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Comparative myology and evolution of marsupials and other vertebrates, with notes on complexity, Bauplan, and ‘scala naturae’

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Abstract

Opossums are frequent subjects of developmental studies because marsupials share developmental features not seen in placentals and because Didelphimorpha is the sister-group of other extant Marsupialia. But is the adult marsupial muscular system markedly different from that of placentals or is it, like the skeletal system, very similar? We provide, for the first time, a brief description of all head and limb muscles of *Didelphis virginiana* based on our dissections and using a unifying nomenclature by integrating the data gathered in our long-term project on the development, homologies and evolution of the muscles of all major vertebrate taxa. Our data indicate that there were many more muscle synapomorphic changes from the last common ancestor (LCA) of amniotes to the mammalian LCA (63) and from this LCA to the LCA of extant therians (48) than from this latter LCA to the LCA of extant placentals (10 or 11). Importantly, *Didelphis* is anatomically more plesiomorphic (only 14 changes from LCA of extant therians) than are rats (37 changes) and humans (63 changes), but its musculature is more complex (193 muscles) than that of humans (only 180 muscles). Of the 194 muscles of *Didelphis*, 172 (89%) are present in rats, meaning that their adult muscle anatomy is indeed very similar. This similarity supports the existence of a common, easy recognizable therian Bauplan, but one that is caused by developmental constraints and by evolutionary change driven by the needs of the embryos/neonates, rather than by a 'goal' towards a specific adult plan/'archetype,' as the name Bauplan suggests.

Keywords: muscles, homology, evolution, comparative anatomy, marsupials, placentals, monotremes, mammals, complexity, scala naturae, Bauplan

Introduction

Marsupial opossums (order Didelphimorpha) are frequently the focus of evolutionary developmental studies because this order is the sister-group of a clade including all other extant marsupials and is therefore a good model to study the origin and early evolution of marsupials as a whole (e.g. Horovitz & Sánchez-Villagra 2003). Moreover, opossums are a very useful model to investigate the diversity of mammalian development because marsupials share very peculiar developmental features that are not seen in placentals, many of which are related to remarkable heterochronic and heterotopic changes in marsupials (Smith, 1994, 2001, 2006; Sánchez-Villagra et al., 2002; Vaglia & Smith, 2003; Keyte & Smith, 2010; Sears, 2004; Kelly & Sears, 2011; Moustakas et al., 2011; Goswami et al., 2012; Hübler et al., 2013; Wakamatsu et al., 2014; Chew et al., 2014). Interestingly, despite these marked developmental differences, the skeletal structures of adult marsupials such as opossums and placentals such as mice are, in general, quite similar (e.g. Smith 2006; Goswami et al., 2012).

With the exception of very few studies (e.g., Cheng 1955, Smith 1994, Keyte & Smith 2010), works on the developmental biology of opossums do not refer to muscles. This lack of muscular developmental studies limits our understanding of mammalian evolution because the scarce data available indicates that muscle development might also differ considerably between marsupials and placentals (e.g. Keyte & Smith, 2010). For instance, in most tetrapods the skeletal and muscular systems develop over the same period, but in marsupials, because so many skeletal elements are delayed in development, some muscles appear far in advance of the skeletal elements that will form their attachment points (Smith, 2006). However, we do not know whether the adult muscular phenotype of marsupials such as opossums is very different from that found in most placentals or is instead fairly similar (as is the skeletal system). The latter case would be a further example of different developmental patterns leading to a similar adult configuration.

In fact, a major weakness of most of the studies on the adult muscles of the Virginia opossum (*D. virginiana*) and other marsupials is that they do not provide comparisons with placentals or other tetrapods. For instance, numerous authors have studied and discussed the hand muscles of marsupials, including *D. virginiana* (e.g. Brandell, 1935; Campbell, 1939; McMurrich, 1903ab; Lewis, 1989; Jouffroy, 1971; Stein, 1981; Coues, 1872; Brooks, 1886a; Young, 1880). However, their lack of comparative context and common nomenclature rendered them confusing or simply “useless”, as stated by Lewis (1989). Moreover, most studies on the muscles of adult opossums mainly refer to a specific region of the head or limbs (e.g. Young, 1880; Huber 1930a, 1930b, 1931; Langworthy, 1932; Haines, 1939; Lightoller, 1940a, 1940b, 1942; Straus, 1941a, 1941b, 1942; Shrivastava, 1962; Brandell, 1965; Hiiemae & Jenkins, 1969; Minkoff et al.,’s, 1979; Grant et al., 2013). Even those studies that focus on both the head and limbs (e.g. Coues 1870, 1872) omit some muscles. For instance, Coues’ (1872) description of the facial and pharyngeal muscles is very incomplete.

In some cases such omissions were deliberate, but in most cases they were due to a strong historical bias regarding marsupials as a perfect intermediate taxon within the ‘scala naturae’ leading to placentals,

and then to humans. The notion of a *scala naturae*, which dates back to thinkers such as Aristotle, represents an evolutionary trend in complexity from “lower” to “higher” taxa, with *Homo sapiens* as the end stage (discussed in Diogo et al., 2015a). For example, many authors described only a few undifferentiated facial muscles in marsupials, more numerous muscles in placentals such as rats, and ‘most complex’ facial musculature in humans (e.g. Huber 1930a, 1930b, 1931; Lightoller, 1940a, 1940b, 1942). Such notions of ‘*scala naturae*’, i.e. of progress towards greater complexity leading to humans, are found not only in works from the 19th and early 20th centuries, but even those from the late 20th century. For instance, Minkoff et al.’s (1979) study of the facial muscles of *D. virginiana* describes 21 muscles of facial expression in this species, including extrinsic ear muscles, i.e., only about 2/3 of the 31 facial muscles found in humans (25 + 6 extrinsic ear muscles: see Table 2). In contrast, we found exactly the same number of facial muscles in *D. virginiana* as in placentals such as rats, which is very similar to the number found in humans, as we will explain below (Table 2).

In our paper (Diogo et al., 2015a) we also discussed related notions, such as that of *Bauplan*, or “body plan”, which was originally related to a pre-evolutionary notion of “archetype” in the sense that it refers to a “plan”. We also argued that the (justified) refutation of old notions such as *scala naturae* does not mean that we should abandon terms such as “phylogenetically basal” and particularly “anatomically plesiomorphic” to refer to groups such as the urodeles within the Tetrapoda, or lemurs within the Primates. Here we investigate whether the term “anatomically plesiomorphic” might also apply to marsupials such as the opossum, within the Mammalia, by providing, for the first time, a rigorous comparative framework between the adult myology of marsupials, placentals, monotremes, and other tetrapods, for both the head and limbs. Specifically, we provide a list and brief description of all the *D. virginiana* head and limb muscles using an updated, unifying vertebrate myological nomenclature to allow more straightforward comparison between marsupials and other taxa. We combine the new anatomical data obtained from our dissections of *D. virginiana* with the myological information obtained in our previous works, and a detailed literature review of works done by others on the myology of marsupials and other mammals. The present work is therefore the culmination of this 20-year project because we include marsupials and also Gambaryan et al.’s (2002) detailed data on monotreme hindlimb musculature in the evolutionary/homology tables (Tables 1-10) elaborated in our previous works (e.g. Diogo, 2007, 2008, 2009; Diogo & Abdala, 2007, 2010; Diogo et al., 2008, 2009a, 2009b, 2013a, 2013b, 2014, 2015a, 2015b, 2015c, 2015d; Diogo & Wood, 2012; Diogo & Molnar, 2014; Diogo & Tanaka, 2014; Diogo & Ziermann, 2014).

Materials and Methods

Five adult specimens of *D. virginiana* (three males and two females) were dissected by RD (RuiDiogoLab specimen #HUDV1-5, preserved in alcohol) for the present work, and compared with one male specimen of *D. albiventris* dissected by GBH (Department of Biology, FFCLRP specimen #XDB1, frozen) and with descriptions of marsupials and other vertebrates from the literature (see Introduction). We found no major

differences between the *D. virginiana* specimens we dissected. Differences between our observations and those of other authors are described in the Results/Discussion below. The homology/evolutionary hypotheses (Tables 1-10) integrate data from our dissections, our previous studies using techniques such as histological sectioning and fluorescent labeling, and the literature. A detailed description of the methodology used to compile these tables, including the hypotheses about the presence/absence of muscles in the last common ancestor (LCA) of specific clades, was provided in Diogo et al., (2015a, 2015d). Importantly, in the present paper when we refer to the LCA of a certain clade (e.g. placentals), we always refer to the LCA of the *extant* members of that clade. Therefore, we use the information available on the extant members of the other clades to make assumptions about the presence/absence of muscles. For instance, if a muscle is present in all extant monotremes and all extant marsupials and missing in all extant placentals, we make the more parsimonious assumption that it was secondarily lost in placentals (1 evolutionary step) rather than independently acquired in monotremes and marsupials (2 evolutionary steps).

As we use the updated, unifying muscle nomenclature for vertebrates developed in our previous works the names commonly used by other authors in the past are given in the Results section, to facilitate comparisons. A list of the other non-primate vertebrate specimens studied by us in our long-term project is provided in Diogo & Abdala (2010), Diogo et al., (2010, 2013a, 2013b, 2014), Diogo & Molnar (2014), Diogo & Ziermann (2014), and Diogo & Tanaka (2014); a list of the studied primates is provided in Diogo & Wood (2012).

Results

The order followed in the descriptions below basically follows Tables 1-10, in which muscles are grouped based on their developmental origins (for more details see Diogo & Abdala, 2010, and Discussion below).

Mandibular muscles (1st branchial arch) (Table 1; Fig. 1)

All muscles in this paragraph insert on the mandible unless otherwise noted. The **mylohyoideus** connects the hemimandibles and is fused to its counterpart at the midline. It has no central raphe or direct attachment onto the hyoid apparatus, but only an indirect attachment via fascia, as described by Coues (1872). The **digastricus anterior** originates on and is continuous with the digastricus posterior and has no well-defined intermediate tendon. The digastricus anterior muscles also attach indirectly through the ‘tendinous arcade’ to the hyoid apparatus. The digastricus anterior is not blended with its counterpart at the midline, although according to Coues (1872) the two muscles might contact each other. The **masseter** (masseter + external adductor of Hiiemae & Jenkins 1969) originates from the squamosal, presphenoid, maxillary, jugal, frontal, parietal, and occipital bones and is blended with the temporalis and the pterygoideus medialis. It is subdivided into an inferior/anterior bundle, a superficial bundle, a deep bundle, and a zygomatico-mandibular bundle. The latter bundle is sometimes considered to be an independent muscle, but it is in fact mixed with the other masseter bundles and partially with the temporalis as well. The **temporalis** (posterior adductor + internal adductor of Hiiemae & Jenkins 1969) originates from the squamosal, presphenoid, parietal, frontal and

occipital. It is subdivided into superficial/anterior and deep/posterior bundles that mainly insert on the lateral and medial surfaces of the coronoid process, respectively, and a pars suprazygomatica that is mainly fused with the deep/posterior bundle. The **pterygoideus lateralis** originates from the presphenoid. It is a very thin muscle, but still slightly differentiated into an inferior head inserting onto the condylar process and a superior head inserting onto the capsule of the squamodentary joint. The much thicker **pterygoideus medialis** originates from the palatine and presphenoid bones. Lastly, the **tensor tympani** and **tensor veli palatini** originate from the temporal and sphenoid bones, respectively. The tensor tympani inserts onto the malleolus, and the tensor veli palatini inserts onto the soft palate.

Hyoid muscles (2nd branchial arch) (Table 2; Fig. 1)

Reiss (2001) coded the **stylohyoideus** as absent in *Didelphis*, but it is actually present, as described by e.g. Coues (1872) and Minkoff et al. (1979). The stylohyoideus and **digastricus posterior** originate from the paroccipital process of the occipital bone. The former inserts on the basihyal, passing deep to - but not being blended with - the latter. The **stapedius** runs from the squamosal and/or surrounding regions to the stapes. The remaining hyoid muscles are muscles of facial expression, and all except the mandibulo-auricularis and extrinsic ear muscles are blended with their counterparts on the opposite side of the body. The **platysma cervicale** and **platysma myoides** (both part of platysma of Minkoff et al., 1979) are fused with each other and with the orbicularis oris. The former muscle originates from a raphe anchored to the ligamentum nuchae and the latter from skin extending as far posteriorly as the acromion of the scapula, and both insert onto the corner of the mouth and the lower lip. The **occipitalis** connects the occipital bone and ligamentum nuchae to the frontalis muscle anteriorly and to the ear and scutiform cartilage anterolaterally and is blended with the auricularis posterior. As is the case in many placentals (e.g. rats), the occipitalis has a medial portion (occipitalis of Lightoller, 1934, and Minkoff et al., 1979) that extends anteriorly to blend with the frontalis and a lateral portion (cervico-auriculo-occipitalis of Lightoller, 1934; cervicoauricularis superficialis + cervicoscutularis of Minkoff et al., 1979) that runs anteroventrolaterally to attach onto the posterior surface of the ear and to the scutiform cartilage. These two portions are fused posteriorly, attaching to the dorsal region of the neck just anterior to the posterior attachment of the auricularis posterior (which mainly corresponds to the cervicoauricularis medius of Minkoff et al., 1979). The **auricularis posterior** runs anterolaterally from the ligamentum nuchae to the ear. It mainly corresponds to the cervicoauricularis medius (which includes the ‘transversus nuchae’, ‘interparietoscutularis’, ‘cervicoauricularis posterior profundus’ and ‘interparietoauricularis’) of Minkoff et al., (1979). Examples of **extrinsic (facial) muscles of the ear** present in marsupials are the obliquus auriculae, transversus auriculae, helicis, tragicus, depressor helicis and/or antitragicus. These muscles, with the exception of the depressor helicis (‘auricularis inferior’) seem to be blended with each other in *D. virginiana*, forming the ‘auricularis externus’ muscle complex of Minkoff et al., (1979). The **mandibulo-auricularis** connects the external ear to the mandible.

The **sphincter colli profundus** (sphincter colli preauricularis + part of sphincter colli superficialis of Minkoff et al., 1979) is mainly deep to the platysma myoides and platysma cervicale. It seems to have no bony attachments and is mingled with the zygomaticus minor of the same side of the body and the sphincter colli profundus of the opposite

side of the body. As shown in figure 7 of Huber (1930a) and figure 413 of Jouffroy & Saban (1971), the ‘sphincter colli superficialis’ of Minkoff et al., (1979) actually corresponds to part of the sphincter colli profundus (the part that extends to the neck, posteroventrally to the platysma myoides) and to part of the platysma (the part that lies more anteriorly, on the ventral region of the head). According to Minkoff et al., (1979), the latter part was fused with the anterior region of the platysma myoides. The **interscutularis** crosses the top of the head transversely between the ears to attach onto the scutiform cartilage of the opposite side of the head, so it is blended with the interscutularis of the opposite side of the body. Although they are blended with each other and with the platysma cervicale and orbicularis oris, the **zygomaticus major** and **minor** (corresponding to part of zygomaticus and/or sphincter colli profundus and to zygomaticus of Minkoff et al., 1979) are separate muscles, contra Minkoff et al., (1979) and Jouffroy & Saban (1971) (Figure 1). They resemble and correspond to the ‘auriculolabialis inferior’ and ‘auricularis superior’ in placentals, respectively, which are similarly blended. The zygomaticus major runs from the ear to the angle of the mouth, as it does in many placentals. The zygomaticus minor is for the most part continuous with the sphincter colli profundus and with the orbicularis oris posteriorly and superiorly. It runs anteriorly and inferiorly to attach onto the angle of the mouth. The **frontalis** (frontalis pars epicranialis of Minkoff et al., 1979) is mainly continuous with the occipitalis posteriorly and with the frontalis of the opposite side of the body through the epicranial aponeurosis. It runs anteriorly and passes between the eyes to fuse anteriorly with the levator labii alaeque nasi. The **auriculo-orbitalis** (frontalis pars palpebralis, or frontoscutularis, of Minkoff et al., 1979) runs from the anterior corner of the external ear, where its fibers originate from the scutiform cartilage, to the frontal bone along the anterior portion of the supraorbital rim deep to the orbicularis oculi. The **auricularis superior** (auricularis anterior superior, or adductor auris medius, or scutuloauricularis superficialis dorsalis, of Minkoff et al., 1979) lies in the region between the scutiform cartilage and the external ear.

