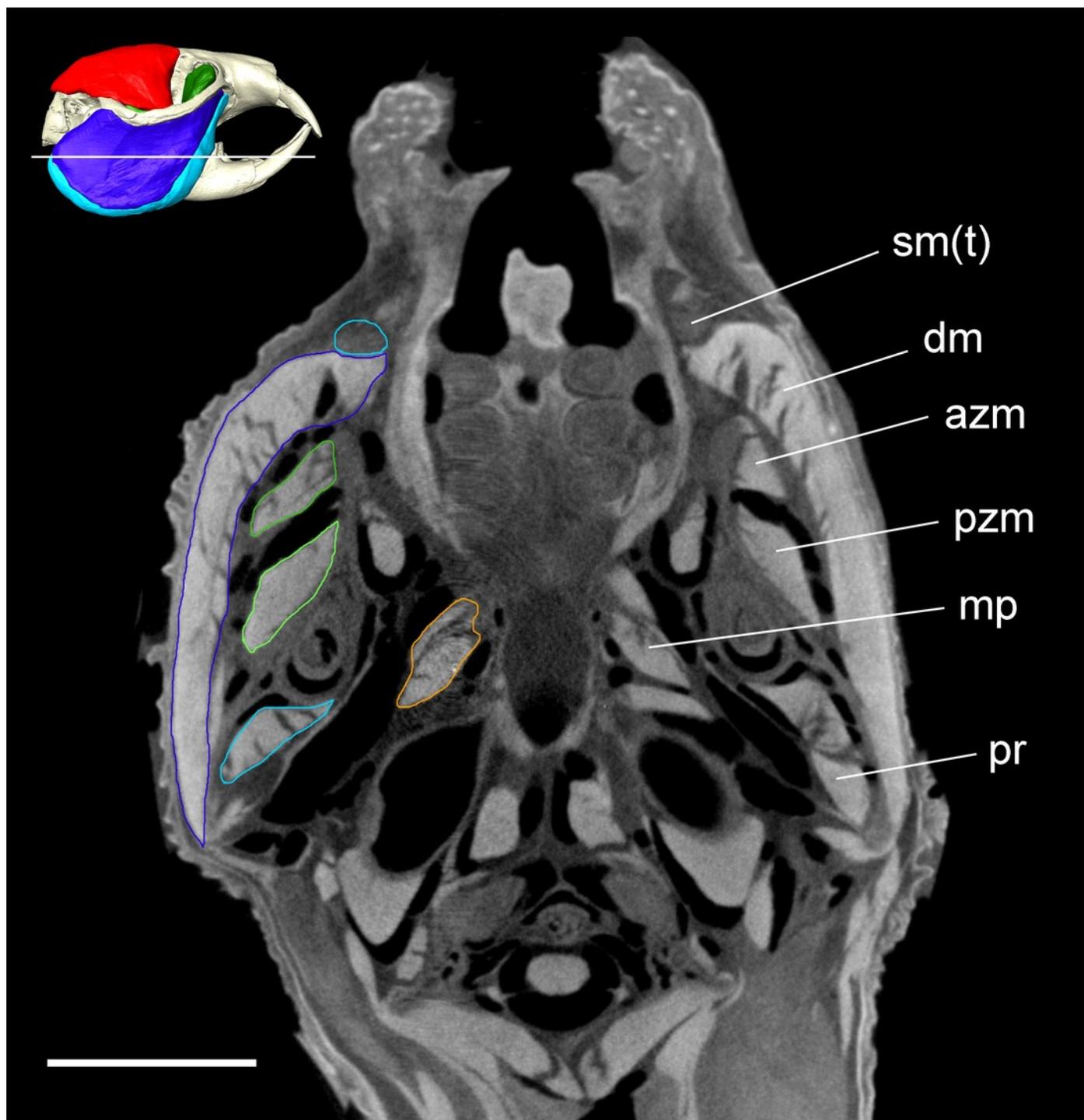


Figure 5

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.

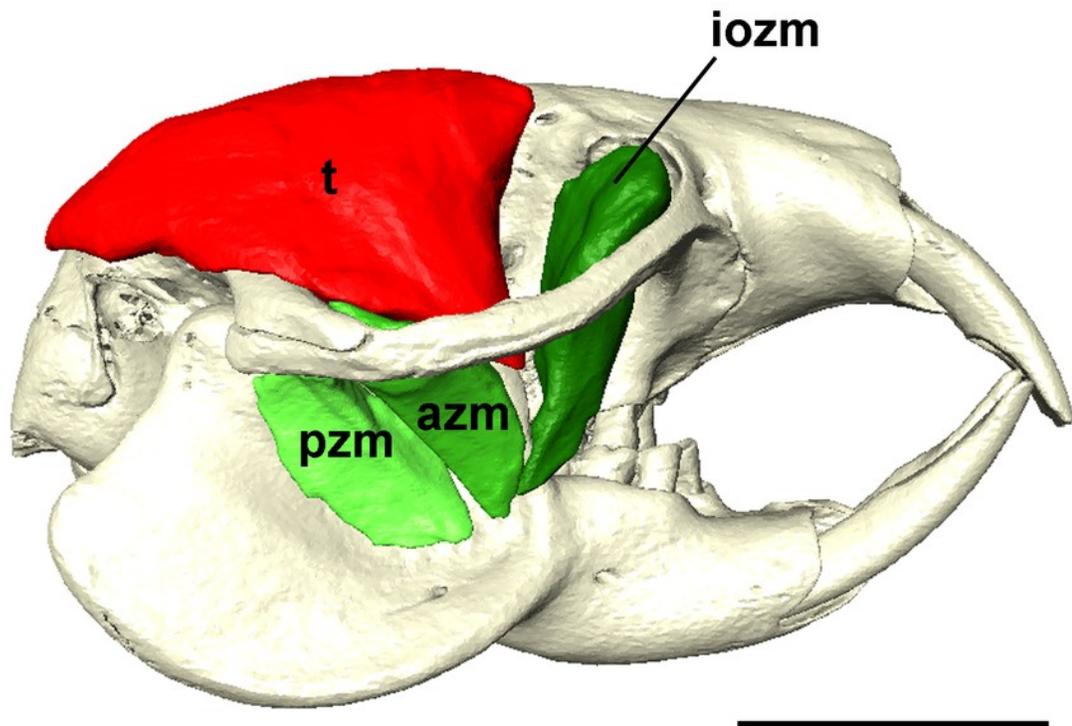


Table 1 (on next page)

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