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Morphology of the jaw-closing musculature in sciurormorph, hystricomorph and myomorph rodents

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**Morphology of the jaw-closing musculature in sciromorph,
hystricomorph and myomorph rodents**

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ABSTRACT

Rodents are frequently separated into three non-monophyletic groups - the sciurormorph, hystricomorph and myomorph forms - based on the morphology of their masticatory muscles. Yet there still exists in the literature a degree of controversy as to the exact morphology and internal architecture of the rodent jaw-closing musculature, particularly with regard to the masseter. This study examined the muscles of mastication in a typical member of each of these groups: a grey squirrel, domesticated guinea pig and brown rat, respectively. Three dimensional reconstructions of the skull, mandible and masticatory muscles of each of these rodents were generated from contrast-enhanced microCT images. The temporalis and pterygoid muscles were found to be broadly similar in all three specimens, although separation of the temporalis into anterior and posterior parts was only evident in the rat and squirrel. The three layers of the masseter - superficial, deep and zygomatico-mandibularis - showed the most variation in morphology. The guinea pig had an enlarged superficial masseter and reduced deep masseter compared to the rat and squirrel. The deep masseter was greatly enlarged in the squirrel and rat, and had expanded forward to take an origin on the rostrum. The deep masseter was also separated into anterior and posterior parts, but no such division could be seen in the guinea pig. The zygomatico-mandibularis was split into anterior and posterior parts in all three specimens by the masseteric nerve, and in the rat and guinea pig it had an additional expansion through the infraorbital foramen on to the rostrum. This infraorbital part of the muscle was particularly well-developed in the guinea pig. The results here give valuable origin, insertion and muscle volume data that will be used in future biomechanical studies of the rodent masticatory apparatus.

Keywords: rodent: sciurormorph; hystricomorph; myomorph; masticatory muscles

INTRODUCTION

The rodents are defined by their masticatory apparatus. The teeth are the diagnostic feature of the order – a pair of enlarged, self-sharpening, ever-growing incisors in both

the upper and lower jaws separated from the cheek teeth by a long diastema (indicating the lost canines and first premolars). In addition, the masticatory muscles of rodents are just as distinctive. The masseter is by far the dominant jaw-closing muscle in the Rodentia, comprising between 60 and 80% of the entire masticatory muscle mass (Turnbull, 1970). In contrast, the temporalis is markedly reduced and forms a similar proportion of the masticatory complex to the pterygoid muscles. Furthermore, the musculature has become specialised to accomplish not only gnawing at the incisors and chewing at the molars, but also propalinal movement of the lower jaw between these two feeding modes (Becht, 1953). These movements are necessary in rodents, because the incisors and cheek teeth cannot both be in occlusion at the same time, and thus incision and mastication have become mutually exclusive activities (Hiimae & Ardran, 1968). Given the unique demands on the masticatory apparatus, it is perhaps unsurprising that the morphology of the jaw-closing muscles, in particular the masseter, has long been used to classify the rodents into subgroups.

It was Brandt (1855) who first used features primarily from the masticatory apparatus to group rodents into squirrel-like (Sciuromorpha), mouse-like (Myomorpha) and porcupine-like (Hystricomorpha) forms (Brandt's fourth group Lagomorpha, the rabbits, hares and pikas, now occupy a separate, albeit closely related, order). These three suborders were largely retained with only minor revisions by most workers for the next century (e.g. Thomas, 1896; Miller & Gidley, 1918), and indeed were still the basis for rodent taxonomy in George Gaylord Simpson's monumental classification of the mammals in 1945. It should be noted, however, that Simpson alludes to a growing dissatisfaction with the three suborders (Simpson, 1945, p.198), but retains them in his work owing to a lack of a better alternative at that time. The problem with the three suborder arrangement can clearly be seen in Simpson's classification: there are a number of rodent families that do not neatly fit into the Sciuromorpha, Myomorpha or Hystricomorpha. In particular, the Anomaluridae (scaly-tailed squirrels), Pedetidae (springhare), Dipodidae (jerboas, jumping mice and birchmice), Bathyergidae (mole-rats) and Ctenodactylidae (gundis) have all posed problems to various workers in the past. A competing classification of rodents, first proposed by Tullberg (1899), split the Rodentia

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3 into two suborders (Sciurognathi and Hystricognathi) based on the morphology of the
4 angular process of the mandible. This system overlaps with the masseter-based
5 classification in some respects, for instance, the fact that all hystricognaths have a
6 hystricomorph muscle arrangement (Lavocat, 1974, Wood 1974); but has notable
7 differences as well, e.g. sciurognaths can possess any of the three masticatory muscle
8 morphologies (Offermans & De Vree 1989).
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16 Neither of the two classifications outlined above has stood the test of time. Although
17 evidence points towards a monophyletic Hystricognathi, the Sciurognathi is almost
18 certainly a paraphyletic grouping, and the idea that the three suborders of Brandt (1855)
19 and Simpson (1945) represent monophyletic groups of rodents is now generally
20 discredited (Adkins et al., 2001; Huchon et al., 2002; Adkins et al., 2003; Blanga-Kanfi et
21 al., 2009). However, the use of the terms sciuiromorph, myomorph and hystricomorph as
22 adjectives describing particular arrangements of jaw-closing muscles has persisted,
23 largely thanks to Wood (1965). In his work, Wood describes the primitive arrangement of
24 rodent masticatory muscles (the 'protrogomorph' condition, found in most pre-Oligocene
25 fossil rodents, and also in the extant mountain beaver, *Aplodontia rufa*), and the three
26 arrangements derived from it. In the sciuiromorph condition, part of the masseter has
27 expanded antero-dorsally to take its origin from the rostrum and the widened root of the
28 zygomatic arch. This arrangement is seen in the Sciuridae (squirrels), Castoridae
29 (beavers) and Geomyoidea (pocket gophers, and kangaroo rats and mice). In the
30 hystricomorph masticatory apparatus, a deeper part of the masseter has extended
31 forwards, through the orbit and the grossly enlarged infraorbital foramen to take an origin
32 on the snout. This morphology is found in the Caviomorpha (South American rodents),
33 Phiomorpha (African mole-rats, cane rats and the dassie rat) and Hystricidae (old world
34 porcupines) as well as the previously mentioned Pedetidae, Anomaluridae, Dipodidae and
35 Ctenodactylidae. Lastly, the myomorphs combine sciuiromorph and hystricomorph
36 features with the origins of both parts of the masseter having migrated on to the rostrum.
37 This condition is seen in the Muroidea (mice and rats) and the Gliridae (dormice).
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The above morphological descriptions have been greatly complicated by the complete lack of consensus on the nomenclature of rodent masticatory muscles, with particular regard to the masseter (hence the lack of specific muscle nomenclature in the previous paragraph). Part of the confusion arises due to the uncertainty of how many layers the masseter divides into, and whether all of these layers should be referred to as the masseter or as entirely separate muscles. The situation is further confounded by the difficulty in identifying homologous muscles in all three groups, and across mammals as a whole.

