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 digs extensive tunnel systems with its incisors. It has been the subject of considerable research in recent years owing to its many highly specialised adaptations. As well as being one of only two eusocial species of mammal, the naked mole-rat is also extremely long-lived, insensitive to acid-induced pain, and almost totally resistant to cancer. 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 very large for a rodent, covering the entirety of the braincase and much of the rear part of the orbit. The morphology of the superficial and deep masseters 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 would generally be considered protrogomorphous rather than hystricomorphous, although it probably evolved from a hystricomorphous ancestor and secondarily lost the rostral portion of the ZM. Overall, the morphology of the masticatory musculature indicates a species with a powerful bite force and a wide gape – both important adaptations for a life dominated by digging with the incisors.

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Introduction

The African mole-rats, or blesmols, (family Bathyergidae) are a highly specialised group of subterranean rodents. According to recent estimates (Faulkes & Bennett, 2013), the family comprises thirty or more species, in six genera, and is inferred to have originated in the Eocene of Africa (Huchon & Douzery, 2001) although the earliest known fossils are Miocene in age (Faulkes *et al*., 2004). On the basis of lower jaw and inner ear morphology, the bathyergids were considered members of the Hystricognathi by Tullberg (1899). Subsequent works tended to preserve this relationship, with the Bathyergidae frequently being closely allied to two other families of Old World hystricognaths, the Thryonomyidae and Petromuridae (Landry, 1957; Wood, 1974). However, a degree of doubt remained over the placement of bathyergids owing to the unusual morphology of their masticatory muscles. Almost all living rodents can be classified as sciuromorph (squirrel-like), myomorph (mouse-like) or hystricomorph (porcupine-like) based on the morphology of the masseter muscle (Brandt, 1855; Wood, 1965). Most members of the Hystricognathi are defined as being hystricomorph, owing to the possession of an enlarged infraorbital foramen, through which a substantial portion of the zygomaticomandibularis (ZM) muscle (or medial masseter) extends to take an origin on the rostrum. However, in the Bathyergidae, the infraorbital foramen is small, simply transmitting the infraorbital artery and the infraorbital branch of the maxillary nerve (Maier & Schrenk, 1987) as in most other mammals, and no part of the masticatory musculature attaches to the rostrum. This morphology is termed protrogomorphous, and is the ancestral condition for rodents seen in many Eocene fossil taxa (Wood, 1965) and the extant mountain beaver, *Aplodontia rufa* (Druzinsky, 2010). It should be noted, however, that a moderate enlargement of the infraorbital foramen is seen in two recently-split extant genera of blesmols, *Cryptomys* (Boller, 1970; Morlock, 1983) and *Fukomys* (Van Daele, *et al*., 2009), as well as in genera from the Miocene of East Africa, (Lavocat, 1973, 1974). In *Cryptomys* and *Fukomys* this enlargement is accompanied by a very limited extension of the ZM through the foramen on to the rostrum (Boller, 1970; Van Daele *et al*., 2009).

Bathyergid protrogomorphy was the subject of some debate for a number of years, particularly with regard to its evolutionary history: Does the bathyergid condition represent retention of the primitive condition or is the morphology secondarily derived? Tullberg (1899) and Wood (1965, 1985) believed that bathyergids show the ancestral condition, and that *Cryptomys* demonstrates nascent hystricomorphy. On the other hand, Landry (1957), Lavocat (1974) and Luckett &

41   Hartenberger (1985) were of the opinion that bathyergids evolved from a hystricomorph ancestor,

42   and that their current morphology represents a reversal to the primitive condition. Maier &

43   Schrenk (1987) added support to the latter view by showing that small bundles of fibres of the

44   ZM muscle protrude through the infraorbital foramen early in development in two genera of

45   blesmols, *Bathyergus* and *Georychus*, but subsequently retreat from the rostrum, and are absent at

46   birth. More recently, molecular analyses have supported a close evolutionary association of the

47   Bathyergidae, Thryonomyidae (cane-rats) and Petromuridae (dassie rat), ~~termed the~~ Phiomorpha,

48   within the Hystricognathi (Blanga-Kanfi *et al.*, 2009; Fabre *et al.*, 2012). Given the clear

49   hystricomorphy of *Thryonomys* and *Petromus* (Woods, 1972), it is more parsimonious to assume

50   that blesmols have evolved from a hystricomorph ancestor, than that they have retained the

51   protrogomorph condition, and that hystricomorphy has evolved at least three times independently

52   within the hystricognath radiation.

53   Despite the controversy surrounding mole-rat muscular morphology, there very few detailed

54   descriptions of the masticatory musculature of bathyergids. In his monumental study of rodent

55   anatomy, Tullberg (1899) included three bathyergid species: *Georychus capensis*, *Georychus*

56   *coecutiens* (now synonymised with *Cryptomys hottentotus* according to Wilson & Reeder [2005])

57   and *Bathyergus* ~~maritinus~~ (now *B. suillus*). As mentioned above, the jaw-closing muscles of

58   *Cryptomys hottentotus* were also described by Boller (1970). Morlok (1983) examined all five

59   genera of bathyergids recognised at the time (*Bathyergus*, *Cryptomys*, *Georychus*, *Heliophobius*

60   and *Heterocephalus*), but only provided detailed descriptions of the musculature of *Cryptomys*.

61   More recently, the jaw-closing muscles of the genus *Fukomys* (formerly part of *Cryptomys* [Kock

62   *et al.*, 2006]) were briefly described by Van Daele *et al.* (2009). The Bathyergidae were excluded

63   from the comparative study of New and Old World hystricomorphs of Woods (1972) owing to

64   their perceived protrogomorphy. This study aims to fill at least part of this gap in the comparative

65   literature by examining the masticatory musculature of one particularly notable absentee, the

66   naked mole-rat.