The **orbicularis oculi** surrounds the eye, and its only bony attachment is to the maxilla. There does not seem to be a separate depressor supercilii, but some fibers of the medial portion of the orbicularis oculi run more vertically (supero-inferiorly) than the rest (Fig. 1) and thus may correspond to at least part of the depressor supercilii of other mammals. The **corrugator supercilii** (superciliaris of Minkoff et al., 1979) originates from the nasofrontal fascia, deep to the frontalis and well separated from the corrugator supercilii of the opposite side of the body. It runs mainly anteriorly and passes deep to the auriculo-orbitalis to reach the upper eyelid near its medial corner, blending with the orbicularis oculi. The **retractor anguli oculi lateralis** (retractor anguli oculi of Minkoff et al., 1979) runs from the temporal fascia, posteriorly, to the posterolateral portion of orbicularis oculi, anteriorly. The **levator labii superioris alaeque nasi** (nasolabialis or levator nasolabialis of Minkoff et al., 1979, and Grant et al., 2013) extends anteriorly to the snout from the region just between the eyes, attaching onto the maxilla, nasal bone and upper lip and lateral wings of the rhinarium. It is mixed with the orbicularis oris of the same side of the body and with the levator labii superioris alaeque nasi of the opposite side of the body. The **buccinatorius** runs from the maxilla to the mandible and upper lip and is fused with the levator anguli oris. The **dilatator nasi** (pars nasalis of maxillonasolabialis of Minkoff et al., 1979) runs from the maxilla to the skin of the nose, upper lip and alar nasal cartilage. It has a strong anterior tendon, as shown in figure 7E of Huber (1930a). The **levator labii superioris** (pars labialis of maxillonasolabialis of Minkoff et

al., 1979; part or totality of maxillolabialis of Grant et al., 2013) connects the maxilla to the upper lip and is blended posteriorly with the dilatator nasi. The **nasalis** (nasolabialis profundus of Grant et al., 2013) lies on the anterior snout region and is associated with vibrissae. The depressor septi nasi may be present as part of the ‘nasolabialis profundus’ of Grant et al., (2013), but neither they nor other authors describe it as a separate muscle, and we did not find it either. In addition to the extrinsic muscles described above, Grant et al., (2013) described various **intrinsic muscles of the snout/vibrissae** in marsupials such as *Monodelphis domestica*. Therefore, it is very likely that such muscles are also present in *D. virginiana* but are too small to be observed in gross dissections. The **levator anguli oris facialis** (pars anterior of maxillonasolabialis of Minkoff et al., 1979) runs from connective tissue of the upper lip and from the buccinatorius to the region between the upper lip and the anterior portion of the snout, passing deep to the levator labii superioris. It is less differentiated from the surrounding muscles than is the same muscle in placental mammals such as mice and rats. The **orbicularis oris** surrounds the mouth and is blended with the orbicularis oris of the opposite side of the body. Minkoff et al., (1979) states that the mentalis is not differentiated, or is poorly differentiated, in *D. virginiana*. We did not find a differentiated mentalis in our dissections, nor do other authors describe it in this species, but Jouffroy & Saban (1971) stated that the mentalis is differentiated in at least some other marsupials.

Branchial muscles (posterior branchial arches) (Table 3)

As noted by Jouffroy & Saban (1971), marsupials often have a broad **stylopharyngeus** running from the paroccipital process of the occipital to the pharynx and thyroid cartilage. The pharyngeal portion (pars pharyngea) is said to be a unique, derived feature of marsupials and to replace functionally the superior constrictor of placentals because it is a circular constrictor (Smith, 1994). The thyroid portion (pars thyroidea) thus corresponds to the entire stylopharyngeus of placentals because it is a longitudinal constrictor. The **ceratohyoideus** runs from the hypohyal to the ceratohyal. Although the trapezius and sternomastoideus complexes are often described as part of the forelimb musculature, they are in fact branchial muscles, derived from the posterior branchial arches (Diogo & Abdala, 2010; Diogo et al., 2015c). The **acromiotrapezius** (anterior trapezius of Stein, 1981) connects the vertebrae, ligamentum nuchae and occipitalis to the spine and acromion of scapula and to the lateral half of the clavicle. The **spinotrapezius** (posterior trapezius of Stein, 1981) runs from vertebrae to the spine of the scapula and is mixed distally with the acromiotrapezius and the deltoideus scapularis. The **cleido-occipitalis** extends from the occipital region to the clavicle. It is blended with the cleidomastoideus but clearly is a separate muscle. The **cleidomastoideus** and **sternomastoideus** connect the mastoid process of the squamosal to the clavicle and sternum, respectively.

The pharyngeal muscles **constrictor pharyngis medius** and **inferior** both originate on the pharyngeal raphe and are blended with each other and with their counterparts on the opposite side of the body. The former muscle inserts on the hyoid apparatus and the latter on the cricothyroid cartilage. The cricothyroideus is secondarily absent in adult marsupials (present in at least some marsupial embryos), probably as a result of the fusion between the cricoid and thyroid cartilages (see, e.g. Jouffroy & Saban, 1971). The **palatoglossus** runs from the soft palate to the tongue, where it blends with the hyoglossus. The **pterygopharyngeus** and **palatopharyngeus** are blended, both extending from the

pterygoid region of the presphenoid (the former muscle also from the tympanic region and the latter from the soft palate) to the pharyngeal musculature. The **musculus uvulae** (medialis veli palatini of Jouffroy & Saban, 1971) lies on the infero-medial region of the soft palate.

The laryngeal muscles **thyroarytenoideus** and **cricoarytenoideus lateralis** connect the unpaired cricothyroid cartilage to the arytenoid cartilage of the respective side. The latter muscle is fused with the unpaired arytenoideus muscle and with the thyroarytenoideus muscle of the same side of the body. The **arytenoideus** attaches to the left and right arytenoid cartilages and also to the unpaired interarytenoid cartilage. As noted by authors such as Symington (1898) and Jouffroy & Saban (1971), the portion going to the interarytenoid cartilage (often designated the procricoideus cartilage) is sometimes named ‘ary-procricoideus.’ Lastly, the **cricoarytenoideus posterior** originates from the unpaired cricothyroid cartilage. As described by Symington (1898), it is differentiated into a ‘cricoarytenoideus posticus internus’ bundle attaching onto the arytenoid cartilage of the same side of the body and the unpaired interarytenoid cartilage and a ‘cerato-crico-arytenoideus posticus’ bundle attaching onto the arytenoid cartilages. The medial margin of this muscle approaches but does not reach its counterpart.

Hypobranchial muscles (somitic tongue and neck musculature) (Table 4)

The **geniohyoideus** and **genioglossus** are blended, both originating from the mandible and inserting on the unpaired basihyal. The latter muscle also inserts on the tongue and is blended with the **hyoglossus**, which connects the hyoid apparatus to the tongue. Edgeworth (1935) and Jouffroy & Saban (1971) suggested that marsupials such as *Didelphis* have a ‘primitive hyoglossus’ that is not separated into the hyoglossus and styloglossus muscles found in most placental mammals. However, Smith (1994) refers to a **styloglossus** in *Monodelphis*, and this muscle was present in our *D. virginiana* specimens, running from the paraoccipital process of the occipital to the tongue but being mixed with the hyoglossus. Osgood (1921) also described a styloglossus in other marsupials. As stated by Saban (1968), the **intrinsic muscles of the tongue** of marsupials are similar to those of placentals in that they include the muscles longitudinalis superior, longitudinalis inferior, transversus linguae and/or verticalis linguae. The **sternohyoideus** and **sternothyroideus**, which do not have clear intersections, are blended with each other and with their counterparts on the opposite side of the body. They originate from the sternum and insert onto the hyoid apparatus and cricothyroid cartilage, respectively. The **omohyoideus** connects the scapula to the hyoid apparatus and has no defined intermediate tendon, only a short tendinous intersection. Lastly, the **thyrohyoideus** runs from the cricothyroid cartilage to the hyoid apparatus and is blended with the sternothyroideus.

Proximal forelimb muscles (pectoral girdle and arm) (Table 5; Fig. 3)

Six muscles connect the axial skeleton to the pectoral girdle. The **serratus anterior** (part of serratus magnus of Coues, 1872; part of serratus ventralis of Jenkins & Weijts, 1979; serratus magnus of Stein, 1981) and **levator scapulae** (part of serratus magnus of Coues, 1872; levator anguli scapulae of Stein, 1981) are fused to form a single, continuous structure that inserts on the scapula. The former originates from ribs 1-7, 1-8, or 1-9 and the latter from C3-C7 or C4-C7 vertebrae. The **levator claviculae** (levator scapulae ventralis of Jouffroy, 1971; atlanto-acromialis of

Stein, 1981, and Coues, 1872) and **atlantoscapularis posticus** (atlanto-scapularis of Stein, 1981, and Coues, 1872) connect the transverse process of the atlas to the lateral and medial third of the scapular spine, respectively. The **subclavius** runs from the first rib to the clavicle, acromion and cleidoacromial ligament and is blended with the cleidoacromialis.

Regarding the pectoral musculature and its derivatives, the **pectoralis major** (ectopectoralis of Lander, 1918; pectoralis superficialis of Langworthy, 1932; part of pectoralis of Coues, 1872, and Jenkins & Weijs, 1979) has a clavicular head originating from the sternum and sometimes also from a small portion of the clavicle, a sternocostal head originating mainly from the sternum and ribs, and an abdominal head originating from the sternum. All insert on the humerus, the clavicular head being superficial to and inserting more distally than the sternocostal head. These three heads are shown as part of the “pectoralis superficialis” of Langworthy (1932: see his figs. 2 and 3). The pectoralis major contacts the pectoralis major of the opposite side of the body at the midline. The **pectoralis minor** (entopectoralis of Lander 1918; pectoralis abdominalis + pectoralis profundus of Langworthy, 1932; part of pectoralis of Coues, 1872, and Jenkins & Weijs, 1979) has three heads running from the xiphoid process and abdominal muscles to the humerus only. Langworthy (1932) stated that the pectoralis minor inserts on the coracoid process of the scapula, but other authors confirmed that it does not (e.g. Coues, 1872, and Jenkins & Weijs, 1979). Lander (1918) stated that the entopectoralis is absent in *Didelphis*. On the contrary, the configuration seen in *D. virginiana* is very similar to that seen in rats: in both species, the entopectoralis has three heads. Because the “pectoralis abdominalis” of Langworthy (1932) is deep to the other two heads (of the “pectoralis profundus” of Langworthy, 1932), it cannot correspond to the abdominal head of the pectoralis major seen in, e.g., humans, which is never deep to the pectoralis minor. This supports the idea of Jouffroy (1971) that the “pectoralis abdominalis” of different authors in different taxa probably corresponds to different structures. That is, when the structure is superficial to the pectoralis minor and adjacent to the main body of the pectoralis major, it corresponds to the abdominal head seen in, e.g., humans, while when the structure is deep to the pectoralis minor and adjacent to the main body of the pectoralis minor, it corresponds to part of the pectoralis minor instead, as seen in, e.g., *Didelphis* and rats. The “fourth pectoral layer” of Langworthy (1932) actually corresponds to the muscle sternalis, not to part of the pectoralis major or pectoralis minor. The pectoralis minor contacts the pectoralis minor of the opposite side of the body at the midline. The **panniculus carnosus** runs from soft tissues such as the abdominal muscles and fascia, its only bony attachment being onto the humerus. It contacts its counterpart at the midline and is blended with the pectoralis minor and the latissimus dorsi of the same side of the body.

The posterior shoulder muscles **infraspinatus**, **supraspinatus**, **teres minor**, **subscapularis**, and **teres major** connect the scapula to the proximal humerus. Coues (1872) stated he could not find a **teres minor**, but as stated by Stein (1981) and Jenkins & Weijs (1979), this muscle is present. The **cleidoacromialis** (probably corresponds to scapulo-clavicularis of Wood, 1870) runs from the distal end of the clavicle to the acromion and lateral third of the scapular spine and also to the superficial aponeurosis of the supraspinatus, as

described by Jenkins & Weijs (1979). It is partially blended with the subclavius. The **deltoideus scapularis** (part of deltoideus of Coues, 1872) runs from the scapula to the humerus. Stein (1981) describes only a 'clavodeltoideus' and a 'spinodeltoideus' in *D. virginiana*. The 'clavodeltoideus' is only partially differentiated into a pars acromialis (from acromion) and a pars clavicularis (from clavicle), which are blended with each other and thus constitute the deltoideus acromialis et clavicularis of the present work. The 'spinodeltoideus' corresponds to the deltoideus scapularis of the present work. The **deltoideus acromialis et clavicularis** (part of deltoideus of Coues, 1872) extends from the acromion of the scapula and the clavicle to the humerus and is blended with the pectoralis major. The **latissimus dorsi** runs from T4 or T5 to L3 or L4 vertebrae to the humerus and is blended with the dorsoepitrochlearis muscle.

Lastly, regarding the arm muscles, as described by Jenkins & Weijs (1979), Coues (1872) and Stein (1981) and as in humans, the **triceps brachii** has scapular, medial and lateral heads, as in humans. It connects the scapula and humerus to the olecranon process of the ulna and is also blended with the dorsoepitrochlearis. As described by these authors, the **dorsoepitrochlearis** (tensor fasciae antebrachii of Stein, 1981; omo-anconeus of Coues, 1872) runs from the latissimus dorsi to the olecranon process of the ulna and adjacent fascia. The **brachialis** (brachialis anticus of Coues, 1872) connects the humerus to the ulna. Coues (1872) suggested that the two heads of **biceps brachii** of *D. virginiana* may both correspond to the short head of other mammals because they mainly originate from the coracoid process and surrounding regions rather than from the capsular ligament of the shoulder joint or the supraglenoid region. However, we agree with Jouffroy (1971) that these heads correspond to the long and short heads of other mammals. Another peculiarity of the biceps is that it runs from the scapula to both the ulna and radius (without a well-defined bicipital aponeurosis). Lastly, the **coracobrachialis** has a single head, which could in theory correspond to the coracobrachialis medius/propius of other mammals. However, it is very short, going from the coracoid process to the proximal humerus, just distal to the lesser tubercle. Moreover, it is mainly deep to, and not fused with, the biceps brachii. Jouffroy agreed with this interpretation, designating this head 'coracobrachialis brevis/profundus' (Jouffroy 1971, Fig. 93).

Distal forelimb muscles (forearm and hand) (Table 6; Fig. 3)

The flexor (ventral) forearm muscle **pronator quadratus** connects the distal ulna to the distal radius. The **flexor digitorum profundus** is fused with the flexor digitorum superficialis, running from the humerus, radius and ulna to the distal phalanges of digits 1, 2, 3, 4 and 5. The **flexor digitorum superficialis** connects the humerus to the middle phalanges of digits 2, 3, 4 and 5, although in a few specimens of this species the muscle seems to go only to digits 2, 3 and 4, as a variant (e.g. Coues, 1872; Stein, 1981). The **palmaris longus** (palmaris longus externus of Jouffroy 1971) and **palmaris longus internus** (of Jouffroy 1971) are fused with each other and also with the flexor digitorum longus and flexor carpi ulnaris, respectively. Both run mainly from the humerus to the palmar aponeurosis. The **flexor carpi ulnaris** connects the humerus and ulna to the pisiform bone and surrounding aponeurosis. This seems to be the common condition for *D. virginiana*, as it is also described by Coues (1872) and Stein (1981), although authors such as Straus (1942) refer to an attachment to both the pisiform and metacarpal 5 in *Didelphis*. The **epitrochleoanconeus** (anconeus internus of Stein, 1981) runs from the medial epicondyle of the humerus to the olecranon process of the

ulna. Straus (1942) suggested that the epitrochleoanconeus of opossums does not correspond to the ‘anconeus internus’ of other mammals, but the ‘anconeus internus’ of authors such as Stein (1981) clearly does correspond to the epitrochleoanconeus of other mammalian taxa. The **flexor carpi radialis** connects the humerus to metacarpal 2. This seems to be the common condition for *D. virginiana*, as it was also reported by Coues (1872) and Stein (1981), but Straus (1942) reported an attachment to both metacarpals 1 and 2 in *Didelphis*. The **pronator teres** runs from the humerus to the middle third or distal two thirds of the radius. A palmaris brevis was not present in the specimens dissected by us or by Coues (1872) and Stein (1981), but Jouffroy & Saban (1971) explain that this muscle is present in some other marsupials.

Among the numerous studies that referred to the hand muscles of *D. virginiana* (see Introduction), Brooks (1886ab) is the most complete and accurate. Brooks describes four lumbricales, an abductor pollicis brevis (ab2), flexor pollicis brevis (superficial head of human anatomy: f2r), adductor pollicis (a1), contrahentes to digits 2 (a2), 4 (a4) and 5 (a5, plus a somewhat distinct bundle, a5a1), an abductor digiti minimi (abd5), a flexor digiti minimi with two heads (a5a and f5u), a flexor brevis digitorum manus to the middle phalanx of digit 5, the usual eight flexores breves profundi plus an adductor pollicis accessorius, and the usual four intermetacarpales. Of the two heads of the flexor digiti minimi brevis, the first (a5a) mainly corresponds to the flexor digiti minimi of humans, while the deeper f5u probably derives from the same anlage that gives rise to the opponens digiti minimi of humans because it is deeper to the main body of the flexor digiti minimi brevis. The adductor pollicis accessorius constitutes a ‘third’ short flexor of digit 1 but seems to derive instead from the adductor pollicis (Brooks 1886ab). Hopefully, by providing one-to-one comparisons with other mammals and tetrapods (Table 6), the present paper will help to solve once and for all the controversy and misunderstandings concerning these muscles. The **flexor brevis digitorum manus** connects the hamate to the middle phalanx of digit 5. The four **lumbricales** run from tendons of the flexor digitorum profundus to the ventral side of the proximal phalanges of digits 2, 3, 4 and 5. They also attach indirectly onto the dorsal side of the middle and distal phalanges of these digits via the extensor expansions. There are three **contrahentes digitorum** other than the adductor pollicis: the contrahens of digit 2 runs from metacarpal 3 to the proximal phalanx of digit 2; contrahens of digit 4 runs from metacarpal 3 to the proximal phalanx of digit 4; and the contrahens of digit 5 runs from metacarpal 3 and capitate to the proximal phalanx of digit 5, having a small, somewhat separate head. The **adductor pollicis** (contrahens of digit 1) connects metacarpal 3 and the capitate to the proximal phalanx of digit 1. The **adductor pollicis accessorius** (‘volaris primus of Henle’) connects the trapezoid and metacarpal 1 to the proximal phalanx of digit 1. All ten **flexores breves profundi** are present, but number 1 corresponds to the flexor pollicis brevis and number 10 corresponds to the flexor digiti minimi brevis, both of which are described below. The other eight flexores breves profundi are: number 2 from trapezium to ulnar side of proximal phalanx of digit 1; numbers 3, 4, 5, 6, 7 and 8 from metacarpals 3, 4, and 5 to the radial and ulnar sides of the respective digit; and number 9 from metacarpal 5 to the radial side of proximal phalanx of digit 5. The **flexor pollicis brevis** connects the trapezium to the radial side of proximal phalanx of thumb, corresponding to the ‘superficial head of the flexor pollicis brevis’ of human anatomy. Although Figure 9.8A of Lewis (1989) suggests that at least some marsupials have an opponens pollicis, the consensus is that this muscle is not present in *D. virginiana*, as noted above.