In most works, the masseter is split into three layers (although not all, see Hiiemae & Houston, 1971). The outermost layer is the least controversial and is almost universally named the *masseter superficialis*, or superficial masseter. Immediately medial to the superficial masseter is the middle layer. This muscle has the most confusing nomenclature, because it has been referred to using two seemingly contradictory terms. Wood (1965), Woods & Howlands (1979), Woods & Hermanson (1985), Olivares et al., (2004), Satoh & Iwaku (2004, 2006, 2009) and Druzinsky (2010) refer to it as the *masseter lateralis* or lateral masseter, because it is lateral to the layer underneath. However, Yoshikawa & Suzuki (1969), Turnbull (1970), Hiiemae & Houston (1971), Weijs (1973), Offermans & De Vree (1989), Ball & Roth (1995), Thorington & Darrow (1996) and Satoh (1997, 1998, 1999) name it the *masseter profundus* or deep masseter, because it is deep compared to the superficial masseter. Woods (1972), following Hill (1937) and Bryant (1945), combines the two nomenclatures and calls this muscle the '*masseter lateralis profundus*'. Similarly, in their anatomy of the guinea pig, Cooper & Schiller (1975) refer to the 'deep lateral masseter'. However, the text and illustrations of this work do not have matching nomenclatures, and the figure labels read simply 'deep masseter'. Neither name for this muscle layer is entirely satisfactory, though 'deep masseter' has the advantage of being consistent with the nomenclature used in most other mammalian orders (e.g. Storch, 1968; Turnbull, 1970; Coldiron, 1977; Janis, 1983).

The dichotomy in nomenclature seen in the middle layer of the masseter persists into the innermost layer. Those workers that refer to the lateral masseter generally label this muscle the *masseter medialis*, or medial masseter (Hill, 1937; Woods, 1972; Cooper &

Schiller, 1975; Woods & Howlands, 1979; Woods & Hermanson, 1985; Olivares et al., 2004. Wood [1965] also calls this muscle the *masseter medialis* in the text of his paper, but confusingly labels it the *masseter profundus* in all the figures). In contrast, most other anatomists who use the name deep masseter for the middle layer, term the innermost layer the zygomatico-mandibularis in reference to its origin and insertion (e.g. Schumacher & Rehmer, 1962; Yoshikawa & Suzuki, 1969; Turnbull, 1970; Weijs, 1973; Offermans & De Vree, 1989; Ball & Roth, 1995; Thorington & Darrow, 1996). However, the other possible combinations of nomenclatures also exist. Satoh & Iwaku (2004, 2006, 2009) name the three masseteric layers as superficial, deep and medial. Conversely, for specific reasons outlined in detail in his study, Druzinsky (2010) refers to the lateral masseter and the zygomatico-mandibularis. Strangely, Hiimeae & Houston (1971) do not mention the deepest layer of the masseter at all – it is suspected that they felt it to be indistinguishable from the deep masseter and thus did not treat it separately. This scheme was also followed in the earlier works of Satoh (1997, 1998, 1999).

The antero-dorsal expansion of the innermost layer of the masseter on to the rostrum in myomorphs and hystricomorphs is generally referred to as the infraorbital part of the zygomatico-mandibularis (Weijs, 1973) or medial masseter (Wood, 1965; Woods & Howland, 1979; Olivares et al., 2004; Satoh & Iwaku 2004, 2006, 2009) depending on what the rest of the muscle has been termed. However, a number of authors describe it as a separate entity, named the maxillo-mandibularis (Becht, 1953; Schumacher & Rehmer, 1962; Yoshikawa & Suzuki, 1969; Turnbull, 1970; Offermans & De Vree, 1989). In those studies in which the zygomatico-mandibularis is undescribed (Hiimeae & Houston, 1971; Satoh, 1998), this part of the muscle is generally referred to as the infraorbital part of the anterior deep masseter.

The other jaw-closing muscle groups, the temporalis and pterygoids, also have competing nomenclatures. The temporalis is often divided into two parts, called the medial and lateral (Ball & Roth, 1995; Thorington & Darrow, 1996), anterior medial and anterior lateral (Weijs, 1973), main part and orbital part (Woods, 1972), *pars posterior* and *pars orbitalis* (Woods & Howlands, 1979) or posterior and anterior (Druzinsky, 2010). It

should be noted that the posterior temporalis of Woods & Howlands (1979) and Druzinsky (2010) is not the same as the posterior temporalis of Woods (1972) and Weijs (1973). The latter two works use posterior temporalis to refer to a small ventral part of the temporalis arising from the dorsal surface of the zygomatic process of the squamosal. Druzinsky (2010) refers to this muscle as the suprazygomatic part of the temporalis. The two pterygoid muscles are usually termed internal and external (Wood, 1965; Woods, 1972; Weijs, 1973; Woods & Howland, 1979; Satoh & Iwaku, 2004; Druzinsky, 2010), but are also known as medial and lateral (Schumacher & Rehmer, 1962; Offermans & De Vree, 1989; Ball & Roth, 1995; Thorington & Darrow, 1996).

The aim of the current paper is to diminish the current confusion in rodent masticatory muscle morphology by providing thorough descriptions of the masseter, temporalis and pterygoid muscles of a typical representative of the sciurormorph, hystricomorph and myomorph forms. All these muscles will be investigated using current imaging techniques, and the results will be compared to and contrasted with previous investigations.

MATERIALS AND METHODS

Sample

Three rodent species were chosen to represent the sciurormorph, hystricomorph and myomorph morphologies. These were, respectively, the Eastern grey squirrel (*Sciurus carolinensis*), the domesticated guinea pig (*Cavia porcellus*), and the brown rat (*Rattus norvegicus*). These species were selected as they have all been well-studied previously, and each represents a typical member of its feeding type (i.e. none is anomalously specialised).

Imaging

To visualize the muscle tissues as well as the bone in a non-destructive manner, a formalin-fixed specimen of each species was imaged using contrast-enhanced microCT (Jeffery et al., 2010). The enhancement uses a solution of iodine (I₂KI) to increase the

differential attenuation of X-rays among soft-tissues and has been shown to demonstrate patterns of muscle fibres and fascicles against the connective tissues (see Fig. 1 and Jeffery et al., 2010). Specimens were fixed in phosphate buffered formal saline (PBFS) solution (polymerized formaldehyde dissolved as a 4% solution in phosphate buffered saline allowing for long term storage with limited tissue shrinkage) and then placed in I₂KI contrast agent for a period of two months. This incubation time was insufficient for passive diffusion throughout the larger *Sciurus* and *Cavia* specimens. Small volumes of the contrast agent were therefore injected into the body of the muscles with a fine grade needle. After further incubation the specimens were imaged with the Metris X-Tek custom 320kV bay system at the EPSRC funded Henry Moseley X-ray Imaging Facility, University of Manchester. Imaging parameters were optimised for each specimen to maximise spatial and contrast resolution as well as data handling. Voxel resolutions varied from 0.033mm to 0.040mm.

