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Gabby Neves Guilhon

MYOLOGICAL EVOLUTION OF NEOTROPICAL MARSUPIALS

Belo Horizonte
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Orientador: Prof. Dr. Fernando Araújo Perini

Coorientadora: Profa. Dra. Stephanie Pierce

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Myological evolution of Neotropical Marsupials

GABBY NEVES GUILHON

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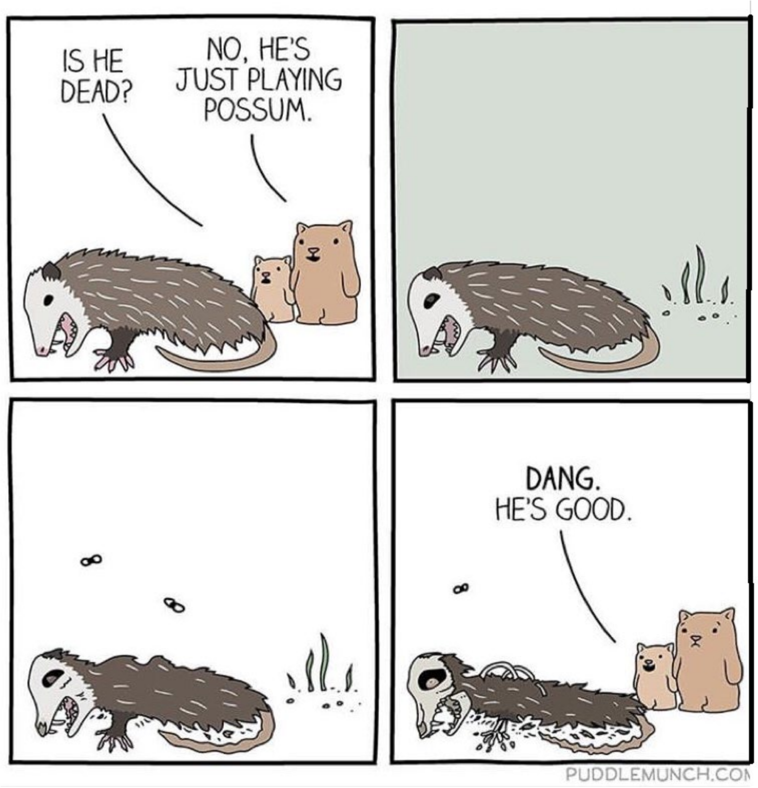

To my inner child, who was already fascinated by natural life (and all the weird stuff that is accompanied by it…).

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"It seems to me that the natural world is the great source of excitement: the greatest source of visual beauty, and it is the greatest source of so much in life that makes life worth living. An understanding of the natural world, and what is in it as a source of not only great curiosity, but great fulfilment." – Sir David Attenborough

RESUMO

A musculatura anatômica dos mamíferos marsupiais é pouco explorada, tendo algumas descrições da espécie mais estudada, o gambá da Virgínia (*Didelphis virginiana*), e poucas outras espécies descritas além desta. O objetivo desta tese é (1) descrever o estado da arte da musculatura dos membros apendiculares de marsupiais Neotropicais; (2) contribuir para o conhecimento existente do grupo com a descrição da miologia dos membros de algumas espécies do grupo e, (3) discutir a relação da morfologia funcional e dos hábitos de locomoção das espécies estudadas. Foi observado que a musculatura apendicular possui diversas modificações morfológicas (inserções, tamanho, peso, quantidade) que podem refletir adaptações ecomorfológicas do grupo. Algumas dessas características, no entanto, parecem estar mais relacionadas a um componente filogenético. É sugerido aqui que a musculatura dos membros dos marsupiais Neotropicais está mais associada com o hábito locomotor de cada espécie do que a filogenia do grupo, porém, faz-se necessário análises evolutivas utilizando os dados de caracteres miológicos aqui descritos para a descrição do estado ancestral e o mapeamento filogenético dessas informações.

Palavras-chave: anatomia, evolução morfológica, morfologia funcional.

ABSTRACT

The muscular anatomy of marsupial mammals is not well explored, with descriptions of the key species *Didelphis virginiana* (the Virginia opossum) and a few more species described. The goal of this thesis is to (1) describe the state of art of the appendicular myology of Neotropical marsupials; (2) contribute to the existing knowledge of the group with new muscular description of rare species, and (3) to discuss the association of functional morphology and locomotion habits of those species. We observed that the appendicular myology presented several morphological variations (insertions, size, weight, quantity) that might reflect ecomorphological adaptations in the group. Some of these features, however, might be more related with a phylogenetic component. We suggest that the appendicular myology of Neotropical marsupials is more associated with their habits than their phylogenetic history, but evolutionary analysis is necessary, using the myological information provided in this study, to describe the ancestral state and the phylogenetic mapping of these information.

Keywords: anatomy, morphological evolution, functional morphology.

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Sumário

THESIS INTRODUCTION

Marsupials are a group of mammals mainly characterized by their distinct reproduction and reproductive features. They have a short gestation period followed by extensive lactation, sometimes inside a pouch, also called marsupium. However, despite what the group name implies, several marsupials do not have a pouch. Their litter is composed by altricial neonates, which means that they have an incipient state of development at birth, being excessively small, blind, and without fur – but with the ability to climb the mother's inguinal region until they find a teat to attach to and complete its development (Tyndale-Biscoe and Renfree 1987, Renfree and Shaw 1996, Guilhon 2020).

Marsupials are divided in two cohorts, "Ameridelphia" and Australidelphia. This division reflect, in part, their geographic distribution, with ameridelphians occurring in the Americas and australidelphians in Australasia. However, this classification is also supported by morphological characters, particularly those associated to tarsal morphology (astragalus and calcaneum), in which Australian marsupials would have a different morphology from American marsupials' tarsals, suggesting that the first group are monophyletic. However, molecular data does not support the monophyly of Ameridelphia, with Australidelphia being recovered as a monophyletic group inserted in an "ameridelphian" radiation (Frankham and Temple-Smith 2012, Mitchell *et al.* 2014, May-Collado *et al.* 2015, Duchêne *et al.* 2018). Australidelphia has five orders: Dasyuromorphia, Diprotodontia, Notoryctemorphia, Peramelemorphia, and Microbiotheria. Most of them occur in the Australasia region (Wilson and Mittermeier 2015), except for Microbiotheria, which, despite belonging to the Australidelphia cohort, occurs only in South America, in southern Andean forests, being sometimes considered an Australian or Antarctic radiation survivor (Guilhon 2020).

Although they are mostly associated to Australia, a significant amount of marsupial diversity occurs in the Americas, with two living orders being exclusive to this continent, Didelphimorphia and Paucituberculata (Wilson and Mittermeier 2015). These two orders, together with Microbiotheria, include all living American marsupials and will be the focus of this thesis. Order Paucituberculata includes a single family, Caenolestidae, with three genera and six species, while Microbiotheria also includes just one family, Microbiotheriidae, which is monotypic (Palma and Valladares-Gómez 2015, Patterson 2015). However, most of extant Neotropical marsupials are included in Didelphimorphia, which also includes a single family, Didelphidae, with 18 genera and 125 species (Voss 2022). Didelphids are widely distributed in almost all biomes of the New World, ranging from southern Canada to Argentinian Patagonia, but occurring mainly in tropical South America. In contrast, caenolestids and microbiotheriids are restricted to the Andean area, occurring in different altitudes in Chile, Ecuador, Peru and Colombia (Eisenberg and Redford 1999, Gardner 2007).

It is interesting to notice that Microbiotheria, despite occurring exclusively in South America, possess an "australidelphian" tarsal morphology, being included in this group (Szalay 1982). More recently, molecular phylogenies suggested that "Ameridelphia" (including only Didelphimorphia and Paucituberculata) is a paraphyletic group, being successive radiations in relation to the monophyletic Australidelphia, and added more evidence for the inclusion of Microbiotheria in the latter (Frankham and Temple-Smith 2012, Mitchell *et al.* 2014, May-Collado *et al.* 2015, Duchêne *et al.* 2018) (**Figure 1**). Therefore, the tarsal morphology that defined "Ameridelphia" is now interpreted as retained primitive characteristics for marsupials (Horovitz and Sánchez-Villagra 2003). Currently, these relationships are supported by both molecular and morphological analyses, as well the monophyly of all the traditionally recognized orders, although there are still some divergencies about the phylogenetic position of Notoryctemorphia and Microbiotheria (Nilsson *et al.* 2003, Asher *et al.* 2004, Ladèveze and Muizon 2010, May-Collado *et al.* 2015). However, these phylogenies frequently use different sources of data, including mitochondrial and nuclear genes in molecular analyses, but mainly dental and osteological characters in morphological analyses. Therefore, myological information is constantly neglected or underused (Horovitz and Sánchez-Villagra 2003), in part due to the lack of knowledge about marsupial muscle anatomy.

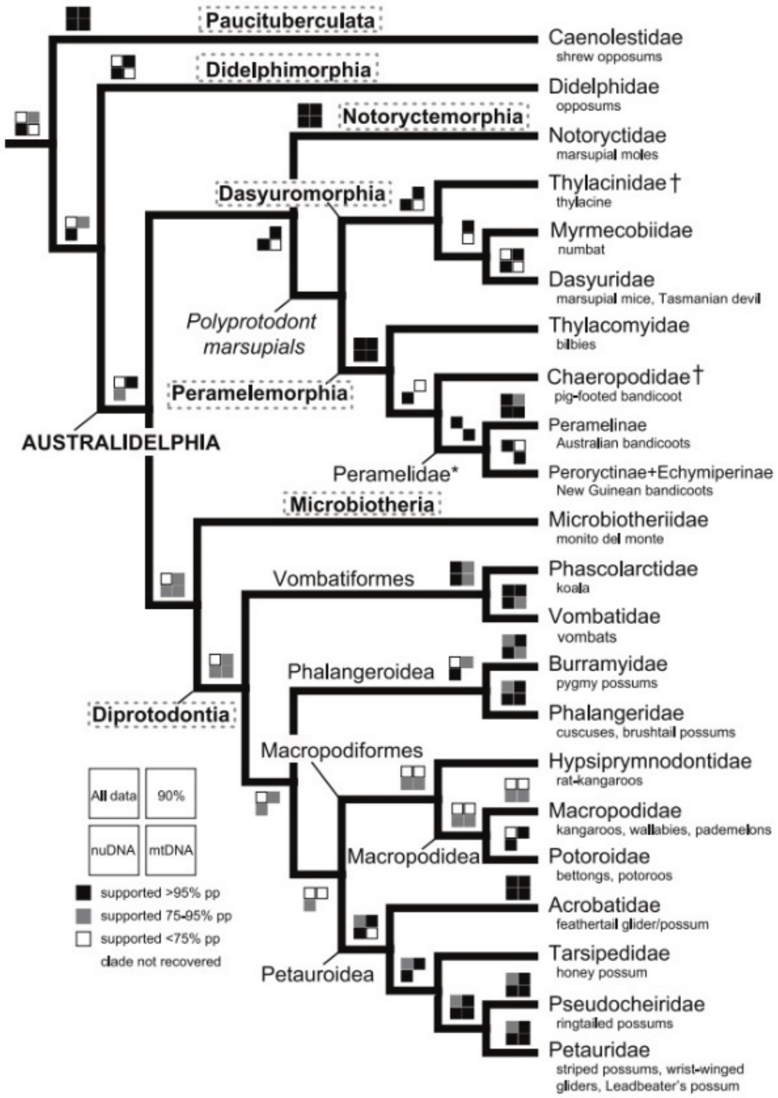


Figure 1. Cladogram adapted from May-Collado *et al.* (2015) summarizing the relationships among marsupial groups. The grey dashed names correspond to the seven main orders cited in the text.

Since the first descriptive anatomical studies of Neotropical marsupials, myological research has been focused on the Virginia opossum, *Didelphis virginiana* (Kerr, 1752) (Tyson 1698, Coues 1872, Elftman 1929, Stein 1981, Hazimihalis *et al.* 2013, Diogo *et al.* 2016). This is probably due to this species being abundant and well represented in mammal collections throughout the world and especially in North America, where a large number of researchers are based, besides being also frequently used as model in several biological and medical studies (Krueger *et al.* 2016, Zecca *et al.* 2020). The few studies of comparative myology that include other didelphids are mostly about differences in functional morphology, for example, between the semi-aquatic *Chironectes minimus* (Zimmermann, 1780) and, once again, the scansorial *Didelphis virginiana* (Stein 1981), or about functional morphology inferences of extinct marsupials (*Mayulestes ferox* and *Pucadelphys andinus*) using extant genera as *Caluromys*, *Monodelphis*, and *Metachirus* as models (Argot 2001, 2002, 2003). Among other American marsupials, there is an early monograph describing a single specimen of *Caenolestes fuliginosus* (Tomes, 1863), a Paucituberculata (Osgood 1921), and there are virtually no myological studies about microbiotherids so far (summary of myological publications of American marsupials, **Table 1**). Most information of marsupial myology available comes from Australasian species, with one of the oldest studies being the description of the forelimbs and hindlimbs of *Dasyurus viverrinus* (Shaw, 1800) (MacCormick 1886, 1887), followed by an anatomical atlas of the brush-tailed opossum *Trichosurus vulpecula* (Kerr, 1792) (Barbour 1963). Since then, several myological descriptions and functional morphology studies of Australian marsupials appeared, as the anatomical description of the tail and the association of muscle proportion of members with sexual dimorphism of the kangaroo *Macropus fuliginosus* Desmarest, 1817 (Dawson *et al.* 2014, Richards *et al.* 2015), types of muscle fibers of short and long members of the quokka *Setonix brachyurus* (Quoy and Gaimard, 1830) (Casinos *et al.* 2016), and many studies of comparative functional morphology of marsupials with different habits as tree-kangaroos (*Dendrolagus* spp.) and the marsupial mole (*Notoryctes typhlops* Stirling, 1889) (Warburton 2006, 2009, 2012, Warburton and Marchal 2017). This emphasis of studies in Australian taxa may be attributed to the higher diversity of marsupials in this region when compared to Neotropical marsupials, more researchers interested in the subject, and more resources to invest in these studies.

Table 1. Myological data references for American marsupials. (*) Species cited were dissected in the context of locomotion reconstruction of extinct species, but with no formal or detailed description. (×) Only flexor tendons of the manus were analyzed.

Order/species	**References**
Didelphimorphia	
Caluromys philander (Linnaeus, 1758)	Argot 2001, 2002, 2003 (*)
Caluromys derbianus (Waterhouse, 1841)	Abdala *et al.* 2006 (×)
Chironectes minimus (Zimmermann, 1780)	Sidebotham 1885, Augustiny 1942, Stein 1981, Abdala *et al.* 2006 (×)
Cryptonanus chacoensis (Tate, 1931)	Abdala *et al.* 2006 (×)
Didelphis virginiana (Kerr, 1752)	Tyson 1698, Coues 1872, Cheng 1955, Jenkins and Weijs 1979, Stein 1981, Diogo *et al.* 2016
Didelphis marsupialis Linnaeus, 1758	Argot 2001, 2002, 2003 (*)
Didelphis albiventris Lund, 1840	Abdala *et al.* 2006 (×)
Lutreolina crassicaudata Desmarest, 1804	Delupi al. 1997, Abdala *et al.* 2006 (×), Guilhon *et al.* (present study)
Marmosa constantiae (Thomas, 1904)*, M. demerarae* (Thomas 1905)*, M. murina* (Linnaeus, 1758)*, M. paraguayanus* (Tate, 1931)*, M. robinsoni* Bangs, 1898	Argot 2001, 2002, 2003 (*), Abdala *et al.* 2006 (×)
Marmosops fuscatus (Thomas, 1896)	Abdala *et al.* 2006 (×)
Monodelphis brevicaudata (Erxleben, 1777)*, M. dimidiata* (Wagner, 1847)*, M. domestica* (Wagner, 1842)	Argot 2001, 2002, 2003 (*), Abdala *et al.* 2006 (×), Wright *et al.* 2022

Metachirus myosurus (É. Geoffroy, 1803)	Argot 2001, 2002, 2003 (*), Abdala *et al.* 2006 (×)
Philander opossum (Linnaeus, 1758)	Abdala *et al.* 2006 (×)
Thylamys elegans (Waterhouse, 1839), *T. venustus* Thomas, 1902, *T. pallidior* (Thomas, 1902), *T. pusillus* (Desmarest, 1804)	Mann-Fischer 1951, 1953, 1956; Abdala *et al.* 2006 (×)
Paucituberculata	
Caenolestes fuliginosus (Tomes, 1863)	Osgood 1921
Microbiotheria	
-	-

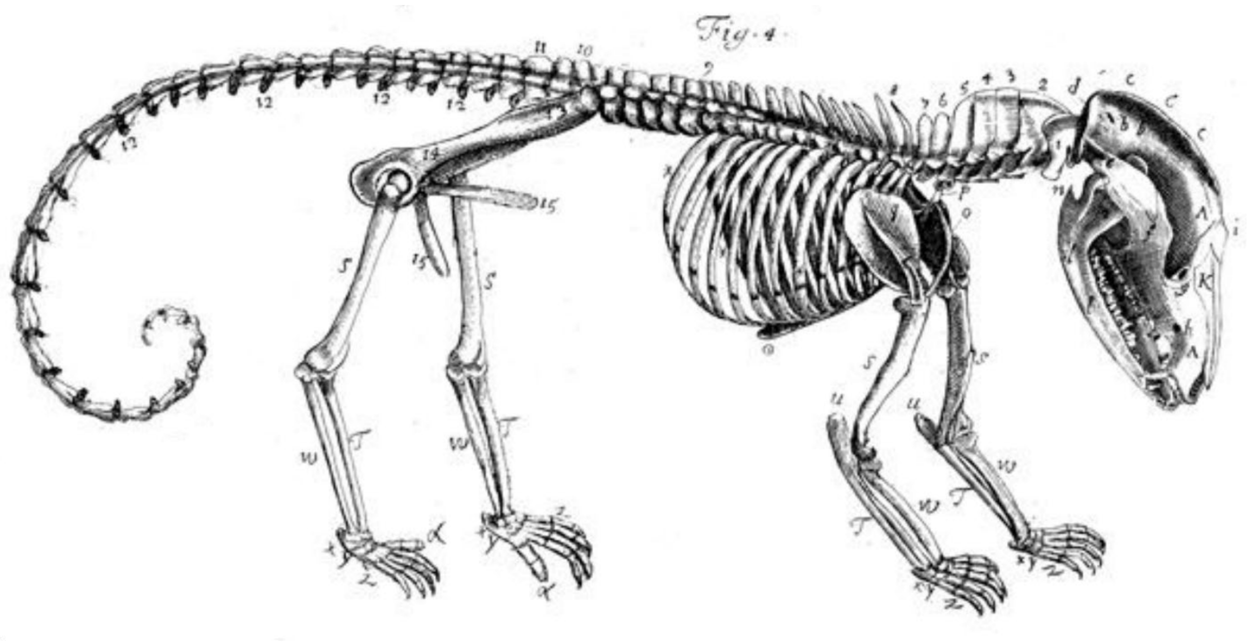
It is possible to gather from this scenario that not only the association of Neotropical marsupial's form and function remains understudied (Astúa and Guilhon 2022), but also their myology, in both descriptive and functional terms, as well its use in biomechanical and phylogenetic analyses. The most recent and complete study concerning evolutionary perspectives about marsupial myology compared their musculature with placental mammals and, in addition, provided standard nomenclature for its muscles (Diogo *et al.* 2016). However, once again, only *Didelphis virginiana* was used as a marsupial model for this study, in a "mammal spectrum" from monotremes to placentals.

It is important to notice that anatomical descriptions allow us to infer myological characteristics which could occur in species with different locomotory habits, including extinct species (Argot 2001, 2002, 2003). American marsupials present a variety of types of locomotion as arboreal, scansorial, terrestrial and semi-aquatic (Paglia 2012, Astúa 2015), the latter being unique among all marsupials (*Chironectes minimus*). It is possible that the group shows a considerable variation in their muscular structure which could be related both to functional adaptations and its evolutionary phylogenetic history. Nonetheless, the comprehension of myology evolution in marsupials is hampered due to the absence of basic descriptive studies.

Currently, molecular data is outdoing morphological data as the preferred source for phylogenetic reconstructions. Among mammals, osteological characters are usually more prevalent when compared to other morphological complexes in phylogenetic studies, despite myological characters showing great potential to generate informative data, or at least as much as osteological or molecular data. Diogo (2012) analyzed 81 bony structures, extracting 198 osteological characters, but also 63 muscular complexes, extracting 122 myological characters. The retention and consistency index for myological characters were higher than osteological data, showing the potential of myology characters for phylogenetic reconstruction. However, musculature remains poorly explored in studies with mammals in general, and marsupials in particular. There is only a single work concerning hominid evolution focused on myology as a major source for phylogenetic characters (Diogo *et al.* 2012). It is possible that this lack of studies occurs due to the resistance of researchers to use myological information given its nomenclature inconsistency, or by the domain of osteological data as the main source of morphological characters (Borden 1999). Besides, the usual modes of mammal preservation do not allow the preservation of the musculature, since usually only skins, skulls and skeletons are kept, with few non-volant mammal specimens stored in spirit collections.

This thesis is divided in three chapters. The first is a review of didelphid marsupial form and function, that has already been published as a book chapter and summarized here in the skeletal and myological topics only. The second chapter is the muscular anatomy description of the appendicular limbs of the thick-tailed opossum *Lutreolina crassicaudata*, which we expect to publish in two papers, one for each limb. The third chapter is a comparative anatomy study of both limbs of three species, one from each American marsupial order: *Dromiciops gliroides* Thomas, 1894 (Microbiotheria), *Lestoros inca* (Thomas, 1917) (Paucituberculata) and *Monodelphis domestica* (Wagner, 1842) (Didelphimorphia). The main objective is to review and contribute to the knowledge of Neotropical marsupial myology.

CHAPTER 1: Morphology, Form and Function in Didelphid Marsupials*



Didelphis virginiana from Tyson, 1698

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Morphology, Form and Function in Didelphid Marsupials

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ABSTRACT

Didelphids are considered morphologically conservative and unspecialized; however, information about different morphological systems is not equally available among its members. Recent research is unveiling variation in their morphology that can be related to function, in the skeleton or soft tissues. Yet, differences in didelphid tribosphenic molars correlate poorly with their diet (possibly due to highly overlapping diets across taxa), and variation in cranial and mandibular shape can be partially related to function, although allometry and phylogeny play major roles in constraining that variation. Morphological variation in post-cranial elements relates mostly to sexual dimorphism and locomotor habits, particularly in larger opossums, and osteological characters have been used for inferences about the locomotion habit of extinct taxa. Didelphid myology is generally similar within the group, but with some variation associated with adaptations to different locomotion habits (e.g., arboreal, scansorial, cursorial, and semi-aquatic). Gross digestive morphology also varies partially with diet in a few studied species, but digestive histology data are still too incipient for adequate form-function estimates. While the knowledge on reproductive anatomy is highly unbalanced, information on reproductive behavior is too scarce and anecdotal for reliable inferences on the function of known reproductive morphology. Overall, while the few better-studied taxa are revealing interesting patterns of relationships between morphology, function, and history in Didelphidae, the family still needs more detailed analyses, given its ecological and taxonomic diversity.

Keywords: Anatomy. Functional morphology. Evolution. Neotropical marsupials.

INTRODUCTION

Opossums (family Didelphidae) have traditionally been considered morphologically conservative, and for a long time, they were considered representative of primitive and unspecialized mammals (especially larger genera and particularly *Didelphis*), to be regarded as models for functional and biomechanical estimates for fossil taxa (Jenkins 1971, Jenkins and Weijs 1979). However, this was mostly based on a few functional systems, mostly cranial and postcranial skeletons and sometimes associated muscles. Even though, more recent and detailed analyses have revealed varying levels of specialization in this so-called unspecialized group, and have emphasized that in many cases the use of some of these taxa as models is inadequate (e.g. Argot 2003, Astúa 2009). Furthermore, didelphids show varied levels of ecological specialization, whether in food use, habitat use, or other niche variables (Astúa 2015, Goin *et al.* 2016, Guilhon 2020, Voss and Jansa 2021). Opossum anatomy has been studied at a greater or lesser depth for several taxa and anatomical systems (e.g., Argot 2001, Santori *et al.* 2004, Flores 2009, Voss and Jansa 2009, Diogo *et al.* 2016), but the morphology has not always been explicitly related to function. How much of the morphology of Didelphidae and its variation could be related to adaptive processes and how much is restricted to evolutionary or functional constraints varies depending on the studied system (e.g. Shirai and Marroig 2010, Chemisquy *et al.* 2015, 2021). How the existing variation in morphology across Didelphidae relates to its functionality has also been a matter of interest more recently (Astúa 2009, Chemisquy *et al.* 2015, Pilatti and Astúa 2017, Chemisquy *et al.* 2021). The amount of existing information on several aspects of the biology and evolution of Didelphidae has been constantly increasing (Astúa 2015), but an adequate review focused on their form, function, and the relationship between them is still lacking. Here we present a summary and review of the existing data on dental, cranial, and postcranial morphology, myology, and other soft systems of Didelphidae and, whenever possible, to relate it to existing information on their functions. For this thesis, this chapter differ from the published material, being presented in an abridged version focused only on aspects of bone and muscle anatomy, which correspond to the part written only by the second author (GNG). It is expected that this contribution could highlight which areas should be prioritized for both increasing the knowledge of the different morphological systems in didelphids and, consequently, for a better understanding of the relationship between form and function in this group, as well those which are paramount to increase and improve the current understanding on the biology, ecology, and evolution of New World opossums.

Post-Cranial Skeleton and Locomotion

As for all tetrapods, the post-cranial skeleton provides support, movement, and balance. In Didelphidae, it has been studied under phylogenetic and functional approaches (Flores 2009) and some of its features have been related to different locomotion habits, while other functions have been postulated for some unique features of opossum skeletons.

The orientation of the occipital condyles of terrestrial genera (e.g., *Metachirus* and *Monodelphis*) is more rostrocaudally oriented than in arboreal genera, such as *Caluromys* and *Marmosa* (Argot 2003). This morphology has been related to different habits and Argot (2003) suggested that it could help the species move in the three-dimensional arboreal substrate (coupled with supposedly forward-facing eyes in *Caluromys*). While this variation in cranial inflection had been also noted by Astúa de Moraes *et al.* (2000), it should be noted that no relation between orbit orientation and locomotion could be detected across all Didelphidae (Pilatti and Astúa 2017). The presence of hypertrophied spinous processes in cervical vertebrae, a synapomorphy of the genus *Didelphis* (Flores 2009), has been hypothesized to have an adaptive function (Giannini *et al.* 2011). While Argot (2003) could not relate such unique morphology to any specific locomotor requirements, Giannini *et al.* (2011) suggested that it would have acted as a protection against placental carnivores that usually kill prey through a bite on the neck. This hypothesis was supported by the existence of three specimens of two *Didelphis* species with signs of cervical fracture later healed. More posteriorly in the vertebral column, the transverse processes in the lumbar vertebrae in *Didelphis* and *Philander* are short and oriented anteriorly, with no prominences, while in *Caluromys* these processes are large and blade-shaped, reducing the available space for ligaments and fibers. This reduces the efficiency of the leverage of the *m. quadratus lumborum*, which provides ventral flexion, and also the efficiency of the epaxial musculature to resist ventral flexion of the spine in the arboreal form (Argot 2003). The number of vertebrae, seven cervical, 13 thoracic, six lumbar, and two sacral, is apparently constant across Didelphidae, with the exception are the caudal vertebrae, which are variable in number, as in other mammals (Argot 2003, Flores 2009). Inamassu *et al.* (2021), however, found considerable variation in vertebral counts when examining a large series of *Didelphis albiventris*, identifying three different vertebral formulae (that varied from five to seven lumbar vertebrae), and caudal vertebrae varying from 24 to 29.