67   The naked mole-rat, *Heterocephalus glaber* (Rüppell, 1842), is found in the hot, dry regions of

68   the Horn of Africa (Honeycutt *et al.* 1991), and is probably the most well-known bathyergid

69   species. It is completely subterranean, and as its name suggests is almost totally hairless (Jarvis

70   and Bennett, 1991). Like all bathyergids except *Bathyergus*, it digs with its incisors and is able to

71   close its lip folds behind the incisors to prevent soil from entering the mouth during tunnelling

72 (Tucker, 1981). The naked mole-rat is the only species within the genus *Heterocephalus*, and

73 phylogenetic analyses indicate ~~that it is the most basal species of the family (Allard & Honeycutt,~~

74 ~~1992), with~~ an estimated divergence from the other bathyergid genera between 40 and 48 million

75 years ago (Faulkes *et al.*, 2004), or 33-35 million years ago, depending on the fossil calibration of

76 the molecular clock (Faulkes *et al.*, 2011; Ingram *et al.*, 2004). On this basis, some authors place

77 the naked mole-rat in its own subfamily, the Heterocephalinae, distinct from the remaining genera

78 in the Bathyerginae (e.g. Wilson & Reeder, 2005). The naked mole-rat first came to prominence

79 scientifically when it was shown that its complex social structure is in fact a rare example of

80 eusociality in vertebrates (Jarvis, 1981, 1991). That is, naked mole-rats have a caste system

81 equivalent to that of ants and termites, with a breeding female, or queen, at the top of the

82 hierarchy, and smaller, non-breeding workers at the bottom. Since then, naked mole-rats have

83 been discovered to possess many other unusual qualities that have placed them in the forefront of

84 research in a number of fields. For example, naked mole-rats appear to be insensitive to acid-

85 induced pain (Smith *et al.*, 2011), extraordinarily resistant to cancer (Seluanov *et al.*, 2009), and

86 extremely long-lived for a small rodent (Buffenstein, 2005).

87 Notwithstanding the many unique adaptations of the naked mole-rat, much of its basic anatomy

88 remains undescribed. This may be in part because the small size of this species renders traditional

89 dissection very difficult. However, the small size also makes the naked mole-rat an ideal

90 candidate for digital dissection via contrast-enhanced micro-computed tomography (microCT)

91 and virtual reconstruction. This technique is based on the work of Metscher (2009) and Jeffery *et*

92 *al*. (2011) and involves the staining of biological specimens with iodine potassium iodide (I_2KI)

93 to enable the visualisation of soft tissues with microCT. The musculature of several rodent

94 species has already been successfully imaged using this technique (Cox & Jeffery, 2011; Hautier

95 *et al*., 2012; Baverstock *et al*., 2013) as well as that of other vertebrates (Tsai & Holliday, 2011;

96 Düring *et al*., 2013; George & Holliday, 2013: Gignac & Kley, 2014; Lautenschlager *et al*.,

97 2014). The aim of this study is to provide a complete description of the jaw-closing musculature

98 of *Heterocephalus glaber*. This will enable comparisons to be made between the naked mole-rat

99 and the other genera of bathyergids already described in the literature. Given the basal position of

100 the naked mole-rat amongst the extant genera, the morphology of the masticatory muscle in this

101 species is a vital piece of evidence in determining whether protrogomorphy is ancestral or

102 secondarily-derived in the Bathyergidae.

Materials and Methods

Sample and scanning

Three naked mole-rat (*Heterocephalus glaber*) individuals were obtained from the collections held by CGF at Queen Mary University of London. The specimens were all non-breeding workers and had been preserved in 95% ethanol for several years. In order to visualise the bony morphology, the specimens were imaged using microCT at the Department of Engineering, University of Hull. Isometric voxel sizes ranged between 0.015 and 0.02 mm. Following the initial scanning, the specimens were immersed in a 5% solution of I_2KI dissolved in phosphate-buffered formal saline for a period of two weeks. The stained specimens were then microCT scanned, again at the Department of Engineering, University of Hull. The scan was performed at 80 kV and 60 µA without a filter, with 4000 projections averaging 2 frames per projection, and using a beryllium target. Voxels were isometric and ranged between 0.022 and 0.024 mm in size.

Digital reconstruction

Only one of the contrast-enhanced microCT scans (corresponding to specimen 'Hetero3') was sufficiently well-resolved to enable virtual reconstruction of the masticatory muscles. This scan was imported as a stacked TIFF into Avizo 8.0 (FEI Visualization Sciences Group, Burlington, MA, USA), and the masticatory muscles of the right side of the head were reconstructed. Although the muscles were clearly visible as individual components, the contrast difference between muscle and bone was not sufficiently different to enable automatic thresholding of the muscles. Thus manual segmentation was employed to produce the 3D muscle reconstructions. The cranium and mandible were also reconstructed to facilitate the visualisation of attachment areas. However, the bony components were reconstructed from the initial unstained scans to allow automatic thresholding. Bone and muscle reconstructions were then brought together and aligned in Avizo 8.0 to produce high resolution figures and movies. Downsampled surface files of the skull, mandible and muscles were combined using Adobe 3D Reviewer (Adobe Systems Inc., San Jose, CA, USA) to produce a 3D interactive PDF (Supplementary File S1) following the method outlined by Lautenschlager (2014). Muscle volumes were calculated by Avizo 8.0 and converted to masses assuming muscle density of 1.0564 g/cm^3 (Murphy & Beardsley, 1974). It was clear from the microCT images that the specimen had suffered extensive soft tissue shrinkage. This is likely to be a result of the iodine staining (Vickerton *et al.*, 2013) as well as the lengthy preservation time. Therefore the absolute mass of each masticatory muscle should be

134 approached with some caution. However, the preservation and staining techniques affect all
135 muscles equally, so the relative proportions of the muscles can be analysed with confidence.
136 Condylobasal cranial length (distance from prosthion to basion) of the specimen was measured as
137 a proxy for size.