The **flexor digiti minimi brevis** runs from the pisiform to the proximal phalanx of digit 5 and has two heads, one superficial and one deep, which correspond topologically to the flexor digiti minimi brevis and to the opponens digiti minimi of humans, respectively. However, the latter head does not seem to be directly homologous to the opponens digiti minimi of humans because this muscle was acquired only later in evolution, within placental mammals. The condition seen in marsupials reflects only a rough evolutionary parallelism because the deeper head attaches onto the proximal phalanx of digit 5 rather than onto the metacarpal 5 as does the opponens digiti minimi of, e.g., humans (see Table 6 and below). The **abductor pollicis brevis** connects the trapezium to the radial side of the proximal phalanx of digit 1. The **abductor digiti minimi** runs from the pisiform to the ulnar side of the proximal phalanx of digit 5. Lastly, there are four **intermetacarpales**: as described by Young (1880), number 1 runs from metacarpal 1 to radial side of proximal phalanx of digit 2; number 2 runs from metacarpals 2 and 3 to ulnar side of proximal phalanx of digit 2 and radial side of proximal phalanx of digit 3; number 3 runs from metacarpals 3 and 4 to ulnar side of proximal phalanx of digit 3 and radial side of proximal phalanx of digit 4; and number 4 connects metacarpals 4 and 5 to the ulnar side of proximal phalanx of digit 4 and radial side of proximal phalanx of digit 5.

There are ten muscles in the extensor layer of the forearm; all originate on the humerus except as otherwise noted. The **extensor carpi radialis longus** and **brevis** insert on metacarpals 2 and 3, respectively (the latter may attach to both, as a variant: see Straus, 1941). The **brachioradialis** (supinator longus of Coues 1872) inserts on the scaphoid, lunate and triquetrum, as described by Haines (1939) and Straus (1941), and contra Coues (1872) and Stein (1981). The **supinator** (supinator brevis of Coues 1872) inserts on the proximal radius. It is a well-defined, separated muscle, as described by Stein (1981), and contra Coues (1872). The **extensor carpi ulnaris** runs from the humerus and ulna to metacarpal 5. The **anconeus** (anconeus externus of Stein, 1981) runs from the lateral epicondyle of the humerus to the olecranon process of the ulna. The **extensor digitorum** (extensor digitorum communis of Coues, 1872) inserts on the distal phalanges of digits 2, 3, 4 and 5 (as a variant the tendon to digit 5 might be missing: e.g. Coues, 1872; Stein, 1981). The **extensor digiti minimi** (extensor digitorum lateralis of Stein, 1981; extensor digitorum ulnaris of Haines, 1939) inserts on the distal phalanges of digits 4 and 5 (and 3, as a variant: e.g. Haines, 1939). The **extensor indicis** (extensor digitorum profundus of Straus, 1941) runs from the ulna to the distal phalanges of digits 1, 2 and 3, as described by Straus (1941) and Haines (1939) (or 1 and 2 only, as a variant: Coues, 1872; Stein 1981). The **abductor pollicis longus** (extensor ossis metacarpi pollicis of Coues, 1872) runs from the ulna and radius to metacarpal 1.

Proximal hindlimb muscles (pelvic girdle and thigh) (Table 7; Fig. 4)

Regarding the pelvic girdle muscles, the **quadratus lumborum** seems to mainly connect the ribs and vertebrae to the pelvic girdle. The psoas minor is not present as a separate muscle. The **gluteus maximus**, which originates from two sacral and first three caudal vertebrae, and the **femorococcygeus**, which originates from the third and fourth caudal vertebrae, are usually blended, as stated by Coues (1872), and insert together on the femur. The **gluteus medius** connects the ilium to the proximal femur, as does the **gluteus minimus**, and is blended with the gluteus minimus, gluteus maximus, and piriformis. The tensor

fasciae latae is not present as a distinct structure. As will be seen below, it is probably a derived feature of placentals because it does not seem to be present in monotremes or marsupials (e.g. Bardeen, 1906; Gregory & Camp, 1918; Appleton, 1928). The **scansorius** (iliofemoralis of Coues, 1872, and Stein, 1981) is a small, very slender fasciculus from the acetabular region to the lesser trochanter of the femur, lying in the same position as the iliofemoral ligament of humans. Stein (1981) could not find this muscle in *D. virginiana*, but other authors have also found it (e.g. Coues, 1872). The **obturator internus** and **externus** connect the ischium and pubis to the proximal femur. The former and is blended distally with the **gemellus superior** and **gemellus inferior**, which connect the ischium to the proximal femur, as does the **quadratus femoris**. The **piriformis** runs from the second sacral and first caudal vertebrae to the proximal femur. Finally, the **iliacus** runs from the ilium to the femur and is blended with the **psoas major**, which connects the last three lumbar and first two sacral vertebrae to the femur.

As noted by Coues (1872) and Stein (1981) there is no distinct vastus intermedius ('crureus') in *D. virginiana* or in other marsupials (Warburton 2003; see also, e.g. Thompson & Hillier, 1905, and Osgood, 1921). Instead, this muscle is mingled with the other parts of the 'quadriceps femoris,' making it a triceps complex. The **rectus femoris** originates from the ilium, the **vastus lateralis** and **vastus medialis** originate from the humerus, and all three are blended distally and insert together on the patella and tibia. The **sartorius** connects the ilium to the patella and tibia. The **pectineus** seems to connect the femur to the ischium, as stated by Stein (1981), but Coues (1872) states that it actually originates from the epipubic bone ('marsupial bone': see Fig. 2), not from the ischium. The **adductor brevis** (adductor parvus of Coues, 1872, and Stein, 1981) runs from the pubis to the femur. The **adductor longus** and **adductor magnus** run from the pubis and ischium to the femur (adductor minimus not present as separate muscle). The **gracilis** runs from the pubis to the tibia. The **biceps femoris** has a single head from the ischium to the fibula and is blended with the semitendinosus. Therefore, the short head of the biceps femoris seems to be absent in *D. virginiana*, as stated by Coues (1872), but the tenuissimus ('bicipiti accessorius') is present in some other marsupials, such as marsupial moles (e.g. Warburton 2003). The **semimembranosus** runs from the ischium to the tibia. The presemimembranosus is not present in *D. virginiana* or, seemingly, in most other marsupials (see, e.g. Osgood 1921), with a few exceptions such as marsupial moles (Thompson & Hillier 1905, Warburton 2003).

Appleton (1928) stated that in marsupials the 'caudofemoralis' often inserts onto the proximal femur and thus designated it 'piriformis.' However, as will be explained in the Discussion, the 'piriformis' muscle described in the opossum by authors such as Coues (1872) and Stein (1981) seems to correspond to the piriformis of placental mammals. Lastly, the **semitendinosus** has an anterior/dorsal head and a posterior/ventral head that correspond respectively to the 'crurococcygeus' and 'semitendinosus' of Coues (1872) and Stein (1981). As recognized by these authors, even if the two heads are clearly differentiated in *D. virginiana*, they are still blended proximally, forming a single muscle. The posterior/ventral head connects the ischium to the tibia as described by Coues (1872) (not the femur, as stated by Stein, 1981),

while the anterior/dorsal head connects the third coccygeal vertebra to the tibia as described by Coues (1872) (not the tibia + fibula as described by Stein, 1981).

Distal hindlimb muscles (leg and foot) (Tables 8, 9, 10; Fig. 4)

Concerning the flexor layer of the leg, the **gastrocnemius internus** connects the femur to the calcaneus. The **gastrocnemius externus** (gastrocnemius externus et soleus of Coues, 1872, and Stein, 1981) runs from the femur (part corresponding to lateral head of gastrocnemius of placental mammals) and fibula (part corresponding to soleus of gastrocnemius of placental mammals) to the calcaneus and is well separated from the gastrocnemius internus. The **plantaris** (tensor fasciae plantaris of Coues, 1872) connects the fibula to the calcaneus and is blended with the gastrocnemius externus. The **flexor digitorum longus** (flexor hallucis longus + flexor digitorum longus of Coues, 1872, and Stein, 1981) connects the fibula and tibia to the distal phalanges of digits 1, 2, 3, 4 and 5. It is mixed with the quadratus plantae, interosseus cruris, tibialis anterior, flexor digitorum brevis, and the four lumbricales. Coues (1872) and Stein (1981) erroneously stated that the ‘flexor hallucis longus’ part of the flexor digitorum longus of the present work does not attach onto digit 1. They considered the muscle that sends a tendon to the distal phalanx of digit 1 to be the ‘flexor brevis hallucis obliquus’ (of Stein, 1981) or ‘flexor brevis pollicis obliquus’ (of Coues, 1872). However, as stated by McMurrich (1903ab) and Lewis (1989), this muscle actually corresponds to the **quadratus plantae** (flexor accesorius of McMurrich, 1905; flexor brevis pollicis obliquus of Coues, 1872; flexor hallucis obliquus of Stein, 1981; accessorius of Lewis, 1989), which originates from the calcaneus and inserts on the tendon of flexor digitorum longus. The **interosseus cruris** (pronator tibiae of McMurrich, 1905; rotator fibulae or popliteus of Lewis, 1989, and Coues, 1872) connects most of the proximodistal length of the tibia and fibula and includes the popliteus, as stated by Stein (1981). A similar condition is found in many non-mammalian tetrapods and in other marsupials as well (e.g. Bardeen, 1906). Lastly, the **tibialis posterior** connects the tibia and fibula to the navicular bone.

All ten **flexores breves profundi** are present in the foot, as they are in the hand (see above). Numbers 1 and 10 correspond to the flexor hallucis brevis (‘superficial or medial head of the flexor hallucis brevis’ of authors such as Coues, 1872) and flexor digiti minimi brevis, respectively, and are described below. The other eight are: number 2 (‘lateral head of flexor hallucis brevis’) from medial cuneiform to proximal phalanx of digit 1; numbers 3 and 4 (‘flexor brevis indicis’) from intermediate cuneiform to proximal phalanx of digit 2; numbers 5 and 6 (‘flexor brevis medii’) from lateral cuneiform to proximal phalanx of digit 3; numbers 7 and 8 (‘flexor brevis annularis’) from cuboid to proximal phalanx of digit 4; and number 9 from metatarsal 5 and cuboid to proximal phalanx of digit 5. There are four **intermetatarsales**: number 1 from metatarsal 1 to the medial side of the proximal phalanx of digit 2; number 2 from metatarsals 2 and 3 to the medial side of the proximal phalanx of digit 3; number 3 from metatarsals 3 and 4 to the lateral side of the proximal phalanx of digit 3 and to the medial side of the proximal phalanx of digit 4; and number 4 from metatarsals 4 and 5 to the lateral side of the proximal

phalanx of digit 4 and to the medial side of the proximal phalanx of digit 5 (see, e.g. figure 16.1 of Lewis, 1989). The **abductor digiti minimi** connects the calcaneus to the proximal phalanx of digit 5. Some authors refer to two heads of the abductor digiti minimi in marsupials (see also figure 16.1 of Lewis, 1989). However, their ‘superficial head’ is similar to the abductor digiti minimi of, e.g., humans, while their ‘deep head’ (‘calcaneo-metatarsales’ of Coues, 1872, and Stein, 1981) is similar to the opponens digiti minimi of, e.g., apes because it goes to metatarsal 5, not to the proximal phalanx of digit 5. Therefore, the ‘deep head’ might well correspond instead to a deeper bundle of the flexor brevis profundus 10 (i.e. flexor digiti minimi brevis) that resembles (homoplastically) the opponens digiti minimi, as seems to be the case in the hand of *D. virginiana* (see above). The **abductor hallucis** connects the navicular bone to the proximal phalanx of digit 1. The **flexor digitorum brevis** inserts on the middle phalanges of digits 2, 3, 4 and 5, as it does in placentals such as humans, but it originates from the tendon of flexor digitorum longus rather than from the calcaneus. Therefore, like their corresponding muscles in the upper limb (flexor digitorum profundus and flexor digitorum superficialis), the flexor digitorum brevis and longus in *D. virginiana* are more blended with each other than they are in humans. The four **lumbricales** originate from the tendons of the flexor digitorum longus and seem to insert onto the middle phalanges of digits 2-5. The four **contrahentes pedis** all originate from metatarsal 3 and insert onto the proximal phalanges of digit 1, 2, 4 and 5, respectively. The **flexor digiti minimi brevis** includes the ‘calcaneo-metatarsales’ of Coues (1872) and Stein (1981) and the ‘abductor ossis metatarsi quinti digiti’ of McMurrich (1906). It connects the calcaneus and cuboid to the base of metatarsal 5 and to the proximal phalanx of digit 5 (see also flexores breves profundi above). Finally, the **flexor hallucis brevis** runs from the medial cuneiform to the proximal phalanx of digit 1. This muscle is the medial head of flexor hallucis brevis of Stein (1981) and thus corresponds to the flexor brevis profundus 1 of the present work (see above).

The last group of muscles to be described here is the extensor layer of the leg. Three blended muscles originate from the fibula: **extensor digitorum longus** (to distal phalanges of digits 2, 3, 4 and 5) and **fibularis brevis** and **longus** to metatarsals 5 and 1, respectively. The **extensor hallucis longus** runs from the fibula to the distal phalanx of digit 1 and is blended with the extensor digitorum brevis. The **tibialis anterior** (flexor tarsi tibialis of Coues 1872) runs from the tibia to the medial cuneiform. The fibularis digiti quarti is not present as a distinct muscle in *D. virginiana*, confirming that it is simply a short extensor to digit 4, because in this taxon the short extensor to digit 4 is fused with the main body of the extensor digitorum brevis, the latter muscle thus going to digits 2, 3 and 4. In fact, Stein (1981) uses the name extensor digitorum brevis in *Didelphis*, while he uses the names ‘fibularis digiti quinti’ and ‘fibularis digiti quarti’ in *Chironectes* (also a marsupial), alternately. The **fibularis digiti quinti** (peroneus tertius of Coues, 1872, and Stein, 1981) runs from the fibula to the distal phalanx of digit 5, and thus corresponds to the short extensor of digit 5 rather than to the fibularis tertius. While the short extensor of digit 5 is also seen in placentals such as rats and mice and always attaches onto the phalanges, the fibularis tertius is a derived structure found only in a few placentals such as humans and often attaches instead onto metatarsal

5. The **extensor digitorum brevis** runs from the fibula to the middle phalanges of digits 2, 3 and 4 (as a variation the tendon to digit 4 can send a thin branch to digit 5 as well: e.g. Coues, 1872), as is often the case in other marsupials (e.g. Osgood 1921). Lastly, the **extensor hallucis brevis** (extensor brevis pollicis of Coues, 1872) connects the lateral malleolus of the fibula to the distal phalanx of digit 1 (and 2, as a variant: e.g. Coues, 1872; Stein, 1981).

Discussion

On the evolution, development, and homologies of the head muscles

In Tables 1-10 we mark the major differences from Diogo & Abdala (2010) using a bold red font.

Regarding the hyoid facial musculature, after comparing marsupials and other mammals, including primates (e.g. Diogo & Wood, 2012), and taking into account new observations by others (e.g. Grant et al., 2013, on opossums; Haidarliu et al., 2012, on mice), we concluded that the nasolabialis gave rise to the levator labii superioris alaeque nasi (not to the levator labii superioris: Table 2). The ‘maxillo-naso-labialis’ mainly corresponds to the levator labii superioris (not to the nasalis/depressor septi nasi), and the ‘naso-labialis profundus’ to the nasalis (which subsequently gave rise to the depressor septi nasi). Intrinsic muscles of the snout are present in rats and mice (e.g. the depressor and levator rhinari: Haidarliu et al., 2012), and similar muscles are also found in marsupials such as opossums (Grant et al., 2013), so they were likely already present in the LCA of placentals and marsupials.