The scapula in smaller didelphids has similar shapes, despite varying locomotion habits. However, larger didelphids present different shapes that can be associated with their locomotion, especially when arboreal and terrestrial genera are compared (Astúa 2009). Arboreal species have scapulae with large acromia cranially displaced, frequently covering the glenoid cavity, and distinct caudal angles with expanded vertebral borders. In terrestrial species, regardless of their size, the acromion is more caudally displaced and scapular blades are more oval-shaped, with the infraspinous fossa and the vertebral borders smaller than in arboreal species (Astúa 2009). The relative scapula total area is greater in *Metachirus* than in *Caluromys*, thus concentrating the muscular weight of the limb at the proximal end of the scapula in the former, reducing the moment of inertia of the limb. This could reflect a curso-saltatorial specialization in *Metachirus* (Argot 2001). In *Chironectes*, the scapula has a very different size and shape when compared with all other Didelphini, with a lower ratio of scapular width to scapular length, considered an adaptation to the semi-aquatic habit (Stein 1981). It also seems to be less influenced by body size as in other Didelphini, as its shape is more similar to smaller species, possibly due to its mostly aquatic foraging pattern (Astúa 2009, Bubadué *et al.* 2019). In smaller species, the similar scapula shape is seemingly efficient to perform several types of locomotion, such as terrestrial or arboreal, due to the similarity of functional challenges in these substrates and could be generally classified as scansorial (Astúa 2009). Despite the association with locomotor habits, the scapula morphology in didelphids is also strongly influenced by phylogenetic history, as phylogenetically close taxa tend to have more similar scapulae, and allometry, as some of the major axes of shape variation in the scapula correlate with size (Astúa 2009, Bubadué *et al.* 2019).

The humeral head and the glenoid cavity have also been related to the locomotion habits across major locomotor types in Didelphidae. *Metachirus* have the most anteroposteriorly elongated humeral head within didelphids, with a more hemispherical glenoid cavity than *Caluromys*, and these features are associated with cursoriality, promoting rapid limb motion (Argot 2001). In arboreal species like *Marmosa* and *Caluromys*, the trochlea has a great anterior development and is separated from the capitulum by a flat area, where the ulna is connected, and along with a widely open trochlear notch, the flexion and extension range of pronation and supination increases, helping them to catch new supports in an unstable substrate. For instance, in *Didelphis*, the trochlear notch is less open in the medial view than in *Caluromys*, suggesting a reduced range of movements in the former, while in *Metachirus* the same structure is also less open than *Caluromys*, but it has a deeper coronoid and olecranon fossa that does not restrict the movements excessively and stabilizes its position (Argot 2001).

The wrist of some arboreal and scansorial mouse opossums (such as *Marmosa* and *Marmosops*) exhibits remarkable sexual dimorphism, with distinct carpal tubercles in males, which are more developed in older individuals: a prominence on the radial side of the palm, supported by a modified prepollex, in *Marmosa*, or on the ulnar side, supported by a spoon-shaped pisiform, in *Marmosops* (Lunde and Schutt 1999). These tubercles have been related to the copulatory behavior, with the male holding the female hanging by the tail (Barnes and Barthold 1969, Harder 1992, Valtierra-Azotla and Garcia 1998). Aside from these genera, the usual morphology of Didelphidae wrists is the same between the sexes (Lunde and Schutt 1999). Arboreal opossums have relatively longer fingers than scansorial and terrestrial ones, and *Marmosa* and *Caluromys* have more prehensile extremities when compared to *Didelphis*, *Philander*, and *Monodelphis*, which has been related to the use of vines and fine branches (Lemelin 1999). These are gripped between the opposable hallux and the other pedal digits, and between the 2nd and 3rd digits of the manus in *Caluromys* and most opossum species. The hallux, however, did not show any variation that could be correlated to the differential use of fine supports. In *Chironectes*, the digits of the manus are slender and more elongated than in *Didelphis*, where they are short and thick (Stein 1981). In *Didelphis*, there are digital pads at the tip of each finger with interdigital pads at their bases, with a specific pattern that increases traction while gripping when the palm is ridged (Stein 1981). In *Chironectes*, the digital skin of the fingertips is remarkably different, with no papillary ridges, instead, it has epidermal finger-like cones radially arranged. The papillary ridge loss is associated with the complete loss of climbing activities, but these cones help the animal in receiving stimuli from any direction and improve its foraging performance underwater by touch (Hamrick 2001). In arboreal species, such as *Marmosa* and *Caluromys*, the digital and plantar pads are apparently larger and with more pronounced papillary ridges than terrestrial species such as *Metachirus*, *Monodelphis*, and *Philander*. This is probably associated with friction, but just a few didelphids presented these differences (Argot 2002, Voss and Jansa 2009). The palmar and plantar morphology varies in some arboreal and terrestrial didelphids, which might reflect the appropriate grip and activity in different substrates, but could also be constrained by phylogeny (Martin 2019).

The pelvic girdle in *Metachirus* is very unique, being the only among didelphids to have an iliac wing as a large blade and its ilium is deflected outwards in the anterior extremity, allowing the great development of the epaxial and rump musculature, associated with the saltatorial habit (Argot 2002, Flores 2009). It also has a deeper, rounded, and less open acetabulum than arboreal species such as *Caluromys* and *Marmosa*, which have a shallower and more open acetabulum (Flores 2009), a morphology that allows for a wider range of movements in the arboreal forms (Argot 2002). Epipubic bones are dimorphic in Didelphidae, with longer and more curved ones in females than in males (Guilhon 2017), but the function of such dimorphism is yet unknown. They are also related to ventilation and locomotion (Reilly *et al.* 2009). The size and shape of these bones allow the separation of arboreal didelphids (*Caluromys* and *Marmosa*), terrestrial (*Metachirus*), scansorial (*Didelphis* and *Philander*), and semi-aquatic (*Chironectes*) (Guilhon 2017). Nevertheless, the association of the epipubic bones with different habits needs further investigation.

The femur of *Chironectes* is remarkably different from *Didelphis*, to which it has been compared. It is relatively shorter in the former than in the latter relative to the total length of the leg. With a shorter femur, *Chironectes* can bring the entire hindlimb limb closer to the body during the stroke phase (Stein 1981), probably associated with bipedal paddling (Fish 1993). The lesser femoral trochanter is associated with locomotion habits in some didelphids, being more prominent medially in the arboreal *Caluromys* than the terrestrial *Metachirus*, where it is more posterior and reduced. Peculiarly, the same morphology can be verified between the small arboreal genus *Marmosa* and the terrestrial *Monodelphis*: the lesser trochanter is also smaller in the terrestrial form. This prominence emphasizes the ability of the medial femur rotation in the arboreal forms (Argot 2002).

All didelphids present a hindfoot with a powerful ability to grasp. In *Caluromys*, the first and the fourth metatarsals are very robust when compared with the others, different from *Metachirus*, with long metatarsals when compared with tibia length. This metatarsal extension increases the stride length when it is running and leaping. Although *Metachirus* is not a highly specialized runner, it could help in its terrestrial locomotion (Argot 2002). The webbed hindfoot in *Chironectes* is remarkably different, being much larger than *Didelphis* and increasing the surface area during swimming. When coupled with a larger length of the hallux, these characteristics result in a more symmetrical shape of the foot, being an advantage for swimming due to equal water pressure applied to the hindfoot while stroking (Stein 1981).

Cranial Muscles and Mastication

Studies on didelphid myology are mainly based on *Didelphis virginiana*. One of the first anatomical descriptions of *D. virginiana*, from the late seventeenth century, mentions only a few muscles and is more focused on external and internal gross anatomy (Tyson 1698). Since then, additional studies on the musculature of this and a few additional species of Didelphidae have been conducted (Coues 1872, Mann-Fischer 1953, Hiiemäe and Jenkins 1969, Minkoff *et al.* 1979, Smith 1994, Delupi *et al.* 1997, Lautenschlager *et al.* 2017). While earlier studies were mostly descriptive with no specific intent to relate the observed morphologies to function, more recent analyses were conducted specifically with this intent in mind, hence the unbalanced amount of information on morphologies when compared to their functional implications. Here the available information on the musculature of didelphids is briefly reviewed, and as in other systems, the existing information on its function is related whenever possible. For consistency, the myological nomenclature of Diogo *et al.* (2016) is mostly adopted.

Cranial muscles can be distinguished by their developmental origin and function. Some are responsible for the facial expression and integument movements, including those of the sense organs - for a more detailed description of the myology and development of these muscles in didelphids, see Minkoff *et al.* (1979) and Smith (1994), respectively. As in other mammals, the main didelphid mandibular muscles (often called muscles of mastication, innervated by the trigeminal nerve – cranial nerve CN V) are the masseter, temporalis, pterygoideus, digastricus (anterior belly; the posterior belly is a hyoid muscle, innervated by the facial nerve – CN VII), and the mylohyoideus. They all have an attachment at the mandible, either as an origin or as an insertion. The *m. masseter* and *m. temporalis* suspend and close the lower jaw against resistance. The *m. masseter* can also produce lateral movements working with the *m. pterygoideus medialis*, while the *m. pterygoideus lateralis* can induce rotation of the mandible. The *m. digastricus* inserts in the medial portion of the mandibular symphysis, lowering the jaw and flooring the mouth, while the mylohyoideus connect the hemimandibles and is indirectly attached to the hyoid via muscular fascia (Coues 1872). The *m. digastricus*, *m. geniohyoideus*, and *m. mylohyoideus* work as accessory masticatory muscles controlling the position of the hyoid bone for swallowing and also act as elevators of the tongue (Hiiemäe and Jenkins 1969). All these muscles not only work in opening and closing the mandible during biting, but also act in hemimandibular long-axis rotation during chewing (Bhullar *et al.* 2019).

The masticatory apparatus of *Thylamys* includes a highly developed supra-zygomatic part of the *m. temporalis*, which would help in the anteroposterior sliding of the lower jaw, a movement that is useful to break rigid elements as the exoskeleton of insects. This feature, absent in *Didelphis*, is useful during the grinding process and was considered a specialization for an insectivorous diet (Mann-Fischer 1953). In *Lutreolina*, the *m. temporalis* is well developed, filling a very deep temporal fossa, and the origin of its deeper part extends to the sagittal crest, being much larger than in *Didelphis*. The heavier muscle mass in *Lutreolina*, along with an increased surface of the temporal fossa, and a shorter of the rostral region, which increases mechanical advantage during biting, suggest *Lutreolina* may be a more efficient hunter than *Didelphis*, with a greater bite force (Delupi *et al.* 1997).

The *m. masseter* in *Thylamys* is separated in superficial and deep masseter but was considered indivisible and simple in *Didelphis* by Mann-Fischer (1953). However, Hiiemäe and Jenkins (1969) and Diogo *et al.* (2016) considered the *m. masseter* separated into two or four portions in *Didelphis*, respectively. The functional properties of this division are influenced by differences in fiber angles, which lead to different jaw movements such as retraction, elevation, and lateral displacement, although further specific studies in didelphids are needed to relate muscle composition and function to diet. In *Lutreolina*, the deep masseter is bulkier and larger than

Didelphis. In the former, it arises by tendinous fibers in the inferior border and in the medial surface of the zygomatic arch, it inserts in the inferior two-thirds in the masseteric fossa with fibers in ventral-caudal direction, and lies posterior to the origin of the *m. levator labii superioris* (lip lifting muscle). In *Didelphis*, the origin is the same, but with a longer insertion in the masseteric fossa, reaching the ventral part of the angular process, and it lies inferior to the lip lifting muscle. The result is that in *Didelphis* the zygomatic labial musculature is located superior to the *m. masseter* while in *Lutreolina* it lies rostral to it (Delupi *et al.* 1997).

The *m. pterygoideus* is separated in internal and external (or lateral and medial) pterygoideus. The lateral pterygoideus is big and thick but smaller than the *m. temporalis* and *m. masseter*, with the function of stabilizing and closing the jaws, and could also be involved in lateral movements. In *Lutreolina*, this muscle has a tendinous portion, and with the superficial masseter, they lie above the *m. mylohyoideus*. This conformation might indicate the bite strength of this genus and was not found in *Didelphis* (Delupi *et al.* 1997).

The myology of the masticatory apparatus in those few didelphids studied to date presents some variation that can be related to their feeding habits. Most specifically, the *m. temporalis* with the superficial size of the temporal fossa seems to hold the main morpho-functional differences, with both *Thylamys* and *Lutreolina* appearing to have adaptations for insectivorous and carnivorous habits, respectively. In *Didelphis*, even with the richness of descriptive studies, there is an assumed condition of “generalized morphology” that could be hampering the recognition of its specializations or similar features with other more specialized genera. Further studies are needed directly associating the *m. masseter*, *m. pterygoideus*, and *m. mylohyoideus* muscle morphology with their function and eating habits in didelphids: despite the morphological variation between the species studied, their functional influence is not well established.

Body Myology and Locomotion

The most comprehensive and comparative analysis of the axial skeleton morphology, which includes myology and functional information for several didelphids, was conducted by Argot (2003). She used *Caluromys* and *Marmosa* as arboreal representatives, *Didelphis* and *Philander* as scansorial, and *Monodelphis* and *Metachirus* as terrestrial.

In the cervical vertebrae, the nuchal muscles as the *m. trapezius*, *m. rhomboideus occipitalis*, *m. splenius*, *m. spinalis capitis*, and the *m. rectus capitis dorsi* insert on the supraoccipital and the occipital crests, which are poorly developed in most didelphids. In *Thylamys*, the atlas ventral arch is not ossified, which affects the development of muscles that are usually attached to this structure. Therefore, the *m. levator claviculae*, *m. atlantoscapularis posticus*, and, especially, the *m. rectus capitis anterior* are leaner and much smaller than in other didelphids, and they are attached to the ligament that replaces the ventral arch ossification, probably the anterior longitudinal ligament (Mann-Fischer 1951). These muscles are responsible for moving and supporting the head, and this neck mobility could be associated with feeding activity, depending on how much the animal uses its jaws or forelimbs to capture prey. Despite putting this hypothesis forward, Mann-Fischer (1951) did not provide additional behavioral data to test it, nor are the current authors aware of any particular behavior in *Thylamys* that would justify such mobility to be different from the remaining didelphids. Another interesting feature is that the orientation of the transverse processes in the cervical vertebrae is variable across examined didelphid taxa, but the same muscle, *m. longissimus cervicis*, is attached to these processes in all of them. In *Metachirus*, its insertion extends more posteriorly than in *Caluromys*, which added to the transverse process orientation, providing higher neck mobility, essential for terrestrial-saltatorial locomotion. An additional variable aspect is the development of an inferior lamella on the ventral edge of the transverse process, where the *m. longus colli* and *m. longus capitis* connect the neck (cervical vertebrae) with the basicranium. This lamella is more prominent posteriorly and longer in *Philander* than in *Caluromys*, giving the former more strength to flex the neck, which could be an advantage for predatory habits (Argot 2003).

The diaphragmatic vertebra separates the lumbar-like vertebrae (posteriorly) and the horizontal plane formed by the zygapophyses (anteriorly). In this posterior part, the *m. longissimus dorsi* muscle originates from strong tendons from the mamillary process, to extend the back and stabilize the vertebral column. On the anterior rib pairs there are the attachments of the *m. pectoralis*, *m. scalenus*, and *m. serratus anterior*, which support the head and the anterior part of the trunk. Both the mamillary process and the anterior pair of ribs are more developed in *Caluromys* than in *Metachirus*, providing more rigidity in the spine necessary for the climbing and bridging behavior in the former. Likewise, in *Caluromys*, all the ribs are expanded anteroposteriorly, with a robust sternum and a remarkably keeled manubrium, which increases the rigidity in the rib cage as well, and reveals the substantial load supported by the *Mm. pectoralis*. In *Marmosa*, despite its arboreality, the ribs are thin as in the other didelphids, and this species is smaller, slenderer, and more agile than *Caluromys* (Argot 2003). This could be related to lighter loads for arboreality in small species, as has been observed in other skeletal structures, such as the scapula (Astúa 2009).

The thoracic and lumbar vertebrae can also be divided into an anterior and posterior region. In *Caluromys* this transitional area is gradual while in *Metachirus* the change is more marked, with shorter and thicker neural processes, compressing this area, which can be attributed to its bounding-running type of locomotion (Argot 2003). Argot (2003) described that the neural processes of the lumbar vertebrae of both *Caluromys* and *Philander* have a posterior inclination, indicating the action of the *m. multifidus* and *m. semispinalis dorsi*, and they do not have an anticlinal vertebra, suggesting less vertebral column flexion. However, in *Metachirus*, the third lumbar vertebra, or the eleventh thoracic, *sensu* Flores (2009) is anticlinal with an anterior inclination in the neural processes, where a well-developed *m. longissimus dorsi* attaches, and this highlights the importance of the lower back musculature for the locomotion of this species.

The caudal vertebrae are reduced and variable in *Monodelphis*, with less than 20 caudal vertebrae, while other didelphids generally have around 30 caudal vertebrae (Flores 2009). *Caluromys* has a well-developed mamillary process in the anterior caudal vertebrae, which also suggests a well-developed multifidus caudae, a short muscle that extends the tail for vertical movements. On the same vertebrae, the transverse processes of the first four caudals form a quadrangular blade, which serves as insertion for the *m. ischio-caudalis* and *m. abductor caudae dorsalis*, forming a robust structure with tendinous and aponeurotic attachments, respectively, while in other didelphids this structure is less robust, the attachments are fleshy and the muscles are poorly developed. These two muscles are responsible for the caudal lateral flexion and balance while moving (Argot 2003). Added to it, the posterior caudal vertebrae of *Caluromys* have ventral keeled hemapophyses, which guarantees a strong attachment for the *m. sacrocaudalis* tendon until the end of the tail. All these features are evidence of the use of the tail as a fifth limb by *Caluromys*, unlike most other didelphids (Argot 2003). Although short-tailed *Monodelphis* species also have fairly robust caudal vertebrae with a highly developed transverse process, that allows them to grasp objects with the tail (Unger 1982), it cannot be used as a fifth limb as in the arboreal *Caluromys*. In *Metachirus* the neural process of the first sacral vertebra is the most developed and functions as a central base for the lumbar epaxial musculature, while the neural process of the second sacral vertebra is abruptly reduced, with the distal caudal vertebrae slender than in *Caluromys*. This suggests that *Metachirus* is also not able to support its body weight by the tail, and its long size may be related to the animal balance during leaping locomotion (Argot 2003).

From the scapula to the digits, the forelimbs have highly complex muscles (**Figure 2**) associated with several behaviors other than locomotion, such as food handling, grooming, or mating (Streilein 1982, Astúa 2015). The scapula is connected to the axial skeleton by several muscles (Argot 2001, Diogo *et al.* 2016), including the *m. serratus anterior* and the *m. levator scapulae*, that are fused and inserted on the scapula. They are probably associated with the animal "hugging" the substrate while climbing (Coues 1872). These two muscular parts also support and transmit the thorax weight to the forelimbs and, in *Caluromys*, the area of insertion of these muscles allows it to absorb and transmit larger forces during tensile efforts (Argot 2001).

The posterior shoulder muscles include the *m. supraspinatus*, *m. infraspinatus*, *m. teres minor*, *m. teres major*, and *m. subscapularis* that connect the scapula to the proximal humerus, and the deltoid group (Diogo *et al.* 2016). The *m. supraspinatus* elevates and abducts the humerus and absorbs part of the impact when the forelimbs contact the floor at the end of leaps (Maynard-Smith and Savage 1956). This could justify the well-developed supraspinatus fossa for the insertion of this muscle in *Metachirus*, an opossum considered specialized for leaping locomotion (Argot 2001). The infraspinatus is an external rotator of the humerus, and its fossa is well developed in *Caluromys*, *Marmosa*, and *Didelphis*, moving the forearm to reach supports in a multidimensional space (Argot 2001). In the semi-aquatic opossum *Chironectes*, there is a larger *m. teres major* than *Didelphis*, which connects the scapula with the humerus. This difference shows a greater motor force in the former than in the latter, possibly to overcome water resistance (Stein 1981). In arboreal species such as *Caluromys*, the origin of the deltoideus muscle is high and robust and has a well-marked insertion area (deltoid ridge), which indicates powerful tractions of the humerus. Besides, this muscle acts synergistically with the *m. pectoralis major*, the most developed muscle in *Caluromys* forelimb, performing powerful adductions in this arboreal genus. This feature was not found in other didelphids (Argot 2001). The *m. coracobrachialis* is also very important for arboreal forms, to pull the body up when the animal is climbing, and it is unique in *Caluromys* with a long head instead of a short head in other didelphids with other locomotory habits (Argot 2001). Comparatively, the forelimb muscles of *Caluromys* are twice the weight of *Metachirus*, when considering the percentage of the total weight of the forelimb musculature.

In the forearm, the olecranon process represents the lever arm of the *m. triceps brachii*, which is very characteristic in terrestrial forms. In *Monodelphis*, this process is very long and allows the insertion of a well-developed triceps, providing powerful extensions of the forearm. In *Metachirus*, another terrestrial genus, the olecranon process is shorter but very robust and proportionally larger than in other didelphids, reflecting the strong traction capacity of this muscle (Argot 2001). Stein (1981) also found myological evidence in *Chironectes* that suggest advantages for its locomotion, such as the presence of a second *m. anconeus*, with the same size and probably the same function, but which allows a more powerful extension of the forearm in this species than in *Didelphis*. However, differences in the musculature of *Chironectes* and its relationship with its unique locomotory adaptations still need further investigation.

Another particularity is the difference between the flexor muscles of the forearm of *Chironectes* and *Didelphis*, especially the *m. flexor digitorum superficialis*. In the former, the muscle has an extensive origin and its fibers converge on the tendon in a pinnate form, which increases the strength that the muscle can exert relative to its overall width (Stein 1981). In the latter, the muscle has parallel fibers instead, which are fleshy until the wrist, increasing the distance that this muscle can contract, but is weaker when compared to *Chironectes*. Stein (1981) suggested that this difference could be related to differences in the form of foraging between these species, but without evidence to support this idea. The *m. flexor digitorum profundus* and the *m. abductor pollicis longus* are important muscles for prehensility function in the manus, originating from the concavity of the radius bone. In *Caluromys*, this bone is slightly concave medially and with a craniocaudal convexity, which increases the space for these muscles, and it is particularly well-developed in arboreal forms, especially when compared with terrestrial ones. *Monodelphis* also presents a notably curved radius, which is unexpected for terrestrial forms, indicating significant mobility of the manus, possibly allowing the manipulation of small prey (Argot 2001). This hand mobility is also enabled by the *m. flexor carpi ulnaris*, which is well-developed in *Metachirus*, increasing its mechanical advantage. This characteristic is associated with the use of the forelimb during galloping and especially leaping (Argot 2001). Despite the diversified locomotion of the group, the flexor tendons pattern of the manus in didelphids has little to no direct association with locomotor behavior. Yet, the associated musculature was useful to discover several novel synapomorphies through character mapping, such as the morphology of the *m. palmaris longus* and *m. flexor carpi ulnaris*, the origin and insertion of the *m. flexor digitorum superficialis*, and the length of the forearm muscular fibers (Abdala *et al.* 2006) that support previous clades proposed by Voss and Jansa (2003). Additionally, Abdala *et al.* (2006) also found that several didelphids possess the “P pattern”, in which the flexor plate is reduced or absent, and tendons pass directly to the terminal phalanges. This morphology might be consistent with a synapomorphy of the didelphids, but needs further investigations with a broader sample.

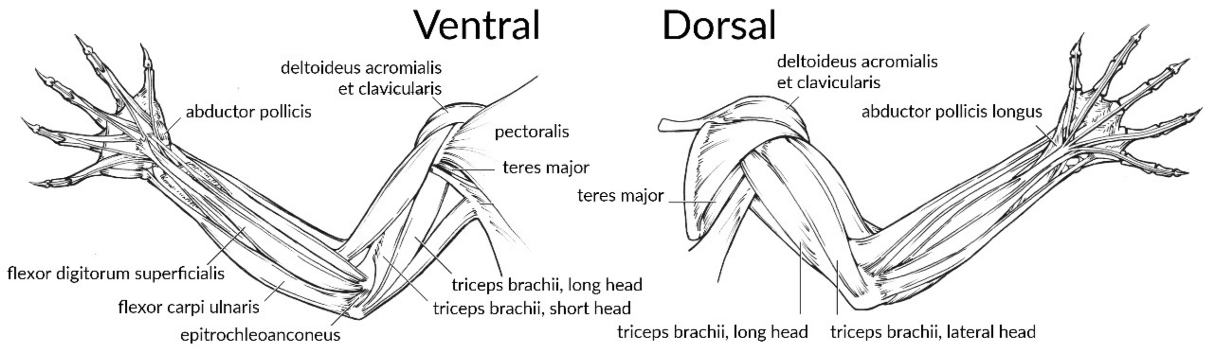

Figure 2. *Didelphis virginiana* forelimb muscles, ventral and dorsal views. Only muscles mentioned in the text are indicated. Modified from the schematic drawing by J. Molnar from Diogo *et al.* (2016). Refer to Diogo *et al.* (2016) for a complete description of all forelimb muscles analyzed.

The pelvic girdle of marsupials possesses a pair of epipubic bones, ventrally projected into the belly, with several muscles associated with it and the abdominal wall, but mainly the *m. pyramidalis* and *m. pectineus*. The first connects the entire bone to the midline of the body and the second connects the base of the bone to the femur. They act as a lever during locomotion, providing support to the body during each step, and also acting synchronically with ventilation (Reilly *et al.* 2009). Despite the recent studies associating these muscles with locomotion, their direct role in didelphid species with different locomotory habits is still unknown.

As for overall body musculature weight, the terrestrial genus *Metachirus* has more muscle mass than any other didelphid and its hindlimbs have twice as much muscle mass as its forelimbs, with high proportions of back extension muscles (Grand 1983). Grand (1983) suggested that as *Metachirus* has a light tail and elongated tibia and fibula, the muscular mass of this species has migrated proximally up the limbs, which is evidence of cursorial adaptation. *Monodelphis* is also terrestrial with a light tail and a high proportion of musculature but it is not concentrated in the low back and thighs as in *Metachirus*, so the former probably do not move so quickly (Grand 1983), and might indicate a different way of moving around (Parchman *et al.* 2003). An alternative explanation for the differences between *Metachirus* and *Monodelphis* could be that *Monodelphis* is less terrestrial, as a recent study in the Pantanal has reported 50% of captures of *Monodelphis domestica* in the understory (Sano *et al.* 2020).

The hip region of *Chironectes* has a well-developed psoas muscle, named *m. psoas tertius*, considered an additional muscle of the iliac group. The psoas group of muscles is responsible for flexing, adducting, and rotating the hip joint. This robust pattern with an extra muscle in the species could be an advantage in overcoming water resistance and involved in its unique hindfeet paddling (Stein 1981, Fish 1993). Similar to the forelimbs, Stein (1981) also describes features in *Chironectes* hindlimbs that might be associated with the semi-aquatic habit, which also needs further investigation. Despite the relatively shorter femur in *Chironectes* than in *Didelphis*, the *m. vastus externus* is larger in mass and smaller in length in *Chironectes*, and this could be evidence of the stronger force exerted by this species when contracting this muscle (Stein 1981).

The *Mm. adductor* of the thigh, including the *m. pectineus*, have a different morphology according to locomotor habits. In *Caluromys*, the insertion of these muscles onto the femur is rugose while in *Metachirus* is less so. Although they are not inserted directly on the *pes*, the adductors, together with the calf, play an important role in stabilizing the foot against arboreal surfaces, especially in medial position, with a broad range of femoral abduction (Argot 2002). The *m. quadratus femoris* is another important muscle, which in *Caluromys* has a wide origin area comprising the whole ischium, and its insertion joins the greater and the lesser trochanters. This feature emphasizes the developed ability of this genus to rotate the femur during arboreal locomotion. In other didelphids, the insertion of this muscle is restricted to the lesser trochanter (Argot 2002).

The hamstrings muscle group in the hindlimbs originates from the ischium and inserts onto the tibia and includes the *m. biceps femoris*, *m. semitendinosus*, and *m. semimembranosus* (**Figure 3**). These muscles comprise a substantial portion of the hindlimb musculature representing about 20% in *Caluromys*, *Marmosa*, and *Didelphis*, and more than 25% in *Metachirus*. Besides the proportion of the hamstrings, in *Metachirus* only the *m. biceps femoris* is massive, representing 11% of the hindlimb musculature, compared to 4% in *Marmosa* and *Caluromys* (Argot 2002). This group of muscles is mainly related to extension movements, providing acceleration during locomotion, and their remarkable development in *Metachirus* could be associated with its cursoriality (Maynard-Smith and Savage 1956). From this group of muscles, the *m. semitendinosus* in *Caluromys* originates from the whole posterior ramus of the ischium, and its dorsal head originates from the transverse process of the fourth caudal vertebra, being much more developed than in other didelphids and is probably related to the use and prehensility of the tail (Argot 2002).