Reconstruction and observations

Three-dimensional reconstructions of all the jaw-closing muscles were created for each specimen using the volume rendering function of Amira 5.2 (Mercury Systems Inc., Chelmsford, MA, USA). Reconstructions of the skull and mandible were also created to facilitate visualisation of the origins and insertions of each muscle. As can be seen from Fig. 1, the difference in contrast between muscle and bone was not sufficient to allow the models to be generated using the threshold function. Hence, the muscle and bone reconstructions were built by manually painting the object of interest in a number of slices and interpolating between them. A smoothing function was used to reduce the blocky appearance of the reconstructions. Amira 5.2 was also able to output the volume of each muscle in each reconstruction, and these are reported in the results section.

RESULTS

In the following descriptions, the three layers of the masseter are referred to as the superficial masseter, the deep masseter and the zygomatico-mandibularis. It is felt that, although all the nomenclatures currently in use have their advantages, the one used here

is at least consistent with the nomenclature used in most other mammalian groups. The rostral expansion of the innermost layer in myomorphs and hystricomorphs is termed the infraorbital part of the zygomatico-mandibularis. Where the temporalis has been split into two parts, they are named the medial and lateral parts, as this is felt to reflect more accurately their anatomical relationship to one another. The pterygoids are referred to as internal and external in reference to their origin in and on the pterygoid fossa.

Superficial Masseter

Fig. 1 demonstrates the enhanced microCT imaging of the muscles in the squirrel. Regions of light (high x-ray attenuation) represent groups of muscle fibres (see Jeffery et al., 2010). Darker bands represent the epimysium and perimysium that separate muscles and fascicles. There is a clear dark band distinguishing the superficial masseter from the posterior deep masseter. From the reconstructions (Fig. 2) it can be seen that the superficial masseter exhibits a fairly consistent morphology across the three specimens. This muscle takes a small origin from a flattened tendon attached to a small tubercle or process just below the infraorbital foramen. The postero-dorsal muscle fascicles then run to the back of the mandible to insert into a small region on the postero-lateral surface of the angle of the jaw ramus. Some of the muscle fascicles on the dorsal edge of this muscle also insert on to the aponeurosis of the lower masseteric layer, making the separation of these two parts of the masseter frequently difficult. The antero-ventral fascicles of the superficial masseter run under the mandible to insert on the medial surface of the jaw in a fossa just ventral to the insertion of the internal pterygoid. In the guinea pig and, to a lesser degree, in the rat, there is also a dorsal elongation of the reflected part of the superficial masseter anterior to the internal pterygoid, so that it inserts on the medial condyloid process. This is termed the '*pars reflexa*' by some workers (e.g. Turnbull, 1970; Woods, 1972; Weijs 1973). It can be seen from Table 1 that the superficial masseter is relatively larger in the guinea pig (Fig. 2a) than in the rat or squirrel (Fig. 2b,c), forming almost half of the jaw-closing musculature. Its dorsal edge is at the level of the zygomatic arch, almost completely obscuring the deeper layers in lateral view. In contrast, the superficial masseter of the rat and squirrel is much more

restricted dorsally, its margin running diagonally from the origin at the front of the zygomatic arch to the tip of the angle, thus revealing the deep masseter behind.

Deep Masseter

This muscle layer, immediately behind the superficial masseter, takes its origin from the ventro-lateral surface of the zygomatic arch. In the squirrel and rat (Fig. 3a,b), the muscle has also spread anteriorly on to the rostrum to originate from the masseteric fossa and the widened inferior root of the zygomatic process of the maxilla. In the rat, the origin of the deep masseter extends as far as the anterior margin of the maxilla, and in the squirrel, beyond this point on to the premaxilla. In the guinea pig (Fig. 3c), this muscle is restricted to the zygomatic arch by the large infraorbital foramen. The deep masseter inserts on the lateral surface of the mandible all along the masseteric ridge from beneath the second molar to the angular process. The microCT images show that this muscle layer clearly divides into two sections in the squirrel and rat (Fig. 4a,b), based on variation in the fascicle direction. There is an anterior part that originates from the rostrum and inserts on the anterior portion of the masseteric ridge, and a posterior part that originates further back on the zygomatic arch and inserts on the mandibular angle. This separation is particularly obvious in the microCT images of the squirrel. No such division is seen in the guinea pig where there is no rostral expansion of the deep masseter. In this species, the deep masseter has been reconstructed as a single muscle. The deep masseter is a relatively much smaller muscle in the guinea pig than in the rat and squirrel, where it forms well over 30% of the masticatory muscle volume (see Table 1).

Zygomatiko-mandibularis

The innermost layer of the masseter runs between the zygomatic arch and the dorsal part of the mandible. More specifically, it originates from the medial surface of the zygomatic arch (largely on the jugal, but also on parts of the maxilla and squamosal), and inserts on the lateral surface of the lower jaw. In squirrels (Fig. 5a), the insertion is in the masseteric fossa, just posterior to the toothrow, ventral and anterior to the mandibular condyle. In rats and guinea pigs (Fig. 5b,c) the zygomatiko-mandibularis inserts on the lateral crest below the second and third molars and on to the coronoid process. As in the previous

layer, the microCT images provide good evidence to justify splitting this muscle into anterior and posterior parts. The anterior part runs ventrally from the medial surface of the jugal to the lateral crest, whereas the posterior part originates on the ventral and medial surface of the zygomatic process of the squamosal and runs antero-ventrally to the coronoid process of the mandible. As well as showing different orientations of their muscle fascicles, the two parts are also clearly separated by the masseteric nerve (Fig. 6). In the rat and the guinea pig, there is a antero-dorsal expansion of the anterior part of this muscle into the orbit and through the infraorbital foramen to take an origin from the rostrum (Fig. 5b,c). The rostral origin is relatively small in the rat, restricted to the area of the maxilla dorsal to the masseteric fossa (the origin of the anterior deep masseter). However, the expansion of this muscle is much greater in guinea pig, extending through the grossly enlarged infraorbital foramen to take a large origin on the premaxilla as well as on the maxilla. In both the rat and guinea pig, the infraorbital part of the zygomatico-mandibularis inserts into a fossa at the anterior end of the lateral crest, ventro-lateral to the first cheek tooth. Overall, the zygomatico-mandibularis makes up a small part of the masticatory musculature - less than 10% in the rat and squirrel (see Table 1). It is slightly greater in the guinea pig (15%) owing to the large infraorbital portion in this species.

Temporalis

The temporalis muscle, which dominates the masticatory musculature in many other mammals, is greatly reduced in comparison to the masseter in all rodents (see Table 1). The medial temporalis takes its origin from the lateral surface of the cranium, extending rostro-caudally from the frontal-parietal suture to the lambdoid crest, and dorso-ventrally from the temporal ridge to the external auditory meatus and zygomatic process of the squamosal. The muscle fascicles from this wide origin converge to a small region on the medial surface of the mandible between the retromolar fossa and the coronoid process. In the squirrel and rat, a separate smaller part, the lateral temporalis, is visible (Fig. 2b,c). This takes its origin from the anterior half of the fascia overlying the medial temporalis and inserts on the coronoid process. This division between medial and lateral was not visible in the guinea pig microCT images and so the temporalis has been reconstructed as a single muscle in this specimen (Fig. 2a).