The branchial laryngeal muscle cricoarytenoideus posterior is divided into two bundles in opossums (see Results) and frequently in other marsupials as well, which are often seen as separate muscles (Jouffroy & Saban, 1971). Because the cricoarytenoideus posterior is also divided into two bundles in some placentals (Jouffroy & Saban 1971), it may have existed as such in the LCA of monotremes and therians. Regarding the branchial pharyngeal muscles, the detailed study of Maier et al. (1996) on *Monodelphis* showed that fibers of the palatopharyngeus and of the pterygopharyngeus are somewhat differentiated into parts that seem to correspond to the superior constrictor of placentals (e.g. the part of the *Didelphis* musculature named ‘superior constrictor’ in Jouffroy & Saban’s (1971) figure 300). However, there is no true, separated superior constrictor muscle with a median raphe as there is in placentals. Instead, in marsupials the ‘pars pharyngea’ of the stylopharyngeus and parts of the palatopharyngeus and pterygopharyngeus seem to partially fulfil the function of the placental superior constrictor. Therefore, the pterygopharyngeus and superior constrictor of therians most likely derive from the anlage that gives rise to the monotreme palatopharyngeus rather than from the anlage that gives rise to the constrictor pharyngis of monotremes (Table 3). House (1953) and Smith (1992) suggested that the pterygopharyngeus of, e.g., rats and mice probably corresponds to part of the constrictor pharyngis superior of modern humans. Recent molecular developmental studies also support the idea that the superior constrictor is developmentally closely related to the palatopharyngeus (e.g. Grimaldi et al., 2015). The levator veli palatini is not present as a well-developed, well-differentiated muscle in marsupials. Maier et al.’s (1996) study of *Monodelphis* showed that fibers of

the palatopharyngeus/pterygopharyngeus seem to correspond to a very poorly differentiated levator veli palatini, but again there is no true, separated muscle like that seen in placentals. The medialis veli palatini - the precursor of the human musculus uvulae (Saban, 1968) - is present in marsupials and therefore was probably present in the LCA of placentals + marsupials (Table 3).

In Diogo & Abdala (2010) we stated that the palatoglossus was most likely derived from the hypobranchial musculature, specifically from the styloglossus, as proposed by Edgeworth (1935) based on his developmental studies and statements by others that the palatoglossus is usually innervated by the hypoglossal nerve (CN XII), including in humans. However, most human atlases state that the palatoglossus is innervated by the vagus nerve (CN X), grouping it with the true pharyngeal muscles rather than with the hypobranchial tongue muscles. In support of Edgeworth's hypothesis, several studies have suggested that in at least some mammals, including non-human primates, the palatoglossus is innervated by the hypoglossal nerve (Sokoloff & Deacon, 1992). However, in their careful study of *Macaca fascicularis*, Sokoloff & Deacon (1992) did not find a pattern of innervation truly similar to that found in other tongue muscles such as the styloglossus. Based on their data and also on developmental data in mice, Sokoloff & Deacon (1992) stated that a palatal or a tongue (or both) origin of the palatoglossus were plausible hypotheses. House (1953) suggested that the palatoglossus derives specifically from the glossopharyngeal part of the superior constrictor of the pharynx, i.e. to the part that inserts onto the tongue, through an anterior migration of the origin of the muscle from the pharyngeal wall/medial raphe to the soft palate/lateral wall of the oropharynx. As noted above, developmental studies also support the idea that the palatoglossus is derived from the palatopharyngeus/superior constrictor musculature (Schaeffer, 1929; Cohen et al., 1993, 1994). In particular, studies of human (e.g. Cohen et al., 1993) and mouse (Grimaldi et al., 2015) development strongly support the idea that the palatoglossus is a pharyngeal muscle and is more closely related, developmentally, to the palatopharyngeus, levator veli palatini and uvulae than to the superior pharyngeal constrictor. Our results and comparisons support this idea because they indicate that the palatoglossus is a well-developed muscle in marsupials, implying that it was already differentiated in the LCA of placentals + marsupials, while the superior constrictor only became differentiated in placentals (Table 3). In summary, although more data are needed to settle the origin of the palatoglossus once and for all, the weight of evidence strongly supports a pharyngeal origin of the palatoglossus, specifically from the primordia that also give rise to the levator veli palatini, palatopharyngeus and musculus uvulae (Table 3).

On the evolution, development, and homologies of the forelimb muscles

By combining our new data with developmental studies (e.g. Cheng 1955), we were able to test the homology/evolutionary hypotheses proposed by Cheng (1955) and Diogo & Abdala (2010), which agree on most points. A major exception is that Diogo and Abdala (2010) considered it more likely that the dorsoepitrochlearis derives from the triceps brachii than from the latissimus dorsi, as suggested by Cheng (1955) (see Table 5). In their recent detailed study on monotreme forelimb musculature, Gambaryan et al.,

(2015) stated that the dorsoepitrochlearis is continuous with the distal portion of a bundle of the latissimus dorsi in *Zaglossus* and *Tachyglossus* and that this condition is probably plesiomorphic for mammals. They thus proposed that the dorsoepitrochlearis derives from the latissimus dorsi, as did Cheng (1955). However, they recognized that the platypus (*Ornithorhynchus*) condition is similar to that found in therians; i.e. the dorsoepitrochlearis is not completely continuous with the latissimus dorsi. Therefore, it is more parsimonious to accept that the *Zaglossus* and *Tachyglossus* condition is derived (one step in branch leading to family Tachyglossidae, including these two genera) than it is to accept that the conditions seen in platypus and therians are homoplastic (2 steps). That is, the Tachyglossidae, while interesting, do not provide new phylogenetic information to challenge the hypothesis that the dorsoepitrochlearis derived from the triceps brachii. This hypothesis is moreover supported by the fact that the dorsoepitrochlearis is usually innervated by the radial nerve (which usually innervates the triceps brachii), and not by the subscapular nerves (which usually innervate the latissimus dorsi; for other pieces of evidence, see Diogo & Abdala 2010).

Regarding the mammalian teres minor (Table 5), the results of our re-analysis contradict Diogo & Abdala (2010) and support Cheng (1955). Diogo & Abdala (2010) stated that the teres minor seems to correspond to part of the deltoideus scapularis of non-mammalian tetrapods and might be directly homologous to the reptilian scapulo-humeralis posterior. This statement was based in part upon Jouffroy's (1971) criticism of the supposed homology between the mammalian teres minor and the scapulo-humeralis anterior proposed by, e.g., Romer (1944) and Cheng (1955). Jouffroy (1971) pointed out two main problems: 1) both the scapulo-humeralis anterior and teres minor are present in monotremes; 2) in reptiles such as lizards the scapulo-humeralis anterior is innervated by a branch of the radial nerve rather than of the axillary nerve, which usually innervates the teres minor in mammals (and the deltoideus scapularis in mammals and reptiles). However, the second piece of evidence is not very strong because in placentals such as humans both the radial and axillary nerves are branches of the posterior cord of the brachial plexus, meaning that the two nerves are closely related to each other. Moreover, the scapulo-humeralis anterior is very likely derived from the procoracohumeralis, which also gave rise to the deltoideus acromialis et clavicularis, and the latter muscle is innervated by the axillary nerve. Regarding Jouffroy's (1971) first piece of evidence, Gambaryan et al., (2015) stated that the structure that is often designated 'teres minor' in monotremes (in addition to the true scapulo-humeralis anterior) corresponds in fact to the infraspinatus, because it is innervated by the supracoracoideus nerve. The authors therefore concluded that monotremes have three muscles derived from the ancestral supracoracoideus - i.e. the infraspinatus, supraspinatus, and the remnant of the original supracoracoideus - and that the latter muscle was lost in eutherians, which have only a supraspinatus and an infraspinatus. Thus, the 'teres minor', 'scapulo-humeralis anterior' and 'infraspinatus' of Diogo & Abdala (2010) correspond to the infraspinatus, teres minor and supracoracoideus of the present work, respectively (Table 5). This updated scenario better combines all the available data on the development (in marsupials, placentals, reptiles and amphibians) and innervation (in these groups and in monotremes) of the shoulder muscles, as pointed out by Gambaryan et al., (2015).

The results of our dissections and comparisons agree with studies such as Jouffroy's (1971) and

Warburton's (2003) that the cleidoacromialis is not derived from the subclavius, but instead is an appendicular muscle derived from the supracoracoid group (i.e., the group that gives rise to the supraspinatus and infraspinatus). This idea was also supported by Cheng's (1955) developmental study of *Didelphis*, which strongly suggested that the cleidoacromialis originated from the supraspinatus. The cleidoacromialis seems to be often absent in placentals, but Wood (1870) stated that at least some rats, and also humans as variants, have a 'scapulo-clavicularis' that might correspond to the cleidoacromialis. Presence of the cleidoacromialis as a distinct muscle in some adult placentals would support the idea that this muscle was present in the LCA of placentals + marsupials (Table 5).

Jouffroy (1971) stated that most (so, supposedly not all) marsupials have a single, undivided rhomboideus, as seen in *Didelphis*. However, most authors agree that marsupials have a single rhomboid, as do monotremes, indicating that this was probably the plesiomorphic condition for both mammals as a whole and for the LCA of marsupials and placentals (Table 5). The presence of a "rhomboideus occipitalis" in anurans is therefore probably homoplastic; see Diogo & Ziermann, (2014).

Our dissections of *Didelphis* revealed a single deltoideus acromialis et clavicularis (rather than two separate deltoid muscles). After dissecting more mice and rats (unpublished observations, Diogo) and reviewing our notes on *Tupaia*, *Cynocephalus* and other mammals, we conclude that this was also the most common condition in mammals and, therefore, very likely the condition in the LCA of placentals and marsupials (Table 5). Also, we conclude that the atlantoscapularis posticus of placentals corresponds to the 'atlanto-scapularis' of marsupials and was thus very likely present in the LCA of placentals and marsupials (Table 5). As shown by Cheng (1955), the atlantoscapularis posticus seems to be derived developmentally from the levator scapulae, not from the levator claviculae. In fact, the levator claviculae itself is derived from the levator scapulae. Our dissections of lizards, particularly *Timon* and *Lacerta* (unpublished observations, Diogo), convinced us that there is a levator claviculae in *Timon* and many lepidosaurs, meaning that it is probably a very ancient muscle (Table 5). Consequently, in the present paper we made a slight change in nomenclature: in Diogo & Abdala (2010) we stated that the levator claviculae gave rise to the atlantoscapularis posticus and anticus of, e.g., *Tupaia*, but here for taxa such as *Tupaia* we simply use the names levator claviculae, levator scapulae, and atlantoscapularis posticus (Table 5). Because the levator claviculae does not seem to have changed much from the LCA of placentals + marsupials to *Tupaia*, we decided not to use the term 'atlantoscapularis anticus' for the levator claviculae of *Tupaia*, as we did in 2010.

Straus (1942) stated that the 'palmaris longus' muscles of placentals + marsupials may be derived from the flexor carpi radialis (as an exception), from the flexor digitorum superficialis (most frequently) and/or from the flexor carpi ulnaris (somewhat frequently). In certain mammals, such as some marsupials and some Carnivora, there are in fact two 'palmaris longus' muscles, probably derived from the flexor carpi ulnaris and/or flexor digitorum superficialis. Windle & Parsons (1897) refer to a 'palmaris longus externus' and a 'palmaris longus internus,' stating that both are present in *D. virginiana* and that the 'palmaris longus externus' is more commonly

found in mammals. McMurrich (1903a) states that the palmaris longus of mammals corresponds to part of the flexor digitorum longus of reptiles. Jouffroy (1971) reviewed the literature and stated that, although some authors refer to three different ‘palmaris longus’ muscles (‘radial’ derived from flexor carpi radialis, ‘intermedius’ from flexor digitorum superficialis and ‘ulnaris’ from flexor carpi ulnaris), the ‘intermedius’ and ‘radialis’ never seem to coexist. Therefore, Jouffroy preferred to refer simply to the ‘palmaris longus externus,’ derived from the flexor digitorum superficialis and often innervated by median nerve, and the ‘palmaris longus internus,’ derived from the flexor carpi ulnaris and often innervated by the ulnar nerve. Our dissections and comparisons support the idea that the palmaris longus internus and externus are both present in *D. virginiana*, and Jouffroy's idea that the presence of a palmaris longus internus (in addition to the palmaris longus) in some marsupials and some placentals is probably due to homoplasy (Table 6). In contrast, the palmaris longus (‘externus’) very likely was present in the LCA of marsupials and placentals because it is also present in other tetrapods (Table 6).

The presence/absence and homologies of some hand muscles in various tetrapods are controversial. Diogo & Abdala (2010) stated that the contrahentium caput longum of amphibians may correspond to part of the flexor digitorum longus of reptiles and monotremes. However, based on the evidence available at the time, they considered it more likely that the former muscle is completely absent in amniotes. On the contrary, the detailed study of the forelimb musculature of monotremes by Gambaryan et al. (2015) revealed that one of the heads of the flexor digitorum longus of monotremes is extremely similar to the contrahentium caput longum of amphibians, lying between structures that clearly seem to correspond to the flexor accessorius lateralis and flexor accessorius medialis of amphibians. Also, while some authors consider the intermetacarpales, contrahentes digitorum and dorsometacarpales to be absent in monotremes (e.g. Howell, 1937; Jouffroy & Lessertisseur, 1971) - an idea followed by Diogo & Abdala (2010) -, Gambaryan et al. (2015) recently described intermetacarpales in the platypus (they did not find them in other extant monotremes, however) (see Table 6). Similarly, following Jouffroy (1971), Diogo & Abdala (2010) stated that monotremes appear to have an adductor pollicis, but in Gambaryan et al.'s (2015) detailed study no such muscle was described, supporting Howell's (1937) suggestion that this muscle is probably absent in monotremes.

A new insight on the evolution, development, and homologies of the hindlimb muscles

The hindlimb muscles present in placentals such as rats/mice and humans are also, with very few exceptions, present in marsupials such as opossums (Tables 7-10). This conclusion runs contrary to previous ideas of ‘scala naturae’ from marsupials to placentals, culminating in humans (see Diogo et al. 2015a), and it also means that the homologies between marsupials and placentals are not particularly difficult to accept. Instead, the main difficulty lies in comparing the muscles of these two groups with those of monotremes, and such comparisons will thus be the main focus of this section. One of the few major controversies about the homologies between the hindlimb muscles of marsupials and placentals concerns the piriformis. Appleton (1928) stated that the muscle often designated ‘piriformis’ in marsupials

corresponds to the caudofemoralis, and not to the piriformis, of other mammals, but Stein (1981) considered the marsupial ‘piriformis’ to be homologous with the placental piriformis. Our dissections and comparisons show that the attachments of the marsupial ‘piriformis’ are similar to those of the placental piriformis (see Results). Considering that the ‘crurococcygeus’ of opossums corresponds to the dorsal/anterior head of the semitendinosus rather than to a true caudofemoralis, it seems that the caudofemoralis was secondarily lost in the opossum (Table 7; N.B., the caudofemoralis does not correspond to the femorococcygeus, as both these muscles are present in other marsupials and in early developmental stages of placentals such as rats). Furthermore, some marsupials have a piriformis, a caudofemoralis, and a ‘crurococcygeus’ (e.g. Osgood 1921, who also stated that the caudofemoralis is not present in *Didelphis* species).

Regarding the origin of and homologies between the hindlimb muscles in monotremes, therians, and other tetrapods, we agree with the homologies proposed by Gambaryan et al., (2002), with the following five major exceptions. First, the sartorius was not described in the platypus by Gambaryan et al., (2002), but it is usually considered to be present in extant monotreme species. It runs from the pubis (and not the ilium as is usually the case in therians) to the tibia (Table 7; see, e.g. Gregory & Camp, 1918; Pearson, 1926; and Jouffroy & Lessertisseur, 1971).

Second, the ‘obturator internus’ is present in monotremes according to Appleton (1928) and in *Ornithorhynchus* but not in *Echidna* according to Gregory & Camp (1918). However, as stated by Jouffroy & Lessertisseur (1971), the structure designated ‘obturator internus’ or ‘gemellus’ (e.g. Gambaryan et al., 2015) in monotremes seems to correspond simply to an undifferentiated ischiotrochantericus, meaning that only one muscle derived from this group is present in the adult platypus (Table 7).

Third, according to Gambaryan et al., (2002), the ‘plantaris’ described in monotremes by authors such as Coues (1870) does not correspond to the therian plantaris, but instead to the flexor hallucis longus (‘flexor digitorum tibialis’). According to Gambaryan et al., (2002), in the platypus the ‘plantaris’ runs from the fibula to the plantar fascia and then to a sesamoid lying on the tibial side of the tarsal region, not the Achilles tendon, and it is broader than the plantaris of many therians. However, the authors did not employ a rigorous comparative anatomical framework, and in particular they did not draw comparisons with marsupials. Coues (1870, 1872), who did compare the opossum with the platypus, designated the platypus muscle ‘plantaris’ because it closely resembles the plantaris of opossums in, e.g., its origin from the fibula and topological relationship with the gastrocnemius externus. Moreover, unlike the plantaris, which was clearly present in the LCA of placentals and marsupials, the flexor hallucis longus is consistently present as a well-separated muscle only in some placental clades (Table 8). Further (as recognized by Gambaryan et al., 2002), in the platypus the flexor digitorum longus goes to digits 1-5. Therefore, it is extremely unlikely that platypus would also have a separate flexor hallucis longus because this muscle is derived from the part of the flexor digitorum longus going to digit 1 (Table 8). Finally, the distal attachment of the plantaris onto the plantar aponeurosis is not surprising because the name ‘plantaris’ refers precisely to the fact that this

muscle is the ‘topological equivalent’ of the palmaris longus in the forelimb, which usually inserts mainly on the palmar aponeurosis.