More posteriorly, the tibia and fibula are broadly separated from each other in *Caluromys*, allowing the considerable development of the *m. flexor digitorum fibularis*, a particular feature of several arboreal forms. However, in *Marmosa*, these bones are very close to each other, being even partially fused in some specimens, suggesting some saltatorial ability of this genus (Argot 2002). In terrestrial marsupials such as *Metachirus*, the most developed muscles in the leg are the *Mm. gastrocnemii* and *m. plantaris*, especially the *m. gastrocnemius externus*. The latter originates from the parafibula while the *m. gastrocnemius internus* originates from the distal femur, and both of them insert on the tip of the calcaneus, being a lever arm of the foot extensors. The *Mm. gastrocnemii* correspond to 8% of *Metachirus* total hindlimb musculature, against less than 4% in *Caluromys*. However, in *Caluromys*, the *m. flexor digitorum fibularis* is more developed, with more than 9% of the hindlimb musculature, being responsible for the grasping foot activity, against only 4% in *Metachirus*. Other muscles that act as levers connecting the fibula and the foot are similarly developed in didelphids, independent of their habits, except for the *m. peroneus longus* (Argot 2002). Comparing this muscle between *Chironectes* and *Didelphis*, in the former it has two heads and bipinnate fibers, while in the latter the muscle has just one head with parallel fibers. These features in *Chironectes* might increase the force that it can exert during hindlimb movement (Stein 1981, Fish 1993). The tendon of this muscle is especially involved in the grasping ability of the hallux, being a strong adductor and flexor of the first metatarsal in arboreal forms (Argot 2002). The genus *Thylamys* appears to be well adapted to climbing as well as *Didelphis*, with very similar morphology between them, both in the forelimbs and hindlimbs (Mann-Fischer 1953), but with no remarkable structures involved in specific functions.

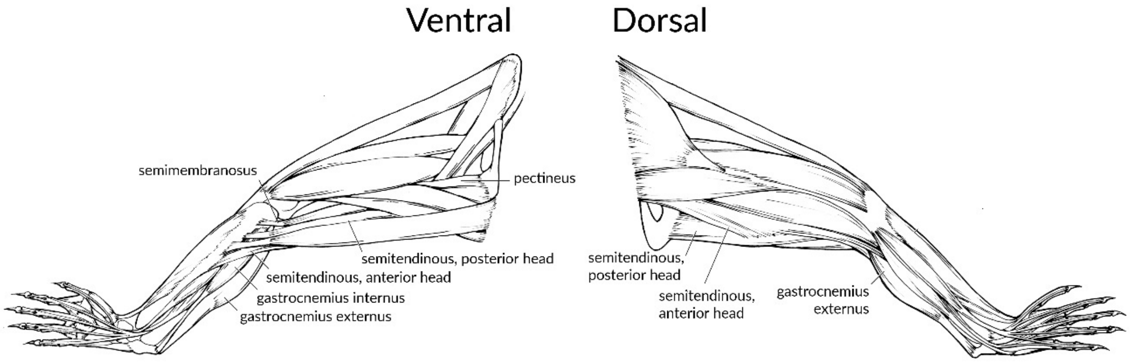

Figure 3. *Didelphis virginiana* hindlimb muscles, ventral and dorsal views. Only muscles mentioned in the text are indicated. Modified from the schematic drawing by J. Molnar from Diogo *et al.* (2016). Refer to Diogo *et al.* (2016) for a complete description of all hindlimb muscles analyzed.

Conclusions and Future Directions

Although some of the studies cited here are very comprehensive, it is evident that more information on most Didelphidae species is still needed for appropriate comparative approaches. Studies with hard tissue are usually directed to species delimitation and phylogeny, while soft tissue works are more descriptive and comparative with different locomotion habits, hardly considering interspecific variation or the phylogenetic context. This occurs especially in all the smaller species outside the Didelphini tribe. The more effort is invested in acquiring this type of data across a taxonomically diverse sample, the more one will be able to assess if morphological characteristics are indeed associated with a specific habit and represent a functional adaptation, or if they are sharing these characteristics phylogenetically, merely reflecting inherited and shared patterns derived from a common ancestor. Focusing on the larger species and the lack of morphological and functional studies of other clades so far limits the understanding of didelphid evolution. As a consequence, the analytic possibilities in integrating bony and soft tissue data in several areas such as natural history, ethology, systematics, and functional morphology are also hindered, and so is the current understanding of the biology of extinct taxa.

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CHAPTER 2: Muscular limb anatomy of the thick-tailed opossum *Lutreolina crassicaudata* (Desmarest, 1804) (Didelphimorphia: Didelphidae)

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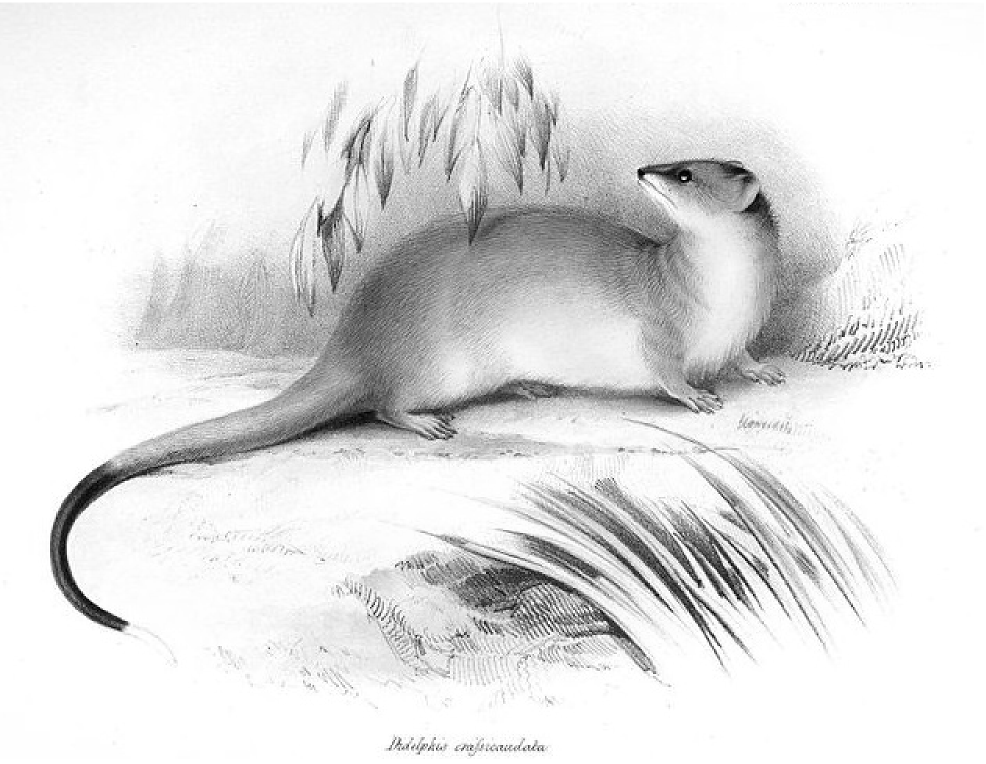

Lutreolina massoia from Darwin, 1838, adapted.

ABSTRACT

Descriptive studies about mammal myology are scarce and frequently outdated, leading to nomenclature instability and sometimes becoming a difficult topic of research. Regarding marsupial myology, the Virginia opossum *Didelphis virginiana* is the model for studies in the group, with a lack of research for most American species. The thick-tailed opossum *Lutreolina crassicaudata* is a Neotropical species that resembles placental carnivors mustelids, as the mink. The aim of this work is to describe the forelimb and hindlimb myology of this species in order to contribute to the understanding of its functional implications. Using standard dissection techniques, equipment, and nomenclature, we compared the myology of *L. crassicaudata* with the literature available for species of *Didelphis virginiana* and *Dasyurus geoffroii*, a carnivorous marsupial from Australia, and also the lesser grison *Gallictis cuja*. Our results showed that *L. crassicaudata* has similar forelimb muscle attachments to *Da. geoffroii*, such as the deltoid group, which are the main muscle involved in arm lifting and influences movement limitation during locomotion. Compared with placental mammals, *L. crassicaudata* shows a similar morphology of the mm. atlantoscapularis muscle with *G. cuja,* important muscles in protraction, considering its short limbs. Regarding the hindlimbs, *L. crassicaudata* has a well-developed hamstrings muscle group, followed by the *m. quadriceps femoris*, both important to a cursorial locomotion. The comparison with other marsupials showed that, in didelphids, the hindlimb muscle mass seems to be much more connected to its locomotion habits, while for Australian marsupials, the phylogenetic component might be stronger than functional morphology. We conclude that most forelimb myology of *L. crassicaudata* is similar to *Da. geoffroii*, and some traits were considered plesiomorphic for both of them. Besides the general body shape and carnivorous habits, we could also consider the myology of *L. crassicaudata* is somewhat convergent with the placental weasel-like mustelid *G. cuja.* Regarding its hindlimb, *L. crassicaudata* muscle mass proportion overlapped almost completely with *Metachirus myosurus*, a terrestrial didelphid, suggesting an adaptation in both species to cursorial locomotion, or even retention of an ancestral feature.

Keywords: Anatomy, limb musculature, marsupial muscles, functional morphology.

INTRODUCTION

Muscular anatomy studies are commonly associated with human or veterinary anatomy due to health and clinical purposes, but it is slowly increasing in recent times for other groups of mammals. Primary and formal muscle descriptions are a requirement for several different analysis, as comparative functional morphology, phylogenetic reconstruction, and also as an integrative information for skeletal and biomechanic investigations (Diogo *et al.* 2012, 2016). Anatomical descriptions are also important to allow us to infer myological characteristics which could occur in different locomotory habits, including extinct species (Argot 2001, 2002, 2003). American marsupials present a variety of types of locomotion as arboreal, scansorial, terrestrial and semi-aquatic (Paglia 2012, Astúa 2015). It is possible that the group shows a considerable variation in their muscular structure which could be related both to functional adaptations and its evolutionary phylogenetic history. Nonetheless, the comprehension of myology evolution in marsupials is hampered due to the absence of basic descriptive studies.

Myological descriptive studies about didelphid marsupials are scarce and frequently outdated, with several issues about translation and nomenclature instability (*e.g.:* Diogo *et al.* 2016 tried to establish the standard nomenclature for marsupials). Part of this problem can be attributed to the most common forms of specimen storage in mammal collections prioritizing mostly skins and cleaned skulls (Cook and Light 2019), and often discarding soft tissues. In the specific case of

New World marsupials, most works about musculature are focused on *Didelphis virginiana* (Kerr, 1792), with a considerable literature containing descriptions and comparative functional morphology investigations (Tyson 1698, Coues 1872, Elftman 1929, Jenkins and Weijs 1979, Stein 1981, Diogo *et al.* 2016). There is also a descriptive study of *Thylamys elegans* (Waterhouse, 1839) (Mann 1953, 1956) and some studies about ecomorphology of *Chironectes minimus* (Zimmermann, 1780) (Sidebotham 1885, Stein 1981), again compared to *D. virginiana*. Finally, there are some studies of functional morphology of living species in the context of comparisons to estimate the locomotion of extinct taxa, including myological analysis of *Caluromys, Marmosa, Metachirus, Philander* and *Monodelphis* (Argot 2001, 2002, 2003), but without a complete characterization of limb musculature. Despite this comparative literature, most of didelphid species lack any description of the musculature whatsoever.

The thick-tailed or lutrine opossum, *Lutreolina crassicaudata* Desmarest (1804), is a species of mammal not particularly common in collections, and difficult to collect in the wild (Ximénex 1967). It is a medium-sized Neotropical marsupial that can be found in two discontinuous non-forest regions of South America, including Brazil, central-eastern Argentina, Paraguay, Uruguay and part of Bolivia (Astúa 2015). It is mostly restricted to open grassland vegetation, swamp/flooded areas, and gallery woodland environments (Marshall 1978, Monteiro-Filho and Dias 1990). *Lutreolina crassicaudata* is frequently considered one of the most carnivorous Neotropical marsupials, eating small vertebrates as birds, rats, rabbits (Monteiro-Filho *et al.* 2006) and many invertebrates, but mainly crustaceans and beetles (Cáceres *et al.* 2002), although consumption of fruits has also been reported (Marshall 1978, Monteiro-Filho and Dias 1990, Astúa de Moraes *et al.* 2003). The dental and cranial morphology of *L. crassicaudata* also suggest a more carnivorous diet (Goin *et al.* 1992, Flores *et al.* 2003, Astúa and Guilhon 2022), added to some aspects of its short facial region and the enlargement of the temporal fossa with corresponding great development of muscular mass of the temporalis muscle, assuring a potent bite when compared to *Didelphis* (Delupi *et al.* 1997). Although there are a few studies correlating the masticatory apparatus with its food habits in the species (Delupi *et al.* 1997, Voss and Jansa 2009) there is no information about limb myology description for *L. crassicaudata*.

Since this species occurs next to water bodies and swamp areas (Monteiro-Filho and Dias 1990), it has been occasionally considered as semi-aquatic due to its facility to dive and swim (Marshall 1978). Even with a stroke frequency and buoyancy ability more similar to those of the water opossum, it was not considered a fully specialized semi-aquatic marsupial (Santori *et al.* 2005) being more commonly considered a terrestrial marsupial (Astúa 2015), due to its elongated body and short legs. More than any other didelphid, it resembles a mustelid and/or a carnivorous placental and are often called “weasel-like” or “mink-opossum” (Lee and Cockburn 1987). However, no descriptive or functional analyses has been made on the limbs of the species to substantiate these assumptions. Considering how the postcranial morphology is visibly divergent compared to other didelphids (Flores 2009), the description of the myology of *L. crassicaudata* could shed new light about the functional morphology of the species, and it may have a significant influence in its locomotion and possibly hunting behavior. Therefore, the aim of this work is to describe the limb myology of *L. crassicaudata* and compare it with other American and Australian marsupial data available in the literature, making inferences about its functionality.

MATERIAL AND METHODS

Myological and osteological material of three specimens of thick-tailed opossum *L. crassicaudata* were used for this study. The material is deposited in the mammal collection of the

Centro de Coleções Taxonômicas da Universidade Federal de Minas Gerais (hereafter only referred as CCT/UFMG), and detailed information about the specimens is available:

Brazil: Minas Gerais: Parque Nacional da Serra da Canastra (UFMG 7989♀, UFMG 7990♀, UFMG 7991♀, UFMG 8001); Poços de Caldas (UFMG 7992♀); Serra do Salitre (UFMG 7993♂).

The frozen specimens were thawed, skinned, eviscerated, and conserved in 70% ethanol due to the advanced decomposition stage. The muscles were dissected, identified, and reflected to confirm points of origin/insertion, and their attachments were listed and described. Muscular mapping and raw dissection photographs of the main muscles were provided. Anatomical nomenclature followed Evans and Lahunta (2013) and Diogo *et al.* (2016), and we used mostly Coues (1872), MacCormick (1886, 1887), Argot (2002), Ercoli (2014) and Warburton and Marchal (2017) for comparative muscle attachments in both marsupials and placentals.

After dissection and description, muscles from two specimens were separated and weighted in a precision scale for muscle mass analysis (Appendix I, **Table 4**). Each muscle was weighted three times, and we used only the average weight for each specimen. Besides providing the raw individual muscle mass, muscles were grouped by its actions, to analyze their functional morphology. Forelimb muscles were grouped following de Souza-Júnior *et al.* (2018): pronators (*m. pronator teres* and *m. pronator quadratus*), supinators (*m. supinator* and *m. biceps brachii*), flexors of forearm (*m. brachioradialis* and *m. biceps brachii*), extensors of forearm (*m. triceps brachii*), flexors of hand/digits (*m. flexor carpi ulnaris* and *m. flexor carpi radialis*, *m. flexor digitorum superficialis, m. flexor digitorum profundus* and *m. flexor pollicis longus*) and extensors of the hand/digits (*m. extensor carpi radialis* and *m. extensor carpi ulnaris, m. extensor digitorum communis, m. extensor digitorum profundus* and *m. extensor digitorum minimus*). Hindlimb muscles were grouped as suggested by Warburton *et al.* (2015): hip flexors (*m. rectus femoris*, *m.*

iliopsoas, *m. sartorius*), gluteal group (all gluteal muscles), deep hip (*m. piriformis*, *Mm. gemelli*, *Mm. obturators*, and *m. quadratus femoris*), quadriceps femoris (*m. rectus femoris*, *Mm. vastii*), adductor group, hamstrings (*m. biceps femoris*, *m. semitendinosus* and *m. semimembranosus*), extensors of *pes* and flexors of *pes*.

We could not find raw weight data of forelimb muscles in the literature, only for hindlimbs. For this reason, besides the muscle proportions by function mentioned above, we used hindlimb raw wet muscle data from the literature to provide broad comparisons with other American and Australian marsupials, with similar and different locomotion habits. In the American group, we compared *L. crassicaudata* (~200 – 910g) with the generalist scansorial opossum *Didelphis marsupialis* Linnaeus, 1758 (~2.4kg), the most specialized arboreal opossum *Caluromys philander* (Linnaeus, 1758) (~390g) and the terrestrial opossum *Metachirus myosurus* (É. Geoffroy, 1803) (~284 – 480g). The approximate genera weight abovementioned is from Astúa (2015), while all raw weight data for analysis was obtained from Argot (2002), except *L. crassicaudata*. In the Australian group, we compared *L. crassicaudata* with the generalist arboreal diprotodont *Trichosurus vulpecula* (Kerr, 1792) (~1.2 – 4.5kg), small hopping macropodid *Setonix brachyurus* (Quoy and Gaimard, 1830) (~3.5 – 4.2kg) and the terrestrial peramelid *Isoodon obesulus* (Shaw, 1797) (~400g – 1.8kg). Approximate genera weight data are from Helgen and Jackson (2015), Eldrigde and Coulson (2015) and Dickman (2015), respectively, while all raw weight data for analysis was extracted from Warburton *et al.* (2015). The relative mean weights of the main hindlimb muscles from *L. crassicaudata*, American (Argot 2002) and Australian marsupials (Warburton *et al.* 2015) were ranked similarly as Argot (2002) (Appendix I, **Table 5**). In Argot (2002), each muscle weight was divided by the whole hindlimb musculature weight, and then ranked accordingly its proportion (% value), using only the average weight of two specimens. In

Warburton *et al.* (2015), all the raw data of the specimens was available, so we considered only the specimen with more similar average weight considered by Wilson and Mittermeier (2015): *I. obesulus* (QKN01) with 1500g, *T. vulpecula* (PKN07) with 2020g and *S. brachyurus* (Q17) with 3800g (Warburton *et al.* 2015, Appendix 1). Both raw data from the present study and Warburton *et al.* (2015) were adapted to fit Argot (2002) ranking values, for a more appropriate comparison between the three studies (**Table 5**).

RESULTS

We described the forelimb and hindlimb myological complex of *Lutreolina crassicaudata* and summarized the attachments and main actions (**Table 2**, **Table 3**).

Table 2. Origin, insertion and actions of the identified forelimb muscles of *Lutreolina crassicaudata*. Main actions according to Miller et al. (2013) and Souza Júnior et al. (2018).

Muscle	Origin	Insertion	Main Action
		Extrinsic muscles of the forelimb	
Trapezius	Cranial base and spine until C4 vertebra; C4–T10	Scapular spine, acromion process and border, deltoid ridge	Elevate, protract and abduct the limb
Atlantoscapularis	Wing of Atlas	Scapula spine and acromion	Scapula rotation
Omohyoideus	Base of the hyoid bone	Cranial angle of scapula border	Depresses the hyoid
Latissimus dorsi	T4-T5	Greater tuberosity of humerus	Retract the limb and flex the shoulder joint
Rhomboideus	Spinous processes C7–T5	Border and scapula spine	Elevate the limb
Serratus	First eight ribs, cranial aspect	Serratus and scapula border	Support of the trunk
Pectoralis major	Greater tuberosity of humerus	Manubrium	Adduct the limb, stability
Pectoralis minor	Greater tuberosity of humerus	Xiphoid process	Adduct the limb, retract the limb caudally, flex the shoulder joint

Muscle	Origin	Insertion	Main Action
Subclavius	Manubrium lateral edge	Acromioclavicular joint	Stabilize the clavicle
Shoulder and arm muscles			
Deltoideus acromialis	Acromion	Deltoid ridge	Flexion of shoulder
Deltoideus clavicularis	Clavicula	Deltoid ridge	Flexion of shoulder
Deltoideus scapularis	Scapular spine	Deltoid ridge	Flexion of shoulder
Supraspinatus	Supraspinous fossa	Greater tubercle of humerus	Extension of shoulder
Infraspinatus	Infraspinous fossa	Greater tubercle of humerus	Flexion of shoulder
Subscapularis	Entire scapula medial aspect	Lesser tubercle of humerus	Adduction of shoulder
Teres major	Caudal angle of scapula	Teres major tuberosity	Flexion of shoulder
Teres minor	Not found	Not found	-
Coracobrachialis	Coracoid process	Teres major tuberosity	Extension of shoulder
Biceps brachii	Supraglenoid tubercle and coracoid process	Ulnar tuberosity	Flexion of elbow and extension of shoulder
Brachialis	Neck of humerus	Ulnar tuberosity	Flexion of elbow
Triceps brachii (medial/short head)	Medial aspect of humerus shaft	Ulnar olecranon process	Extension of elbow
Triceps brachii (lateral head)	Lateral aspect of proximal humerus diaphysis	Ulnar olecranon process	Extension of elbow
Triceps brachii (long head)	Caudal border of scapula, in the distal third	Ulnar olecranon process	Extension of elbow and flexion of shoulder
Triceps brachii (accessory head)	Caudal aspect of proximal humerus diaphysis	Ulnar olecranon process	Extension of elbow
Dorsoepitrochlearis	Caudal scapula border	Olecranon process	Abduct the limb
Anconeus lateralis	Distal humerus epiphysis	Anconeus process	Extension of elbow
Forearm muscles			
Pronator teres	Medial epicondyle of humerus	Radial diaphysis	Pronation
Flexor carpi radialis	Medial epicondyle of humerus	Carpal radial bone	Flexion of carpal joint
Flexor carpi ulnaris	Medial epicondyle of humerus	Accessory carpal bone	Flexion of carpus
Flexor digitorum superficialis	Medial epicondyle of humerus	Phalanges of II–V digits	Flexion of the digits II–V
Flexor digitorum profundus	Medial epicondyle of humerus	Palmar + Phalanges of all digits	Flexion of all digits

Muscle	Origin	Insertion	Main Action
Pronator quadratus	Radial interosseous space	Ulnar interosseous space	Pronation
Brachioradialis	Supracondylar crest	Carpal radial bone	Supination
Extensor carpi radialis	Supracondylar crest	II–III Metacarpal bone	Extension of carpal joint
Extensor carpi ulnaris	Lateral epicondyle of humerus	Hamate bone	Extension of lateral hand
Extensor digitorum communis	Supracondylar crest (more distally)	Dorsal aspect of all phalanges	Extension of all digits
Extensor digiti minimi	Supracondylar crest	Distal phalange of digits IV and V	Extension of digits IV and V
Extensor digitorum profundus	Lateral aspect of ulna	Dorsal aspect of digits I–IV	Extension of all digits
Supinator	Lateral epicondyle of humerus	Cranial aspect of radius (2/3)	Supination
Abductor pollicis longus	Lateral epicondyle of humerus	I Metacarpal bone	Extension and abduction of digit I

Description of forelimb myology

Extrinsic muscles of the pectoral limb

Mm. trapezius (**Figure 6**)

The most superficial muscle in dorsal view is the *Mm. trapezius*. We identified three parts of this muscle (*pars capitis*, *pars cervicis* and *pars thoracica*) although this division is not physical, but subtle and only considering the gradually thickening and change of fiber orientation. The *pars capitis* and *pars cervicis* are mostly blended, originating via fleshy fibers in the occipital bone and continuing along the spine, ending in the fourth cervical vertebrae. Its cranial part is a bit thicker, and a small part reaches the proximal part of the clavicle (similar with *m. brachiocephalicus* insertion in domestic animals) and continues to insert via fleshy fibers along the scapula spine until the acromion process, and also via small tendinous fibers in the dorsal border of the scapula and in the deltoid ridge. The fibers near the acromion process elongates superficially to the *m. deltoideus acromialis* and inserts alongside the *m. deltoideus clavicularis*. The *pars thoracica* originates via fleshy fibers from the same fourth cervical vertebrae, blending with the *pars cervicis* origin and extends until the tenth thoracic vertebra. Their insertion is also blended in the dorsal half of the scapular spine, but the *pars thoracica* continues distally of the scapular spine and then inserts via fleshy fibers on the dorsal border of the scapula.

M. atlantoscapularis anticus (m. levator claviculae, *m. omotransversus)* (**Figure 4**, **Figure 6**).

Both *m. atlantoscapularis anticus* and *m. atlantoscapularis posticus* are well delimited ribbon-like muscles lying in front of *m. supraspinatus*. The former originates via fleshy fibers from the wing of the atlas (C1) and inserts by small tendinous fibers in the third part of the scapula spine, in the metacromion process border.

M. atlantoscapularis posticus (m. levator scapulae posticus) (**Figure 4**, **Figure 6**).

This muscle has a very deep origin via fleshy fibers in the wing of the atlas (C1) and it inserts by small tendinous fibers in the first third (1/3) of the scapula spine, in between *m. rhomboideus* (proximal side) and *m. atlantoscapularis anticus* (distal side).

Figure 4. Raw images of *L. crassicaudata m. rhomboideus*, *m. atlantoscapularis anticus* and *m. atlantoscapularis posticus* dissection. Lateral view on both images.

M. omohyoideus (**Figure 5**, **Figure 6**).

This is a very long ribbon shaped muscle, arising from the base of the hyoid bone and lying together with the neck muscles until its insertion in the cranial angle of the scapula border. Since it is impossible to remove the entire thoracic limb without removing this muscle, it was considered as part of the group of extrinsic muscles of the thoracic limb.

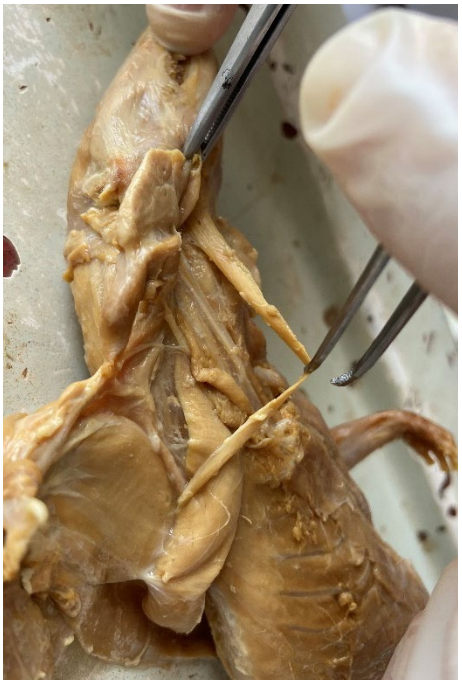

Figure 5. Raw images of *L. crassicaudata m. omohyoideus* dissection. Ventral view of opened right forearm.

M. latissimus dorsi

This thin and flattened muscle has a very long origin. It arises via fleshy fibers under the *m. trapezius pars thoracica* insertion, from the third thoracic vertebrae to the third lumbar vertebrae. It has a double insertion, both via tendinous fibers: the first it is more caudally displaced, in the teres major tuberosity, the tendon being mixed with part of the *m. teres major*, going under the medium third of the *m. biceps brachii*, almost in the cranial shaft of humerus; the second insertion is cranially blended with the *m. pectoralis minor*, near its insertion, in the pectoralis ridge.

M. rhomboideus (**Figure 4**, **Figure 6**).

This muscle splits in *rhomboideus pars capitis* + *pars cervicis* (blended) and *pars thoracica*. The first has a thin ribbon-like origin from the occipital bone and nuchal crest and lies above the *m. splenius* from the C2 until the C7 vertebrae, and inserts in the scapula border, just above the *m. rhomboideus pars thoracica* and *serratus ventralis* insertion. The *m. rhomboideus pars thoracica* is shorter, thicker and more robust than the *m. rhomboideus pars cervicis*, and its origin is subtle and continuous with the pars cervicis insertion, running from the spinous process of T1 – T3 to the scapula border, between the *m. rhomboideus pars cervicis* and *m. serratus ventralis*.

M. serratus ventralis (M. serratus anterior) (**Figure 6**)

This muscle is commonly divided in two parts, *m. serratus ventralis pars cervicis* and *pars thoracica*, although they are blended together. The former originates in the transverse process of C2–C7 and inserts in the *facies serrata.* The latter is a fan-shaped muscle with a broader origin in the cranial side of the first eight ribs, and it inserts at the serratus tuberosity of the ribs and the dorsal border of scapula.