138 The arrangement of jaw-closing muscles in the naked mole-rat revealed by contrast-enhanced
139 microCT was compared to previously published descriptions of masticatory muscles in other
140 bathyergids: Tullberg (1899); Boller (1970); Morlock (1983) and Van Daele *et al.* (2009). In
141 addition, to understand how naked mole-rat masticatory muscles relate to rodents more generally,
142 the results here were compared to published descriptions of other rodents including
143 sciuromorphs, myomorphs and hystricomorphs, as well as the only living protrogomorph,
144 *Aplodontia rufa*. The literature consulted was as follows: Müller, 1933 (*Hydrochaerus*); Greene,
145 1935 (*Rattus*); Schumacher & Rehmer, 1962 (*Cavia*, *Rattus*); Wood, 1965 (*Marmota*, *Myocastor*,
146 *Ondatra*); Turnbull, 1970 (*Sciurus*, *Rattus*, *Hystrix*); Woods, 1972 (*Proechimys*, *Echimys*,
147 *Isothrix*, *Mesomys*, *Myocastor*, *Octodon*, *Ctenomys*, *Erethizon*, *Cavia*, *Chinchilla*, *Dasyprocta*,
148 *Thryonomys*, *Petromus*); Weijs, 1973 (*Rattus*); Woods & Howland, 1979 (*Capromys*,
149 *Geocapromys*, *Plagiodontia*, *Myocastor*); Woods & Hermanson, 1985 (*Capromys*, *Geocapromys*,
150 *Plagiodontia*, *Myocastor*, *Echimys*, *Octodon*, *Erethizon*, *Coendou*, *Dasyprocta*, *Atherurus*,
151 *Thryonomys*, *Petromus*); Offermans & De Vree, 1989 (*Pedetes*); Ball & Roth, 1995 (*Sciurus*,
152 *Microsciurus*, *Sciurillus*, *Tamiasciurus*, *Tamias*, *Glaucomys*); Thorington & Darrow, 1996
153 (*Aplodontia*, *Paraxerus*, *Funisciurus*, *Myosciurus*, *Heliosciurus*, *Protoxerus*, *Funambulus*,
154 *Calliosciurus*, *Tamiops*, *Xerus*, *Atlantoxerus*, *Ratufa*); Olivares *et al.*, 2004 (*Aconaemys*,
155 *Octomys*, *Tympanoctomys*, *Spalacopus*, *Octodon*, *Octodontomys*); Satoh & Iwaku, 2004
156 (*Cricetulus*, *Mesocricetus*, *Phodopus*, *Tscherkia*); Satoh & Iwaku, 2006 (*Onychomys*); Satoh &
157 Iwaku, 2009 (*Neotoma*, *Peromyscus*); Druzinsky, 2010 (*Aplondontia*, *Cynomys*, *Tamias*,
158 *Marmota*, *Ratufa*, *Sciurus*, *Thomomys*); Hautier & Saksiri, 2009 (*Laonastes*); Hautier, 2010
159 (*Ctenodactylus*); Cox & Jeffery, 2011 (*Cavia*, *Rattus*, *Sciurus*); Baverstock *et al.*, 2013 (*Mus*).

160 **Results**
161 The cranial length of the scanned specimen of *Heterocephalus* glaber was 18.9 mm. The absolute
162 masses and relative proportions of the jaw-closing muscles are given in Table 1. Although the
163 absolute muscle masses are of limited use, given the extensive muscle shrinkage that has taken

164 place, it should be noted that the total muscle masticatory mass (0.242 g) is 75% of that reported

165 for the rat (Cox & Jeffery, 2011), despite the naked mole-rat skull being under half the length of

166 the rat skull. Thus, even before muscle shrinkage has been accounted for, the naked mole-rat

167 clearly has very large jaw-closing muscles compared to other rodents. The muscles of mastication

168 are described below and shown in Figs. 1-5. A 3D interactive reconstruction is provided in the

169 supplementary PDF (Supplementary File S1) and a rotating reconstruction is given in the

170 supplementary movie (Supplementary File S2).

171 *Superficial masseter*

172 The superficial masseter is a moderately large muscle in the naked mole-rat, forming just under a

173 quarter of the masticatory muscle mass (Table 1). It takes a small tendinous origin from the

174 ventral surface of the anterior zygomatic arch where it meets the skull. From its origin, the

175 superficial masseter runs postero-ventrally along the anterior border of the deep masseter, and

176 inserts along the ventral margin of the mandible. It also wraps around the mandibular margin and

177 extends over the medial mandibular surface. This section of the superficial masseter, known as

178 the *pars reflexa* (Turnbull, 1970), pushes backwards, around the insertion of the medial

179 pterygoid, almost reaching the posteriormost tip of the medial surface of the angle (Figs. 2 and 3).

180 The microCT images of the naked mole-rat do not indicate that the superficial masseter is

181 separated into smaller divisions. In most other descriptions of hystricomorph rodents, the

182 superficial masseter is either divided into a *pars horizontalis* and a *pars reflexa* (e.g. Turnbull,

183 1970), or into a main section and a small *pars anterior* that originates from the mesial edge of the

184 tendon (e.g. Woods, 1972; Woods & Hermanson, 1985; Hautier & Saksiri, 2009; Hautier, 2010).