Fourth, Gambaryan et al., (2002) did not describe a flexor digitorum brevis in monotremes, but this muscle is clearly present in these mammals (e.g. Coues 1870) (see Table 8). Instead, they divide the flexor digitorum brevis into two muscles: the part attaching to digits 2 and 3 and the part attaching to digit 4. They explain that the first part (‘flexor digitorum intermedii’) originates from the tendon of the flexor digitorum longus rather than from the calcaneus, which is the origin of the flexor digitorum brevis in most therians. Accurately identifying it as one of the short the short flexors of the foot, they named the second part, which does originate from the calcaneus, ‘flexor digiti quarti brevis.’ However, had the authors compared the platypus with other marsupials such as *D. virginiana*, they would have found that entire flexor digitorum brevis originates from the tendon of the flexor digitorum longus. Therefore, the condition seen in the platypus, in which the muscle originates partly from the calcaneus and partly from the tendons of the flexor digitorum longus, is intermediate between the condition seen in opossums and that seen in many placentals.

Fifth, Gambaryan et al., (2002) misinterpreted the homologies of various foot muscles. Once again, their errors resulted from lack of comparison with other mammals, particularly marsupials. For example, they stated that the flexor digiti minimi brevis is absent in the platypus, but they described an ‘abductor metatarsi V’ and an ‘abductor hallucis brevis’ in this taxon. Their description of the flexores breves profundi and of the intermetacarpales was extremely confusing, and they erroneously used the names ‘interossei plantares’ and ‘interossei dorsales,’ respectively, for these muscles. However, the homologies of these foot muscles had already been resolved prior to Gambaryan et al’s 2002 study. Earlier homology hypotheses (e.g., Coues 1870, Jouffroy & Lessertisseur 1971) were very simple and are well supported by recent work, including the present study: the foot muscles found in the platypus are essentially the same as those found in *D. virginiana*, the only exception being that the platypus lacks a distinct abductor hallucis brevis (Table 9).

Despite their lack of an in-depth comparative anatomical framework, the work by Gambaryan et al. (2002) is extremely valuable because it describes all the hindlimb muscles of monotremes and resolves some points of controversy. For instance, enormous confusion has surrounded the homologies of the extensor muscles of the leg in monotremes. However, taking into account the configuration of muscles, particularly their distal attachments, in phylogenetically basal (see Diogo et al., 2015a) therians such as opossums, it is clear that the patterns in monotremes are actually very similar to those found in many therians. Our homology hypotheses largely agree with those proposed by Gambaryan et al., (2015); their ‘extensor digitorum superficialis’ and ‘extensor digitorum profundus’ correspond to the extensor digitorum longus and extensor digitorum brevis of the present work (Table 10). Thus, the muscle that inserts onto the distal phalanx of digit 5 corresponds to the fibularis digiti quinti of therians, which is very likely a short extensor to digit 5. That is why in therians the extensor digitorum brevis, formed by the short extensors, normally does not include a tendon to digit 5; this condition is e.g. seen in the opossum. It is not clear

whether the extensor digitorum brevis in the platypus sends a tendon to digit 5. Gambaryan et al., (2002) state on their page 18 that it does, but then on page 19 they state that it only sends tendons to digits 1-4 and that the individual tendon to digit 5 is the tendon of the fibularis digiti quinti, as is the case in many therians. If the extensor digitorum brevis does send a tendon to digit 5, part of the short extensor to digit 5 might be included in this muscle in the platypus, or, alternatively, the short extensor to digit 4 might send a tendon to digit 5, as such evolutionary changes are not uncommon in tetrapods.

Several pieces of evidence suggest that the ‘superficial extensor’ of the platypus corresponds to the extensor digitorum longus of other mammals. For one thing, this muscle does not go to digit 1; the portion going to digit 1 is differentiated into an extensor hallucis longus, as is the case in other mammals. Also, the tendons of both this ‘superficial extensor’ to digits 2-5 and of the extensor hallucis longus to digit 1 pass superficial to the tendons of the ‘extensor profundus’ in the platypus. Likewise, in other mammals the tendons of the extensor digitorum longus are superficial to the tendons of the short extensors (which form the extensor digitorum brevis and extensor hallucis brevis). In fact, the overall number of extensor muscles and the pattern of their distal attachments in the platypus is almost the same as that found in *D. virginiana*, with only two differences (Table 10): 1) the extensor hallucis brevis is not differentiated as a separate muscle in monotremes; 2) the proximal attachment of the short extensors (i.e. of the extensor digitorum brevis) migrated proximally, all the way to the proximal leg. The latter configuration is also found in the hindlimb of therians (fibularis digiti quinti and fibularis digiti quarti) and in the short forelimb flexors of therians (flexores breves superficiales), which are integrated into the long forearm muscle flexor digitorum superficialis (see Table 6).

Muscle synapomorphies of the head, forelimb and hindlimb of mammals, therians and placentals

Our results, summarized in Tables 1-10, allow us to provide a very detailed list of muscle synapomorphies shared by extant marsupials, extant therians, and extant mammals as a whole. Based on this comparison, extant mammals share 63 muscle synapomorphies, for the head (34 in total), forelimb (11 in total) and hindlimb (18 in total). These numbers illustrate the utility of studying muscles to characterize certain clades, and pave the way for paleontological, developmental and functional works that investigate the specific evolutionary time of origin/loss and developmental mechanisms that led to the characteristic muscle anatomy of each clade and their functional implications.

There are ten synapomorphies of the mandibular muscles of extant mammals: differentiation of mylohyoideus, digastricus anterior, masseter, temporalis, pterygoideus lateralis, pterygoideus medialis, tensor tympani, and tensor veli palatini; loss of adductor mandibulae A2-PVM and of dorsal mandibular muscles (Table 1). There are 12 for the hyoid muscles: differentiation of styloideus, stapedius, platysma cervicale, platysma myoides, extrinsic ear muscles, sphincter colli superficialis, sphincter colli profundus, orbicularis oculi, naso-labialis/levator labii superioris alaeque nasi, buccinatorius, orbicularis oris, and mentalis (Table 2). There are 11 for the branchial muscles: differentiation of acromiotrapezius,

spinotrapezius, dorsocutaneous, cleidomastoideus, sternomastoideus, constrictor pharyngis, cricothyroideus, palatopharyngeus, thyrocricoaerytenoideus, and arytenoideus; loss of constrictor laryngis (Table 3). There is only one for the hypobranchial muscles: differentiation of sternothyroideus (Table 4). Therefore, in the transitions that led to the LCA of extant mammals, all major groups of head muscles experienced drastic changes with the exception of the much more conserved hypobranchial muscles of somitic origin, which experienced a single synapomorphic change.

Regarding the proximal (girdle/arm) forelimb muscles, during these transitions there were eight synapomorphic changes: differentiation of pectoralis major, pectoralis minor, panniculus carnosus, infraspinatus, supraspinatus, subscapularis, teres major, and dorsoepitrochlearis (Table 5). In contrast, there were only three synapomorphic changes within the distal (forearm/hand) forelimb muscles: loss of palmaris profundus 1, of flexores breves superficiales as a group, and of dorsometacarpales (Table 6). These differences between fore- and hindlimb are probably related to major changes in posture and locomotion from stem amniotes to basal mammals, including the major rotations/torsions of the proximal region of the limbs that produced the characteristic mammalian posture (see, e.g. Kardong, 2011). While most non-mammalian tetrapods use a sprawling gait in which the legs are splayed out to the side and the spine undulates mediolaterally, most mammals use a parasagittal gait in which the legs are held beneath the body and the spine bends dorsoventrally (e.g. Schilling, 2011). Most of the synapomorphic changes in the hindlimb leading to the LCA of extant mammals occurred in the proximal region, supporting this hypothesis (16 synapomorphic changes in the proximal (girdle/thigh) hindlimb region - differentiation of psoas minor, gluteus medius, gluteus minimus, gluteus maximus, rectus femoris, vastus lateralis, vastus medialis, iliopsoas, pectineus, adductor magnus, adductor longus, obturator externus, semimembranosus, biceps femoris, semitendinosus, and quadratus femoris (Table 7) -, as opposed to only two changes in the distal (leg/foot) hindlimb muscles - differentiation of plantaris and extensor hallucis longus (Tables 8-10)). However, there were fewer total changes in the two limbs (=29) than in the head (=34, or 54% (34/63) of all changes). In addition to describing the drastic changes that occurred in both the head and limbs during the transitions leading to extant mammals, our results also show mosaic evolution because some subregions of these three major regions experienced many more changes than others. For example, the hypobranchial and distal limb muscles changed less than those of other subregions.

There were 48 synapomorphic changes from the LCA of extant mammals to the LCA of extant therians: 28 in the head, 11 in the forelimb, and nine in the lower limb. There were no changes within the mandibular muscles (Table 1), 18 within the hyoid muscles (differentiation of stylohyoideus, digastricus posterior, occipitalis, auricularis posterior, mandibulo-auricularis, interscutularis, zygomaticus major, zygomaticus minor, frontalis, auriculo-orbitalis, auricularis superior, corrugator supercilii, retractor anguli oculi lateralis, dilatator nasi, levator labii superioris, nasalis, and levator anguli oris facialis; loss of remaining of original interhyoideus: Table 2), eight within the branchial muscles (differentiation of cleido-occipitalis, constrictor pharyngis medius, constrictor pharyngis inferior, palatoglossus, pterygopharyngeus,

musculus uvulae, thyroarytenoideus, and cricoarytenoideus lateralis: Table 4) and only two within the hypobranchial muscles (differentiation of styloglossus and thyrohyoideus: Table 4). These results indicate that the origin of therians was particularly marked by evolutionary changes in facial muscles, pharyngeal muscles, and laryngeal muscles. These changes were probably related to specializations in facial and vocal communication through movements of both the larynx and pharynx and new ways of feeding, including mastication and suckling.

Among the therian limb synapomorphies, four relate to the proximal (girdle/arm) forelimb muscles (differentiation of atlantoscapularis posticus and cleidoacromialis; loss of costocoracoideus and of remaining of original supracoracoideus: Table 5), seven to the distal (forearm/hand) forelimb muscles (differentiation of flexor digitorum superficialis, palmaris longus, palmaris brevis, flexor brevis digitorum manus, extensor carpi radialis longus, and extensor carpi radialis brevis: Table 6), nine to the proximal (girdle/thigh) hindlimb muscles (differentiation of piriformis, scansorius, femorococcygeus, tenuissimus, psoas major, iliacus, obturator internus, gemellus superior, and gemellus inferior: Table 7), and none to the distal (leg/foot) hindlimb muscles (Tables 8-10). Therefore, unlike the transitions to mammals, those leading to therians were more dramatic in the distal forelimb, consistent with the idea that the former were mainly related to posture and locomotion. However, in the hindlimb all synapomorphic changes occurred in the proximal region. There are fewer therian than mammalian synapomorphies - about 4/5, or 77% (i.e. 48/63) -, but still there were substantial changes in the head (almost as many as seen in the transitions from stem amniotes to mammals: 28 vs. 34), forelimb (as many as seen in the transitions to mammals: 11 vs. 11), and hindlimb. However, there were only half as many changes in the hindlimb in the transitions leading to therians as in those leading to mammals (nine vs. 18). Overall, changes in the head accounted for a slightly greater percentage of total changes in the transitions to therians 58% (28/48) than in the transitions to mammals (53%, 34/63).

There are ten or 11 synapomorphies of extant placentals: three or four in the head, three in the forelimb, and four in the hindlimb. None concerns the mandibular and hypobranchial muscles (Tables 1, 4), none or just one concern the hyoid muscles (possibly the differentiation of depressor septi nasi: Table 2), and three concern the branchial muscles (differentiation of constrictor pharyngis superior, levator veli palatini, and salpingopharyngeus: Table 3). Thus, except for the pharyngeal muscles, the head muscles changed very little from the LCA of extant therians to the LCA of placentals. These changes probably related to further specializations of the movements of the larynx (moved by the pharyngeal muscle salpingopharyngeus) and pharynx for vocal communication and/or feeding mechanisms. There were no synapomorphic changes in the distal (forearm/hand) forelimb either, and only three in the proximal (girdle/arm) forelimb (differentiation of rhomboideus major, rhomboideus minor, and rhomboideus occipitalis), two in the proximal (girdle/thigh) hindlimb (differentiation of tensor fasciae latae and vastus intermedius) and two in the distal (leg/foot) hindlimb (differentiation of soleus; loss of interosseus cruris). Therefore, the few synapomorphic changes from the LCA of extant therians to the LCA of extant placentals

are distributed more or less equally among the three major anatomical regions (head three or four, forelimb three, hindlimb four). These numbers provide empirical support for a well-defined therian body plan, which can still be easily recognized in most extant placentals and marsupials except very specialized taxa such as bats and whales. Interestingly, over the transitions from the LCA of extant therians to the LCA of extant placentals, changes in the head account for only about 1/3 of the total (three or four within ten or 11), i.e. a much smaller ratio than that seen in the transitions to mammals (53%) and the transition to therians (58%).

General comments: mammalian evolution, complexity, scala naturae, body plans, and developmental constraints

In order to discuss morphological complexity and make myological comparisons such as those listed in Table 1-10, we must be able to measure complexity. For the present work we will do so using the concept of gross morphological complexity, or “pure complexity” of McShea & Brandon (2010).

However, we are of course aware that there are other ways to measure complexity. For instance, one of the more notable features that distinguish mammals from other tetrapods is the reorganization of the motor nuclei of the brainstem, and in particular the development of discrete motor nuclei to special branchiomic muscles (Smith, 1992). In other tetrapods these motor nerves are arranged in a broadly overlapping column with a variety of functional components, while in mammals the motor nucleus of the facial nerve and especially the nucleus ambiguus are spatially distinct nuclei with a large series of branchiomic muscles as their efferent targets. Therefore, it is likely that general functional changes in the organization of motor systems occurred during the transition to and evolutionary history of mammals, and that in at least some cases fewer musculoskeletal structures have finer motor control due to a more complex neural organization. Also, in recent years we have been working to develop alternative ways of measuring morphological complexity in the organization of body parts, such as anatomical network analyses that take into account not only the number and presence/absence of anatomical structures but also their topological connectivity (e.g. Esteve-Altava, 2015ab; Diogo et al., 2015b). We plan to apply such tools to the data obtained in the present paper in future works, in order to compare “pure complexity” from comparative studies with the organizational complexity of systems biology data obtained from anatomical network analyses.

However, for the specific purposes of the present paper, which already includes discussion on a wide range of various different topics, we will analyze complexity by using the simplest and most objective way to do so in view of the anatomical data obtained here. That is, we use a morphological definition of complexity of Eble (2004, 2005), which corresponds to “pure complexity” as defined by McShea & Brandon (2010). These authors argued that “pure complexity” - i.e. the number of part types (e.g. different muscles) within a body - is a much more appropriate, and practical, measure of complexity than is “colloquial complexity,” which refers not only to part types but also their functionality, sophistication and integration, among other characteristics. According to them, “colloquial complexity” is so vague, subjective, and/or difficult to measure that it has become the source of many problems in biology, including

the paucity of empirical treatments of complexity in the biological literature. Therefore, here we refer to “pure complexity” by taking into account the number of muscles found in *Didelphis*, rats/mice and humans with the number most likely found in the LCA of these three taxa (i.e., of marsupials + placentals), and the number of evolutionary changes that probably occurred between the LCA of marsupials + placentals and each of those three taxa. This method is particularly valuable because 1) the number of muscles is an objective measure (e.g., most researchers/anatomical atlases describe the same number of muscles in the human body); and 2) it combines macroevolutionary and developmental definitions of complexity, including the notion that each muscle is the result of parcellation, i.e. of innovation through differentiation leading to a morphogenetic semi-independence of the muscle (e.g. Bonner 1988, Wagner & Altenberg, 1996, Vermeij, 1973).

There are nine mandibular muscles in the LCA of therians, eight in *Didelphis*, nine in rats, and eight in *Homo*. *Didelphis* and *Homo* acquired a single change from the LCA (lost intermandibularis anterior), and rats acquired none (Table 1). There are 27 hyoid muscles in the LCA of therians, 25 in *Didelphis* and rats, and 27 in *Homo*. From the LCA of extant therians there were two changes to *Didelphis* (loss of sphincter colli superficialis and mentalis), seven to rats (gain of sternofacialis, frontalis + auriculo-orbitalis + auricularis superior formed orbito-temporo-auricularis, loss of corrugator superioris, loss of retractor anguli oculi lateralis, gain of procerus, gain of depressor septi nasi, loss of mentalis), and 14 to humans (loss of platysma cervicale, loss of mandibulo-auricularis, gain of risorius, loss of sphincter colli profundus, loss of sphincter colli superficialis, loss of interscutularis, gain of temporoparietalis, gain of depressor supercillii, loss of retractor anguli oculi lateralis, gain of procerus, loss of dilatator nasi, gain of depressor septi nasi, gain of depressor labii inferioris, gain of depressor anguli oris) (Table 2). There are 19 branchial muscles in the LCA of extant therians, 17 in *Didelphis* and *Homo*, and 21 in *Rattus*. From this LCA there were two changes to *Didelphis* (loss of dorsocutaneous, loss of cricothyroideus), 6 to rats (loss of dorsocutaneous, gain of superior constrictor, loss of palatoglossus, gain of levator veli palatini, gain of salpingopharyngeus, gain of cricoarytenoideus alaris), and ten to humans (loss of ceratohyoideus, acromiotrapezius and spinotrapezius formed trapezius, loss of dorsocutaneous, loss of cleido-occipitalis, sternocleidomastoideus and cleidomastoideus formed sternocleidomastoideus, gain of superior constrictor, loss of pterygopharyngeus, gain of levator veli palatini, gain of salpingopharyngeus, and gain of arytenoideus obliquus) (Table 3). Lastly, there were eight hypobranchial muscles in the LCA of extant therians and in *Didelphis*, *Homo* and rats, with no change from the LCA to any of the latter three taxa (Table 4).