M. pectoralis major (*M. pectoralis superficialis*) (**Figure 6**, **Figure 8**)

This fan-shaped muscle originates via fleshy fibers from the manubrium until half of the sternum bone body. A tiny portion of its insertion reaches the supraglenoid tubercle of the scapula, near the clavicle (which is positioned in this tubercle). Both descendent and transverse part of the *pectoralis major* insert in the humeral crest (pectoralis ridge).

M. pectoralis minor (M. pectoralis profundus) (**Figure 8**)

This muscle originates from the fourth manubriosternal junction until the xiphoid process, partially covered and blended with the *m. pectoralis superficialis*, as it gets closer to the insertion. It inserts via fleshy fibers from the beginning until the half of pectoral tuberosity, caudally to the *m. pectoralis major* insertion.

M. subclavius (**Figure 6**)

It is a ribbon-like muscle that originates by fleshy fibers from the lateral edge of the manubrium, right above the first costal notch. It runs deep to the clavicle, and only truly attaches with fleshy fibers on the dorsal side of the last quarter of the clavicle. It gets a bit larger and triangular and inserts partially via fleshy fibers in the acromion process of the scapula and also via tendinous fibers in the fascia of the *m. supraspinatus*.

Shoulder muscles

M. deltoideus (group) (**Figure 6**)

It was divided in three parts, *pars scapularis*, *acromialis* and *clavicularis*. The *pars scapularis* originates via fleshy fibers in the first third of scapular spine, blended with *m. infraspinatus*, and inserts via tendinous fibers in the deltoid ridge of the humerus. The *pars acromialis* originates via fleshy fibers from scapular acromion and inserts, via fleshy fibers, in the distal end of the deltoid ridge of the humerus, besides the other deltoid insertions. It lies below a small portion of *m. trapezius pars cervicis*. The *m. deltoideus clavicularis* originates via fleshy fibers in the medial half of the clavicle, and it inserts from fleshy fibers medial to *m. deltoideus acromialis* in the deltoid ridge.

M. supraspinatus (**Figure 6**)

It is a massive muscle completely attached by fleshy fibers in the entire dorsal superior aspect of the scapula, covering the supraspinatus fossa and going a bit further after the scapula bone. A small tendon inserts the muscle in the cranial part of the greater tuberosity of the humerus.

M. infraspinatus (**Figure 6**)

This muscle attaches only by flesh fibers in the entire dorsal inferior aspect of the scapula, covering the entire infraspinatus fossa. It inserts by a small tendon in the caudal part of the greater tuberosity of the humerus.

M. teres major (**Figure 6**)

It is a flat and well-developed muscle that originates via fleshy fibers in the caudal angle of scapula border and inserts via tendinous fiber in the teres major tuberosity, in the humerus.

M. teres minor

This muscle was not found in *L. crassicaudata.*

M. subscapularis (**Figure 6**)

Its origin is completely fleshy in the entire medial aspect of the scapula, and it inserts via tendinous fibers in the lesser tubercle of humerus.

M. coracobrachialis (**Figure 6**)

This small and thin muscle originates via fleshy fibers in the coracoid process. It inserts via long and narrow tendinous fibers near and cranially of the teres major tuberosity. We did not find the long head of this muscle in *L. crassicaudata.*

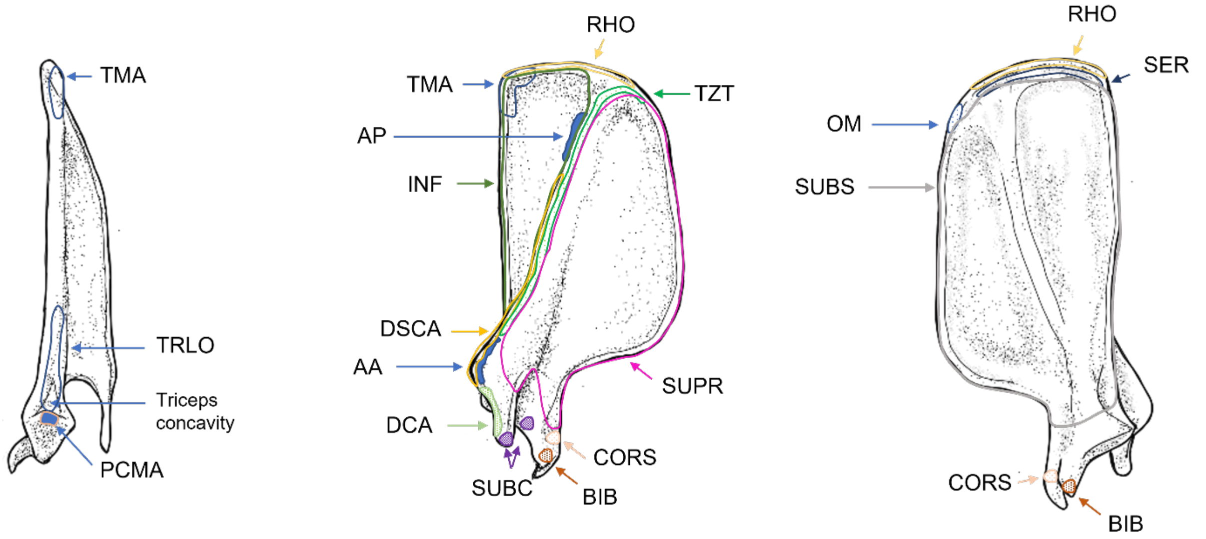

Figure 6. Muscle mapping of *Lutreolina crassicaudata* scapula. Abbreviations: (AA, AP) Atlantoscapularis Anticus, Atlantoscapularis Posticus, (BIB) Biceps Brachii, (BRA) Brachialis, (CORS) Coracobrachialis, (DCA, DSCA) Deltoideus Acromialis, Deltoideus Scapularis, (INF) Infraspinatus, (OM) Omohyoideus, (RHO) Rhomboideus, (SER) Serratus, (SUBC) Subclavius, (SUBS) Subscapularis, (SUPR) Supraspinatus, (TMA) Teres Major, (TZT) Trapezius Thoracic, (TRLO) Triceps Long head.

Brachium muscles

M. biceps brachii (**Figure 6**, **Figure 7**, **Figure 9**)

This muscle has two heads with different origins and share the same insertion. The medial/ supraglenoid head originates in a very well-developed tuberosity in the supraglenoid tubercle. The lateral/coracoid head originates cranio-medially in the greater tubercle of humerus. Both heads have a long tendinous insertion from the supracondylar lateral crest until the cranial aspect of the ulnar tuberosity, being more distal than the *m. brachialis* insertion.

M. brachialis (**Figure 7**, **Figure 8**, **Figure 9**)

It originates lateral-caudal in the greater tubercle, in the neck of the humerus (*collum humeri*). Its insertion is long and via tendinous fibers from the supracondylar lateral crest until the cranial aspect of the ulnar tuberosity, being more proximal than the *m. biceps brachii* insertion.

The insertion of *m. brachialis* and *m. biceps brachii* divides the medial and lateral groups of forearm muscles.

M. triceps brachii (**Figure 6**, **Figure 7**, **Figure 8**, **Figure 9**)

This muscle has the three common heads, short, lateral and long, plus an accessory head, with different origins but the same insertion to all of them. The short (also medial) head is thin and originates via fleshy fibers medially in the humerus shaft and it inserts via fleshy fibers in the olecranon tuberosity. The long head is robust and originates via fleshy and tendinous fibers in the distal third of the caudal border of scapula. *L. crassicaudata* possess a small concavity in the scapular neck (**Figure 6**), in the caudal border of scapula, for the insertion of this long head. It inserts via tendinous fibers in the olecranon tuberosity. The lateral and the accessory head have the same origin in the proximal region of the humerus diaphysis. However, the lateral head is in the lateral aspect, and the accessory head is in the caudal aspect of the *collum humeri.* Both of them also insert via tendinous fibers in the olecranon tuberosity. We also found an accessory head that originates from the caudal shaft of the proximal region of the humerus diaphysis, and its insertion is merged with the others in the olecranon tuberosity.

M. dorsoepitrochlearis (**Figure 9**)

This elongated and thin muscle originates via tendinous fibers on the edge of the caudal border of scapula, in contact and passing above the *m. latissimus dorsi*. It inserts via tendinous fibers in the extremity of olecranon tuberosity.

M. epitrochleoanconeus (or anconeus internus) (**Figure 7**, **Figure 9**)

Its origin is blended with the *m. flexor carpi ulnaris* origin, in the olecranon. It goes obliquely to inserts in the medial epicondyle of the humerus.

M. anconeus lateralis (anconeus externus) (**Figure 8**, **Figure 9**)

It originates in the caudal aspect of the distal epiphysis and its position is caudal to the lateral supracondylar crest. It inserts in the lateral aspect of the olecranon and the anconeus process.

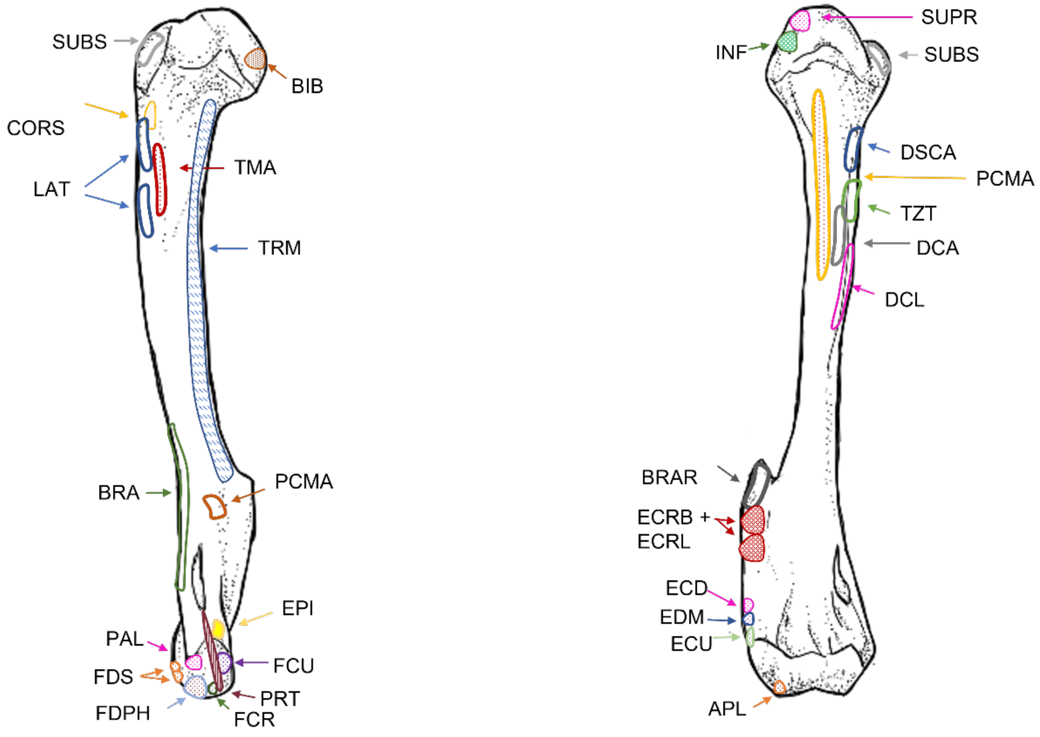

Figure 7. Muscle mapping of *Lutreolina crassicaudata* humerus, medial (left) and cranial (right) views. Abbreviations: (APL) Abductor Pollicis Longus, (BIB) Biceps Brachii, (BRA) Brachialis, (BRAR) Brachioradialis, (CORS) Coracobrachialis, (DCA + DCL + DSCA) Deltoideus Acromialis, Deltoideus Clavicularis and Deltoideus Scapularis, (EPI) Epitrochleoanconeus, (ECRB + ECRL) Extensor Carpi Radialis brevis and Extensor Carpi Radialis longus, (ECU) Extensor Carpi Ulnaris, (EDM) Extensor Digiti Minimi (EDC) Extensor Digitorum Communis, (FCR) Flexor Carpi Radialis, (FCU) Flexor Carpi Ulnaris, (FDPH) Flexor Digitorum Profundus Humeral head, (FDS) Flexor Digitorum Superficialis, (INF) Infraspinatus, (LAT) Latissimus, (PAL) Palmaris Longus, (PCMA) Pectoralis Major, (PRT) Pronator Teres, (SUBS) Subscapularis, (SUPR) Supraspinatus, (TMA) Teres Major, (TZT) Trapezius Thoracic, (TRM) Triceps Medial.

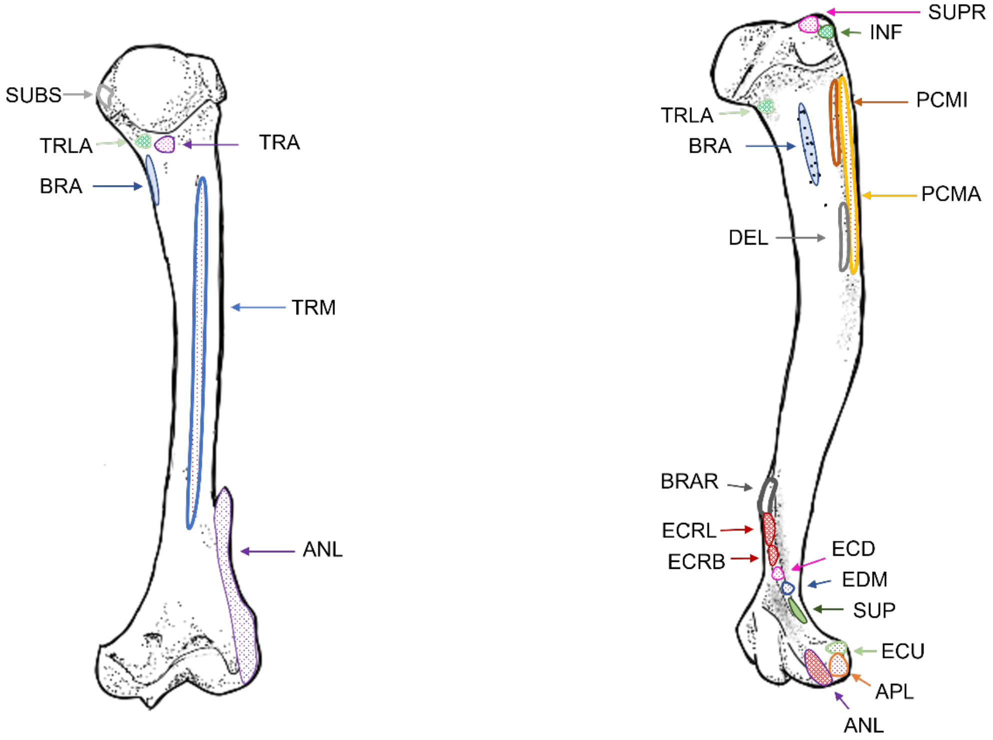

Figure 8. Muscle mapping of *Lutreolina crassicaudata* humerus, caudal (left) and lateral (right) views. Abbreviations: (APL) Abductor Pollicis Longus, (ANL) Anconeus Lateralis, (BRA) Brachialis, (BRAR) Brachioradialis, (DEL) Deltoideus ridge, (ECRB+ECRL) Extensor Carpi Radialis brevis and Extensor Carpi Radialis longus, (ECU) Extensor Carpi Ulnaris, (EDM) Extensor Digiti Minimi, (ECD) Extensor Digitorum Communis, (INF) Infraspinatus, (PCMA + PCMI) Pectoralis Major and Pectoralis Minor, (SUBS) Subscapularis, (SUP) Supinator, (SUPR) Supraspinatus, (TRA + TRLA + TRM) Triceps Accessory head, Triceps Lateral head and Triceps Medial head.

Antebrachium muscles – Ventral division (ventral/medial)

M. pronator teres (**Figure 7**, **Figure 9**)

In the ventral view of the forearm, this muscle origin has two forms: one is tendinous in the inferior and caudal view of the entire medial epicondyle of the humerus, while the fleshy origin is more cranial, and it goes until the border of the supracondylar foramen. It has a thin, long and tendinous insertion, from the cranial aspect of the medium third of the radius until the distal region of the radial diaphysis.

M. flexor carpi radialis (**Figure 7**)

This muscle has a fleshy origin covered by other muscles, in between the *m. pronator teres* origin (above/lateral side) and the *m. flexor digitorum profundus* origin (below/medial). The entire muscle is covered up by the *m. palmaris longus*. The muscle has two indivisible heads, the first coming from the medial epicondyle of humerus and the other is a bit more distally, originating closer of the trochlea. It inserts in the proximal carpal bone of the radius.

M. flexor carpi ulnaris (**Figure 7**, **Figure 9**)

This muscle has two heads that do not split completely, with a broad origin, from the medial epicondyle of humerus until the olecranon tuberosity. It caudally covers all the deep flexor muscles in the medial side of the forearm and inserts in the accessory carpal bone.

M. palmaris longus (**Figure 7**)

It is a robust muscle with two divisible bellies, one slender and the other is ticker, covering both ulnar and radial heads of *m. flexor digitorum profundus*. Its origin is fleshy from the medial epicondyle of humerus, and it has a very strong tendinous insertion in the carpal fascia.

M. flexor digitorum superficialis (**Figure 7**)

This muscle was incompletely divisible in two heads, medial and lateral. Both heads arise via thin and long tendinous fibers from the medial epicondyle of humerus, forming a muscle belly in the middle of this tendon. The medial head inserts in the palmar aspect on the first metacarpal bone. The lateral head inserts in the phalanges of digits II-V.

M. flexor digitorum profundus (**Figure 7**, **Figure 9**)

This muscle is massive, with three heads: humeral, radial and ulnar. The humeral head fills all the space from the medial epicondyle until the olecranon, just above the *m. flexor carpi ulnaris*. The second head comes from the medial aspect of the radius shaft and third arises from the olecranon tuberosity. The muscle inserts dorsally in the phalanges of digits I – IV.

M. pronator quadratus (**Figure 9**)

This muscle is deep and the last one left in the forearm dissection. It originates in the most distal and anterior end of the ulna and has a small belly running to the distal and anterior end of radius, filling the interosseous space.

Antebrachium muscles – Dorsal division (dorsal/lateral)

M. brachioradialis (**Figure 7**, **Figure 8**, **Figure 9**)

It originates via tendinous fibers from the lateral supracondylar crest and inserts by very thin tendinous fibers in the dorsal aspect of the radial carpal bone.

M. extensor carpi radialis (**Figure 7**, **Figure 8**)

This muscle has two incompletely separable heads (*longus* and *brevis*), with the same origin and different insertions. It originates in the lateral supracondylar crest, one besides the other. The *longus* head inserts in the dorsal aspect of the distal region of the second metacarpal bone, while the *brevis* head inserts in the dorsal aspect in between the second and the third metacarpal bone.

M. extensor digitorum communis (**Figure 7**, **Figure 8**)

It originates in the most distal part of the lateral supracondylar crest and it inserts via tendinous fibers dorsally in the phalanges of all digits.

M. extensor digiti minimi (**Figure 7**, **Figure 8**)

It originates in the most proximal part of the lateral epicondyle of humerus, and it inserts in the distal phalanges of the digits IV and V.

M. extensor digitorum profundus (**Figure 9**)

This muscle originates proximally in the lateral aspect of ulna, near the olecranon tuberosity, and it inserts dorsally in the phalanges of digits I-IV.

M. extensor carpi ulnaris (or ulnaris lateralis) (**Figure 7**, **Figure 8**)

It originates in the most distal part of the lateral epicondyle of the humerus, and it inserts in the carpal-ulnar palmar process/hamate bone.

M. supinator (**Figure 8**, **Figure 9**)

This muscle originates from the lateral epicondyle of humerus and inserts in the cranial aspect of the medium third of the radius bone.

M. abductor pollicis longus (**Figure 7**, **Figure 8**, **Figure 9**)

This muscle is flattened, well-developed and very deep, originating from the lateral epicondyle of humerus, lying in the radius-ulna lateral interosseous space. It inserts in the first metacarpal bone.

Manus

We were not able to dissect and properly identify these muscles due to extreme alcohol dehydration and also taxidermy processes, including cutting the edges of most phalanges.

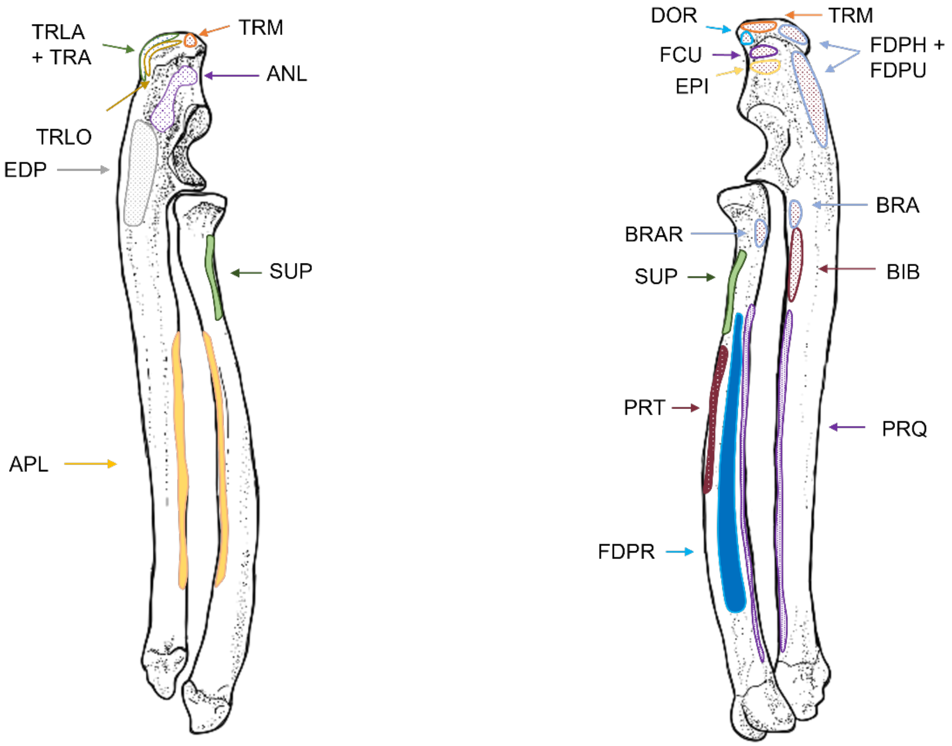

Figure 9. Muscle mapping of *Lutreolina crassicaudata* radio and ulna, lateral (left) and medial (right) views Abbreviations: (APL) Abductor Pollicis Longus, (ANL) Anconeus Lateralis, (BIB) Biceps Brachii, (BRA) Brachialis, (BRAR) Brachioradialis, (DOR) Dorsoepitrochlearis, (EPI) Epitrochleoanconeus, (EDP) Extensor Digitorum Profundus, (FCU) Flexor Carpi Ulnaris, (FDP) Flexor Digitorum Profundus head (H, R, U: humeral, radial, ulnar heads), (PRQ) Pronator Quadratus, (PRT) Pronator Teres, (SUP) Supinator, (TRA, TRLA, TRLO, TRM) Triceps Accessory, Lateral, Long and Medial heads.

Description of hindlimb myology

Table 3. Origin, insertion and actions of the identified muscles of the hindlimb of *Lutreolina crassicaudata*. Main actions according to Miller et al. (2013) and Souza Júnior et al. (2018). (*) Quadriceps femoris muscle group.

Muscle	Origin	Insertion	Main Action
	Extrinsic muscles of the hindlimb		
Iliopsoas	L5 and iliac crest	Femur lesser trochanter	Flexion of hip joint
	Gluteo group		
Gluteus superficialis	Iliac crest + I sacral – II caudal	Femur greater trochanter	Medial rotation of hip
Gluteus medius	Iliac crest and I sacral	Femur greater trochanter	Medial rotation of hip
Gluteus profundus	Ilium, ischiatic spine and I–II caudal vertebrae	Femur greater trochanter	Extension of hip joint
Obturator internus	Obturator foramen (dorsal)	Femur intertrochanteric fossa	Lateral rotation of hip joint
Gemelli	Ischium body, dorsally	Femur intertrochanteric fossa	Lateral rotation of hip joint
Quadratus femoris	Ischium tuberosity	Femur intertrochanteric fossa	Extension and lateral rotation
	Thigh muscles (cranial, medial and caudal)		
Sartorius	Cranial border of iliac crest	Tibial tuberosity	Flex and stifle the hip
Rectus femoris*	Ilium body, near acetabulum	Tibial tuberosity	Extensor of stifle joint +
Vastus lateralis*	Femur greater trochanter	Tibial tuberosity	Tension of fascia cruris +
Vastus medialis*	Femur head	Tibial tuberosity	Flexion of the hip
Vastus intermedius*	Femur shaft	Tibial tuberosity	(the three above)
Gracilis	Ischiatic symphysis	Tibia (2/3)	Adduction of the limb
Pectineus	Epipubic bone	Femur shaft	Adduction of the thigh
Adductor cranial	Pubic symphysis	Femur shaft (2/3)	Adductor and extension +
Adductor intermedius	Ischiatic symphysis	Femur medial condyle	of the +
Adductor caudal	Ischiatic arch	Intercondylar fossa	hip joint
Obturator externus	Obturator foramen (ventral)	Femur intertrochanteric fossa	Lateral rotation of hip joint
Biceps femoris	Ischiatic tuberosity	Lateral epicondyle + half calf	Hip + tarsal joint extensor
Abductor cruris caudalis/tenuissimus	Transverse process of the fourth caudal vertebra	Tibia (2/3), contributes for the common tendon	Tarsus flexion
Semitendinosus	Ischiatic tuberosity	Tibia (2/3)	Extension of hip + tarsal joints
Semimembranosus	Ischiatic arch and tuberosity	Medial tibia border	Hip extensor

Muscle	Origin	Insertion	Main Action
Muscles of the leg (cranial, lateral and caudal)			
Tibialis cranialis	Lateral in tibia tuberosity	First metatarsal bone	Lateral paw rotation
Extensor digitorum longus	Craniolaterally in the fibula head	Digits II–V	Extension of the digits, flexion of the tarsus
Extensor digitorum lateralis	Laterally in the fibula head	Digits IV–V	Extension of the digits
Fibularis	Fibula head	Palmar aspect of V metatarsal bone	Flexor of tarsus
Peroneus digiti quinti	Not found	Not found	-
Tríceps surae	Femur epicondyle	Common Achilles tendon	Extends the tarsal joint
Flexor digitorum superficialis	Fibula head	Digits I–V	Flexion of the digits
Flexor digitorum profundus	Fibula head	Digits I–V	Flexion of the digits
Popliteus	Fibula head (medial)	Caudal aspect of tibia shaft	Medial rotation of femur
Tibialis caudalis	Fibula head (medial)	First metatarsal bone	Extension of tarsus

Extrinsic muscles of the pelvic limb

M. iliopsoas (*iliacus* + *psoas major*)

M. iliacus

It originates laterally in the ilium crest and through all the iliac crest. It inserts in the lesser trochanter. Its insertion is blended with the *m. psoas major*.

M. psoas major

It originates in the ventral aspect of the vertebral body of the L5, lying there until the lesser trochanter of the femur. Its insertion is blended with the *m. iliacus*.

Gluteal region

M. tensor fasciae latae (**Figure 10**)

This is a triangular muscle, surrounded by the *fascia latae*, in the middle of it. Its origins and insertions are blended with other muscles below it. The dorsal part is mixed with the *fascia gluteal*. The cranial edge of the muscle lies in the *m. rectus femoris* in the distal part, while the *fascia latae* prolongates until the femoral trochlea in the knee, and the caudal part is near to the *m. gluteus superficialis* insertion, with the *fascia latae* inserting in the *m. vastus lateralis*.

M. gluteus superficialis (**Figure 10**)

This muscle has two parts, cranial and caudal. Its caudal part is also considered as *m. gluteofemoral* or *m. agitator caudae*. The cranial part originates from the iliac crest, including the spinal process of the first and second sacral and caudal vertebrae. It inserts distally and right above the greater trochanter of the femur. The caudal part originates from the fourth until the seventh caudal vertebrae, more specifically in its articular processes. It inserts in the medium third of the lateral aspect of the femur, in its rough surface, approximately 2cm above the condyles.

M. gluteus medius (**Figure 10**)

It originates via fleshy fibers in the iliac crest and a tendinous part originates from the first sacral vertebra. It inserts in the greater trochanter of femur.