Internal pterygoid

This muscle is well-developed in rodents, and has a similar morphology in all three species in this study. It is particularly notable in the guinea pig in which it accounts for almost a fifth of the jaw-closing musculature (see Table 1 and Fig. 7). It originates in the pterygoid fossa posterior to the molar toothrow. In squirrels, it also has an origin on the lateral surface of the pterygoid process. From here, the internal pterygoid runs ventro-laterally and fans out to make a wide insertion on the medial surface of the angular process, dorsal to the reflected insertion of the superficial masseter.

External pterygoid

This muscle, like the internal pterygoid, varies little in its morphology between the three rodents. However, it is a much smaller muscle compared to its internal counterpart, forming just 3-4% of the jaw-closing musculature (see Table 1 and Fig. 7). It originates along the ventral margin of the skull in the orbito-temporal region on the alisphenoid bone and lateral pterygoid process. It runs postero-dorsally to insert on the medial condyloid process, just below the condyle.

DISCUSSION

The morphology of the rodent masticatory musculature has been the source of considerable controversy in the scientific literature for a number of years. Much of this controversy may be attributed to the difficulty of dissection owing to the small size of the specimens and the need to remove outer muscle layers, and in some cases bone, to reveal the deeper layers. The contrast-enhanced micro CT technique used in the present study has enabled visualisation of the masticatory muscles without the serial destruction of the skull necessary in gross dissection. These images have revealed a sufficient level of detail to allow accurate three-dimensional reconstructions of both the bone and muscle to be created.

In all three specimens, the masseter has been shown to divide into three distinct layers: the superficial masseter, the deep masseter and the zygomatico-mandibularis. The superficial masseter runs from a small origin on the maxilla ventral to the infraorbital foramen and inserts on the margin and the medial surface of the angular process of the mandible. Some workers have divided this muscle into two parts based on the two insertion areas on the lateral and medial surfaces of the mandible (Greene, 1935; Yoshikawa & Suzuki, 1969; Woods, 1972; Woods & Howland, 1979; Woods & Hermanson, 1985). However, this study, along with many others (e.g. Wood, 1965; Weijs, 1973; Ball & Roth, 1995; Thorington & Darrow, 1996; Druzinsky, 2010), retains it as a single muscle mass as there is no clear separation seen in the contrast-enhanced microCT images.

The deep masseter originates on the lateral surface of the zygomatic arch and inserts on the ventral part of the lateral surface of the mandible. In the guinea pig, it is a single entity, restricted to the zygomatic arch, but in the squirrel and rat it has expanded forward on to the rostrum and is divided into anterior and posterior parts. The division into anterior and posterior parts seen in this study is also made by certain other researchers, generally working on sciurormorphs and myomorphs (Yoshikawa & Suzuki, 1969; Weijs, 1973; Ball & Roth, 1995; Thorington & Darrow, 1996; Druzinsky, 2010). This is less common in studies of the hystricomorphs where there is no expansion of the muscle on to the rostrum. Woods (1972) splits his *masseter lateralis profundus* into anterior and posterior parts, but this study does not support such a split. Indeed, the *pars posterior* appears to be an amalgamation of the superficial masseter and the deep zygomatico-mandibularis.

The zygomatico-mandibularis runs from the medial surface of the zygomatic arch to the coronoid process and lateral crest of the mandible. It is split into anterior and posterior parts separated by the masseteric nerve in all three species. In the rat and guinea pig, a third division of the zygomatico-mandibularis exists (the infraorbital part) which extends through the lower part of the orbit and the enlarged infraorbital foramen to attach to the rostrum. A number of authors (Woods, 1972; Weijs, 1973; Woods & Howland, 1979;

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3 Ball & Roth, 1995; Satoh & Iwaku, 2004, 2006, 2009) have divided the zygomatico-
4 mandibularis into anterior and posterior parts, separated by the masseteric nerve, as has
5 been done in this study. In a few studies on hystricomorph rodents, a third part of this
6 muscle is distinguished running almost horizontally from the lateral jugal fossa of the
7 zygomatic arch to the post-condyloid process on the mandible. Woods (1972) and Wood
8 (1974) refer to it as the '*masseter lateralis profundus, pars posterior*, deep division';
9 subsequent studies (Woods & Howlands, 1979; Woods & Hermanson, 1985; Offermans
10 & De Vree, 1989; Olivares et al., 2004) use the less cumbersome 'posterior masseter'.
11 Druzinsky (2010) describes the posterior masseter in *Aplodontia rufa* and a sciurormorph,
12 but notes that, owing to its more vertical course in these rodents, it is very difficult to
13 separate it from the posterior zygomatico-mandibularis. No evidence of a separate
14 posterior masseter was found in any of the contrast-enhanced microCT images used in
15 this study, and it is suspected that the 'posterior masseter' reported by the above authors
16 is simply part or all of the posterior zygomatico-mandibularis.
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30 The temporalis muscle runs from a wide origin on the lateral surface of the cranium to a
31 relatively small insertion on the medial surface of the coronoid process of the mandible.
32 In the squirrel and rat, it is divided into a large medial temporalis and a smaller lateral
33 temporalis which overlays the anterior half of the medial layer. This division could not be
34 seen in the guinea pig images. The division of the temporalis into two parts has been
35 made by a number of authors (Weijs, 1973; Ball & Roth, 1995; Thorington & Darrow,
36 1996; Druzinsky 2010), including some working on hystricomorphs (Woods, 1972;
37 Woods & Howlands, 1979). A number of authors (Woods, 1972; Weijs, 1973) separate
38 the ventralmost fibres of the temporalis to create a third division, the posterior temporalis
39 (called the suprazygomatic by Druzinsky, 2010). This usually consists of those fibres
40 originating from the caudal region of the squamosal (Weijs, 1973), or in some cases, just
41 those fibres taking origin from the zygomatic process of the squamosal (Woods, 1972;
42 Druzinsky, 2010). This division was not clearly separable in any of the three species
43 under study here.
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The morphology of the pterygoid musculature showed little variation between the three rodents. The internal pterygoid runs from the pterygoid fossa to insert on the medial surface of the angular process of the mandible. The external pterygoid originates on the ventral surface of the alisphenoid and the lateral pterygoid process and inserts on the medial condyloid process of the mandible. This morphology has also been noted by other researchers in sciurormorphs (Ball & Roth, 1995; Thorington & Darrow, 1996), hystricomorphs (Woods, 1972; Offermans & De Vree , 1989) and myomorphs (Hiimae & Houston, 1971; Weijs, 1973).