Regarding the forelimb muscles, there are 23 proximal (pectoral girdle/arm) muscles in the LCA of extant therians, 23 in *Didelphis* and rats, and 18 in humans. There were no changes from LCA to *Didelphis*, four to rats (rhomboids minor differentiated, rhomboid occipitalis differentiated, atlantoscaphularis posticus lost, cleidoacromialis lost), and seven to humans (rhomboid minor differentiated, levator claviculae lost, atlantoscaphularis posticus lost, panniculus carnosus lost, cleidoacromialis lost, two deltoids fused into one, dorsoepitrochlearis lost) (Table 5). The number of distal (forearm/hand) muscles was 48 in the LCA of

extant therians, 45 in *Didelphis*, 44 in *Rattus*, and 41 in *Homo*. *Didelphis* has four changes since the LCA of extant therians (gain of palmaris longus internus, loss of palmaris brevis, fusion of extensor digiti minimi and extensor digiti quarti into extensor digiti minimi, fusion of extensor digit 3 proprius, extensor indicis and extensor pollicis longus into extensor indicis), rats have six (loss of flexor brevis digitorum manus, loss of one contrahens, loss of adductor pollicis accessorius, gain of opponens digiti minimi, loss of brachioradialis, undifferentiated extensor digiti 3 proprius), and humans have 12 (gain of flexor pollicis longus, loss of epitrochleoanconeus, loss of flexor brevis digitorum manus, loss of contrahens to digit 2, loss of contrahens to digit 4, loss of contrahens to digit 5, intermetacarpales integrated into interossei, gain of opponens pollicis, gain of opponens digiti minimi, undifferentiated extensor digiti quarti, undifferentiated extensor digiti 3 proprius, gain of extensor pollicis brevis) (Table 6).

Regarding the hindlimb muscles, the number of proximal (pelvic girdle/thigh) muscles is 30 in the LCA of extant therians, 27 in *Didelphis* and rats, and 26 in humans. There were three changes from the LCA of extant therians to *Didelphis* (lost tenuissimus, presemimembranosus and caudofemoralis), seven changes to rats (loss of scansorius in adults, gain of tensor fasciae latae, loss of femorococcygeus in adults, loss of tenuissimus in adults, differentiation of vastus intermedius, loss of sartorius in adults, loss of presemimembranosus in adults), and eight changes to *Homo* (loss of psoas minor in most/many individuals, loss of scansorius, gain of tensor fasciae latae, loss of femorococcygeus, loss of tenuissimus, differentiation of vastus intermedius, loss of presemimembranosus, loss of caudofemoralis) (Table 7). Finally, the number of distal (leg/foot) muscles was 42 in the LCA of extant therians, 40 in *Didelphis*, 37 in rats, and 35 in *Homo*. There were two changes from LCA of extant therians to *Didelphis* (popliteus not differentiated, fibularis digiti quinti not differentiated), seven changes to rats (single gastrocnemius muscle, separate soleus muscle, interosseus cruris not differentiated, abductor hallucis not differentiated, contrahens to digit 1 lost, contrahens to digit 4 lost, extensor hallucis brevis not differentiated), and 11 changes to humans (single gastrocnemius muscle, separate soleus muscle, flexor hallucis longus differentiated, interosseus cruris not differentiated, contrahens to digit 2 lost, contrahens to digit 4 lost, contrahens to digit 5 lost, intermetacarpales incorporated into dorsal interossei, fibularis brevis differentiated, fibularis digiti quinti lost, fibularis digiti quarti not differentiated) (Tables 8-10).

The total numbers of head and limb muscles are 206 for the LCA of extant therians, 193 for *Didelphis*, 194 for rats, and 180 for humans. Therefore, our results show no support for a scala naturae or increase in pure morphological complexity of McShea & Brandon (2010) between the LCA of therians and humans. In fact, if a trend exists it is toward fewer muscles and less pure complexity. Specifically, serial muscles such as the contrahentes, intermetacarpales, and flexores breves profundi were lost or fused, leading toward anisomerism (see Diogo et al., 2015d). Humans have 26 fewer muscles than the LCA of extant eutherians, 14 fewer muscles than placentals such as rats and 13 fewer muscles than marsupials such as *Didelphis*. These results contradict previous reports, which suggested that humans have many more muscles than opossums (e.g. Coues, 1872; Huber, 1930a, 1930b, 1931; Minkoff et al., 1979: see

Introduction). Similarly, Grant et al., (2013) showed that, contrary to previous assumptions, all major muscles present in the snout region of placentals such as mice are actually present in opossums. From the LCA of extant eutherians there were 14 changes to *Didelphis*, 37 to rats, and 63 to humans. Although even more muscles were lost during the evolution of humans than during that of opossums or rats, some muscles also became differentiated, for a total of 63 changes but only 26 fewer muscles than the LCA.

These numbers are important, because they show that being anatomically plesiomorphic and being morphologically less complex are two very different things. This distinction is often neglected or downplayed by evolutionary biologists, partly because of the continuing profound effect of scala naturae in our field (Diogo et al., 2015a). Anatomical changes do not always produce greater complexity, particularly in phylogenetically derived animals such as mammals. *Didelphis* provides an illustrative example, because it is anatomically more plesiomorphic (only 14 changes from LCA of extant therians) than are rats (37 changes) and humans (63 changes), but in terms of pure morphology its musculature is more complex than that of humans. Thus, *Didelphis* is a very good model for the LCA of extant therians. Similarly, our previous study showed that monotremes provide a very good model for the head and forelimb musculature of basal mammals (Diogo et al., 2015a). Another recent study independently reached the same conclusion: “among living tetrapods, one cannot find a better model for reconstruction of the locomotorium of the mammal-like reptiles than monotremes” (Gambaryan et al., 2015: p. 54).

Of the 194 muscles of *Didelphis*, 172 (89%) are present, with exactly the same one-to-one identity/homology, in rats. Therefore, the adult anatomy of placentals and marsupials is very similar despite their very different early developmental patterns, which include not only marked heterochronies but also differences in gene upregulation and expression between the two taxa (e.g. Hübler et al., 2013). As explained in the introduction, this pattern is also seen in the skeletal system. For instance, marsupials have a transient shoulder arch that results from the fusion of several elements of the shoulder girdle complex, including the scapula, coracoid, and manubrium. This structure provides support and important muscle attachment sites for the crawl to the teat (reviewed in e.g. Hübler et al., 2013). The early morphology of the marsupial shoulder arch is thus similar to that seen in embryonic and adult monotremes. Immediately before or shortly after parturition, the coracoid often detaches from the sternum, leading to the adult marsupial shoulder girdle morphology. This morphology is shared with placentals, despite the fact that placentals never form a shoulder arch during their ontogeny (Hübler et al., 2013). A similar pattern is seen in the nervous system. Spinal nerve outgrowth into the opossum forelimb bud is expanded, including cervical nerves 3-8 and thoracic nerves 1-2. This outgrowth is probably related to the expansion of the opossum forelimb early in development (Keyte & Smith, 2010). The contribution from the third cervical nerve is then reduced later in ontogeny, resulting in an adult phenotype like that of adult placentals such as mice in which there is no connection between C3 and the brachial plexus (Keyte & Smith, 2010). This result led Keyte & Smith (2010: 4290) to point out that “although the anterior expansion of the forelimb bud seems to

have impacted early spinal nerve growth into the limb, this is corrected as development proceeds so that in the adult a typical mammalian pattern is observed.”

Smith (2001:129) also briefly referred to this topic, using marsupials and placentals to suggest “that development, even at its earliest stages, is highly plastic; the observation of significant early plasticity, even in animals in which the adults are quite similar, is important for understanding the ways in which development and evolution interact.” This is because the changes observed in marsupials “occur immediately before, during and after the phylotypic stage and include shifts in some of the major patterning events in the body; these observations thus confirm recent studies that suggest that the degree of conservation at a phylotypic stage in vertebrates has been overestimated” (Smith, 2001:129). Such examples actually reinforce the notion of a Bauplan (body plan), although this interpretation is something of a paradox because the “phylotypic stage” is precisely the stage at which earlier authors might have thought that a Bauplan would be most obvious. Smith’s (2001) case study shows that many evolutionary changes are driven by the needs of the embryos/neonates rather than by a ‘goal’ towards a specific adult plan or ‘archetype.’ That is, within a clade like Theria, even drastic changes in early development do not produce, except in very rare cases (e.g. whales and bats), major changes in the adult body plan. This new Evo-Devo interpretation of Bauplan is not adult-driven, as suggested by its name, related to a pre-evolutionary notion of “archetype” and “plan”. It is instead development-driven in that it emphasizes the importance of developmental constraints. These constraints are so prevalent and interdependent (e.g., through well-defined genomic networks) that even drastic developmental changes, such as those seen in early marsupial ontogeny, do not break the whole “spider web” created by the constraints and lead to completely new phenotypes (see, e.g. Wagner, 2014). Therefore, the ancient term “Bauplan” with its modern Evo-Devo interpretation does reinforce both the importance of developmental constraints and the existence of a common, easily identifiable phenotype shared by some clades, but not by others.

Such developmental constraints may explain why the adult musculature of marsupials is similar to that of other mammals even though their development is very different. For example, consider the specificity of fate of, e.g. muscle insertions through neural crest cells of the first and second arches. Muscles derived from each of these arches tend to attach to skeletal elements of the same arch via connective tissues derived from crest cells from the same arch, as seen e.g. in chickens and mice (reviewed in Smith, 2001). The neural crest cells from the midbrain and first rhombomere are found throughout the first arch, while those from the second rhombomere are localized in the posterior margin of the first arch. The cells from the midbrain produce distal elements (e.g. Meckel’s cartilage), while the cells from the first two rhombomeres produce proximal elements (e.g. articular, quadrate, squamosal). In opossums, because of the marked heterochrony characteristic of marsupial early development, the bulk of migration occurs before there is any hint of morphological differentiation of these regions of the brain. As noted by Smith (2001), one possible scenario is that these regions exhibit genetic differentiation early relative to morphological differentiation: if genetic differentiation was advanced then, even though the midbrain, forebrain and first two rhombomeres

are not morphologically distinguishable at the time of neural crest migration, they would possess region specific genetic identity.

An alternative scenario would be that in marsupials the populations of neural crest do not exhibit the region-specific identity seen in chicken and mice. When we compare the first arch muscles of mice and humans, as well as the two second arch muscles that normally insert either directly or indirectly onto the hyoid bone in these taxa (stylohyoideus and digastricus posterior, i.e. all except the muscles of facial expression) with those of opossums, it is clear that the specificity fate constraint is not at all broken in marsupials despite the characteristic peculiarities of early marsupial development. This is because all mandibular muscles of *D. virginiana* still attach onto hard/soft tissues derived from the first arch, and the stylohyoideus and digastricus posterior are still deeply associated with the hyoid apparatus, either directly or indirectly (the latter muscle is attached via aponeurotic/connective tissue, as is also the case in mice and humans: see Results). Therefore, the adult phenotypic insertions of all these muscles are identical in the three taxa because of the strong specificity of fate constraints, despite the differences in early ontogeny seen in placentals and marsupials, and also the more than 150 million years that have passed since these two major groups split (Luo et al., 2011).

Of course, even among therians that conform to the characteristic therian Bauplan (i.e., not including highly specialized forms such as bats or whales), there are minor differences in adult phenotype, particularly between taxa from different higher clades such as placentals vs. marsupials. For instance, the larynx in marsupials is clearly derived: the cricoid and thyroid cartilages are fused, leading to absence of the cricothyroid muscles and an articulation between the two arytenoid cartilages (N.B. the articulation between these cartilages and an interarytenoid cartilage seems to be plesiomorphic for mammals). Even so, many of these specific, “minor” differences among adults of different taxa seem to be related to needs of the embryos and/or neonates. For instance, Symington (1898) explained that these differences in larynx morphology might be related to the fact that marsupials remain in the pouch for a long time attached to the teat and thus need to, for instance, have safer ways to drink and breathe simultaneously. This requirement might also explain the expansion of the palatopharyngeus muscle/connective tissue, and perhaps the expansion of the pars pharyngea of the stylopharyngeus, which are also derived characters within marsupials. As noted above, we plan to apply tools such as anatomical network analysis to the data obtained in the present paper in future works, in order to compare different ways of discussing complexity. Therefore, it is hoped that our long-term project will contribute toward the multidisciplinary data needed for an integrative synthesis of the anatomical macroevolution of vertebrates and for future functional and developmental comparative studies.

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Figure legends

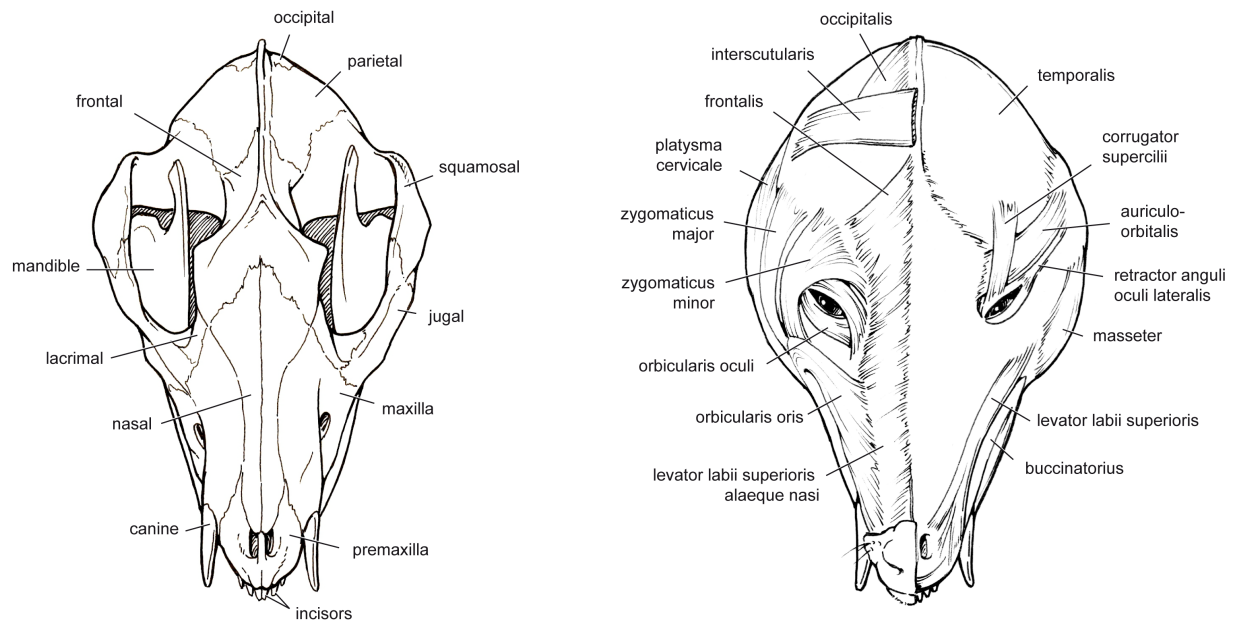


Fig. 1. Schematic drawing (by J. Molnar) of head skeleton and muscles of *D. virginiana*.