M. gluteus profundus (**Figure 10**)

This muscle is massive, and it is hard to split its three parts (*cranial*, *intermedius* and *caudal*) that have different origins but the same insertion. The cranial part originates in the gluteal aspect of the base of the ilium blade. The *intermedius* part originates from the ischiatic spine. The caudal part originates in the traverse process of the first and second caudal vertebrae. The three parts insert together in the greater trochanter of the femur.

M. femoroccocygeus (**Figure 10**)

It has a triangular shape, originating from the second sacral vertebra and the first caudal vertebra. It inserts in the proximal part of femur.

M. obturator internus

It originates in the most caudal and medial extremity of the ischium board, surrounding the internal side/dorsal aspect of the obturator foramen. It inserts in the trochanteric fossa of the femur.

Mm. gemelli

It originates in the dorsal region of the ischium body, in the lesser ischiatic notch, and inserts in the intertrochanteric fossa of the femur.

M. quadratus femoris

It has a short and restrict origin in the ischium border, just ventral to the ischiatic tuberosity. It inserts in the caudal region of the femur proximal epiphysis, in between the greater and lesser trochanter, superficial to the intertrochanteric fossa.

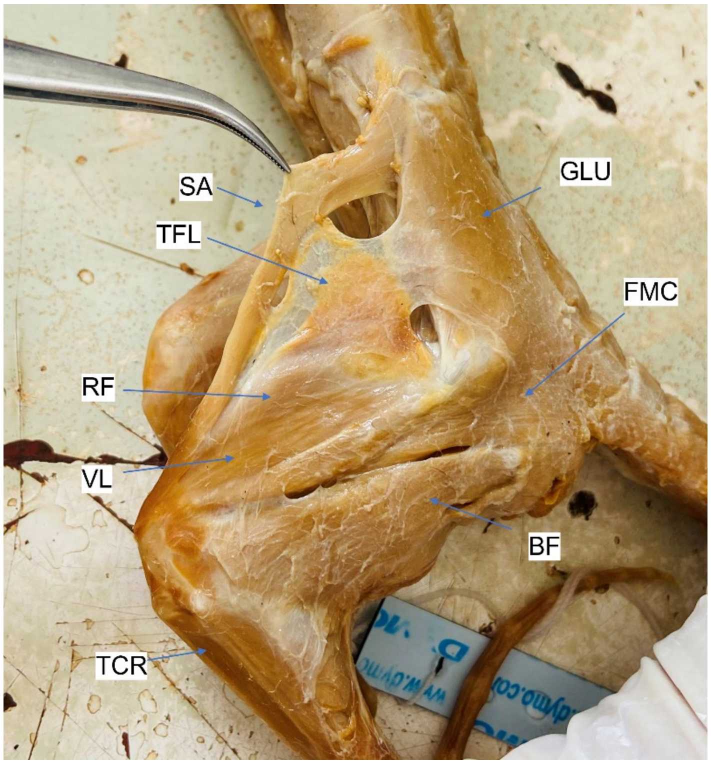

Figure 10. Raw dissection photo of *Lutreolina crassicaudata* right hindlimb, lateral view. Abbreviations: (SA) Sartorius, (TFL) Tensor fascia latae, (RF) Rectus femoris, (VL) Vastus lateralis, (TCR) Tibialis cranialis, (BF) Biceps femoris, (FMC) Femoroccocygeus, (GLU) Gluteus group.

Cranial thigh

M. sartorius (**Figure 10**)

It showed the usual strap-like morphology of the most external muscle of the cranial thigh. It originates in the most cranial part of the iliac crest and inserts in the tibial tuberosity.

M. quadriceps femoris (**Figure 11**)

It is a group of four well-developed and robust muscles with different origins and same tendinous insertion in the tibial tuberosity. It includes the *m. rectus femoris*, *m. vastus lateralis*, *m. vastus medialis* and *m. vastus intermedius*. The *m. rectus femoris* originates in the latero-ventral aspect of the ilium body, near the cranial portion of the acetabulum. The *m. vastus lateralis* originates in the greater trochanter of the femur, the *m. vastus medialis* originates ventrally in the femur neck and the *m. vastus intermedius* originates via strong fleshy fibers in the lateral aspect of the femur shaft, deep to *m. vastus lateralis*.

Medial thigh

M. gracilis

It has a clear and visible origin, reaching the entire ischiatic symphysis from the middle to the caudal part. It inserts via a strong tendon, in the medium third of the tibia, in the cranial, medial and caudal aspects, reaching the three sides diagonally.

M. pectineus (**Figure 11**)

It originates in the lateral tubercle of the epipubic bone and has two incompletely divisible heads (superficial and deep). Both heads insert in the medium third of the femur medial aspect, near the lesser trochanter of the femur.

Mm. adductors (**Figure 11**)

This is a very strong group of muscles, with three different bellies, cranial, intermedius and caudal head. The cranial head is slender and longer than the caudal head, and its origin is ventrally in the pubic symphysis, and semi divisible in two parts. The first part originates from the base of the epipubic bone, but not attached to it (the bone does not move when the muscle is pulled), only to the pubis, while the second part is restricted to the pubic symphysis only. It inserts in the medium third of the femur shaft and runs until the distal epiphysis, above the condyles. The *intermedius* head is the slenderest of all the three heads, and it originates in the most cranial region of the ischiatic symphysis, just caudal to the cranial head. It has a long and more distal insertion in the medial part of the femur shaft, with a thin tendon reaching the medial condyle. Finally, the caudal head is the thickest and robust of all three heads, originating from the entire ischiatic arch, lateral edge and a tiny part of the ischiatic symphysis. Its dorsal part did not reach the ischiatic tuberosity. It inserts in the caudal-lateral aspect of the femur, from half of the femur shaft until the intercondylar fossa, being the only head that indeed occupies the fossa.

M. obturator externus

It originates from the most caudal and medial part of the ischium board, ventrally surrounding the obturator foramen. It inserts in the intertrochanteric line of the femur.

Caudal thigh

M. biceps femoris (**Figure 10**)

Despite its name, it has only one head, and the muscle does not attach in the femur. It originates in the ischium tuberosity, and it inserts with a large and well-developed fascia from the lateral epicondyle until the lateral aspect and middle of the calf. In this same fascia a part of the *m. semitendinosus* is inserted.

M. abductor cruris caudalis (m. tenuissimus)

It originates superficially to the origin of *m. semitendinosus*, in the transverse process of the fourth caudal vertebra. It has two insertions, the most cranial in the lateral aspect of the medium third of tibia and the most caudal in the same medium third, but in the medial aspect. Both insertions contribute for the calcaneal common tendon (“Achilles’ tendon”).

M. semitendinosus

It originates in the ischiatic tuberosity, deep into the *m. abductor cruris caudalis*. It splits in two heads, but both inserting in the medium third of the medial aspect of tibia.

M. semimembranosus (**Figure 11**)

This muscle has two heads, one above the other. The most superficial is also the slenderest and originates in the ischiatic arch near the ischiatic tuberosity. The deeper head is more robust and originates more distal from the ischiatic tuberosity, still in the ischiatic arch but closer from the ischiatic symphysis. The superficial head inserts distally in the medial border of the tibia, while the deep head inserts just above this region, more proximal to the femur.

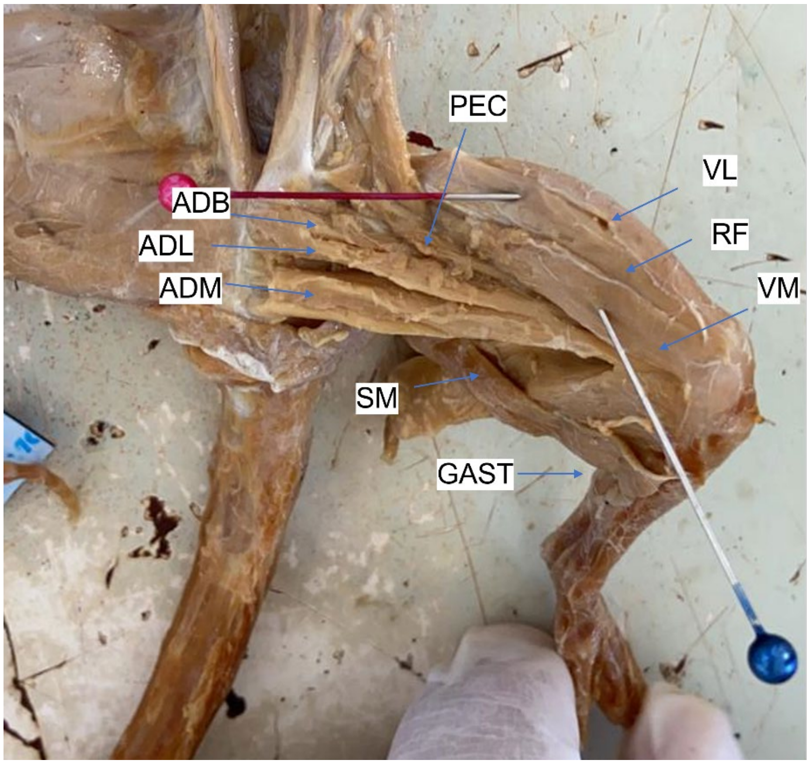

Figure 11. Raw photo dissection of *Lutreolina crassicaudata* left hindlimb, ventral view. Abbreviations: (PEC) Pectineus, (ADB) Adductor brevis, (ADL) Adductor longus, (ADM) Adductor magnus, (SM) Semimembranosus, (GAST) Gastrocnemius, (VM) Vastus medialis, (RF) Rectus femoris, (VL) Vastus lateralis.

Muscles of the leg

M. tensor of the crural fascia

This is a very well-developed fascia that unites with the fascia of other muscles to contributes with the calcaneal common tendon ("Achilles' tendon").

Cranial leg

M. tibialis cranialis (**Figure 10**, **Figure 12**)

This muscle is the larger and more robust in the cranial part of tibia. It originates right below the lateral condyle of tibia, filling the depression of the lateral aspect of the tibial tuberosity. It inserts via strong tendinous fibers in the first metatarsal bone.

M. extensor digitorum longus (**Figure 12**)

It is a slender muscle a thin belly, originating in the cranial-lateral aspect of the fibula head and inserting via tendinous fibers in digits II-V.

M. extensor digitorum lateralis

This is a very thin muscle, originating from the lateral aspect of the fibula head and lying on the interosseous membrane. The insertion is the same found in Warburton *et al.* (2015), via tendinous fibers passing along that of the long digital extensor fascia, over the middle and distal phalanges of digits IV and V.

Lateral leg

M. peroneus [fibularis] longus (**Figure 12**)

It originates in the fibula head and it inserts in the lateral-palmar aspect of the fifth metatarsal bone.

M. peroneus [fibularis] brevis (**Figure 12**)

The muscle origin is in the fibula head, being one of the largest muscles that originates in this region, in a very lateral aspect. The tendon passes below the lateral malleolus and it inserts in the base of the fifth metatarsal bone.

M. peroneus digiti quinti

This muscle was not found in *Lutreolina crassicaudata.*

Caudal leg

Mm. triceps surae (**Figure 11**, **Figure 12**)

It is a group of muscles composed by *m. soleus* and both heads of *Mm. gastrocnemii*, with different origins but the same and unique insertion, by strong tendinous fibers forming the calcaneal common tendon ("Achilles' tendon"). The *m. soleus* originates caudally in the fibula head, near to the *fabellae*. The lateral head of the *m. gastrocnemius* originates in the lateral epicondyle of femur, while the medial head originates in the medial epicondyle of femur.

M. flexor digitorum superficialis (M. plantaris + M. flexor digitorum brevis)

This muscle originates in the medial head of the fibula and its insertion splits in two tendons that separates in the plantar aspect. One tendon goes to the fifth digit while the other branches off to digits I-IV.

M. flexor digitorum profundus (M. flexor digitorum longus)

It originates in the medial aspect of the fibula head, distally near to the *m. flexor digitorum superficialis* insertion. It divides in four thick and flat tendons that inserts in digits I-IV.

M. popliteus

It is apparently a very small muscle, originating in the medial aspect of the fibula head and inserting in the cranial aspect of the proximal region of the tibia shaft.

M. tibialis caudalis (M. tibialis posterior)

This muscle originates in the medial aspect of the fibula head, lying in all interosseous membrane, which appears to be muscular. It inserts via tendinous fibers in the first metatarsal bone (digit I), almost in the proximal phalange.

M. interosseous

It lies in between the tibia and fibula, in the medial aspect of the leg. It runs from the distal part of femur until the tarsal proximal region. This muscle is robust and apparently splits in a minor and distal part in the lateral aspect of the interosseous space.

Intrinsic muscles of the foot

We were not able to dissect and properly identify these muscles due to extreme alcohol dehydration and also taxidermy processes, including cutting the edges of most phalanges.

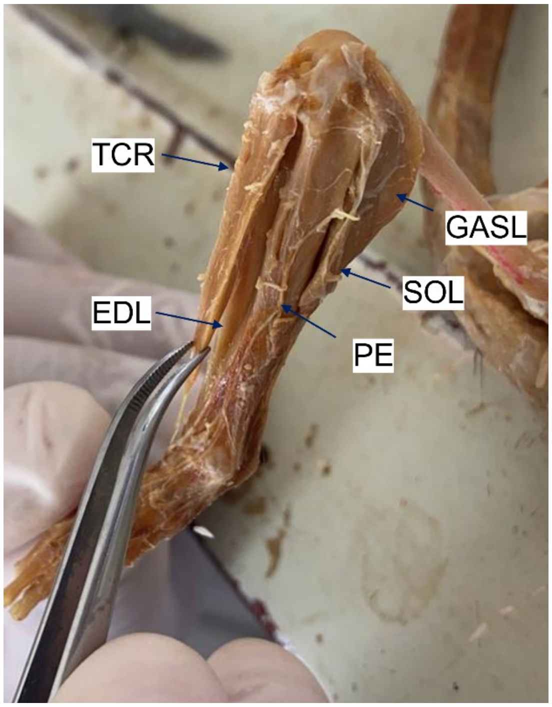

Figure 12. Raw photo dissection of *Lutreolina crassicaudata* left hindlimb, lateral view. Abbreviations: (TCR) Tibialis cranialis, (EDL) Extensor digitorum longus, (PE) Peroneus: brevis and longus, (SOL) Soleus, (GASL) Gastrocnemius lateralis.

Limb muscle weight analysis

Forelimb

The muscle mass proportions were separated by muscle movements/functions: pronator, supinator, flexors of forearm, extensors of forearm, flexors of hand/digits and extensors of the hand/digits (**Figure 13**). For detailed information of the muscle arrangements, see Material and Methods section. The greatest muscle mass was in the extensors of the forearm, represented by the triceps muscle (**Figure 13**, left). Since its weight prevented a detailed view of the weight distribution of the other muscles, we removed it from the second analysis, in which it is possible to observe that the second major muscle mass are the flexors of the hand and digits, followed by its extensors, the supinator, flexors of the forearm and finally, the pronator muscle, with the smallest mass (**Figure 13**, right).

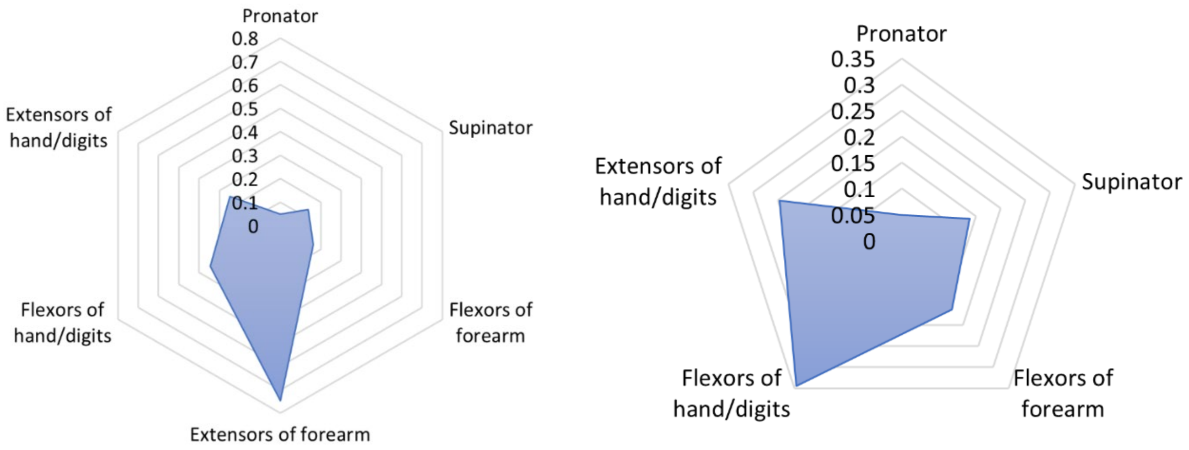

Figure 13. Mean forelimb muscle proportions of *L. crassicaudata* grouped by muscle actions calculated from wet muscle weights. Left, all muscles; right, removing the extensors of forearm (*m. triceps brachii*).

Hindlimb

The muscle mass proportions were separated by muscle movements/functions: hip flexors, gluteal group, deep hip, quadriceps femoris, adductor group, hamstrings, extensors and flexors of pes (**Figure 14**). For detailed information of the muscle arrangements, see Material and Methods section. The greatest muscle mass was in the hamstrings group, followed by the *m. quadriceps femoris*. In sequence, the gluteal group is slightly heavier than the hip flexors, but both groups are considerable smaller than the quadriceps and the hamstrings. Lastly, the flexors and extensors of *pes* and the deep hip muscle group have the smaller mass in the hindlimb musculature.

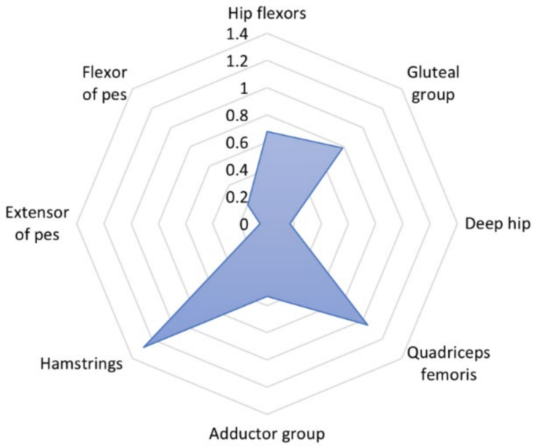

Figure 14. Mean hindlimb muscle proportions of *L. crassicaudata* grouped by muscle actions calculated from wet muscle weights.

The wet muscle data was used to provide broad comparisons with other American and Australian marsupials. In the American group, we compared *L. crassicaudata* with the generalist scansorial opossum *Didelphis marsupialis*, the most specialized arboreal opossum *Caluromys philander* and the terrestrial opossum *Metachirus myosurus* (**Figure 15**). The quadriceps group (*m. rectus femoris*, *m. vastii*), *Mm. glutei*, *m. semimembranosus*, adductors and *m. pectineus* comprised the greatest muscle components in all species, but in small proportions in *C. philander*, which presented less variation in general muscle mass than the other species (**Figure 15, A**). *Lutreolina crassicaudata* was also similar to *D. marsupialis,* but the latter presented more muscle mass in the adductors and *m. pectineus*, and less muscle mass in *Mm. glutei*, *m. biceps femoris* and *m. semimembranosus* than the former (**Figure 15, B**). All muscles were larger in *L. crassicaudata* and *M. myosurus*, in which the muscular masses in the radar graph almost completely overlaps, due to similarities in the muscle proportions, except for a bit more semimembranosus muscle mass in *L. crassicaudata* than *M. myosurus* (**Figure 15, C**).

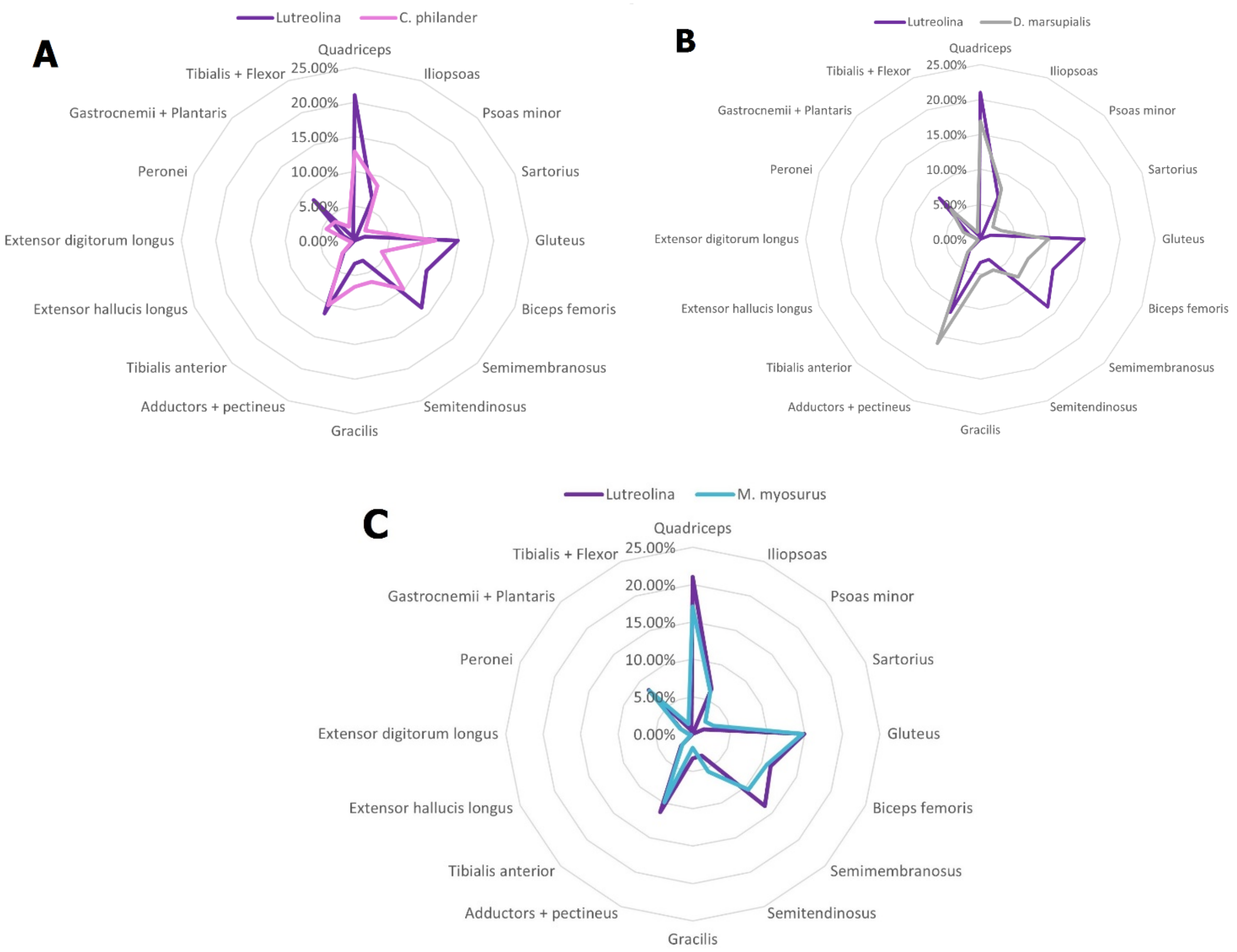

Figure 15. Muscle weight comparative analysis. (A) *Lutreolina crassicaudata* and *Caluromys philander* (arboreal); (B) *L. crassicaudata* and *Didelphis marsupialis* (scansorial) and (C) *L. crassicaudata* and *Metachirus myosurus* (terrestrial). In the three imagens, the purple radar is always *L. crassicaudata*. The main similarity is between *L. crassicaudata* and *M. myosurus*, in which the radar almost completely overlaps.

We also compared *L. crassicaudata* with some Australian marsupials, the generalist arboreal diprotodont *Trichosurus vulpecula*, a small saltatorial macropodid *Setonix brachyurus* and the terrestrial peramelid *Isoodon obesulus*. The same muscles (quadriceps and gluteus group, *m. semimembranosus*, adductors and *m. pectineus*) of the American marsupials also comprised the greatest muscle components in all species of the Australian marsupials, with the mass distribution generally similar to *L. crassicaudata*. In *T. vulpecula* there is less *m. semimembranosus*, *Mm.*

gastrocnemii + m. plantaris, but more *m. gracilis* mass than *L. crassicaudata* (**Figure 16, A**). In *S. brachyurus*, there is a very distinct increase of the gluteal muscles, and also more adductors, *m. pectineus* and *m. biceps femoris* muscle mass than *L. crassicaudata*, while considerably less in *m. semimembranosus* and *m. semitendinosus* (**Figure 16, B**). *Isoodon obesulus* has more *m. biceps femoris* muscle mass but less adductors, *m. pectineus* and quadriceps than *L. crassicaudata* (**Figure 16, C**) Their muscle mass distribution pattern was generally more similar among them than the other Australian species, but it does not overlap as much as the American species *M. myosurus* (**Figure 15, C**).

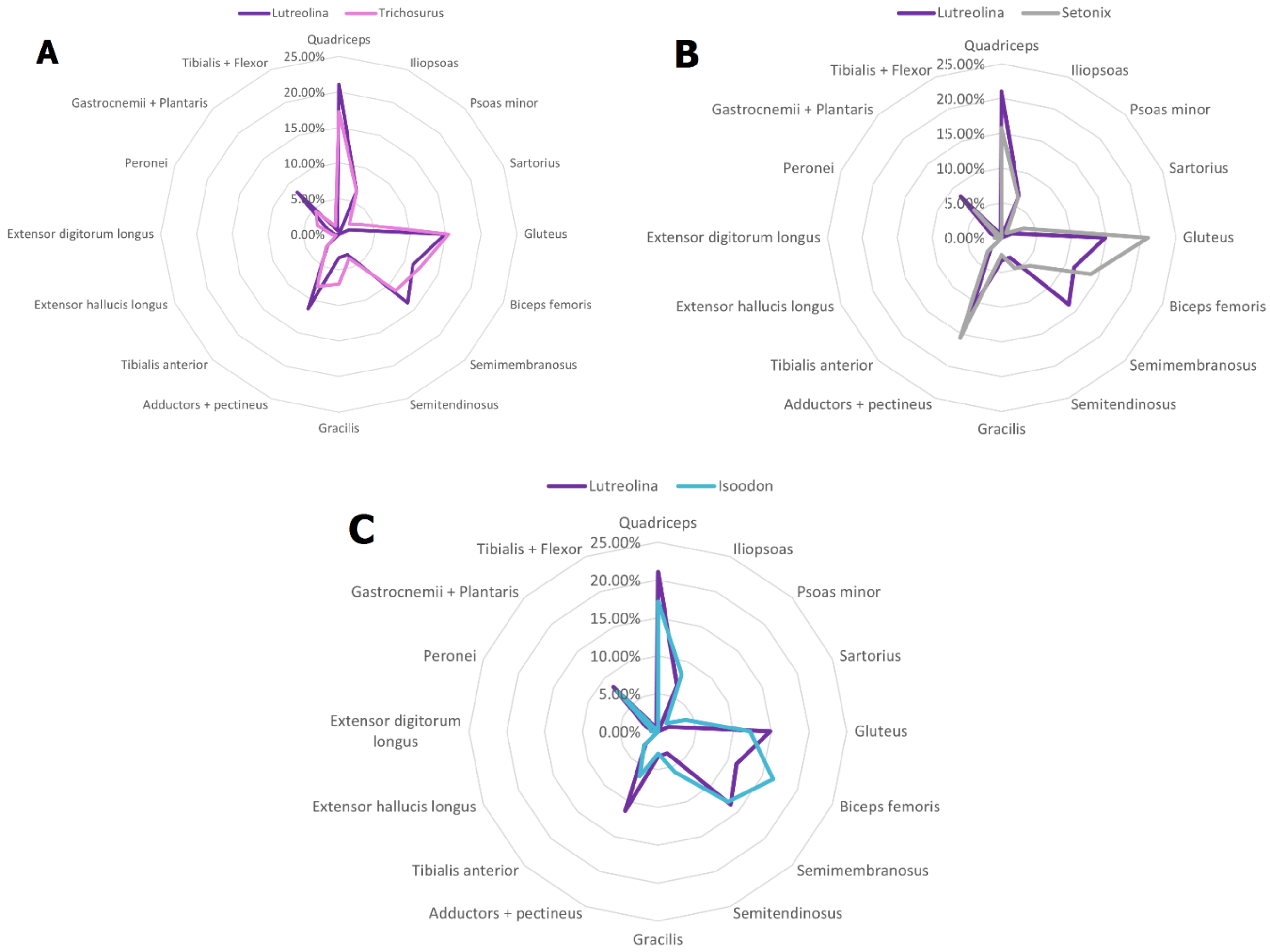

Figure 16. Muscle weight comparative analysis. (A) *Lutreolina crassicaudata* and *Trichosurus vulpecula* (arboreal); (B) *L. crassicaudata* and *Setonix brachyurus* (hopping) and (C) *L. crassicaudata* and *Isoodon obesulus* (terrestrial).