From the descriptions and the muscle volumes in Table 1, the similarities and differences between the three rodents can be highlighted. The superficial masseter is a much larger component in the guinea pig, forming approximately 45% of the masticatory musculature, compared to about 30% in the squirrel and rat. This is partly due to the morphology of the deep masseter in the hystricomorph arrangement. In sciurormorphs and myomorphs, the extension of the deep masseter on to the rostrum appears to restrict the superficial masseter dorsally. In hystricomorphs, where no such extension exists, the superficial masseter is free to enlarge to the height of the zygomatic arch so that it almost completely covers the more medial layers. Conversely, the deep masseter is a much more important component of the musculature in the squirrel and rat (~35%) than it is in the guinea pig (>10%), largely owing to the lack of rostral expansion in the latter species. Overall, the zygomatico-mandibularis plays a much more prominent role in the guinea pig (15%) than it does in the squirrel and rat (6-9%). This is largely attributable to the large infraorbital part in hystricomorphs which, although present, is not so greatly developed in myomorphs and is completely absent in sciurormorphs. Regarding the temporalis, it is the squirrel and guinea pig that are the most similar, with this muscle being relatively small (10-12%). In contrast, the rat has a much larger temporalis, accounting for just over a quarter of the masticatory musculature. Lastly, it is the guinea pig that stands out once again when examining the pterygoid muscles. All three species have a fairly similar sized external pterygoid, but the internal pterygoid is substantially larger in the guinea pig, forming almost 20% of the masticatory musculature, compared to approximately 10% in the squirrel and rat.

Comparison with previous work on rodents shows a high degree of correspondence in the relative proportions of the masticatory muscles. The relative volumes of the muscles of the guinea pig calculated here (see Table 1), match closely those given by Schumacher & Rehmer (1962). Although the values for the temporalis differ by 10%, the values for all the other muscles correspond to within 5%. Similarly, the percentage values for the masseter, temporalis, internal pterygoid and external pterygoid of the squirrel are very similar to those measured by Ball & Roth (1995). Unfortunately, the correspondence in percentage mass of the component layers of the masseter cannot be checked as Ball & Roth (1995) do not give these values. There is also a close match of the muscular proportions of squirrel measured in this study with the values given by Turnbull (1970), except for a somewhat reduced superficial masseter and increased temporalis. However, this discrepancy may be attributable to the use of *Sciurus niger* rather than *Sciurus carolinensis* in the latter study. The percentage muscle volumes measured for the rat correspond very closely to the percentage masses given in both Schumacher & Rehmer (1962) and Hiiemae (1971), assuming that the 'deep masseter' of Hiiemae (1971) encompasses both the deep masseter and zygomatico-mandibularis as defined in this work. The one notable difference between these works and the current study is the infraorbital part of the zygomatico-mandibularis which Schumacher & Rehmer (1962) measure to be 14% of the masticatory musculature, compared to 3.6% in this study. The former figure seems incredibly unlikely, especially as the value stated for the same muscle in the guinea pig in the same paper is only 6%. It is probable that Schumacher and Rehmer (1962) have misattributed part of the deep masseter to the infraorbital part of the zygomatico-mandibularis, and so their value is erroneously high.

The functional consequences of the variation in rodent masticatory muscle morphology have been widely debated in the literature. Thorington & Darrow (1996) suggest that the expansion of the anterior deep masseter forward on to the maxilla and premaxilla in sciurormorphs results in an increase in strength of the incisor bite. Woods (1972), building on Maynard Smith & Savage (1959), proposes that the function of the zygomatico-mandibularis is to stabilise the mandible during chewing, and that the infraorbital

expansion of this muscle in hystricomorphs serves to increase this stability. Wood (1965) postulates that the myomorph arrangement, with its combination of sciuromorph and hystricomorph characters, gives the greatest antero-posterior component of any of the rodent musculature arrangements, and may be a contributing factor to the overwhelming success of the Muroidea. These ideas and others will be tested in further work, using the results of this study, which will examine the biomechanical consequences of the sciuromorph, hystricomorph and myomorph musculature arrangements.

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REFERENCES

Adkins RM, Gelke EL, Rowe D, Honeycutt RL. 2001. Molecular phylogeny and divergence time estimates for major rodent groups: evidence from multiple genes. *Mol Biol Evol* 18: 777-791.

Adkins RM, Walton AH, Honeycutt RL. 2003. Higher-level systematics of rodents and divergence time estimates based on two congruent nuclear genes. *Mol Phylogenet Evol* 26: 409-420.

Ball SS, Roth VL. 1995. Jaw muscles of new-world squirrels. *J Morphol* 224: 265-291.

Becht G. 1953. Comparative biologic-anatomical researches on mastication in some mammals. *Proc Kon Ned Akad Wet, Ser C* 56: 508-527.

Blanga-Kanfi S, Miranda H, Penn O, Pupko T, Debry RW, Huchon D. 2009. Rodent phylogeny revised: analysis of six nuclear genes from all major rodent clades. *BMC Evol Biol* 9: 71.

Brandt JF. 1855. Untersuchungen über die craniologischen Entwicklungsstufen und Classification der Nager der Jetzwelt. *Mém Acad Imp Sci St Pétersbourg, Sér 6* 9: 1-365.

Bryant MD. 1945. Phylogeny of Nearctic Sciuridae. *Am Midl Nat* 33: 257-390.

Coldiron RW. 1977. On the jaw musculature and relationships of *Petrodomus tetradactylus* (Mammalia, Macroscelidea). *Am Mus Novit* 2613: 1-12.

Cooper GC, Schiller AL. 1975. Anatomy of the Guinea Pig. Cambridge, Massachusetts: Harvard University Press. pp.417.

- Druzinsky RE. 2010. Functional anatomy of incisal biting in *Aplodontia rufa* and sciurormorph rodents - Part 1: Masticatory muscles, skull shape and digging. *Cells Tissues Organs* 191: 510-522.
- Greene EC. 1935. Anatomy of the rat. *Trans Am Phil Soc* 27: 1-370.
- Hiiemae K. 1971. The structure and function of jaw muscles in rat (*Rattus norvegicus* L.). III. The mechanics of the muscles. *Zool J Linn Soc* 50: 111-132.
- Hiiemae K, Ardran, GM. 1968. A cinefluorographic study of mandibular movement during feeding in the rat (*Rattus norvegicus*). *J Zool* 154: 139-154.
- Hiiemae K, Houston WJB. 1971. The structure and function of jaw muscles in rat (*Rattus norvegicus* L.). I. Their anatomy and internal architecture. *Zool J Linn Soc* 50: 75-99.
- Hill JE. 1937. Morphology of the pocket gopher mammalian genus *Thomomys*. *Univ Calif Publ Zool* 42: 81-171.
- Huchon D, Madsen O, Sibbald MJJB, Ament K, Stanhope MJ, Catzeflis F, de Jong WW, Douzery EJP. 2002. Rodent phylogeny and a timescale for the evolution of glires: Evidence from an extensive taxon sampling using three nuclear genes. *Mol Biol Evol* 19: 1053-1065.
- Janis CM. 1983. Muscles of the masticatory apparatus in two genera of hyraxes (*Procavia* and *Heterohyrax*). *J Morphol* 176: 61-87.
- Jeffery NJ, Stephenson R, Gallagher JA, Jarvis JC, Cox PG. 2010. Micro-computed tomography with iodine staining resolves the arrangement of muscle fibres. *J Biomech. In submission.*
- Lavocat R. 1974. What is an hystricomorph? In: Rowlands IW, Weir BW, editors. *The Biology of Hystricomorph Rodents. Symp Zool Soc Lond* 34: 7-20.
- Maynard Smith J, Savage RJG. 1956. The mechanics of mammalian jaws. *Sch Sci Rev* 141: 289-301.
- Miller GS, Gidley JW. 1918. Synopsis of the supergeneric groups of rodents. *J Washington Acad Sci* 8: 431-448.
- Offermans M, De Vree F. 1993. Electromyography and mechanics of mastication in the springhare, *Pedetes capensis* (Rodentia, Pedetidae). *Belg J Zool* 123: 231-261.
- Olivares AI, Verzi DH, Vassallo. 2004. Masticatory morphological diversity and chewing modes in South American caviomorph rodents (family Octodontidae). *J Zool* 263: 167-177.
- Satoh K. 1997. Comparative functional morphology of mandibular forward movement during mastication of two murid rodents, *Apodemus speciosus* (Murinae) and *Clethrionomys rufocanus* (Arvicolinae). *J Morphol* 231: 131-142.
- Satoh K. 1998. Balancing function of the masticatory muscles during incisal biting in two murid rodents, *Apodemus speciosus* and *Clethrionomys rufocanus*. *J Morphol* 236: 49-56.
- Satoh K. 1999. Mechanical advantage of area of origin for the external pterygoid in two murid rodents, *Apodemus speciosus* and *Clethrionomys rufocanus*. *J Morphol* 240: 1-14.
- Satoh K, Iwaku F. 2004. Internal architecture, origin-insertion, and mass of jaw muscles in Old World hamsters. *J Morphol* 260: 101-116.
- Satoh K, Iwaku F. 2006. Jaw muscle functional anatomy in Northern grasshopper mouse, *Onychomys leucogaster*, a carnivorous murid. *J Morphol* 267: 987-999.