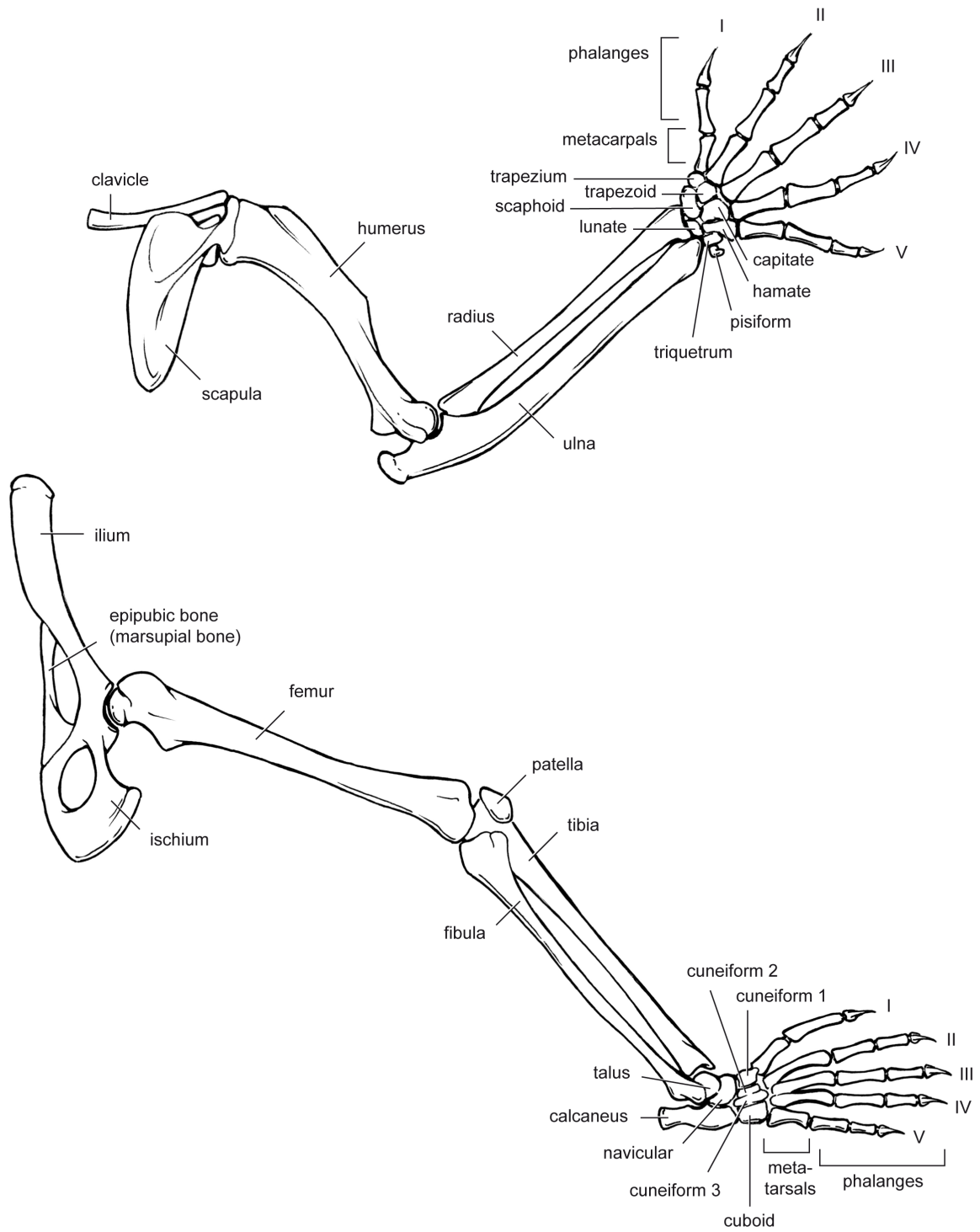


Fig. 2. Schematic drawing (by J. Molnar) of fore and hind limb skeleton of *D. virginiana*.

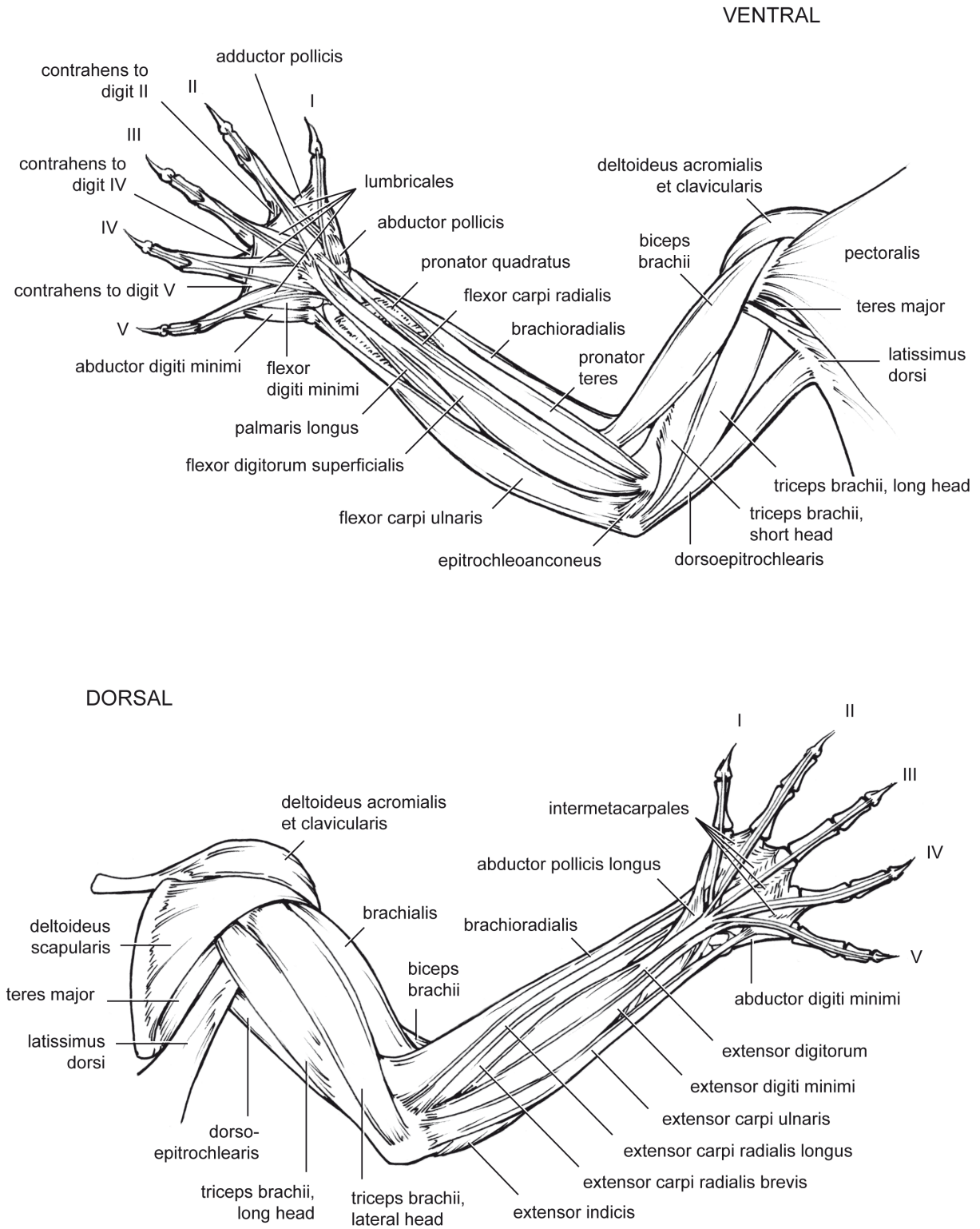


Fig. 3. Schematic drawing (by J. Molnar) of forelimb muscles of *D. virginiana*.

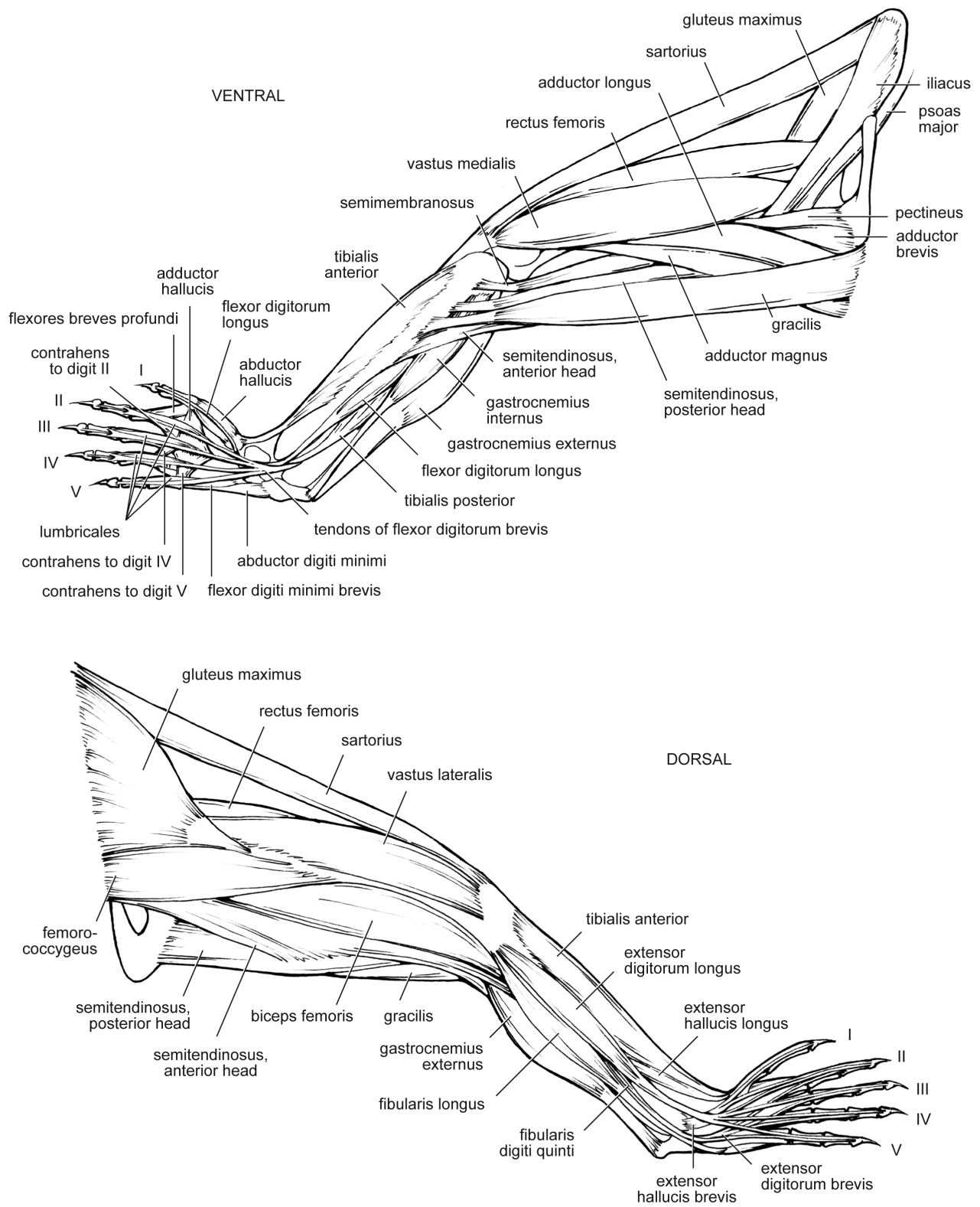


Fig. 4. Schematic drawing (by J. Molnar) of hindlimb muscles of *D. virginiana*.

Table 1. Scheme illustrating the authors' hypotheses regarding the homologies of the mandibular muscles of adults of representative sarcopterygian taxa and the probable condition for the last common ancestor (LCA) of marsupials + placentals. Data from evidence provided by our own dissections and comparisons and by a review of the literature; muscles and other terms in bold and red highlight cases in which the homology and/or evolutionary hypotheses of Diogo & Abdala (2010) were updated, in the present paper, in face of the new data obtained in our dissections and review of the literature. The black arrows indicate the hypotheses that are most strongly supported by the evidence available; the grey arrows indicate alternative hypotheses that are supported by some of the data, but overall they are not as strongly supported by the evidence available as are the hypotheses indicated by black arrows. VENTRAL, DORSAL means ventral musculature and dorsal constrictor musculature of the mandibular arch of Edgeworth (1935). ad. adductor; dig. digastricus; intern. intermandibularis; lat. lateralis; le. levator; m. muscles; man. mandibulae; mandib. mandibularis; psoa. present in some amphibians; psor. present in some other reptiles. Numbers in parentheses are the total number of mandibular muscles found in each respective taxon.

	Actinistia: <i>Lamimeria chalumnae</i> (Coelacanth) (7 m.)	Dipnoi: <i>Lepidosirens paradoxus</i> (South American lungfish) (5 m.)	Amphibia: <i>Ambystoma ordinarius</i> (Michoacan stream salamander) (6 m.)	Reptilia: <i>Timon lepidus</i> (Ocellated lizard) (11 m.)	Mammalia LCA (Monotremata): <i>Ornithorhynchus anatinus</i> (Platypus) (9 m.)	Marsupials + placentals (9 m.)	Mammalia (Marsupialia): <i>Didelphis virginiana</i> (Virginian opossum) (8 m.)	Mammalia (Rodentia): <i>Rattus norvegicus</i> (Norwegian rat) (9 m.)	Mammalia (Scandentia): <i>Tupaia</i> sp. (Tree-shrew) (9 m.)	Mammalia (Dermoptera): <i>Cynocephalus volans</i> (Philippine colugo) (8 m.)	Mammalia (Primates): <i>Homo sapiens</i> (Modern human) (8 m.)
VENTRAL	Intern. posterior	Intern.	Intern. posterior	Intern. posterior	Mylohyoideus	Mylohyoideus	Mylohyoideus	Mylohyoideus	Mylohyoideus	Mylohyoideus	Mylohyoideus
	---	---	---	---	Dig. anterior	Dig. anterior	Dig. anterior	Dig. anterior	Dig. anterior	Dig. anterior	Dig. anterior
	Intern. anterior	---	Intern. anterior	Intern. anterior	---	Intern. anterior	Intern. anterior	Intern. anterior	---	---	
ADDUCTOR MANDIBULAE	Ad. man. A2	Ad. man. A2	Ad. man. A2	Ad. man. A2	Massefer	---	---	---	---	---	---
	---	---	---	---	Detrahens man.	---	---	---	---	---	---
	---	---	---	---	Temporalis	Temporalis	Temporalis	Temporalis	Temporalis	Temporalis	Temporalis
	---	---	---	---	Pterygoideus lat.	Pterygoideus lat.	Pterygoideus lat.	Pterygoideus lat.	Pterygoideus lat.	Pterygoideus lat.	Pterygoideus lat.
	---	Ad. man. A2-PVM	Ad. man. A2-PVM	Ad. man. A2-PVM	---	---	---	---	---	---	---
	---	Retractor ang. oris	---	---	---	---	---	---	---	---	---
	---	---	---	Le. anguli oris mandib.	---	---	---	---	---	---	---
	Ad. mand. A3'	Ad. man. A3'	Pseudotemporalis	Pseudotemporalis	---	---	---	---	---	---	---
	Ad. mand. A3''	---	---	---	---	---	---	---	---	---	---
	---	---	---	--- [psoa.]	Pterygomandibularis	---	---	---	---	---	---
DORSAL	Le. arcus palatini	---	---	Le. pterygoidei	---	---	---	---	---	---	---
	---	---	---	Protractor pterygoidei	---	---	---	---	---	---	---
	---	---	---	---	Tensor tympani	Tensor tympani	Tensor tympani	Tensor tympani	Tensor tympani	Tensor tympani	Tensor tympani
	---	---	---	---	Tensor veli palatini	Tensor veli palatini	Tensor veli palatini	Tensor veli palatini	Tensor veli palatini	Tensor veli palatini	Tensor veli palatini
	---	---	---	---	---	---	---	---	---	---	---
	Ad. mand. A ω	---	---	Ad. man. A ω	---	---	---	---	---	---	---

Table 2. Scheme illustrating the authors' hypotheses regarding the homologies of the hyoid muscles (see caption of Table 1). ad. adductor; al. alaeque; aur. auricularis; bra. + cerat. branchiohyoideus + ceratomandibularis; de. depressor; ex. extrinsic; hyo. hyomandibulae; inf. inferioris; int. intrinsic; le. levator; m. muscles; man. depressor mandibulae; operc. operculi; poam. present in other adult marsupials; poap. present in some other adult placentals; poar. present in other adult reptiles; prof. profundus; sup. superioris; supe. superficialis; tra. transversus.