185 No *pars anterior* was identified in the naked mole-rat, but an extensive *pars reflexa* was clearly

186 visible. Two previous descriptions of bathyergid musculature have reported a superficial masseter

187 morphology not found in any other rodent species. Boller (1970) described a large superficial

188 masseter in *Cryptomys* that completely covered the deep masseter in lateral view. He divided this

189 muscle into three parts, termed M2, M1a and M1b, the latter being essentially synonymous with

190 the superficial masseter described here. This configuration was also reported for *Fukomys* by Van

191 Daele *et al*. (2009), although the authors note that the separation between the superficial masseter

192 and deep masseter is very difficult. In this study, no part M1a or M2 could be identified, with all

193 musculature dorsal to the superficial masseter being assigned to the deep masseter – a view

194 shared by Morlock (1983).

195 *Deep masseter*

196 The deep masseter is very similar in size to the superficial, forming 25% of the total muscle mass

197 (Table 1). It lies immediately dorsal to the superficial masseter, originating along the length of the

198 ventral border of the zygomatic arch and inserting on the masseteric ridge on the ventral part of

199 the mandibular ramus (Fig. 4). As mentioned above, the deep masseter is not covered by part M2

200 of the superficial masseter as described by Boller (1970) and Van Daele *et al*. (2009), but is

201 clearly visible in lateral view (as noted by Morlock, 1983). Thus, the naked mole-rat has a

202 morphology similar to that seen in many hystricomorph taxa, such as *Proechimys* (Woods, 1972),

203 *Myocastor* (Woods & Howlands, 1979) and *Plagiodontia* (Woods & Hermanson, 1985). In some

204 rodents, particularly sciuromorphs and myomorphs, the deep masseter has often been split into

205 anterior and posterior portions (e.g. Greene, 1935; Weijs, 1973; Ball & Roth, 1995; Thorington &

206 Darrow, 1996; Cox & Jeffery, 2011), but, in common with many descriptions of

207 hystricomorphous rodents (e.g. Müller, 1933; Offermans & De Vree, 1989; Woods, 1972; Woods

208 & Hermanson, 1985), no division of the deep masseter was found in *Heterocephalus glaber*.

209 *Zygomaticomandibularis*

210 The ZM is the deepest of the three muscle layers running from the zygomatic arch to the

211 mandible. In the naked mole-rat, as in *Fukomys* (Van Daele *et al*., 2009) it is clearly separable

212 from the deep masseter that lies immediately lateral to it. The iodine-enhanced microCT images

213 in this study show three distinct portions of the ZM (Fig. 5): a posterior ZM originating from the

214 glenoid fossa and running antero-ventrally to insert on the middle of the lateral mandibular

215 ramus; an anterior ZM with an origin spanning the jugo-squamosal suture on the medial surface

216 of the zygomatic arch, and an insertion on the ventral part of the coronoid process; and an

217 infraorbital ZM that originates from the anteriormost part of the orbit where the zygomatic arch

218 meets the skull and the small infraorbital foramen pierces the maxilla, and inserts at the base of

219 the coronoid process lateral to the distal molar. No part of the infraorbital ZM was seen to pass

220 through the infraorbital foramen, but this muscle was so named as it appears to be homologous

221 with the infraorbital ZM in other rodents (e.g. Weijs, 1973; Olivares *et al*., 2004; Satoh & Iwaku,

222 2006, 2009; Cox & Jeffery, 2011).

223 The above description of the ZM largely matches that of Tullberg (1899) and Morlock (1983),

224 differing only in nomenclature. Tullberg (1899) clearly identifies three portions of the medial

225　masseter (equivalent to the ZM here), but assigns the rostral two portions to the '*Portio anterior*

226　*masseteris medialis*' rather than splitting them into infraorbital and anterior sections. Morlock

227　(1983) identifies the three parts as (caudal to rostral): posterior ZM, anterior ZM and '*maxillo-*

228　*mandibularis*'. There are, however, substantial differences in the muscle arrangement described

229　by Boller (1970) in *Cryptomys*. The middle part, here identified as the anterior ZM, was

230　determined by Boller (1970) to be a ventral extension of the temporalis muscle (*pars zygomatica*)

231　on to the lateral surface of the mandible. This was not thought to be the case in the naked mole-

232　rat specimen scanned for this study as, in common with Morlock (1983) no clear connection

233　between this muscle and the temporalis could be seen.

234　*Temporalis*

235　The temporalis is the largest jaw-closing muscle in *Heterocephalus glaber*, forming around 32%

236　of the masticatory muscle mass (Table 1). Its origin covers the entire parietal bone and much of

237　the frontal and squamosal as well, extending from the dorsal midline down to the zygomatic

238　process of the squamosal, and from the occipital bone across the braincase well into the orbit

239　(Fig. 5). The insertion is on the tip and widely across the medial surface of the coronoid process.

240　Amongst the rodents, only the rat has been reported to possess a temporalis comprising over 30%

241　of the masticatory musculature (Turnbull, 1970). Most of the closer relatives of the blesmols in

242　the Hystricognathi have much smaller temporal muscles comprising between 5% and 15% of

243　masticatory muscle mass (Müller, 1933; Turnbull, 1970; Cox & Jeffery, 2011).

244　*Medial pterygoid*

245　The medial pterygoid is split into two branches at its origin – a small branch that attaches to the

246　lateral surface of the pterygoid plate, and a much larger branch that originates deep within the

247　pterygoid fossa. The fossa opens into the braincase in *Heterocephalus glaber* as it does in all

248　bathyergids and all hystricognaths except *Hydrochoerus* (Woods, 1972). The two branches of the

249　medial pterygoid unite and run caudally, ventrally and laterally to take a long, narrow insertion on

250　the medial surface of the angle of the mandible (Fig. 3). As mentioned above the insertion of the

251　medial pterygoid is almost completely surrounded by the *pars reflexa* of the superficial masseter

252　(Figs. 2 and 3).