- Satoh K, Iwaku F. 2009. Structure and direction of jaw adductor muscles as herbivorous adaptations in *Neotoma mexicana* (Muridae, Rodentia). *Zoomorphol* 128: 339-348.
- Schumacher GH, Rehmer H. 1962. Über einige Unterschiede am Kauapparat bei Lagomorphen und Rodentia. *Anat Anz* 111: 103-122.
- Simpson GG. 1945. The principles of classification and a classification of mammals. *Bull Am Mus Nat Hist* 85: 1-350.
- Storch G. 1968. Funktionsmorphologische Untersuchungen an der Kaumuskulatur und an korrelierten Schädelstrukturen der Chiropteren. *Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft*. 517: 1-92.
- Thomas O. 1896. On the genera of rodents: an attempt to bring up to date the current arrangement of the order. *Proc Zool Soc, London* 1896: 1012-1028.
- Thorington RW, Darrow K. 1996. Jaw muscles of old world squirrels. *J Morphol* 230: 145-165.
- Tullberg T. 1899. Über das System der Nagethiere, eine phylogenetische Studie. *Nova Acta Reg Soc Sci Upsala, Ser 3* 18: 1-514.
- Turnbull WD. 1970. Mammalian masticatory apparatus. *Fieldiana (Geol)* 18: 147-356.
- Weijs WA. 1973. Morphology of muscles of mastication in the Albino Rat, *Rattus norvegicus* (Berkenhout, 1769). *Acta Morphol Neerl-Scand* 11: 321-340.
- Wood AE. 1965. Grades and clades among rodents. *Evol* 19: 115-130.
- Wood AE. 1974. The evolution of Old World and New World hystricomorphs. In: Rowlands IW, Weir BW, editors. *The Biology of Hystricomorph Rodents*. Symp Zool Soc Lond 34: 21-60.
- Woods CA. 1972. Comparative myology of jaw, hyoid, and pectoral appendicular regions of New and Old World hystricomorph rodents. *Bull Am Mus Nat Hist* 147: 115-198.
- Woods CA, Hermanson JW. 1985. Myology of hystricognath rodents: an analysis of form, function and phylogeny. In: Luckett EWO, Hartenberger J-L, editors. *Evolutionary Relationships among Rodents: A Multidisciplinary Analysis*. New York: Plenum Press. pp 515-548.
- Woods CA, Howland EB. 1979. Adaptive radiation of capromyid rodents: anatomy of the masticatory apparatus. *J Mammal* 60: 95-116.
- Yoshikawa T, Suzuki T. 1969. The comparative anatomical study of the masseter of the mammal (III). *Anat Anz* 125: 363-387.

Morphology of the jaw-closing musculature in sciurormorph, hystricomorph and myomorph rodents

Philip G. Cox & Nathan Jeffery

TABLES

Table 1. Volumes (mm³) and percentages of jaw-closing muscles.

	Squirrel		Guinea pig		Rat	
Muscle	Volume	%	Volume	%	Volume	%
Superficial masseter	116.50	27.37	207.13	45.35	60.85	20.42
Deep masseter	153.41	36.04	34.60	7.57	102.32	34.34
Anterior	93.50	21.96			44.43	14.91
Posterior	59.91	14.07			57.90	19.43
Zygomatico-mandibularis	37.04	8.70	67.36	14.75	20.15	6.76
Anterior	28.82	6.77	27.16	5.95	4.73	1.59
Posterior	8.22	1.93	6.04	1.32	2.80	0.94
Infraorbital			34.17	7.48	12.62	4.24
Temporalis	51.21	12.03	48.62	10.65	80.05	26.86
Lateral	5.98	1.40			23.02	7.73
Medial	45.23	10.63			57.02	19.14
Pterygoid	67.54	15.87	99.03	21.68	34.62	11.62
External	18.33	4.30	13.70	3.00	9.93	3.33
Internal	49.22	11.56	85.33	18.68	24.69	8.29
Total	425.70	100.00	456.75	100.00	297.99	100.00