In the three imagens, the purple radar is always *L. crassicaudata*. All species are generally similar, but most between *L. crassicaudata* and *I. obesulus*, in which the radar overlaps more congruently.

DISCUSSION

The anatomical position of the muscles in *L. crassicaudata* limbs is generally similar to the morphology described for the North American opossum *Didelphis virginiana* (Coues 1872, Stein 1981, Diogo *et al.* 2016) and also Australian carnivore marsupials such as species of *Dasyurus* (MacCormick 1886, 1887, Warburton and Marchal 2017) and *Antechinus* (Warburton and Marchal 2017). We also compared its morphology to the water opossum *Chironectes minimus* (Sidebotham 1885, Stein 1981) due to the common relation of *L. crassicaudata* and sometimes inferred semi-aquatic habits. Finally, we also found some similarities with the placental weasel-like mustelid *Gallictis cuja* (Ercoli *et al.* 2014). The present discussion focused on the descriptive and comparative analysis of the main muscle groups, especially those with morphologies known as functionally relevant.

Forelimb morphology variation and comparative anatomy

The *Mm. trapezius* was almost indivisible in *L. crassicaudata* and it is considered very variable within marsupials. The split of the muscle in cervical and thoracic part is mentioned in *Didelphis virginiana* by Diogo *et al.* (2016) as *m. acromiotrapezius* and *m. spinotrapezius*, respectively, but with no details of how many cervical and thoracic vertebrae this muscle attaches. Such division was not considered for *Di. virginiana* by other authors (Coues 1872, Jenkins and Weijs 1979, Stein 1981). The origin of the thoracic part of the *m. trapezius* is also variable, being until the T10 in *L. crassicaudata*, T13 in *Di. virginiana* (Jenkins and Weijs 1979), T12 or T13 in *Dasyurus geoffroii* and also T10 in *Antechinus flavipes* (Warburton and Marchal 2017). Another distinct feature was the insertion of the thoracic part of the *Mm. trapezius*, which in *L. crassicaudata* runs until the deltoid ridge, in the humerus. This morphology is apparently uncertain for *Di. virginiana*, being considered present by Coues (1872) and Diogo *et al.* (2016) but absent by Jenkins and Weijs (1979) and Stein (1981). However, this long insertion until the humerus also occurs in *Da. Geoffroii* (MacCormick 1886, Warburton and Marchal 2017) and was considered a plesiomorphic condition among Australidelphia marsupials (Warburton and Marchal 2017) and might reflect this condition within Didelphidae.

The condition of two ribbon-like muscles as the *m. atlantoscapularis anticus* and *m. atlantoscapularis posticus* (also named as *m. omotransversarius* and *m. rhomboideus profundus*, respectively), which connect the scapula to the axial skeleton, is the same for *L. crassicaudata, Di. virginiana* (Coues 1872, Diogo *et al.* 2016) and *Da. geoffroii* (Warburton and Marchal 2017). Despite two myological descriptions of *C. minimus* (Sidebotham 1885, Stein 1981), neither described these muscles in detail, the former lacking schematic drawings and the correlation between them, and the latter describing just one and without mentioning its origins. The morphological variation of these muscles in Australian marsupials were accurately discussed by Warburton and Marchal (2017), in which their reduction or absence would be related to terrestrial-to-digging species and considered as a derived condition amongst marsupials, as well as their presence would represent a plesiomorphic condition not only in dasyuromorphians, but mammals in general. In this way, these muscles in *L. crassicaudata* would be another example of retention of a plesiomorphic feature for didelphid marsupials. Considering placental mammals, this feature was also registered in mustelids as important muscles in protraction, considering its short limbs, which could also absorb the body impact while bounding (Ercoli *et al.* 2014). This also suggests some degree of convergence in form and locomotion behavior between *L. crassicaudata* and *G.*

cuja. Further, this morphology including two separated *m. atlantoscapularis* muscles added to a quadrangular and elongated scapula it was also considered characteristic of terrestrial-to-cursorial species of marsupials as *M. myosurus* (Argot 2001) and placental viverrids such as *Ichneumia albicauda* (G. Cuvier, 1829) (Taylor 1974). This could be another evidence of the preferable classification as a terrestrial and/or cursorial species for *L. crassicaudata* than a semi-aquatic locomotion when compared with the water-opossum *C. minimus*.

The *m. omohyoideus* is an extrinsic forelimb muscle that retracts and depresses the hyoid bone (Evans and Lahunta 2013). Despite Coues (1872) that described it in detail, this muscle is present in the descriptions in the literature with little or no attention (Sidebotham 1885, Stein 1981, Diogo *et al.* 2016, to cite a few). In *C. minimus*, Sidebotham (1885) describes it in a manner similar to our study, but very superficially. Also concerning *C. minimus*, Stein (1981) described its attachments of two different forms, arising from the craniodorsal angle of the scapula and not lying superficial to *m. supraspinatus* or in between the *Mm. atlantoscapularis*, in the scapula spine. These are considerable distant attachment spots for a muscle from the same species, and hard to interpret without a schematic drawing or more detailed information. The first description of Stein (1981) fits what we found for *L. crassicaudata*, in which the muscle lies ventrally the *m. subscapularis*, until its origin in the hyoid bone, different from what is described by the same author for *D. virginiana.* Other authors only mentioned their attachments from the hyoid to the scapula (Diogo *et al.* 2016) or did not describe it at all (Argot 2001, Warburton and Marchal 2017). The *m. omohyoid* is the longer muscle that runs from the ventral-neck area and the only one to reach the scapula, and in marsupials it appears to also work as a third elevator of the scapular angle (Coues 1872). For that reason, we suggest that the *m. omohyoideus* might be also linked to general predatory habits and could be acting as an extra muscle providing strength and accuracy in lateral and ventral head movements while hunting large prey, just as both *Mm. atlantoscapularis* (*m. omotransversarius* and *m. rhomboideus profundus*) in the lesser grison *G. cuja* (Ercoli *et al.* 2014), suggesting a similar hunting behavior in *L. crassicaudata* and *G. cuja.* If this same feature indeed occurs throughout Didelphidae, it could be related to the need of this strength and accuracy during the rapid strike of the head and neck from an almost stationary position for hunting, followed by the handling prey behavior, commonly seem in opossums.

The origin of the *m. latissimus dorsi* is variable but occurring mostly between the third and fourth lumbar vertebrate (Coues 1872, Jenkins and Weijs 1979, Stein 1981). This muscle is apparently similar in all marsupials, and Cheng (1955), Diogo et al (2016) and Warburton and Marchal (2017) reported the same origin and the same blending of its insertion with the *m. dorsoepitrochlearis* for *Di. virginiana* and *D.a geoffroii*, respectively. Some authors considered that the *m. dorsoepitrochlearis* actually merges with the *m. triceps brachii* (Diogo and Abdala 2010). Both conditions remain open to discussion, but it seems that the blending between *m. dorsoepitrochlearis* and *m. triceps brachii* is the more likely to be the plesiomorphic condition for most mammals (Diogo *et al.* 2016).

For the *m. serratus* muscle, *L. crassicaudata* presents a different and larger origin and insertion (C2-C7 until the first eight ribs) than *Di. virginiana* and *C. minimus* (Stein 1981), being more similar to *Da. geoffroii*. The same occurs with the *m. rhomboideus pars cervicis* insertion (Warburton and Marchal 2017).

The deltoid group of *L. crassicaudata* was subdivided in three, *m. deltoideus acromialis*, *m. deltoideus clavicularis* and *m. deltoideus scapularis*, similar to *Dasyurus* (MacCormick 1886, Warburton and Marchal 2017). In *Didelphis* this muscle group was considered with two divisions, *m. deltoideus acromialis et clavicularis* and *m. deltoideus scapularis* (Diogo *et al.* 2016) or with no divisions (Coues 1872, Jenkins and Weijs 1979, Stein 1981). This division of three deltoids was considered a plesiomorphic condition when compared with other morphologies with some fusion of this muscle (Shrivistava 1962). However, as discussed by Warburton and Marchal (2017), the recent classification of Diogo *et al.* (2016) considered the two deltoids of *Didelphis* as plesiomorphic for all mammals, and if this is true, this complex of three well-divided deltoids present both in *L. crassicaudata* and *Dasyurus* could be considered a derived morphology, but this should require more investigation.

The origin of the *m. teres major* in *L. crassicaudata* is similar to *Di. virginiana* (Coues 1872, Jenkins and Weijs 1979, Stein 1981, Diogo *et al.* 2016). However, the same insertion of this muscle, specifically in the teres major tuberosity, was only found in *Da. geoffroii* (Warburton and Marchal 2017). The same situation occurred with *m. coracobrachialis*, in which its origin in the coracoid process is well established in the literature for all the species compared here, but its insertion was similar only to *Da. geoffroii*, in the *m. teres major* insertion (Warburton and Marchal 2017). In *Didelphis* the insertion of the *m. coracobrachialis* seemed variable: it could be in the humeral head close to *m. triceps brachii* (Coues 1872) or in the lesser tubercle deep to the *m. biceps brachii* (Stein 1981, Diogo *et al.* 2016). Either way, in all these species the *m. coracobrachialis* is considered a single muscle, typical for most marsupials (Diogo *et al.* 2016).

Interestingly, the *m. teres minor*, which flexes and laterally rotates the humerus/shoulder joint (De Iuliis and Pulera 2006, Evans and Lahunta 2013) was not found in *L. crassicaudata*. Considering Neotropical marsupials, this muscle was also not found in *Di. virginiana* (Coues 1872) neither the shrew-opossum *Caenolestes fuliginosus* (Osgood 1921). In *C. minimus* it was found by Sidebotham (1885) and Stein (1981). Diogo *et al.* (2016) stated it as present in *Di. virginiana* using Stein (1891) and Jenkins and Weijs (1969) as references, but both found her own identification doubtful, the former with the expectation of confirming it in future studies through muscle innervation. Argot (2001) did not mention this muscle at all. In Australian marsupials, Barbour (1963) found it present in the arboreal common brushtail Australian opossum *Trichosurus vulpecula* and also reviewed this muscle condition in several other Australian marsupials as absent or very reduced. Ercoli *et al.* (2014) discussed that a reduced *m. teres minor* is expected in half-bounder species as weasels due to their propulsion being given mostly by vertebral column extensors. Despite the similarities found here in between *L. crassicaudata* and *G. cuja* (Ercoli *et al.* 2014), this does not seem to be the case in marsupials, since the muscle absence/reduction occurs in different morphotypes and locomotion habits. Besides its reduced function in the group, this feature might be phylogenetic related, and it needs further studies in a broader comparative sample of marsupials.

The *m. biceps brachii* was not mentioned in *Di. virginiana* by Jenkins and Weijs (1979), and Coues (1872) considered its two heads almost fully divided in two different muscles, due to its insertion in both ulna and radius. In *L. crassicaudata* the muscle was well recognized as a single muscle, and both heads attach only in the ulnar tuberosity. In *Da. geoffroii* the muscle also inserts distally to the *m. brachialis* insertion (Warburton and Marchal 2017). The *m. brachialis* connects the humerus and the ulna and was also not mentioned by Jenkins and Weijs (1979) and not discussed by Diogo *et al.* (2016) neither Stein (1981) in *Di. virginiana*. In *L. crassicaudata*, the origin of this muscle lies closer to the insertion of the *m. triceps brachii*, while in *Di. virginiana* and *Da. geoffroii* it is closer to the *Mm. deltoideus* (Coues 1872, Warburton and Marchal 2017). However, the insertion of *m. brachialis* is the same in *L. crassicaudata* and *Da. geoffroii*, near to the m. *biceps brachii* insertion.

The *m. triceps brachii* connects the scapula, humerus and ulna, and is robust and well developed in *L. crassicaudata* but apparently variable in some marsupials. In *Didelphis,* this muscle might blend with the *m. dorsoepitrochlearis* (Diogo *et al.* 2016), but this was not observed by other authors. The origin of both short and lateral head was the same in *L. crassicaudata* and *Di. virginiana* as stated by Coues (1872), near the insertion of the *m. brachialis*. It also has a well-developed accessory head. This was not reported in other marsupials mentioned in the literature, including other carnivorous marsupials (Warburton and Marchal 2017). This feature is more common and highly variable in placental carnivores, and it helps and the stabilization of the limb during cursorial locomotion (Evans and Lahunta 2013, Julik *et al.* 2012). Considering also the poorly developed pronators and supinators (Figure 4), we can suggest that *L. crassicaudata* presents a morphology consistent with terrestrial locomotion, due to minor requirements in manus rotation. This could also implicate that the species is adapted to capture prey of small sizes with their hands (Julik *et al.* 2012). However, we also found a longer *m. omohyoideus* muscle, which function is to depress the hyoid bone and help in the swallowing process. This could indicate that this species may be able to subjugate the prey with its mouth, needing a stronger and longer muscle to perform this action. Unfortunately, there are no behavior studies available for the species to compare with this data.

Additionally, *L. crassicaudata* has both *m. epitrochleoanconeus* (*m. anconeus internus*) and *m. anconeus lateralis* (*m. anconeus externus*). Coues (1872) did not find these muscles in *Di. virginiana.* Diogo *et al.* (2016) cited the *m. anconeus externus* as present in *Di. virginiana* using Stein (1981) as reference. However, she stated this muscle as absent in *Di. virginiana* and present only in *C. minimus* (Stein 1981, p. 118 and 130), suggesting that the presence of the *m. anconeus externus* in *C. minimus* provide powerful extensions of the forearm. This could also be the case in *L. crassicaudata,* reinforcing, once again, its strong extension of the forearm together with the triceps muscle. The *m. anconeus externus*/*lateralis* was also present in Australian carnivorous marsupials and considered a derived character (Warburton 2015).

The flexor muscles of the forearm of *L. crassicaudata* are similar with previous descriptions for *Didelphis*, except for the *m. flexor digitorum superficialis* and *m. flexor digitorum profundus*, apparently more similar with *C. minimus.* Stein (1981) provided an extended differentiation of the *m. flexor digitorum superficialis* and a brief description of the *m. flexor digitorum profundus* between *Di. virginiana* and *C. minimus*. She described the *m. flexor digitorum superficialis* as a large muscle belly that fully occupies the medial surface of the forearm, overlying *m. pronator teres* and *m. flexor carpi radialis*. Her description matches our description of the three robust bellies of the *m. flexor digitorum profundus*, and our *m. flexor digitorum superficialis* is a slender muscle almost adhered to the *m. flexor digitorum profundus*, incompletely divisible. Considering this association between these muscles, we agree with her that this feature might be related to different modes of foraging (Stein 1981). Even though other morphological characteristics of *L. crassicaudata* are more closely associated with a terrestrial locomotion habit, its preferable habitat is swamp areas, foraging in the water and probably consuming similar itens as the water-opossum, but with a different tactile adaptation, which could reflect a more detailed digit movement of *L. crassicaudata*.

Hindlimb morphology variation and comparative anatomy

The *m. sartorius* is one of the first extrinsic muscles of the forelimb to be dissected and part of it was very closely attached to the skin, visible during taxidermy. Its insertion could be uncertain, due to the patellar structure. Denyer *et al.* (2020) suggested the presence of a fibrocartilage patella in *L. crassicaudata,* as in all didelphid analyzed in their work, while Argot

(2002) and Flores (2009), which analyzed only skeletons, stated that there is no patella in didelphid marsupials. In our dissections, we did not find any structure that might resembles a patella, patelloid or fibrocartilage structures, only muscle tendons that fills the groove between the condyles, or tendons running towards the tibial tuberosity, where we found the *m. sartorius* insertion. This insertion was found in the patella of *Di. virginiana* (Diogo *et al.* 2016) or in the fascia covering the patella apparently in both *Di. virginiana* and *C. minimus* (Stein 1981), but neither publication mentioned if they found an ossified or fibrocartilage structure. This exact situation also occurs with the quadriceps group, in which its insertion runs also to the tibial tuberosity in *L. crassicaudata,* but it was considered attached to the patella (Diogo *et al.* 2016) or *ligamentum patellae* (Stein 1981) in the same abovementioned species. Coues (1872) dissected *Di. virginiana* and described the patella as rudimentary/deficient, in some cases with absolutely no trace of this bone. This suggests that the presence, absence and organic composition (bone/fibrocartilage) of the patella might be variable in didelphids, as their respective muscular attachments.

The *Mm. quadriceps femoris* group is regularly composed by four muscles, *m. rectus femoris* and the three *m. vasti* (*m. vastus lateralis*, *m. vastus intermedius* and *m. vastus medialis*), all well separated in *L. crassicaudata*, but its division is also inconsistent compared with other marsupials. Considering didelphids, it might be considered a “triceps complex” without the *m. vastus intermedius*, also named as *m. crureus* (Coues 1872, Stein 1981, Argot 2002, Diogo *et al.* 2016). In Australian marsupials, the four muscles were found in the common brushtail possum (*Trichosurus vulpecula*), the eastern quoll (*Dasyurus viverrinus*) and peramelids as *Isoodon obesulus* and *Macrotis lagotis* (Barbour 1963, MacCormick 1886, Warburton *et al.* 2015). It was also found in the placental mustelid lesser grison (*Gallictis cuja*) (Ercoli *et al.* 2013). Since all these species exhibit several different habits, the quadriceps muscle group division does not appear to have a connection with different forms of locomotion.

The hamstrings group (*m. biceps femoris*, *m. semitendinosus* and *m. semimembranosus*) morphology is similar to previous muscular descriptions of didelphids. The *m. biceps femoris* muscle name suggests that it has two heads as commonly seen in dogs (Evans and Lahunta 2013), but there is only one and indivisible head in *L. crassicaudata*. This morphology and also the *m. biceps femoris* attachments was similarly to that found in *Di. virginiana* (Coues 1872, Stein 1981, Diogo *et al.* 2016), but slightly different of *C. minimus,* the latter with a less extensive and more proximally insertion in the fibula (Stein 1981). The hindlimb morphology of *L. crassicaudata* seems much less variable and similar to other marsupials than the forelimb.

Forelimb weight analysis

The forelimb weight analysis was limited to *L. crassicaudata* data only, due the absence of forelimb weight raw data of other marsupial species in the literature. This could be an interesting point for future research in functional morphology and comparative studies.

We observed that *L. crassicaudata* has its main muscle mass concentrated in the extensors of the forearm (**Figure 13**, left), due to its massive *m. triceps brachii*, including its accessory head. This feature of a large *m. triceps brachii* is typical in terrestrial forms (Grand 1983, Argot 2001), producing strong forelimb extensions. The *m. triceps brachii* has also an additional head, which Ercoli (*et al.* 2014) considered helpful during crouching locomotion in galleries and increasing the range of positions it could perform. Following the *m. triceps brachii*, the flexors of the forearm, hand and digits presented more mass than the supinator and pronator muscles (**Figure 13**, right). This is interesting due to previous flexors muscle patterns associated with Didelphidae, as the “P pattern”. In this case, there is no flexor plate (an aponeurotic extension amidst the *m. flexor digitorum profundus* and tendons inserting in the digits and reaching the sesamoids), or it is very reduced, which means the tendons runs straightforwardly to the terminal phalanges (Abdala *et al.* 2006). In their work, despite they are suggesting that this “P pattern” has little or no direct association with locomotor behavior, they also further stated that this pattern might be related to grasping abilities that could make climbing possible (Abdala *et al.* 2006). Although *L. crassicaudata* is mostly associated with terrestrial habits (Astúa 2015), this could represent the general scansorial capacity of didelphids, in lesser or larger scale. Concerning the grasping ability, despite didelphids being not capable of individual mobility of the digits (Coues 1872), the great muscle mass of flexors in *L. crassicaudata* could indicate its prey capture strength or even that the species has some level of prey manipulating behavior, that is also present in other terrestrial didelphids as *M. domestica* (Argot 2001).

Hindlimb weight analysis

The hindlimb weight analysis included the wet weight of other marsupials available in the literature besides the data collected from *L. crassicaudata* in the present study. From Neotropical marsupials, it was possible to use the data of the arboreal species *Caluromys philander*, the scansorial *Didelphis marsupialis* and the terrestrial *Metachirus myosurus* from Argot (2002). From Australian marsupials, we gathered the weight data of the arboreal *Trichosurus vulpecula*, the hopping *Setonix brachyurus* and the terrestrial *Isoodon obesulus* from Warburton *et al.* (2015).

In *L. crassicaudata*, the hamstrings were the muscle group with greater muscle mass, followed by the quadriceps femoris group. The hamstrings can perform several movements as extending and rotating the hip joint and bending the knee joint, being essential during walking, running and jumping. The quadriceps group will also participate in these functions but extending the knee and providing hip flexion (Evans and Lahunta 2013). A large mass proportion of this muscle is expected in a terrestrial/cursorial species, adding more evidence for *L. crassicaudata* main and/or truly habit, despite its supposed ability to swim. However, we lack direct weight comparison with the only truly semi-aquatic marsupial species, *Chironectes minimus.* Stein (1981) dissected and compared *Di. virginiana* and *C. minimus* myology but without providing muscle mass, or any quantitative data that would allow us to compare both species directly. Regardless, the muscle mass proportion between *L. crassicaudata* and the other extreme cursorial species, *M. myosurus*, overlapped almost completely (**Figure 15, C**), which may be a major indicative of *L. crassicaudata*'s functional morphology adapted for cursorial locomotion. *Metachirus myosurus* is a well-studied species in terms of its habit, being always considered as terrestrial/cursorial and even saltatorial after running in high speeds due to its concentrated gluteal muscle mass (Grand 1983, Paglia *et al.* 2012, Astúa 2015), but it was never considered as near as semi-aquatic as *L. crassicaudata*. The striking difference between *L. crassicaudata* and *C. philander* hindlimb proportion (**Figure 15, A**) is certainly interesting, showing the more balanced weight distribution in *C. philander,* which may be a main morphological adaptation in arboreal species due to its need to keep their balance during locomotion (Argot 2001, 2002). Finally, in the weight analysis comparisons with Australian marsupials (**Figure 16**), it seems that ecology might play a less important role in the muscle mass distribution, since the three species have a very different locomotion, but their muscle masses were mostly similar. It is easy to notice the drastic gluteal and adductors muscle mass in *S. brachyurus*, typical in macropodids for its extreme hopping adaptation. However, others muscle groups are not that different among the three species, even though *T. vulpecula* is an arboreal species and *I. obesulus* is exclusively terrestrial.

CONCLUSIONS

The main observations in the forelimb myology of *Lutreolina crassicaudata* showed its correspondence with both *Didelphis virginiana* and *Dasyurus geoffroii*. *Didelphis* is frequently considered as the most generalist and primitive species within Didelphimorphia, however, this could be due to the major focus of study in this genus and the lack of information for other genera. *Dasyurus* forelimb morphological traits is apparently very similar with *L. crassicaudata*, which might be due to their association with carnivorous habits. Even if frequently treated as a possible semi-aquatic marsupial, *L. crassicaudata* shared very few muscular characteristics with the water-opossum *C. minimus* and exhibited more similarities with terrestrial/cursorial species. *Lutreolina crassicaudata* also shows some myological convergent features with the placental lesser grison *Gallictis cuja,* which added more morphological traits to the species that may support its denomination as a weasel-like opossum. In the hindlimb, *L. crassicaudata* showed a great muscle mass in the hamstrings and quadriceps femoris muscle groups, directly related to terrestrial and cursorial locomotion, and its general muscle mass distribution was almost the same as *M. myosurus,* another very cursorial didelphid. The weight analysis suggests that Neotropical marsupials' ecology might have a stronger influence muscle distribution than in Australian marsupials. Lastly, the basic description of more marsupial species for comparative data it is essential, so it would be possible to track if the muscular morphology for a determined species reflects its common ancestor, shared phylogenetically with the group, or if it is associated with a specific habit, even representing a functional adaptation. This gap in knowledge of marsupial myology limits our understanding of their natural history, functional morphology, systematics and evolution.

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APPENDIX I – Weighting

Table 4. Raw data of *L. crassicaudata* forelimb wet muscle masses for individual specimens used in the analysis. All masses are present in grams (g).

	L. crassicaudata	*L. crassicaudata*
Specimen code	UFMG7992	UFMG7990
Body mass		
M. trapezius	0.379	0.371
M. atlantoscapularis anticus	0.091	
M. atlantoscapularis posticus	0.06	
M. omohyoideus		
M. latissimus dorsi	0.584	0.426
M. romboideus	0.171	
M. serratus	0.479	
M. pectoralis	0.663	0.576
M. subclavius	0.030	
M. deltoideus	0.139	0.097
M. supraspinatus	0.0.318	
M. infraspinatus	0.147	
M. subscapularis	0.254	
M. teres major	0.102	0.074
M. coracobrachialis	0.007	0.010
M. biceps brachii	0.128	0.101
M. brachialis	0.071	0.051
M. triceps	0.746	0.648
M. dorsoepitrochlearis	0.040	
M. anconeus lateralis	0.029	
M. epitrochleoanconeus	0.009	0.007
M. pronator teres	0.043	

M. flexor carpi radialis	0.022	0.028
M. flexor carpi ulnaris	0.096	0.088
M. palmaris longus	0.083	
M. flexor digitorum superficialis		0.102
M. flexor digitorum profundus	0.226	0.235
M. pronator quadratus	0.005	
M. brachioradialis	0.035	0.047
M. extensor carpi radialis	0.162	
M. extensor digitorum communis	0.030	
M. extensor digiti minimi	0.021	
M. extensor digitorum profundus	0.012	
M. extensor carpi ulnaris (lateralis)	0.021	
M. supinator	0.009	0.006
M. abductor pollicis longus	0.033	

Table 5. Relative mean weights (g) of the main hindlimb muscles of marsupials. Except for *Lutreolina crassicaudata*, all data were collected and adapted from Argot (2002) and Warburton *et al.* (2015).

	Lutreolina crassicaudata	*Caluromys philander*	*Didelphis marsupialis*	*Metachirus myosurus*	*Isoodon obesulus*	*Trichosurus vulpecula*	*Setonix brachyurus*
Quadriceps	1 (21.02%)	1 (12.90%)	1 (16.90%)	1 (17.10%)	1 (17.15%)	1 (17.24%)	2 (15.79%)
Ilio-psoas	7 (6.55%)	5 (8.55%)	4 (7.80%)	7 (6.15%)	5 (8.14%)	7 (6.47%)	5 (6.17%)
Psoas minor	15 (0.00%)	14 (2.10%)	13 (2.50%)	10 (2.35%)	12 (1.55%)	13 (2.06%)	13 (0.92%)
Sartorius	12 (1.55%)	11 (3.30%)	10 (3.20%)	9 (2.95%)	9 (3.97%)	10 (3.56%)	9 (3.41%)
Gluteus	2 (14.87%)	2 (11.65%)	3 (9.70%)	2 (14.65%)	4 (12.21%)	2 (15.37%)	1 (21.00%)
Biceps femoris	5 (11.23%)	9 (4.20%)	6 (7.35%)	3 (10.70%)	2 (16.47%)	3 (12.28%)	4 (13.80%)
Semimembranosus	3 (13.64%)	4 (9.90%)	5 (7.60%)	4 (10.50%)	3 (13.08%)	4 (11.25%)	6 (5.75%)
Semitendinosus	9 (3.11%)	7 (6.45%)	9 (4.75%)	8 (5.45%)	8 (5.81%)	9 (3.66%)	8 (4.79%)
Gracilis	8 (3.30%)	6 (6.65%)	8 (5.35%)	12 (1.85%)	10 (2.91%)	6 (7.03%)	11 (2.45%)
Adductors + Pectineus	4 (11.34%)	3 (10.10%)	2 (16.10%)	5 (9.90%)	7 (6.40%)	5 (7.97%)	3 (15.57%)
Tibialis anterior (T. cranialis)	10 (2.24%)	12 (2.55%)	12 (2.60%)	11 (2.00%)	11 (2.62%)	12 (2.53%)	10 (2.87%)
Extensor hallucis longus	15 (0.00%)	16 (0.50%)	16 (0.35%)	15 (0.25%)	15 (0.00%)	16 (0.47%)	15 (0.00%)
Extensor digitorum longus	13 (0.55%)	15 (0.95%)	15 (0.55%)	14 (0.65%)	14 (0.97%)	15 (1.03%)	12 (1.06%)
Peronei	11 (1.75%)	8 (4.40%)	11 (2.65%)	12 (1.85%)	13 (1.16%)	11 (3.28%)	14 (0.85%)
Gastrocnemius + Plantaris	6 (8.33%)	10 (3.90%)	7 (6.00%)	6 (8.20%)	6 (7.56%)	8 (4.59%)	7 (5.57%)
Tibialis posterior (T. caudalis) + Flexor digitorum tibialis	14 (0.52%)	13 (2.20%)	14 (1.10%)	13 (1.45%)	15 (0.00%)	14 (1.22%)	15 (0.00%)

Legend 1. Only muscles found in the present work were considered. As Argot (2002), the first number represents the rank of the muscle, the second is the weight divided by the whole hindlimb musculature weight.