253　Compared to many other rodents such as *Sciurus* (Turnbull, 1970; Ball & Roth, 1995; Cox &

254　Jeffery, 2011) and *Cavia* (Schumacher & Rehmer, 1962; Cox & Jeffery, 2011), the medial

255 pterygoid is a relatively small component of the naked mole-rat masticatory system, forming just

256 6% of the muscle mass (Table 1). However, it is comparable to the size of the medial pterygoid

257 measured in *Cryptomys* (Boller, 1970) and *Rattus* (Schumacher & Rehmer, 1962; Cox & Jeffery,

258 2011).

259 *Lateral pterygoid*

260 The lateral pterygoid originates from the lateral pterygoid plate and part of the alisphenoid bone,

261 dorsal to the smaller branch of the medial pterygoid. From its origin it extends postero-laterally,

262 in an almost horizontal plane, to the medial surface of the mandibular condyle (Fig. 3). The

263 insertion is immediately dorsal to the *pars reflexa* of the superficial masseter. The muscle forms

264 around 2% of the masticatory musculature (Table 1), somewhat less than many other rodents (e.g.

265 Turnbull, 1970; Ball & Roth, 1995; Cox & Jeffery, 2011; Baverstock, 2013).

266 **Discussion**

267 ~~The naked mole-rat, *Heterocephalus glaber*, is a particularly fascinating and unusual mammal,~~

268 ~~even within its own family, the Bathyergidae. It has been the subject of a great deal of scientific~~

269 ~~enquiry regarding its social structure (Jarvis, 1981), cancer resistance (Seluanov *et al.*, 2009),~~

270 ~~pain insensitivity (Smith *et al.*, 2011), and longevity (Buffenstein, 2005). Despite the large~~

271 ~~amount of research devoted to this species, little has been published on its anatomy.~~ Here, the

272 technique of iodine-enhanced microCT scanning (Metscher, 2009; Jeffery *et al.*, 2011) was used

273 to visualise the jaw-closing musculature of the naked mole-rat. Although the specimen studied

274 had been preserved for several years in ethanol and undergone a substantial amount of muscle

275 shrinkage, the iodine potassium iodide staining was very successful in revealing the different

276 layers and sections of the masticatory muscles.

277 The masticatory system of the naked mole-rat is relatively very large compared to other rodents

278 (Cox & Jeffery, 2011). In fact, Jarvis & Sherman (2002) report that the jaw muscles constitute

279 around a quarter of the entire muscle mass of the naked mole-rat. The masticatory complex is

280 dominated by three muscles: the superficial masseter, the deep masseter and the temporalis. The

281 superficial and deep masseters form together almost 50% of the masticatory musculature.

282 Gorniak (1977) and Byrd (1981) suggested that these muscles have an important role in the

283 closing and power strokes of biting at both the incisors and molars. Thus, the large masseter in

284   the naked mole-rat is likely to deliver a large bite force. In addition, the strong horizontal

285   component of pull in the superficial masseter is likely to make this muscle the main protractor of

286   the lower jaw, as proposed by Hiiemae (1971). The microCT images revealed a large portion of

287   the superficial masseter that wraps around the ventral margin of the mandible and inserts on the

288   medial surface of the ramus, known as the *pars reflexa*. Various functions have been suggested

289   for this part of the muscle, including fine control of jaw opening (Weijs & Dantuma, 1975) and

290   increase in the resting length of the muscle to facilitate wider gapes (Satoh & Iwaku, 2004).

291   The relative size of the temporalis muscle in the naked mole-rat (32% of masticatory muscle

292   mass) is one of the largest seen in rodents. In particular, the temporalis is very large compared to

293   other species in the Hystricognathi, extending across the entire temporal region of the cranium

294   and also pushing into the orbit. The highly reduced size of the eye in the naked mole-rat may

295   have partly facilitated the increase in size of the temporalis, enabling its anterior expansion into

296   the relatively unoccupied orbit (Lavocat, 1973). Such a large temporalis is likely to be acting as a

297   powerful elevator of the jaw (Hiiemae, 1971), producing large forces at the teeth. The

298   ventralmost fibres of temporalis running along the zygomatic process of the squamosal have a

299   largely horizontal direction of pull, and thus are likely to act as a strong retractor of the jaw.

300   Therefore, it appears that overall two largest masticatory muscles of the naked mole-rat provide it

301   with the ability to generate large bite forces, strong movements of the mandible in the antero-

302   posterior plane (propaliny) and potentially a wide gape. All these are highly useful characteristics

303   in a subterranean dwelling species that digs extensive tunnels with its incisors, as the naked mole-

304   rat does (Stein, 2000). Brett (1991) radio tracked individuals in a colony of 87 naked mole-rats

305   and found that the total length of tunnels was an impressive 3-4 km, corresponding to 3.6-4.5

306   tonnes of soil being excavated in a single year - the equivalent of 2.3-2.9 km of new tunnels.

307   These are impressive statistics for a small rodent of average 34 g body mass. Such endeavours

308   impose high costs with respect to tooth wear, yet incisor growth is not dissimilar to that of other

309   rodents (Berkovitz & Faulkes, 2001), and perhaps offset as a limiting factor by the social

310   behaviour of the naked mole-rat, where digging activity is distributed among a large workforce

311   with strong jaws.

312   The iodine-enhanced microCT scans show very clearly that the zygomaticomandibularis muscle

313   does not pass through the infraorbital foramen and on to the rostrum in *Heterocephalus glaber*.