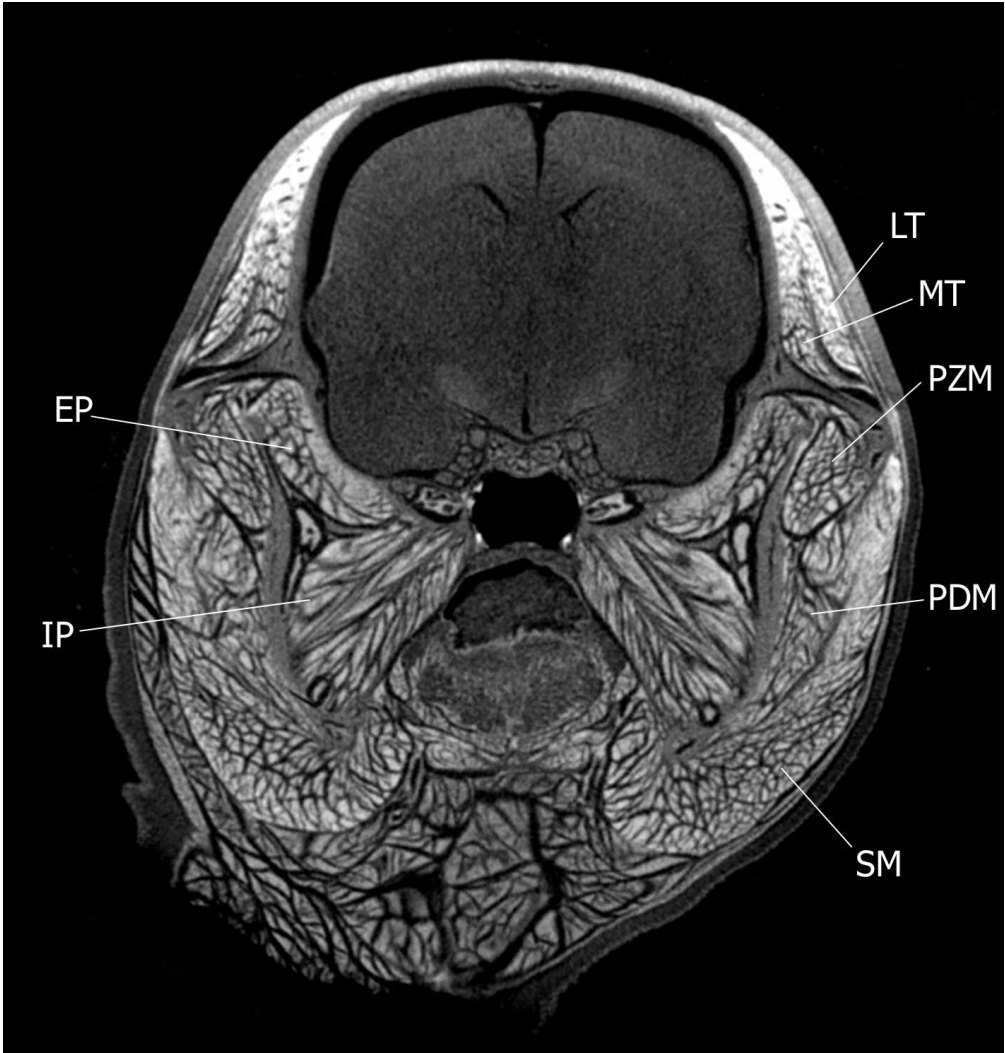


Fig. 1. Enhanced-contrast microCT image of a squirrel in coronal view. EP, external pterygoid; IP, internal pterygoid; LT, lateral temporalis; MT, medial temporalis; PDM, posterior deep masseter; PZM, posterior zygomatico-mandibularis; SM, superficial masseter.
59x62mm (600 x 600 DPI)

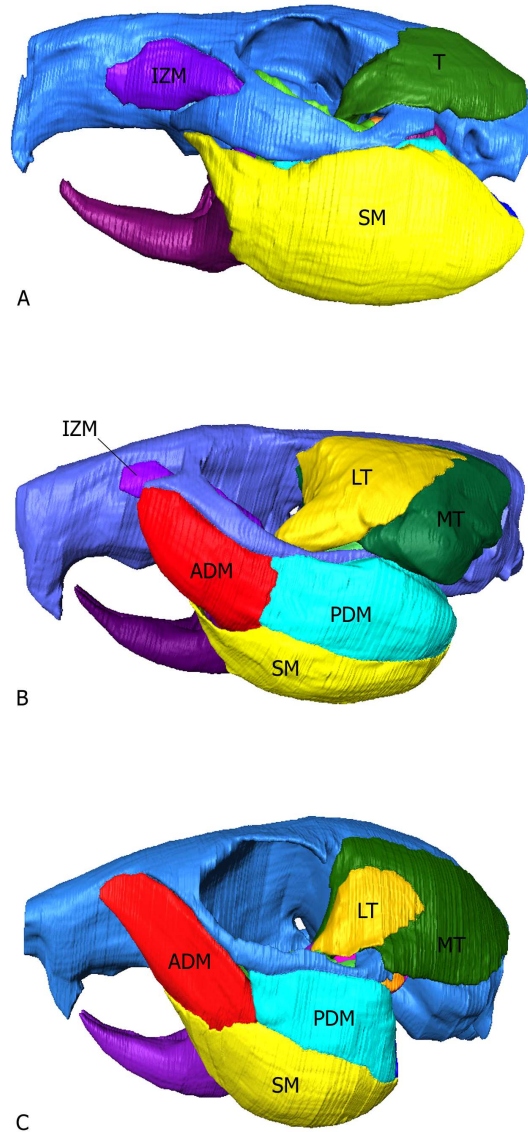


Fig. 2. Left lateral view of 3D reconstructions of the skull, mandible and masticatory muscles of: a) guinea pig; b) rat; c) squirrel. ADM, anterior deep masseter; IZM, infraorbital part of the zygomatico-mandibularis; LT, lateral temporalis; MT, medial temporalis; PDM, posterior deep masseter; SM, superficial masseter; T, temporalis.
59x120mm (600 x 600 DPI)

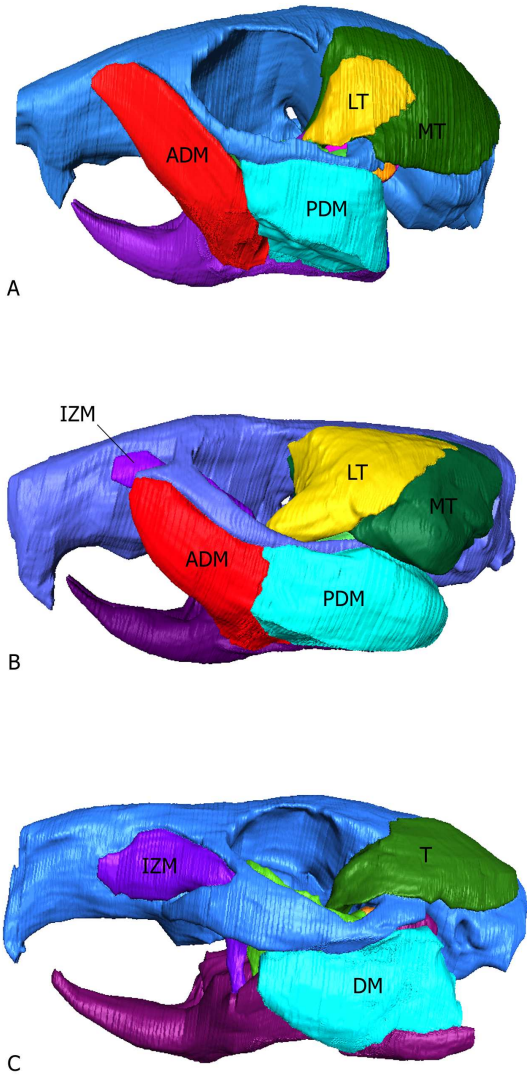


Fig. 3. Left lateral view of 3D reconstructions of the skull, mandible and masticatory muscles of: a) squirrel; b) rat; c) guinea pig. ADM, anterior deep masseter; DM, deep masseter; IZM, infraorbital part of the zygomatico-mandibularis; LT, lateral temporalis; MT, medial temporalis; PDM, posterior deep masseter; T, temporalis. 59x120mm (600 x 600 DPI)