CHAPTER 3: Comparative limb muscular anatomy of representatives of the three orders of South American marsupials: *Dromiciops gliroides* (Microbiotheria), *Lestoros inca* (Paucituberculata) and *Monodelphis domestica* (Didelphimorphia).

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Didelphis virginiana anatomy, created by Artificial Inteligence

INTRODUCTION

As mentioned in both previous chapters, comparative myology studies are well represented by human or domestic/veterinary animals anatomy, frequently with health and clinical purposes, while for wild species these studies are few and constantly outdated. Luckily, there has been a recent increase in such studies for mammals and more specifically and recently, marsupials. A qualitative muscle description is useful for several analyses, such as comparative functional morphology, phylogenetic reconstruction, and as integrative information for skeletal and biomechanical investigations (Diogo *et al.* 2012, 2016). Anatomical descriptions are also essential for inferring myological characteristics in different locomotory habits, to estimate extinct species locomotion based on extant species (Argot 2001, 2002). Regarding American marsupials, as detailed in Chapter 1, they are frequently considered as having a basic and similar *bauplan*, although exhibiting different sizes and a variety of locomotion habits such as arboreal, scansorial, terrestrial, and semi-aquatic (Paglia 2012, Astúa 2015). To the best of our knowledge, almost all studies of Neotropical marsupial myology have used larger species, with the exception of Mann-Fischer (1951, 1953, 1956) who studied the elegant fat-tailed mouse opossum *Thylamys elegans* (Waterhouse, 1839) with a head-body size of 9–13.7cm (Astúa 2015). Therefore, small species remain unexplored. Following the former idea, we have assessed myology variation and convergence of a larger species like the thick-tailed lutrine opossum *Lutreolina crassicaudata* Desmarest, 1804 (<300g) through comparative muscular anatomy and weight only, in Chapter 2. Here, we focused on investigate the myology of small marsupials (>150g) to verify if their myology will be following the expected unspecialized *bauplan,* or if they will have significant differences as larger species might have; and also provide a comparative perspective in between the three orders of Neotropical marsupials (Didelphimorphia, Microbiotheria and Paucituberculata).

The analysis of the muscle architecture includes both qualitative and quantitative description of muscle morphology and its mechanical properties, which can provide insight into the functional and anatomical adaptations of different species. It has also been a useful tool for understanding the mechanics of movement and for predicting muscle performance in different contexts, such as locomotion, prey capture or escape behavior (P.-H. Fabre *et al.* 2017, Rankin *et al.* 2018, Tarquini *et al.* 2023). Additionally, muscle architecture data can be integrated with muscle physiology, biomechanics, and evolutionary history, to gain a more comprehensive understanding of muscle function and evolution (Diogo *et al.*, 2016). The use of muscle architecture analysis has been instrumental in advancing our understanding of the functional adaptations of muscles in different species and will continue to be an important tool in the study of vertebrate locomotion and evolution.

The myological limb anatomy are composed by striated muscle tissue, which is the type of muscle attached to the whole skeleton. In this muscle type the smallest functional and contractile unit is called sarcomeres that will be linked to form myofibrils. The myofibrils will be parallel to each other to form the muscle fiber, and the muscle fiber bundle will form the muscle fascicles, which several of them form the muscle belly (Squire 1997), similar with a “Russian doll” organization. The muscle fascicle is the first and the easiest to visualize after a longitudinal cut in the muscle belly, being the most used in muscle measurements. The number and the length of a muscle fascicle plus the angle formed by the fascicles relative to the axis of force production (pennation angle) will determine the muscle’s contractile property and its cross-sectional area, that reflects the capacity for force production (Gans e de Vree 1987). For this reason, the calculation of the physiological cross-section area, also known as PCSA, will represent the muscle force, while the fascicle length will represent the length excursion of a determined muscle (Martin *et al.* 2020). In this way, these measures can help us to understand how much work (force x distance) an individual muscle can perfom and also how much power (work/time) can be produced (Allen *et al.* 2011). As expected, both attributes can not be concomitantly maximized in one muscle, so the occurrence of muscle specialization for specific functional demands is well accepted (Rosin and Nyakatura 2017).

The aim of this study was to evaluate the muscle architecture of forelimbs and hindlimbs across three small marsupial species representing the three American marsupial orders, inferring possible muscle adaptations regarding locomotion habits. These descriptions and comparisons may contribute to the understanding of the evolution of morphological and locomotor characteristics in marsupials and provide new evidence for the establishing of evolutionary patterns within the group.

MATERIAL AND METHODS

2.1 Study species

We used one marsupial species from each of the three American marsupial orders, Microbiotheria, Paucituberculata and Didelphimorphia.

Dromiciops gliroides Thomas, 1894, also known as the monito del monte, is an arboreal species found in the temperate rainforests of South America, primarily in Chile and Argentina. It is the only surviving member of the order Microbiotheria and it is usually considered a “living fossil” due to its ancient lineage and also the “missing link” status as a connection between American and Australian marsupials’ radiation to Oceania. The monito del monte is primarily nocturnal and arboreal, feeding on insects, fruits, and small vertebrates. They have the unique ability for marsupials of hibernating during winter months, storing fat within an enlarged tail and reducing their metabolism (Palma e Valladares-Gómez 2015). 

Lestoros inca (Thomas, 1917), also known as the Incan shrew opossum, is a semi-fossorial species found in the Andean region of South America, primarily in Peru, Bolivia, and Chile. It is the only member of the genus *Lestoros*, and member of the Paucituberculata order. It is morphologically characterized by its elongated snout, lip flaps and procumbent lower incisors, which is used for stab and capturing prey, being highly insectivorous and also consuming other small invertebrates. It has very small eyes, associated with the semi-fossorial habit, and they can also store fat in their tail to help surviving during winter as *D. gliroides* (Patterson 2015). 

Monodelphis domestica (Wagner 1842), known as the grey short-tailed opossum, is a terrestrial species found in South America, primarily Brazil and Argentina, and a member of the Didelphimorphia order. It has nocturnal habits and a highly insectivorous diet but can also consume fruits and small vertebrates. It is commonly used as a laboratory subject and marsupial research model, due to the ease with which it breeds in captivity, and it have proven to be an important model organism for biomedical research, contributing to advancements in fields such as genetics, neurobiology, and immunology (Astúa 2015). 

2.2 Specimens analyzed

Two specimens of *D. gliroides* and one of *L. inca* were used in this study. All specimens were adult males with full eruption of the fourth molar and are deposited in the mammal collection of the Field Museum of Natural History (FMNH), Chicago, USA, and were loaned to Harvard University. **Specimens analyzed**: *Lestoros inca*: **Perú**: Cuzco (FMNH 172050); *Dromiciops gliroides*: **Chile**: La Araucania (FMNH 22677); Los Lagos (FMNH 127452).

Additionally, we used one frozen specimen of *Monodelphis domestica*, previously analyzed by Wright *et al.* (2022). We collected new data for forelimb analysis and included their data for hindlimb anatomy.

2.3 Digital data collection

Dromiciops gliroides and *L. inca* specimens were first scanned without iodine to ensure the best skeleton 3D visualization, and this material will be deposited in MorphoSource database (**Figure 17**). After that, we started the contrast-stained microcomputed tomography (uCT) using Gray (2023) protocol, which consists in immersing the specimen in a 1.25% I_2KI solution of buffered iodine to enhance soft tissue contrast. The material was kept in a closed recipient, agitated regularly and scanned weekly for 1.5 months using a Bruker Skyscan 1173: *D. gliroides* voxel size 18µm, 110 kV, 136 µA, and 0.3 mm copper filter; *L. inca* forelimbs voxel size 18µm, 120 kV, 125 µA, and 0.5 mm copper filter, hindlimbs voxel size 22µm, 120 kV, 125 µA, and 0.5 mm copper filter; *M. domestica* voxel size 33.71 mm^3, 139 kV, 71 µA, and a 1.0 mm aluminum filter (Wright *et al.* 2022). Final tomograms were reconstructed using Skyscan NRecon software as TIFF image stack and imported into Mimics v19 software (Materialise, Leuven, Belgium) for segmentation of one forelimb and hindlimb of each species. In the case of *M. domestica*, the stain methodology included phosphomolybdic acid (PMA) and the reconstructed forelimb files were shared by Wright *et al.* (2022) for segmentation, as well as their segmented hindlimb data for comparative analysis.

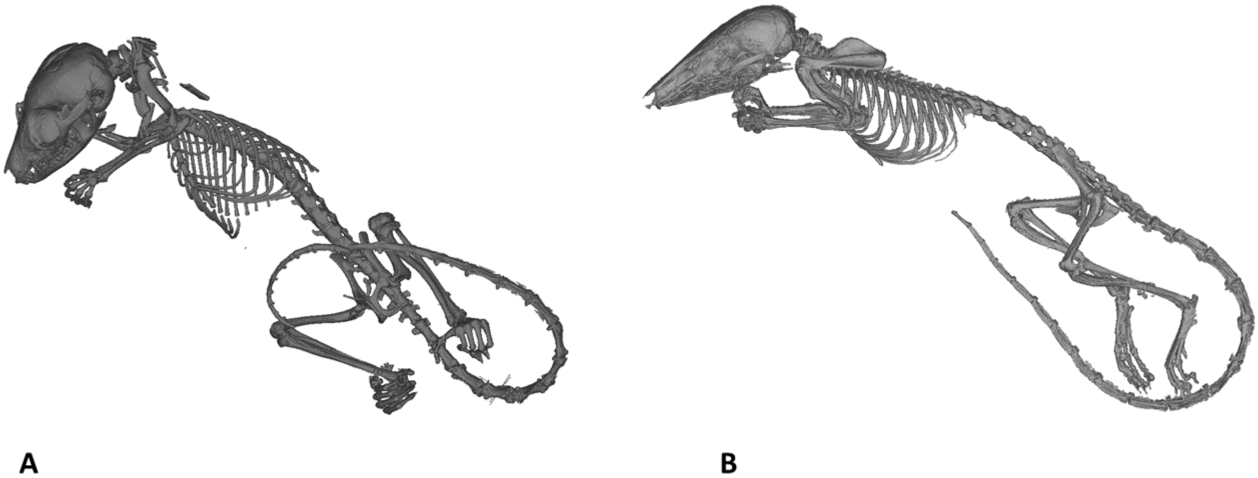

Figure 17. CT Scan of specimen skeletons. A) *Dromiciops gliroides* FMNH127452, head-body 106mm, tail 102mm; B) *Lestoros inca* FMNH172050, head-body 115mm, tail 117mm.

2.4 Physical data collection

Dromiciops gliroides and *L. inca* specimens were fixated in formalin when collected and conserved in 70% ethanol since then. Specimens were skinned, gutted and, during limb dissection, muscles were identified, photographed, weighted and measured. Some specific hand muscles were not dissected due extreme dehydration caused by long time conservation in alcohol. Muscular mapping will be provided in the future publication of this work, but we provide raw digital illustrations (**Figure 21**, **Figure 22**, **Figure 23**). The anatomical nomenclature followed Diogo *et al.* (2016), and we used mainly the marsupial anatomical literature available for comparative analysis, with occasional comparisons with other mammals. Muscles were photographed in situ to help identification and weighted on an Intelligent-Lab Precisa XB 220A scale to obtain individual muscle mass. Muscles were photographed again both isolated and after a longitudinal cut to expose the muscle fascicles, to allow digital measurements of the muscle belly, muscle fascicle and pennation angle. All the morphometric measurements were performed on ImageJ software (Rasband 1997-2015). Each muscle architecture was measured five times, and the average of both limbs of the three species are provided in Appendix I, **Table 6**, **Table 7**, and **Table 8**, and actions of the main muscles identified are provided in **Table 9**. Considering that *M. domestica* is three times bigger than *L. inca* and *D. gliroides*, we scaled the measurements to body mass assuming isometric scaling: lengths through $BM^{1/3}$, areas through $BM^{2/3}$ and masses through BM^1 and then log normalized, following Wright *et al.* (2022). This allowed us to obtain the quantitative data necessary to calculate the standard physiological cross-sectional area (PCSA) through the following equation:

$$\text{PCSA} = \frac{(MM)(cosPA)}{(FL)(\rho)}$$

where (MM) is the muscle mass in g, (PA) is the pennation angle in degrees, (FL) is the fascicle length in mm and (ρ) is the mammal skeletal density as 0.001056 g/mm^{-3}, previously established from Ward and Lieber (2005). However, we observed that since the fascicle length is included in the PCSA equation and, at the same time, usually plotted against the PCSA to dispose muscle dispersion, it would automatically form a half parabole with almost no muscle achieving the plot right superior quadrant where powerful muscles should be represented (**Figure 18, A**). If instead we plot the fascicle length against the muscle mass multiplied by the pennation angle, we can visualize a distribution where higher fascicle length will represent great muscle excursion, higher muscle mass (.cosϴ) will represent great force, and in between them, higher power, based on Power = Force x Acceleration (**Figure 18, B**). This was the method used for PCSA calculation in this chapter, and how we will consider "PCSA" from here onwards.

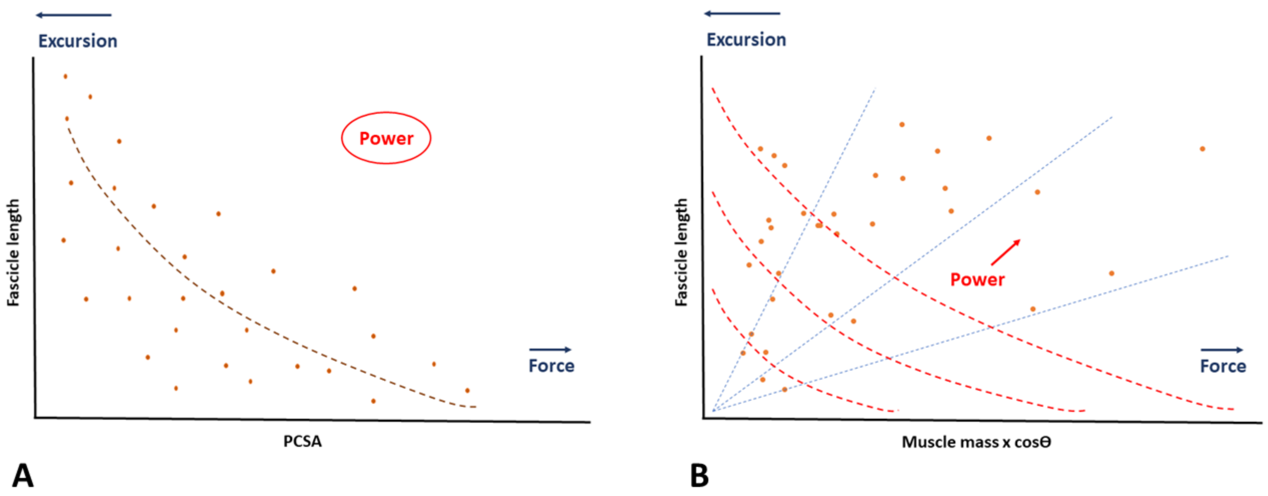

Figure 18. Two ways of PCSA analysis. A) PCSA plotted directly against the fascicle length producing a half parabole with unachievable powerful muscles; B) Muscle mass x cosϴ plotted against fascicle length allowing the visualization of powerful muscles through Power = Force x Acceleration. The blue lines represent the gradual change in between muscles with high excursion and high force, while the redlines represent the gradual increase of muscle power.

Similar with Chapter 2 of this thesis, we grouped the muscles according to their movements/functions, which allow us to compare the muscles weight in a more functional than individual muscle perspective.

Forelimb functional groups based on de Souza-Júnior et al. (2018)

Shoulder extensors	Supraspinatus and coracobrachialis
Shoulder flexors	Infraspinatus, deltoideus, teres major and teres minor
Elbow extensors	Epitrochleoanconeus, anconeus lateralis and triceps brachii
Elbow flexors	Biceps brachii and brachialis
Extensors of the carpus	Extensor carpi radialis
Flexors of the carpus	Flexor carpi radialis, flexor carpi ulnaris
Digit extensors	Extensor digitorum communis and lateralis, abductor pollicis longus
Digit flexors	Flexor digitorum superficialis and flexor digitorum profundus
Supinator muscles	Supinator and brachioradialis
Pronator muscles	Pronator teres and pronator quadratus

Obs.: **1**. Since m. subscapularis can provide both shoulder extension and flexion, medial shoulder stabilization and also adduction of the limb with the m. pectoralis (Evans and DeLahunta, 2013), this muscle was removed from the functional groups (de Souza-Júnior *et al.* 2018).

Hindlimb functional groups based on Warburton et al. (2015)

Hip flexors	Rectus femoris, iliacus, psoas major, sartorius
Gluteal group	All gluteal muscles
Deep hip	Piriformis, Gemelli, obturators and quadratus femoris
Quadriceps femoris	Rectus femoris, vastus lateralis, v. intermedius and v. medialis
Adductor group	All adductors' muscles and pectineus
Hamstrings	Biceps femoris, semitendinosus and semimembranosus
Extensors of *pes*	Extensor digitorums
Flexors of *pes*	Flexor digitorums

RESULTS

We were able to identify 241 muscles in all specimens analyzed: 111 muscles in *D. gliroides* limbs (some represented in **Figure 21**); 81 muscles in *L. inca* limbs (some represented in **Figure 22**); and 49 muscles in the forelimb of *M. domestica* (some represented in **Figure 23**). For *M. domestica*, we used Wright *et al.* (2022) hindlimb muscle data with 34 entries (Appedix I, **Table 8**). During physical dissections, we were able to confirm muscles identified in the digital segmentation and identify extra muscles there were not visible in the digital dissection.

3.1 Qualitative forelimb myology and osteological remarks between species

M. coracobrachialis longus

We were able to find both short and long heads of coracobrachialis muscle (also know as *articulari humeri*) only in the arboreal species *D. gliroides* (**Figure 19**). Both heads originate from the coracoid process of the scapula by a well-defined thick tendon. The short head was completely covered by the long head and inserted proximally and medially in the humerus neck. The long head insertion is much more distally and medially in the humerus shaft half, between lateral and medial supracondylar ridge.

Figure 19. Right forelimb of *Dromiciops gliroides* in medial view. Coracobrachialis longus muscle separated by the two needles.

M. triceps brachii

The most peculiar feature of marsupial forelimbs observed so far in this study is the morphological variation in the triceps muscle. When comparing the three species, *D. gliroides* showed the normal arrangement of three heads in the triceps muscle. However, both *M. domestica* and *L. inca* showed extra triceps heads. The former showed one small accessory head, well separated from the other three, while the latter had the unique presence of a fifth head in the triceps muscle (**Figure 20**). In the medial view of the forearm (**Figure 20**, **A**), we can identify the long, medial and accessory triceps head, and also the biceps brachii muscle on the left side of the needle. After removing the triceps long head, we can discern the lateral head on the needle's left side, covering the medial head, and the brachialis muscle is on the needle's right side. We can also observe an extremely well-developed deltoid crest that merges with the pectoralis rigde, forming a solid bar dividing medial and lateral sides of the forearm (**Figure 20**, **B**). Finally, after removing the brachialis muscle, triceps lateral and medial heads, there is still one extra head left, completely caudally attached to the humerus, with a very broad and proximal head that originates with the brachialis, but independent. We can also identify the biceps brachii muscle with its two heads incompletely separated by the needle (**Figure 20**, **C**).

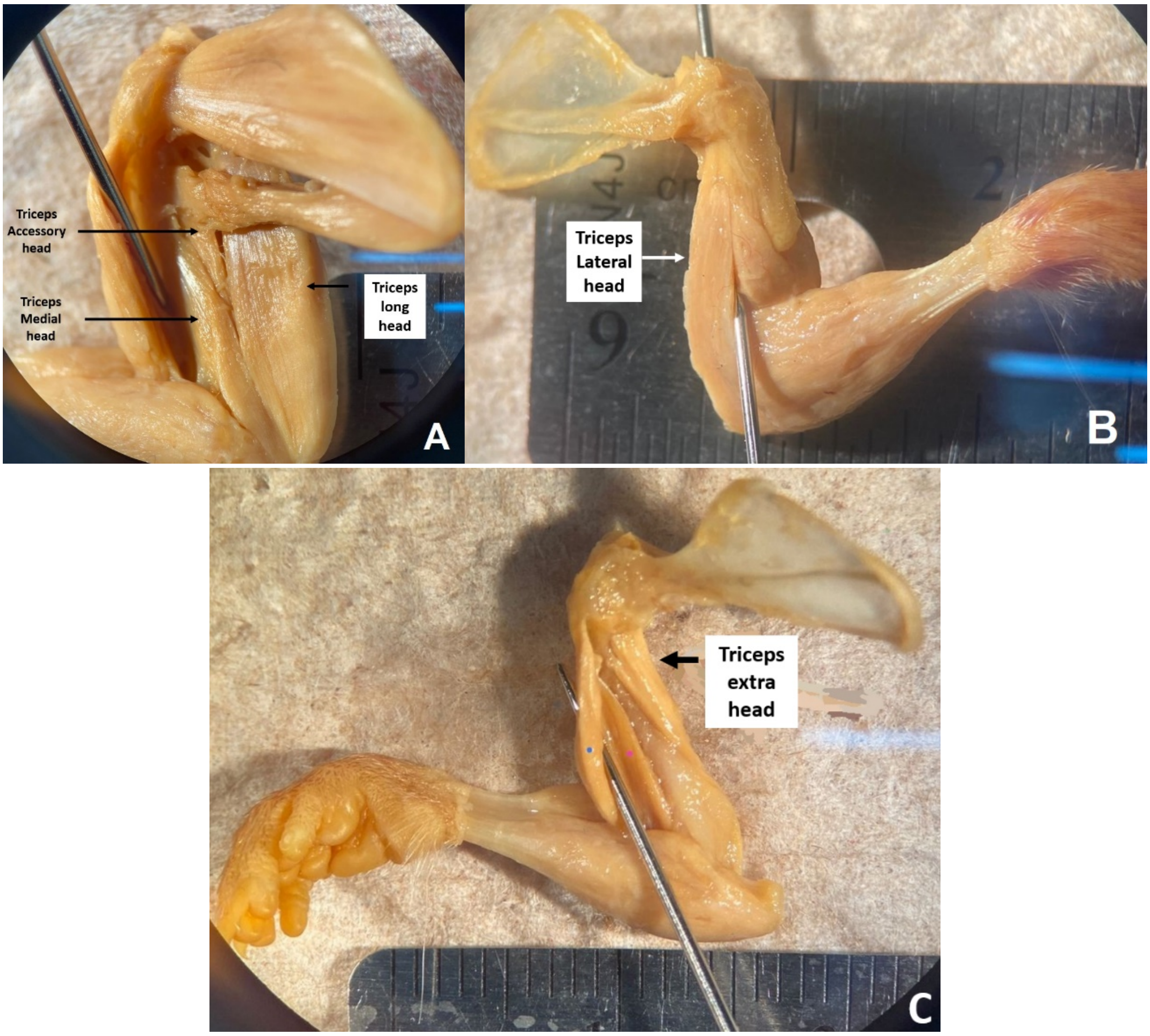

Figure 20. Right forelimb of *Lestoros inca*, medial (A, C) and lateral (B) views. (A) It is possible to identify *m. triceps brachii* long, medial and accessory heads, the last one medially and loose from the humerus; (B) It is possible to identify *m. triceps brachii* lateral head and *m. brachialis*, separated by the needle; (C) It is possible to identify an extra head of *m. triceps brachii*, completely caudally and fully attached to the humerus, and, more cranially, the two heads of *m. biceps brachii* separated by the needle.

3.2 Qualitative hindlimb myology and osteology remarks in between species

Hindlimb myological characteristics were harder to observe in *D. gliroides* and *L. inca* due to certain amount of damage to all specimens of both species, but some features could be observed using data already published by Wright *et al.* (2022) for *M. domestica* as guidance. We noticed that, in *D. gliroides,* the tibia and fibula do not touch completely, allowing the existence of a space between them where we find the interosseous cruris membrane (**Figure 21**, **C**), while in *L. inca* tibia e fibula were completely connected and almost fused at its distal portion where this membrane were supposed to be (**Figure 22 C, D**). *Monodelphis domestica* seems to have a considerable space in between tibia and fibula (Wright *et al.* 2022, Figure 2) but the authors did not record the presence of this membrane (Appendix I, **Table 8**).

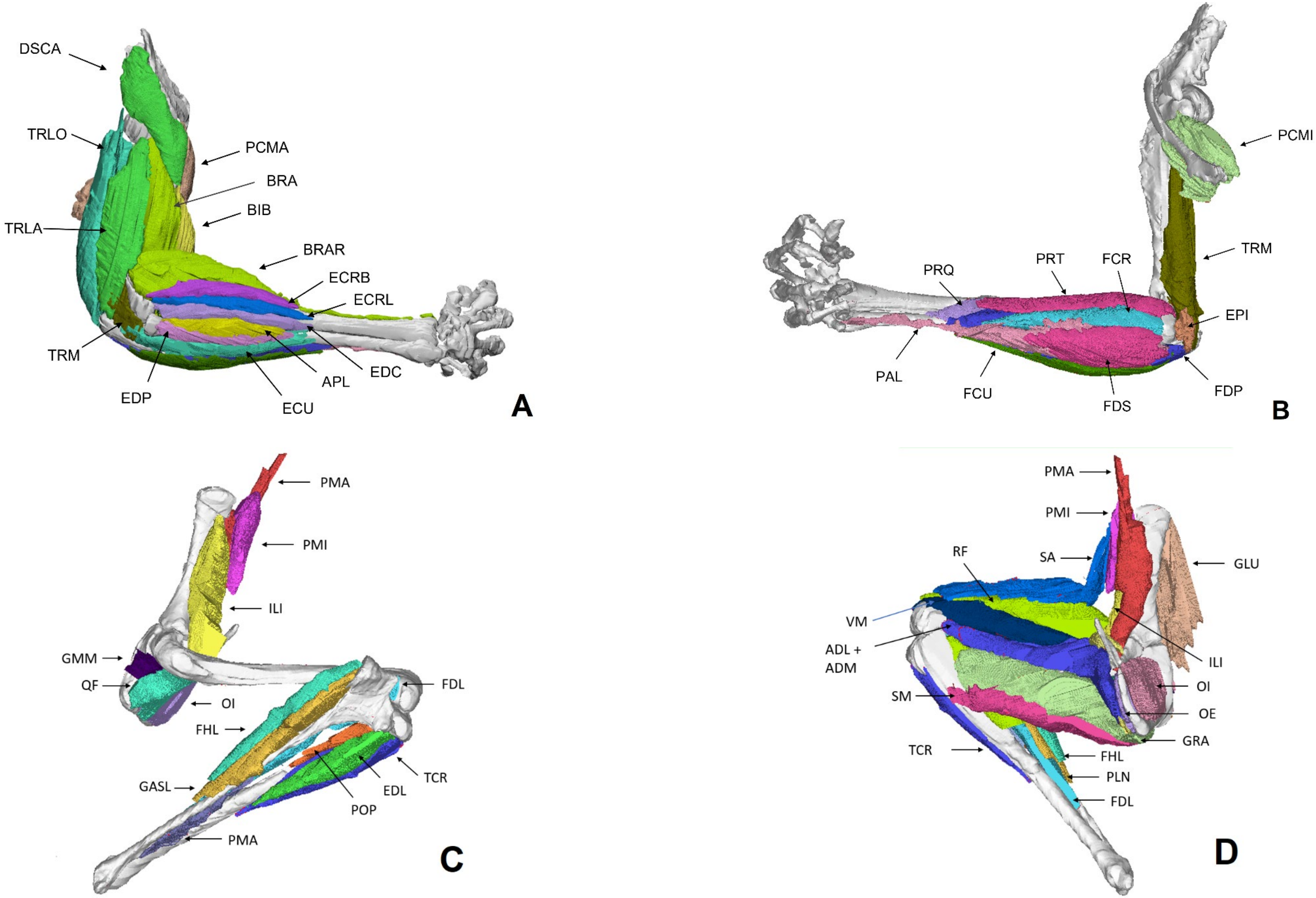

Figure 21. Segmented muscles of *Dromiciops gliroides*. A) Superficial forelimb muscles in lateral view; B) Deep forelimb muscles in medial view; C) Deep hindlimb in lateral view; D) Superficial hindlimb muscles in medial view. See caption of **Figure 24** and **Figure 25** for muscle abbreviations.