314   Thus, the naked mole-rat displays the protrogomorphous morphology (Wood, 1965), as do most

315   other genera in the Bathyergidae (Tullberg, 1899), although there is a very small extension of the

316   ZM through the infraorbital foramen in *Cryptomys* (Boller, 1970; Morlock, 1983) and *Fukomys*

317   (Van Daele *et al*., 2009). In contrast, all other families in the Hystricognathi are hystricomorph

318   i.e. they possess a large infraorbital portion of the ZM that passes through an enlarged infraorbital

319   foramen and takes a wide origin on the rostrum. However, the 3D reconstructions demonstrate

320   that, although the naked mole-rat is technically protrogomorphous, it is very different in

321   morphology to the other extant protrogomorph, *Aplodontia rufa*. In the mountain beaver, the

322   origin of the ZM is restricted to the medial surface of the zygomatic arch and the internal surface

323   of the maxillary root of the zygoma (Druzinsky, 2010). It does not have the wide attachment at

324   the front of the orbit seen in *Heterocephalus glaber*. The morphology of the ZM in the naked

325   mole-rat very much resembles the hystricomorphous condition without the extension on to the

326   rostrum. Whether the naked mole-rat represents a protrogomorphous rodent that is in the process

327   of evolving hystricomorphy, or a hystricomorphous rodent that is secondarily losing the rostral

328   extension of the ZM is difficult to tell from the morphology alone. However, given the position of

329   the Bathyergidae within the rodent phylogeny (Fabre *et al*., 2012), and the presence of

330   hystricomorphy in fossil genera (Lavocat, 1973) and ontogeny (Maier & Schrenk, 1987), it is

331   likely that the African mole-rats evolved from a hystricomorph ancestor. It appears that most of

332   the blesmols, *Heterocephalus* included, have undergone a shortening of the rostrum (Landry,

333   1957), which may account for the retreat of the ZM from the snout. The loss of the infraorbital

334   ZM may also have been an adaptation to increasing gape for incisor digging.

335   In conclusion, the naked mole-rat has evolved an enlarged set of masticatory muscles,

336   particularly the superficial masseter and temporalis. These large muscles enable the powerful bite

337   force and wide gape necessary for digging with the incisor teeth. The overall morphology is

338   protrogomorphous, but appears to have been evolved from a hystricomorphous ancestor, with the

339   infraorbital portion of the zygomaticomandibularis having been lost possibly through rostral

340   shortening. The contrast-enhanced microCT technique has been shown to be a highly effective

341   tool for the visualisation of soft tissues, especially muscle. It is hoped that it will become part of

342   the standard toolkit of anatomical investigation in the future.

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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**

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.
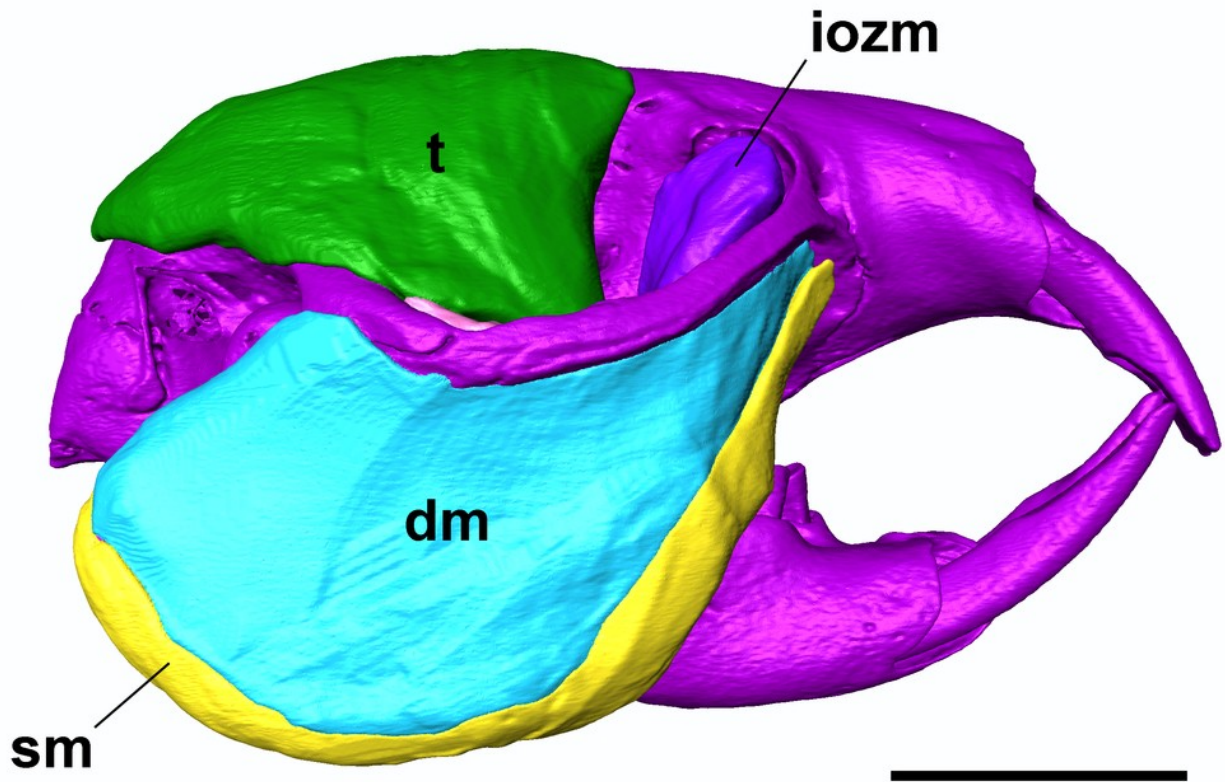
Figure 2