	<i>Latimeria</i> (5 m.)	<i>Lepidosiren</i> (3 m.)	<i>Ambystoma</i> (4 m.)	<i>Timon</i> (3 m.)	<i>Ornithorhynchus</i> (12 m. - not ex. ear/int. snout*)	<i>Ex. marsupials + placentals</i> (27 m. - not ex. ear/int. snout*)	<i>Didelphis</i> (25 m. - not ex. ear/int. snout*)	<i>Peromyscus</i> (25 m. - not ex. ear/int. snout*)	<i>Caracropus</i> (26 m. - not ex. ear/int. snout*)	<i>Chirocephalus</i> (23 m. - not ex. ear/int. snout*)	<i>Hesperomys</i> (27 m. - not ex. ear*)
hyo. Y		---	---	---	---	---	---	---	---	---	---
arcus palatini		---	---	---	---	---	---	---	---	---	---
	De. man.		De. man.	De. man.	Styloideus	Stylohyoideus	Stylohyoideus	Stylohyoideus	Stylohyoideus	---	Stylohyoideus
	---	---	---	---	---	---	---	---	Jugulohyoideus	Jugulohyoideus	---
	---	---	---	---	---	Digastricus posterior	Digastricus posterior	Digastricus posterior	Digastricus posterior	Digastricus posterior	Digastricus posterior
meria's 'le.oper.'	---	---	---	---	---	---	---	---	---	---	---
operculi	---	---	---	---	---	---	---	---	---	---	---
	---		Bra.+Cerat.	---	---	---	---	---	---	---	---
	Le. hyoideus		Le. hyoideus	Le. hyoideus poar.)	Stapedius	Stapedius	Stapedius	Stapedius	Stapedius	Stapedius	Stapedius
	---	---	---	Cervicomandibularis	Platysma cervicale	Platysma cervicale	Platysma cervicale	Platysma cervicale	Platysma cervicale	Platysma cervicale	---
	---	---	---	---	Platysma myoides	Platysma myoides	Platysma myoides	Platysma myoides	Platysma myoides	Platysma myoides	Platysma myoides
	---	---	---	---	---	Occipitalis	Occipitalis	Occipitalis	Occipitalis	Occipitalis	Occipitalis
	---	---	---	---	---	Auricularis posterior	Auricularis posterior	Aur. posterior	Auricularis posterior	Auricularis posterior	Auricularis posterior
	---	---	---	---	Ex. ear mus.*	Ex. ear mus.*	Ex. ear mus.*	Ex. ear mus.*	Ex. ear mus.*	Ex. ear mus.*	Ex. ear mus.*
	---	---	---	---	Mandibulo-auricularis	Mandibulo-auricularis	Mandibulo-auricularis	Mandibulo-auricularis	Mandibulo-auricularis	---	---
	---	---	---	---	---	---	---	---	---	---	Risorius
hyoideus	Interhyoideus		Interhyoideus	Interhyoideus	Interhyoideus prof.	---	---	---	---	---	---
	---	---	---	---	Sphincter colli supe.	Sphincter colli supe.	---	Sphincter colli supe.	Sphincter colli supe.	---	---
	---	---	---	---	---(colli prof. in Echidna)	Sphincter colli prof.	Sphincter colli prof.	Sphincter colli prof.	Sphincter colli prof.	Sphincter colli prof.	---
	---	---	---	---	---	---	---	Stemofacialis	---	---	---
	---	---	---	---	Cervicalis tra.	---	---	---	---	---	---
	---	---	---	---	---	Interscutularis	Interscutularis	Interscutularis	---	---	---
	---	---	---	---	---	Zygomaticus major	Zygomaticus major	Zygomaticus major	Zygomaticus major	Zygomaticus major	Zygomaticus major
	---	---	---	---	---	Zygomaticus minor	Zygomaticus minor	Zygomaticus minor	Zygomaticus minor	Zygomaticus minor	Zygomaticus minor
	---	---	---	---	---	Frontalis	Frontalis	Orbito-temporo-aur.	Frontalis	---	Frontalis
	---	---	---	---	---	Auriculo-orbitalis	Auriculo-orbitalis	---	Auriculo-orbitalis	Auriculo-orbitalis	Temporoparietalis
	---	---	---	---	---	---	---	---	---	---	Auricularis anterior
	---	---	---	---	---	Auricularis superior	Auricularis superior	---	Auricularis superior	Auricularis superior	Auricularis superior
	---	---	---	---	Orbicularis oculi	Orbicularis oculi	Orbicularis oculi	Orbicularis oculi	Orbicularis oculi	Orbicularis oculi	Orbicularis oculi
	---	---	---	---	---	---	---	Zygomatico-orbicularis	Zygomatico-orbicularis	---	---
	---	---	---	---	---	---	---	---	---	---	De. supercillii
	---	---	---	---	---	Corrugator supercillii	Corrugator supercillii	---	Corrugator supercillii	Corrugator supercillii	Corrugator supercillii
	---	---	---	---	---	Re. anguli oculi lateralis	Re. anguli oculi lateralis	---	---	---	---
	---	---	---	---	Naso-labialis	Le. labii sup. al. nasi	Le. labii sup. al. nasi	Le. labii sup. al. nasi	Le. labii sup. al. nasi	Le. labii sup. al. nasi	Le. labii sup. al. nasi
	---	---	---	---	---	---	---	---	---	---	Procerus?
	---	---	---	---	Buccinatorius	Buccinatorius	Buccinatorius	Buccinatorius	Buccinatorius	Buccinatorius	Buccinatorius
	---	---	---	---	---	Dilatator nasi	Dilatator nasi	---	---	---	---
	---	---	---	---	---	Le. labii sup.	Le. labii sup.	Le. labii sup.	Le. labii sup.	Le. labii sup.	Le. labii sup.
	---	---	---	---	---	Nasalis	---	---	---	---	Nasalis



Table 4. Scheme illustrating the authors' hypotheses regarding the evolution and homologies of the hypobranchial muscles of adults of representative sarcopterygian taxa (see caption of Table 1). 'GENIOHYOIDEUS', 'RECTUS CERVICIS' mean 'geniohyoideus' and 'rectus cervicis' groups of Edgeworth (1935). in. to. intrinsic muscles of the tongue; m. muscles.

	<i>Latimeria</i> (2 m.)	<i>Lepidosiren</i> (2 m.)	<i>Ambystoma</i> (6 m. - not in. to.)* (5 m. - not in. to.)*	<i>Timon</i> (6 m. - not in. to.)*	<i>Ornithorhynchus</i> (6 m. - not in. to.)*	<i>CA marsupials + placentals</i> (8 m. - not in. to.)* (8 m. - not in. to.)*	<i>Didelphis</i> (8 m. - not in. to.)*	<i>Rattus</i> (8 m. - not in. to.)*	<i>Tupaia</i> (8 m. - not in. to.)*	<i>Cynocephalus</i> (6 m. - not in. to.)* (8 m. - not in. to.)*	<i>Homo</i> (8 m. - not in. to.)*
Coracohyoideus 'GENIOHYOIDEUS'	mandibularis	Coracomandibularis	Geniohyoideus	Geniohyoideus	Geniohyoideus	Geniohyoideus	Geniohyoideus	Geniohyoideus	Geniohyoideus	Geniohyoideus	Geniohyoideus
	---	---	Genioglossus	Genioglossus	Genioglossus	Genioglossus	Genioglossus	Genioglossus	Genioglossus	Genioglossus	Genioglossus
	---	---	In. mus. tongue*	In. mus. tongue*	In. mus. tongue*	In. mus. tongue*	In. mus. tongue*	In. mus. tongue*	In. mus. tongue*	In. mus. tongue*	In. mus. tongue*
	---	---	Hyoglossus	Hyoglossus	Hyoglossus	▶ Hyoglossus	Hyoglossus	Hyoglossus	Hyoglossus	Hyoglossus	Hyoglossus
	---	---	---	---	---	▲ Styloglossus	Styloglossus	Styloglossus	Styloglossus	Styloglossus	Styloglossus
	---	---	Interradialis	---	---	---	---	---	---	---	---
Sternohyoideus 'RECTUS CERVICIS'	Sternohyoideus	Sternohyoideus	Sternohyoideus	Sternohyoideus	Sternohyoideus	▶ Sternohyoideus	Sternohyoideus	Sternohyoideus	Sternohyoideus	Sternohyoideus	Sternohyoideus
	---	---	Omohyoideus	Omohyoideus	Omohyoideus	Omohyoideus	Omohyoideus	Omohyoideus	Omohyoideus	Omohyoideus	Omohyoideus
	---	---	---	---	Sternothyroideus	◀ Sternothyroideus	Sternothyroideus	Sternothyroideus	Sternothyroideus	Sternothyroideus	Sternothyroideus
	---	---	---	---	---	▲ Thyrohyoideus	Thyrohyoideus	Thyrohyoideus	Thyrohyoideus	Thyrohyoideus	Thyrohyoideus

Table 8. Hypotheses on the evolution/homologies of ventral/flexor leg muscles of adults of representative tetrapod taxa (see caption of Table 7). d. digits; fle. flexor; fle.cr.ta.fi. flexor cruris tarsi fibularis; fle.cr.ta.ti. flexor cruris tarsi tibialis; fle.dig.com. flexor digitorum communis; fle.ti.ex. flexor tibialis externus; ga. gastrocnemius; ga.in. gastrocnemius internus; h. head(s); ha.fa. fascial insertion of hamstring muscles; Inc. includes; mar.+pla. marsupials + placentals; sup. superficial.

	<i>Ambystoma</i> (6 m.)	<i>Timon</i> (6 m.)	<i>Ornithorhynchus</i> (6 m.)	<i>LCA mar. + pla.</i> (8 m.)	<i>Didelphis</i> (7 m.)	<i>Rattus</i> (7 m.)	<i>Homo</i> (8 m.)
(fle.cr.ta.ti. seems included in ischioflexor (fl).cr.ta.ti. seems included in fl.ti.ex. & eventually ga.in)	---	---	---	---	---	---	---
	---	Gastrocnemius internus ('femorotibial')	Gastrocnemius internus	Gastrocnemius internus	Gastrocnemius internus	Gastrocnemius (medial and lateral)	Gastrocnemius (med.&lat. h.)
	---	Gastrocnemius externus ('femoral'; sup. & deep h.)	Gastrocnemius externus	Gastrocnemius externus	Gastrocnemius externus	Soleus	Soleus
	---	---	---	Plantaris	Plantaris	Plantaris	Plantaris
fle.dig.com. (d.1-5; includes fl.c.ta.fi.)	---	Fle. digitorum longus (d. 1-5)	Fle. digitorum longus (d. 1-5)	Fle. digitorum longus (d. 1-5)	Fle. digitorum longus (d. 1-5)	Fle. digitorum longus (d. 1-5)	Fle. digitorum longus (d. 2-5)
long flexor	---	---	---	---	---	---	Fle. hallucis longus (d. 1)
	Fle. accessorius medialis	--- ('fle. accessorius' is distal h. of fle. digitorum longus)	Quadratus plantae	Quadratus plantae	Quadratus plantae	Quadratus plantae (or derived from foot m.?)	Quadratus plantae (med.&lat. h.)
	Fle. accessorius lateralis	---	---	---	---	---	---
	Contraheantum caput longum	---	---	---	---	---	---
	---	Popliteus	Popliteus	Popliteus	---	Popliteus	Popliteus
	Interosseus cruris	Interosseus cruris	--- (but present in Echinidnas)	Interosseus cruris	Interosseus cruris	---	---
	Tibialis posterior ('pronator profundus')	Tibialis posterior ('pronator profundus')	Tibialis posterior	Tibialis posterior	Tibialis posterior	Tibialis posterior	Tibialis posterior

Table 9. Hypotheses regarding the evolution and homologies of the foot muscles of adults of representative tetrapod taxa (see caption of Table 7). d. digits; di. digitorum; fle. flexor; fles. flexores; f.b.p. flexores breves profundi; h. heads; int. interossei; interm. intermetatarsales; m. muscle(s); mar. + pla. marsupials and placentals; me. metatarsal(s); obli. oblique.

	<i>Ambystoma</i> (32 m.)	<i>Timon</i> (25 m.)	<i>Ornithorhynchus</i> (21 m.)	<i>LCA mar.+ pla.</i> (25 m.)	<i>Didelphis</i> (25 m.)	<i>Rattus</i> (22 m.)	<i>Homo</i> (19 m.)
fles. breves superficiales (5 m. d.1-5)	Fles. breves superficiales (5 m. d.1-5)	Fles. breves superficiales (3 m. d.1-3)	Fle. digitorum brevis (d.2-4)	Fle. digitorum brevis (d.2-5)	Fle. digitorum brevis (d.2-5)	Fle. digitorum brevis (d.2-4)	Flexor digitorum brevis (d.2-5)
	---	Lumbricales (2 m. d.3-4)	---	Lumbricales (4 m. to d.2-5)	Lumbricales (4 m. to d.2-5)	Lumbricales (4 m. to d.2-5)	Lumbricales (4 m. to d.2-5)
abductor digiti minimi (me. 5)	Abductor digiti minimi (me. 5)	Abductor digiti minimi (me. 5)	Abductor digiti minimi (d.5)	Abductor digiti minimi (d.5)	Abductor digiti minimi (d.5)	Abductor digiti minimi (d.5)	Abductor digiti minimi (d.5)
	---	---	---	Abductor hallucis (d.1)	Abductor hallucis (d.1)	---	Abductor hallucis (d.1)
	---	---	---	---	---	---	Interosseus plantaris hallucis (d.1)
contrahentes pedis (5 m. to d.1-5)	Contrahentes pedis (5 m. d.1-5)	Contrahentes pedis (5 m. to d.1-5)	Contrahentes pedis (5 m. to d.1-5)	Contrahentes pedis (4 m.; d.1, 2, 4, 5)	Contrahentes pedis (4 m. to d.1, 2, 4, 5)	Contrahentes pedis (2 m. to d.2, 5)	---
	---	---	---	---	---	---	Adductor hallucis (obli.&transverse h.)
	---	---	Fle. digiti minimi brevis (=f.b.p.10, to d.5)	Fle. digiti minimi brevis (to d.5)	Fle. digiti minimi brevis (2h. to d.5)	Fle. digiti minimi brevis (d.5)	Fle. digiti minimi brevis (d.5)
	---	---	Fle. brevis profundus 2 (fibular side d.1)	Flex. brevis profundus 2 (d.1)	Fle. brevis profundus 2 (d.1)	Fle. brevis profundus 2 (d.1)	Fle. brevis profundus 2 (d.1)
	---	---	Fle. hallucis brevis (=f.b.p.1, tibial side d.1)	Fle. hallucis brevis (d.1)	Fle. hallucis brevis (=f.b.p.1, d.1)	Fle. hallucis brevis (d.1)	Fle. hallucis brevis (d.1)
es. digitorum minimi (4 m. d.2-5)	---	---	---	---	---	---	---
terphalangis digiti 3 (1 m. d.3)	---	---	---	---	---	---	---
terphalangis digiti 4 (2 m. d.4)	---	---	---	---	---	---	---
es. breves profundi (10 m. d.1-5)	Fles. breves profundi (10 m. d.1-5)	Fles. breves profundi (10 m. d.1-5)	Fles. breves profundi (3-9; 7 m. to d.2-5)	Fles. breves profundi (3-9; 7 m. to d.2-5)	Fles. breves profundi (3-9; 7 m. to d.2-5)	Fles. breves profundi (3-9; 7 m. to d.2-5)	Int. plantares (3 f.b.p. to d.3,4,5, =3 m.)
	---	---	---	---	---	---	Int. dorsales (4 f.b.p. + 4 interm. =4 m.)
intermetatarsales (4 m. between m.1-5)	Intermetatarsales (4 m. between m.1-5)	Intermetatarsales (4 m. between m.1-5)	Intermetatarsales (4 m. between d.1-5)	Intermetatarsales (4 m. between d.1-5)	Intermetatarsales (4 m. between d.1-5)	Intermetatarsales (4 m. between d.1-5)	---

Table 10. Hypotheses regarding the evolution and homologies of the dorsal/extensor muscles of adults of representative tetrapod taxa (see caption of Table 7). abd. abductor; d. digits; digito, digitorum; ex. extensor; exs. extensores; h. heads; m. muscle(s); mar. + pla. marsupials and placentals; me. metatarsal(s); monot. monotremes.

	<i>Ambystoma</i> (9 m.)	<i>Timon</i> (9 m.)	<i>Ornithorhynchus</i> (6 m.)	<i>LCA mar. + pla.</i> (9 m.)	<i>Didelphis</i> (8 m.)	<i>Rattus</i> (8 m.)	<i>Homo</i> (8 m.)
ex. digito. longus (me. 1-5)	---	---	Ex. hallucis longus (d. 1)	Ex. hallucis longus (d. 1)	Ex. hallucis longus (d. 1)	Ex. hallucis longus (d. 1)	Ex. hallucis longus (d. 1)
	---	Ex. digito. longus (me. 2-3)	Ex. digito. longus (d. 2-5)	Ex. digito. longus (d. 2-5)	Ex. digito. longus (d. 2-5)	Ex. digito. longus (d. 2-5)	Ex. digito. longus (d. 2-5)
	---	---	---	---	---	---	Fibularis tertius (me. 5)

long extensors	Ex. tarsi tibialis	Tibialis anterior	Tibialis anterior	Tibialis anterior	Tibialis anterior	Tibialis anterior	Tibialis anterior
	Ex. cruris tibialis	---	---	---	---	---	---
short extensors	Ex. cruris et tarsi fibularis	Fibularis longus	Fibularis longus	Fibularis longus	Fibularis longus	Fibularis longus	Fibularis longus
	---	Fibularis brevis	---	Fibularis brevis	Fibularis brevis	Fibularis brevis	Fibularis brevis
	---	---	---	Fibularis digiti quarti (me.4/d.4)	---	Fibularis digiti quarti (me.4 rats; d.4 mice)	---
	---	---	Fibularis digiti quinti (short ex. d. 5; me.5/d.5)	Fibularis digiti quinti (me.5/d.5)	Fibularis digiti quinti (d.5)	Fibularis digiti quinti (me.4 rats; d.5 mice)	---
	Ex. digito. breves (4m. to d.2-5)	Exs. digito. breves (4 m. d. 2-5 + part short ex. d.1)	Ex. digito. brevis (1 m. to d. 1-4 or 1-5?)	Ex. digito. brevis (1 m. to d.2-3)	Ex. digito. brevis (1m.; d.2-4)	Ex. digito. brevis (1 m. to d. 2-3)	Ex. digito. brevis (1 m. d.2-4)
	---	---	---	Ex. hallucis brevis (d.1)	Ex. hallucis brevis (d.1)	---	Ex. hallucis brevis (d.1)
abd. et ex. digiti 1 (d.1; abd.+ short ex. d.1)	Abd. et ex. digiti 1 (d.1; abd. + part short ex. d.1)	---	---	---	---	---	