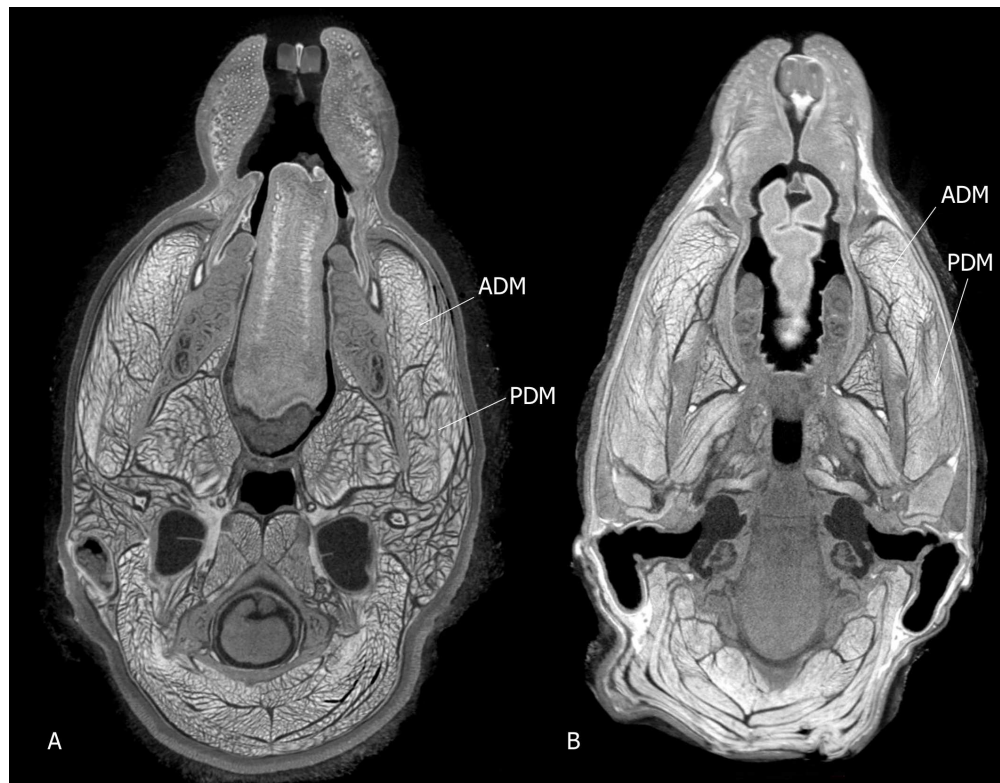


Fig. 4. Axial microCT images of a) squirrel and b) rat showing division between anterior deep masseter (ADM) and posterior deep masseter (PDM). 90x70mm (600 x 600 DPI)

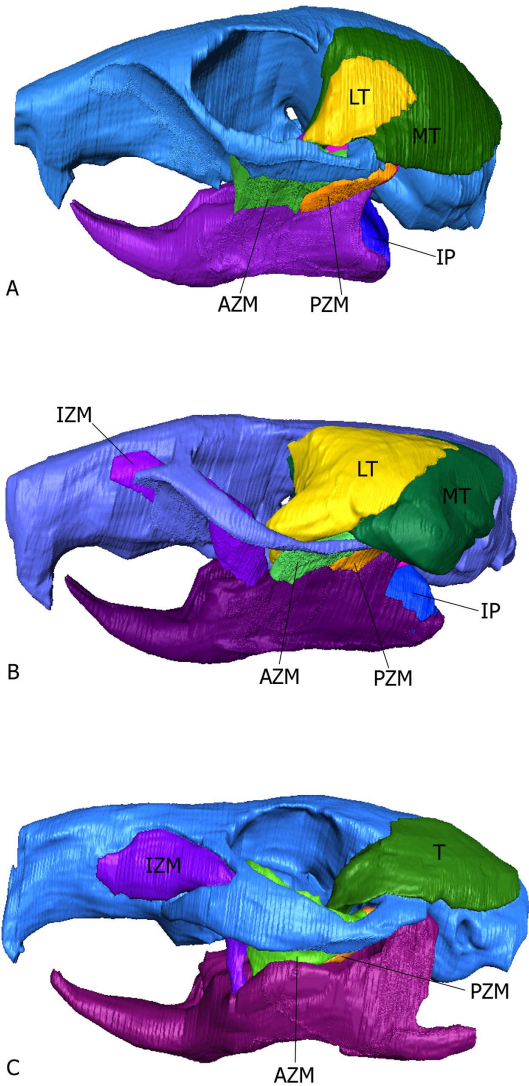


Fig. 5. Left lateral view of 3D reconstructions of the skull, mandible and masticatory muscles of: a) squirrel; b) rat; c) guinea pig. AZM, anterior zygomatico-mandibularis; IP, internal pterygoid; IZM, infraorbital part of the zygomatico-mandibularis; LT, lateral temporalis; MT, medial temporalis; PZM, posterior zygomatico-mandibularis.
60x120mm (600 x 600 DPI)



Fig. 6. Coronal microCT image of squirrel showing division of zygomatico-mandibularis into anterior and posterior parts (AZM and PZM) by masseteric nerve (MN).
59x62mm (600 x 600 DPI)

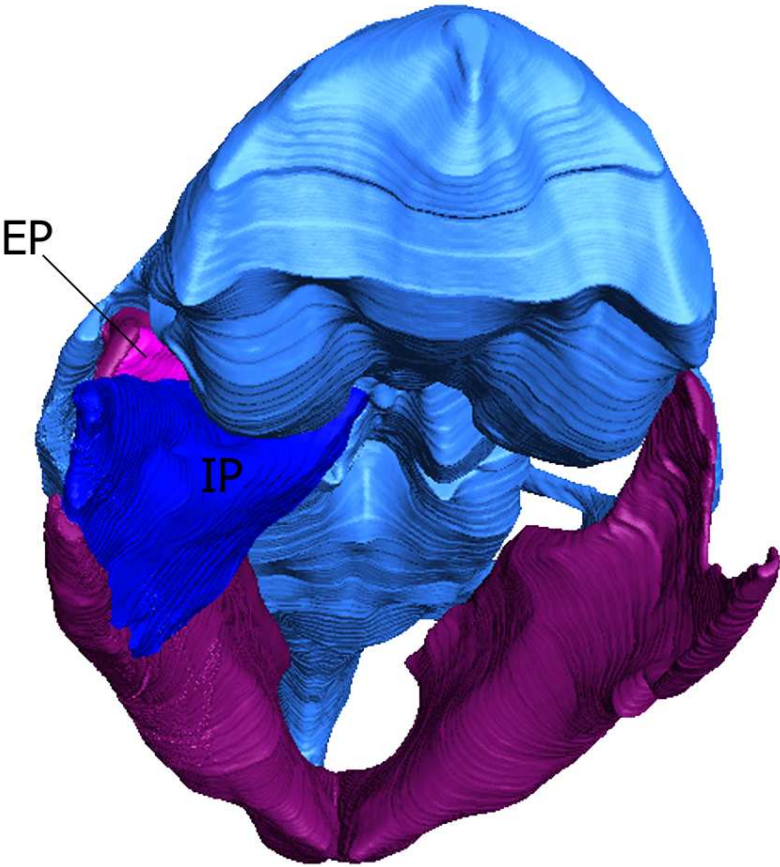


Fig. 7. Postero-ventral view of 3D reconstruction of guinea pig showing pterygoid muscles. EP, external pterygoid; IP internal pterygoid. 45x50mm (600 x 600 DPI)