Iodine-enhanced microCT scan of *Heterocephalus glaber*

Coronal microCT slice through the head of *Heterocephalus glaber*. Abbreviations: d, digastric (not reconstructed); dm, deep masseter; lp, lateral pterygoid; m, mandible; mp, medial pterygoid; pr, pars reflexa of the superficial masseter; sm, superficial masseter; t, temporalis. White line on 3D reconstruction shows position of slice. Scale bar = 5mm.
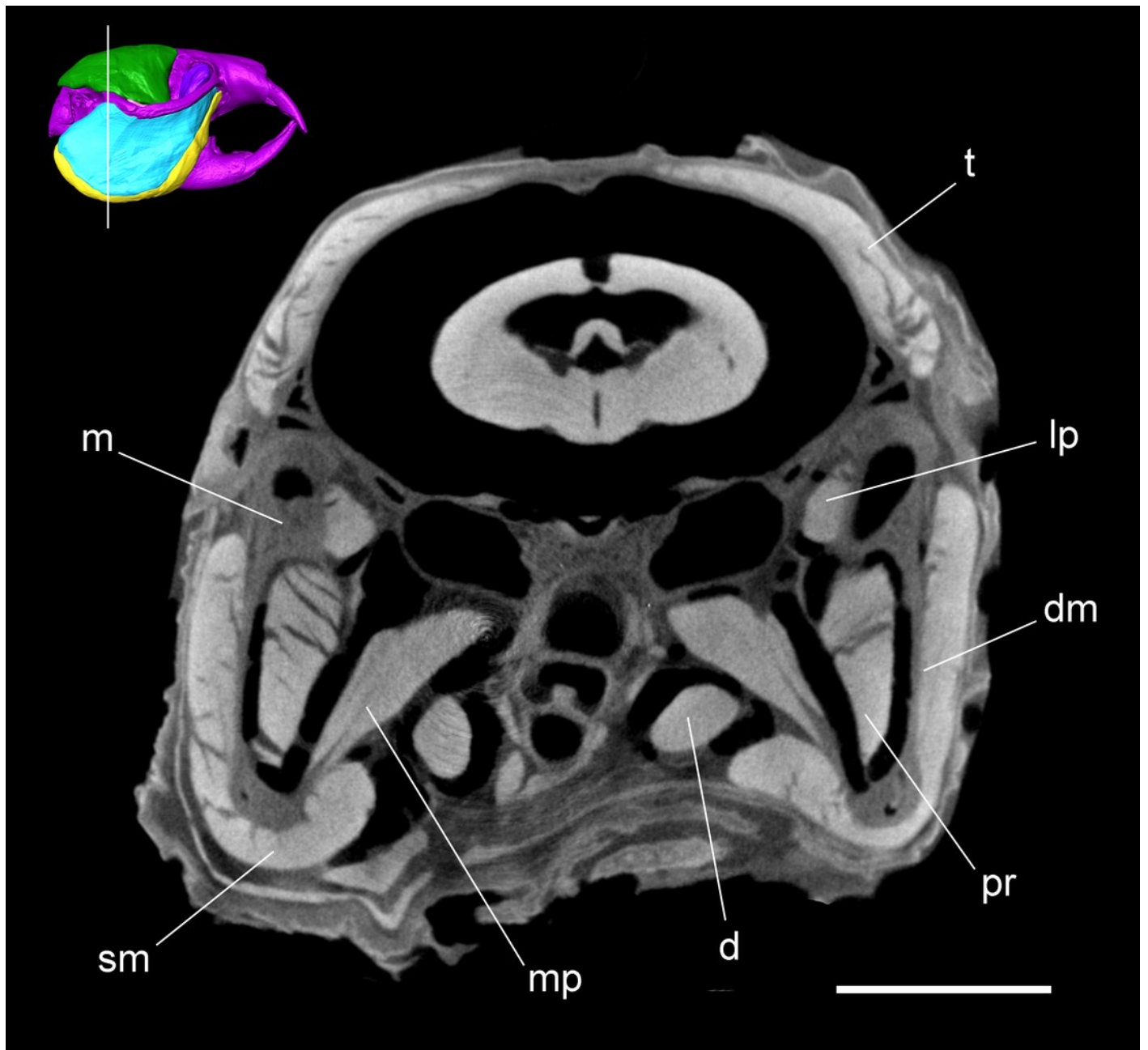
Figure 3

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; sm, superficial masseter. Scale bar = 5mm.
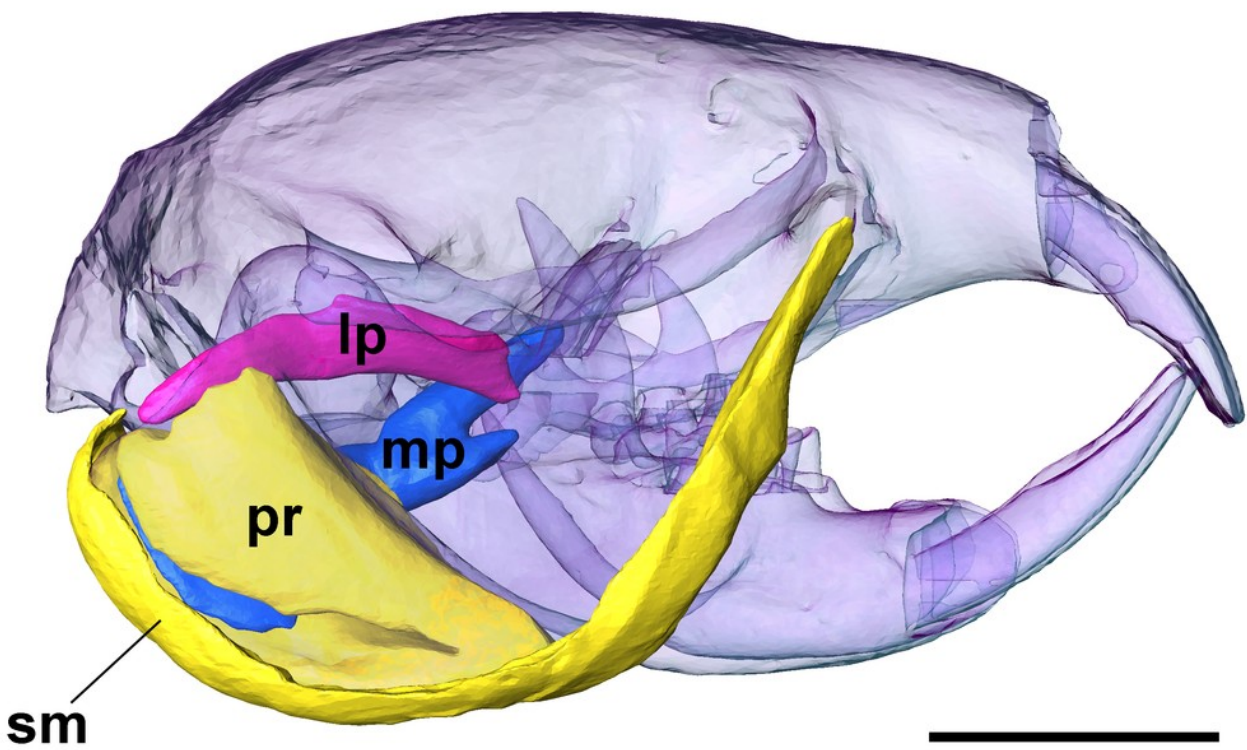
Iodine-enhanced microCT scan of *Heterocephalus glaber*

Transverse microCT slice through the head of *Heterocephalus glaber*. Abbreviations: azm, anterior zygomaticomandibularis; dm, deep masseter; mp, medial pterygoid; pr, pars reflexa of the superficial masseter; pzm, posterior zygomaticomandibularis; sm, superficial masseter. White line on 3D reconstruction shows position of slice. Scale bar = 5mm.
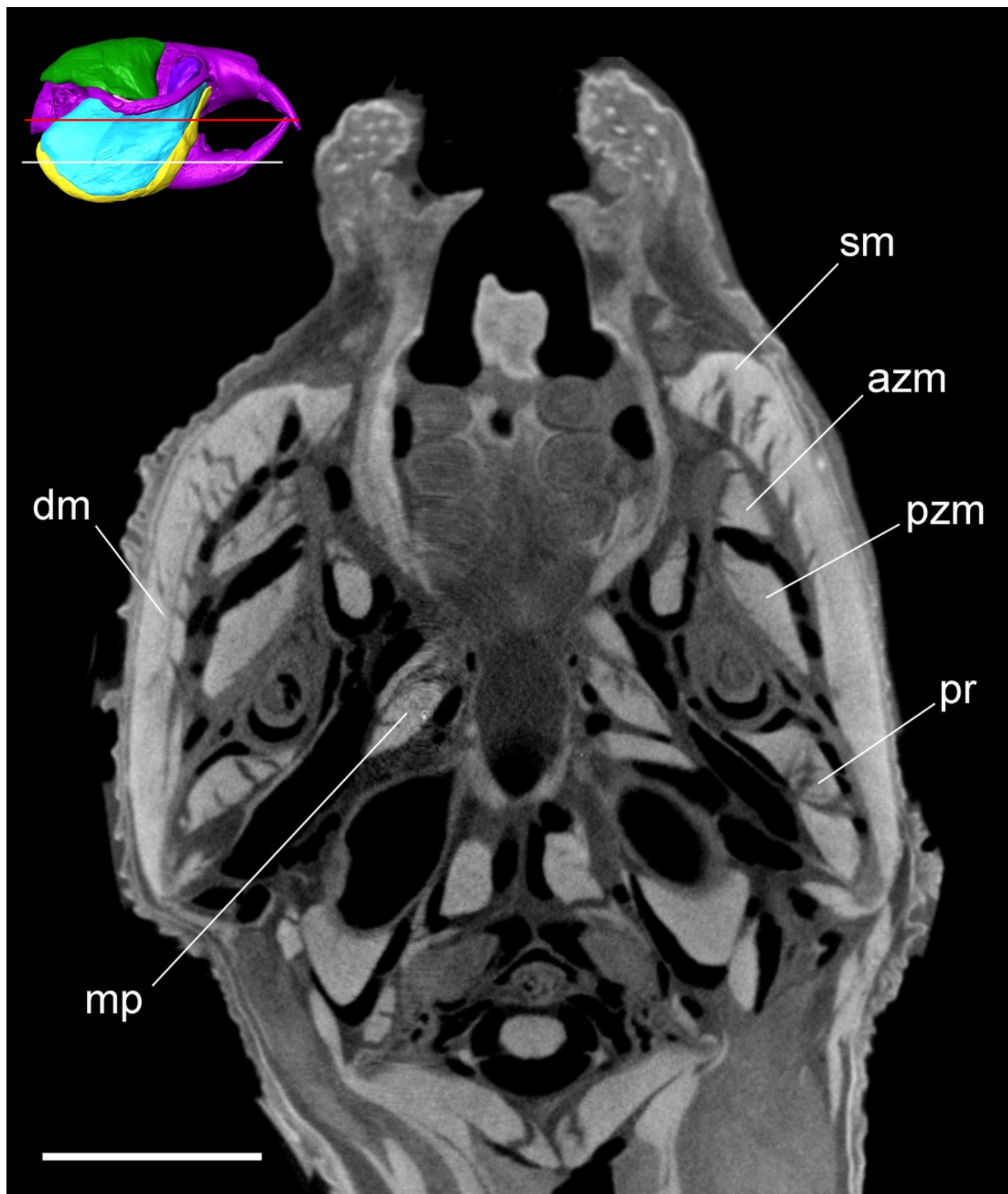
Figure 5

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.