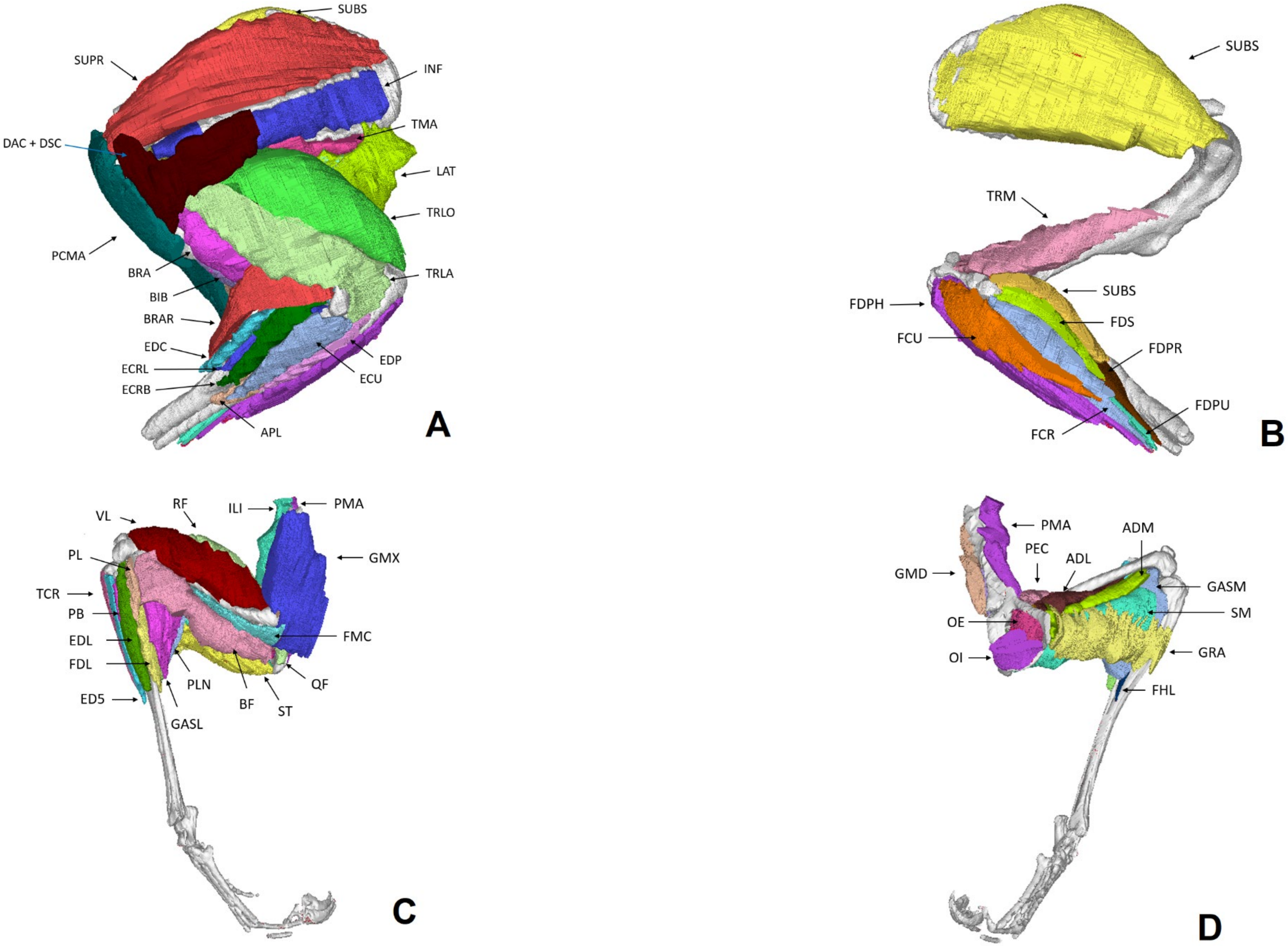

Figure 22. Segmented muscles of *Lestoros inca*. A) Superficial forelimb muscles in lateral view; B) Deep forelimb muscles in medial view; C) Superficial hindlimb in lateral view; D) Deep hindlimb muscles in medial view. See caption of **Figure 24** and **Figure 25** for muscle abbreviations.

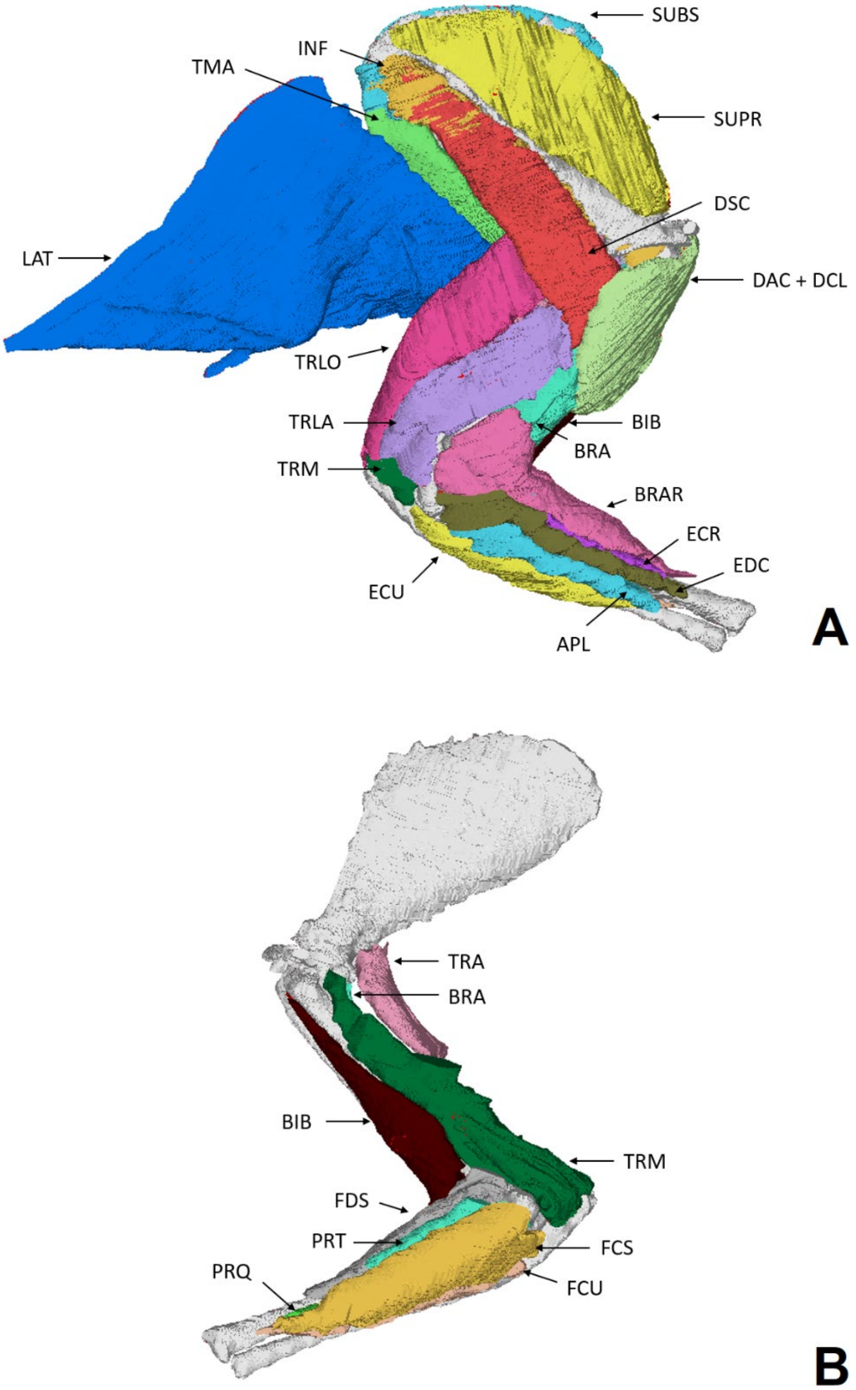

Figure 23. Segmented muscles of *Monodlephis domestica* forelimb. A) Superficial forelimb muscles in lateral view; B) Deep forelimb muscles in medial view. See caption of **Figure 24** and **Figure 25** for muscle abbreviations.

3.3 Quantitative analysis of muscle architecture

3.3.1 PCSA

We analyzed the PCSA from *D. gliroides*, *L. inca* and *M. domestica* forelimb (**Figure 24 A**, **B** and **C**, respectively) and hindlimbs (**Figure 25 A, B** and **C**, respectively). To avoid an exhaustive and minimal comparative discussion, we will focus on the main anatomical remarks in each limb of each species. In *D.*

gliroides forelimb, we found very powerful *m. flexor digitorum profundus* (FDP) and *m. biceps brachii* (BIB), and also a very fast excursion of two extensors (ECRB and EDC). In its hindlimb, the most powerful muscles were the *rectus femoris*, *semitendinosus*, *semimembranosus* and the *adductor magnus* (RF, ST, SM and ADM respectively). In *L. inca* forelimb, more powerful muscles were found in those with pennated fibers, like *latissimus*, *subscapularis*, *supraspinatus* and *pectoralis*, and a powerful triceps long head muscle (LAT, SUBS, SUPR, PCMA and TRLO, respectively), while in the hindlimb, the *semimembranosus* and *biceps femoris* were remarkably powerful, followed by the *vastus lateralis* muscle (SM, BF and VL). Finally, in *M. domestica* forelimb, the *m. trapezius cervicis* was very powerful (TZC) along with the other very pennated muscles as *m. latissimus*, *m. serratus* and *m. pectoralis* (LAT, SER, PCMA), and in the hindlimbs, the gluteal group muscle mass was the most powerful after *m. semimembranosus* (GLU, SM).

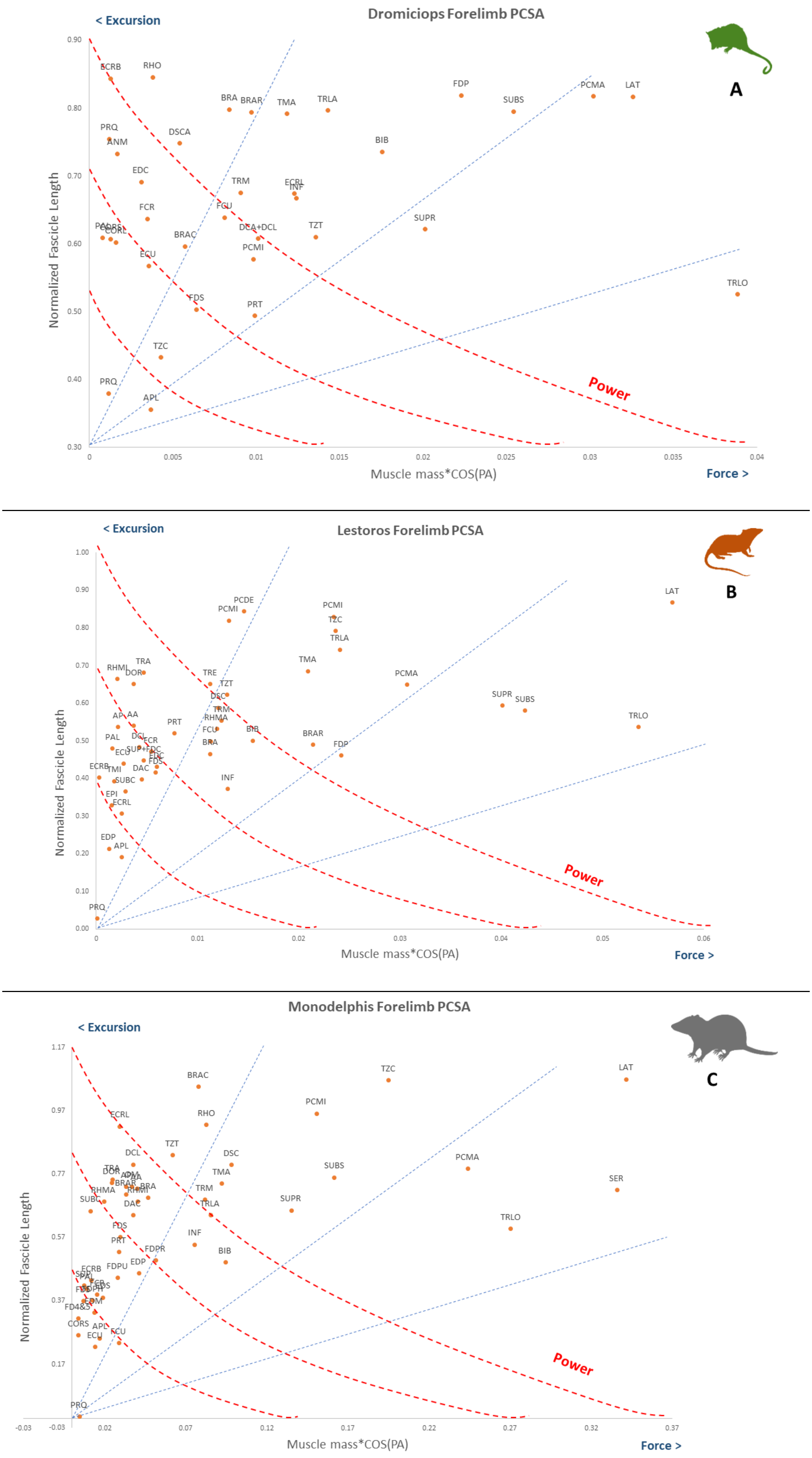

Figure 24. Forelimb PCSA analysis of the three species, *D. gliroides* (A), *L. inca* (B) and *M. domestica* (C). The x axis represents muscle mass multiplied (*) by the cosine of the pennation angle (PA) calculation, while the y axis is the normalized value of the fascicle muscle length. Blue dotted lines represent the gradual change in between muscles with high excursion and high force, while the redlines represent the gradual increase of muscle power. **Abbreviations:** (APL) Abductor Pollicis Longus, (ANL) Anconeus Lateralis, (AA, AP) Atlantoscapularis Anticus + Posticus, (BIB) Biceps Brachii, (BRA) Brachialis, (BRAC) Brachiocephalicus, (BRAR) Brachioradialis, (CORL, CORS) Coracobrachialis Longus and Coracobrachialis Short, (DCA + DCL + DSCA) Deltoideus Acromialis + Clavicularis + Scapularis, (DOR) Dorsoepitrochlearis, (EPI) Epitrochleoanconeus, (ECRB+L) Extensor Carpi Radialis (brevis + longus), (ECU) Extensor Carpi Ulnaris, (EDM) Extensor Digiti Minimi (EDC) Extensor Digitorum Communis, (EDP) Extensor Digitorum Profundus, (EDS) Extensor Digitorum Superficialis, (FCR) Flexor Carpi Radialis, (FCU) Flexor Carpi Ulnaris (FD4&5) Flexor Digitorum IV&V, (FDP) Flexor Digitorum Profundus (+H, R, U, humeral, radial, ulnar), (FDS) Flexor Digitorum Superficialis, (FD5) Flexor Digitorum V, (INF) Infraspinatus, (LAT) Latissimus, (OM) Omohyoideus, (PAL) Palmaris Longus, (PCMA + PCMI) Pectoralis Major + Minor, (PRQ) Pronator Quadratus, (PRT) Pronator Teres, (RHMA + RHMI) Rhomboideus Major + Minor, (SER) Serratus, (SUBC) Subclavius, (SUBS) Subscapularis, (SUP) Supinator, (SUPR) Supraspinatus, (TMA + TMI) Teres Major + Minor, (TZC + TZT) Trapezius Cervicis + Thoracic, (TRA, TER, TRLA, TRLO, TRM) Triceps Accessory, Extra, Lateral, Long and Medial.

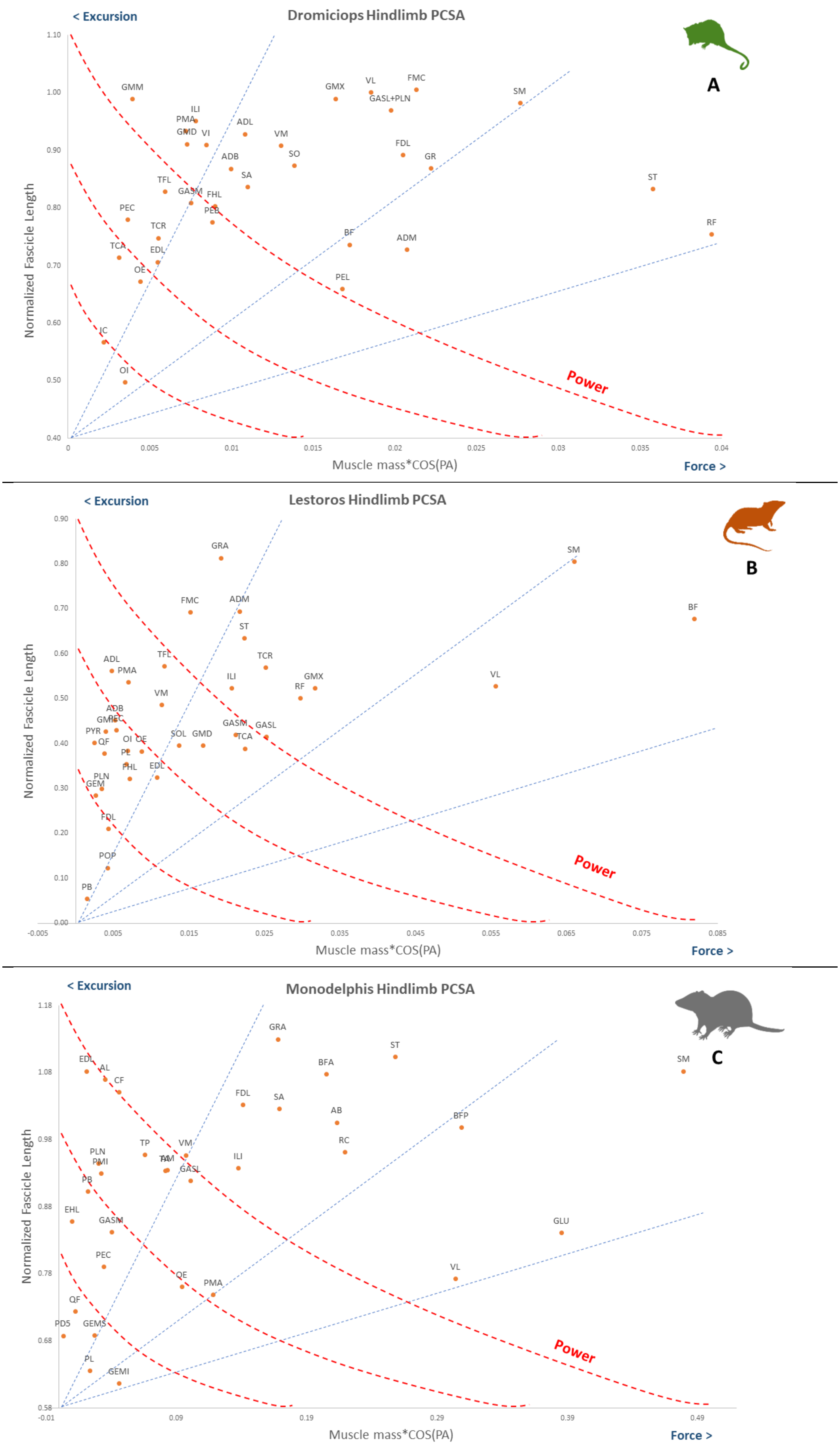

Figure 25. Hindlimb PCSA analysis of the three species, *D. gliroides* (A), *L. inca* (B) and *M. domestica* (C). The x axis represents muscle mass multiplied (*) by the cosine of the pennation angle (PA) calculation, while the y axis is the normalized value of the fascicle muscle length. Blue dotted lines represent the gradual change in between muscles with high excursion and high force, while the redlines represent the gradual increase of muscle power. **Abbreviations:** (ADB) Adductor Brevis, (ADL) Adductor Longus, (ADM) Adductor Magnus, (AL) Adductor longus, (AM) Adductor magnus, (BF) Biceps Femoris, (CF) Caudofemoralis, (EDL) Extensor Digitorum Longus, (EHL) Extensor Hallucis Longus, (FDL) Flexor Digitorum Longus, (FHL) Flexor Hallucis Longus, (FMC) Femorococcygeus, (GASL + GASM) Gastrocnemius Lateralis + Medialis, (GEM) Gemmellus, (GMD, GMI, GMX) Gluteus Medius + Minimus + Maximus, (GRA) Gracilis, (IC) Interosseous Cruris, (ILI) Iliacus, (OE, OI) Obturator Externus + Internus, (PEB, PEL, PT) Peroneus Brevis + Longus + Tertius, (PD5) Peroneus Digiti Quinti, (PEC) Pectineus, (PLN) Plantaris, (PMA + PMI) Psoas Major + Minor, (POP) Popliteus, (PYR) Pyramidalis, (QF) Quadratus Femoris, (RF) Rectus Femoris, (SA) Sartorius, (SM) Semimembranosus, (SOL) Soleus, (ST) Semitendinosus, (TCA) Tibialis Caudalis, (TCR) Tibialis Cranialis, (TFL) Tensor Fascia Latae, (TP) Tibialis Posterior, (VI, VL, VM) Vastus Intermedius + Lateralis + Medialis.

3.3.2 Muscle mass proportion of functional groups

Similar to Chapter 2, we used the muscle mass to compare the functional groups in between the species (**Figure 26**, **Figure 27**). In the forelimbs of the three species, the greatest muscle mass was the elbow extensors, which is expected due to the greater mass of triceps muscle. However, we observed some subtle differences. In *D. gliroides* (**Figure 26**, **A**), shoulder flexors presented twice the mass of shoulder extensors, followed by an almost identical proportion of elbow and digit flexors. The remaining groups (extensors and flexors of the capus, digit extensors, supinator and pronator muscles) also presented a smaller but similar muscle weight distribution. In *L. inca* (**Figure 26**, **B**), the proportion of shoulder extensors and flexors were much more balanced than elbow extensors and flexors, followed by the digit flexors muscle mass that was the biggest between the small muscle groups. The flexors of the carpus muscle were much bigger than its extensors, and supinators were slightly bigger than pronator muscles. In *M. domestica* (**Figure 26**, **C**), shoulder extensors were much smaller than shoulder flexors, but both were bigger than elbow flexors. The digit flexors group was perceptibly bigger than the other small muscle groups, that were all with a similar muscle proportion: extensors and flexors of the carpus, digit extensors, supinators and pronators.

The hindlimbs of the three species presented a more contrasting pattern of muscle group proportion. In *D. gliroides* (**Figure 27**, **A**), we found a very distinct distribution than the other two species, with a great muscle mass of hip flexors, quadriceps femoris and hamstrings, followed by the adductor group, flexors of *pes* and gluteal group, and the smallest groups were the deep hip and extensors of *pes*. In *L. inca* and *M. domestica* the muscle mass distribution was very similar, but *L. inca* (**Figure 27**, **B**) showed almost the same proportion between hip flexors and gluteal group and also between extensors and flexors of *pes*; while *M. domestica* (**Figure 27**, **C**) presented a little bit bigger hip flexors than gluteus and more flexors than extensors of *pes*.

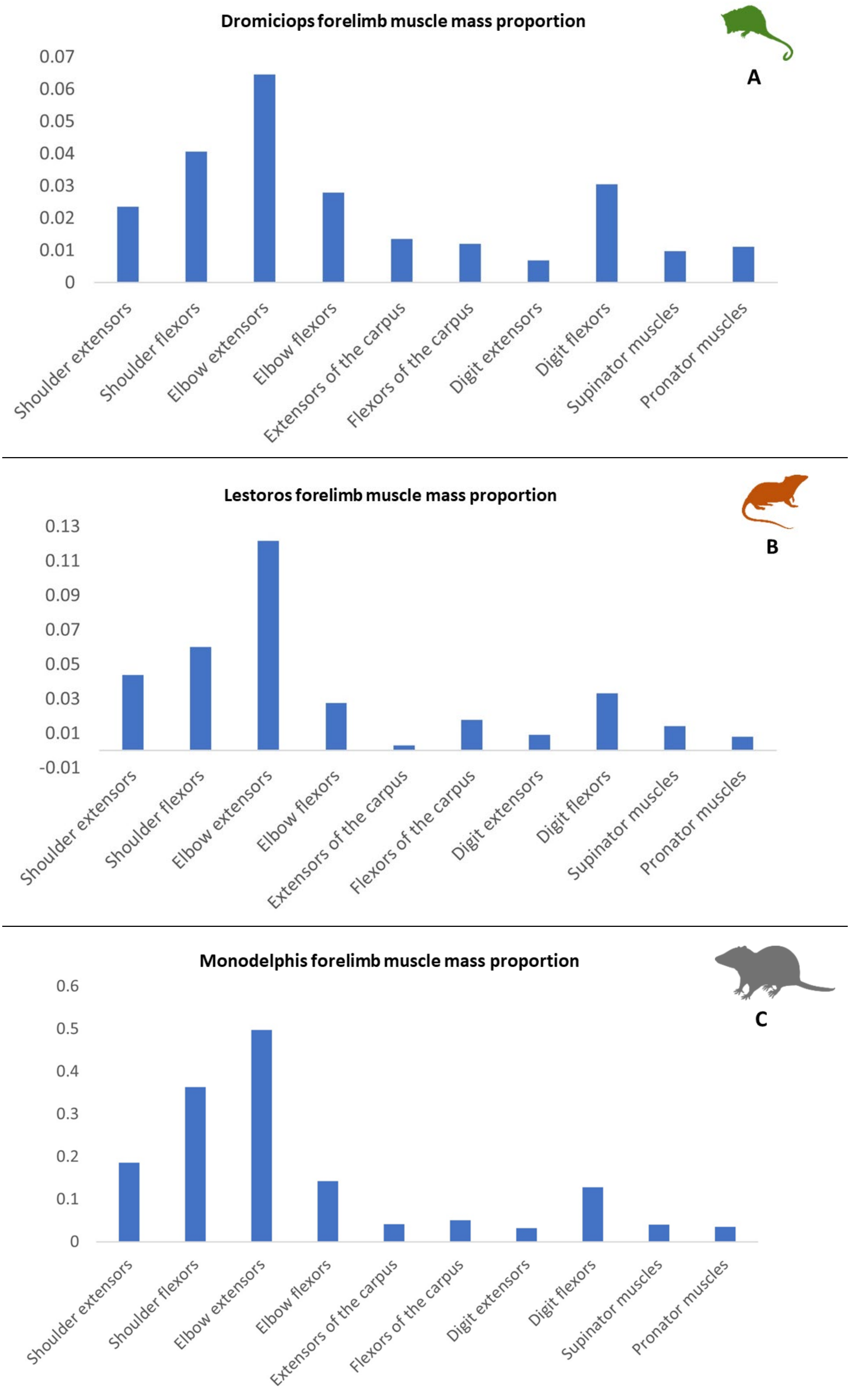

Figure 26. Forelimb muscle mass proportion of the functional muscle groups of the three species, *Dromiciops* (A), *Lestoros* (B) and *Monodelphis* (C). The x axis contains each muscle group and the y axis the muscle mass proportion. **Muscle groups:** Shoulder extensors (supraspinatus + coracobrachialis) and shoulder flexors (infraspinatus + deltoideus + teres major and teres minor); elbow extensors (epitrochleo anconeus + anconeus lateralis + triceps brachii) and elbow flexors (biceps brachii + brachialis); extensors of the carpus (extensor carpi radialis) and flexors of the carpus (flexor carpi radialis, flexor carpi ulnaris); digit extensors (extensor digitorum communis and lateralis, abductor pollicis longus) and digit flexors (flexor digitorum superficialis + flexor digitorum profundus); supinator muscles (supinator + brachioradialis); pronator muscles (pronator teres + pronator quadratus).

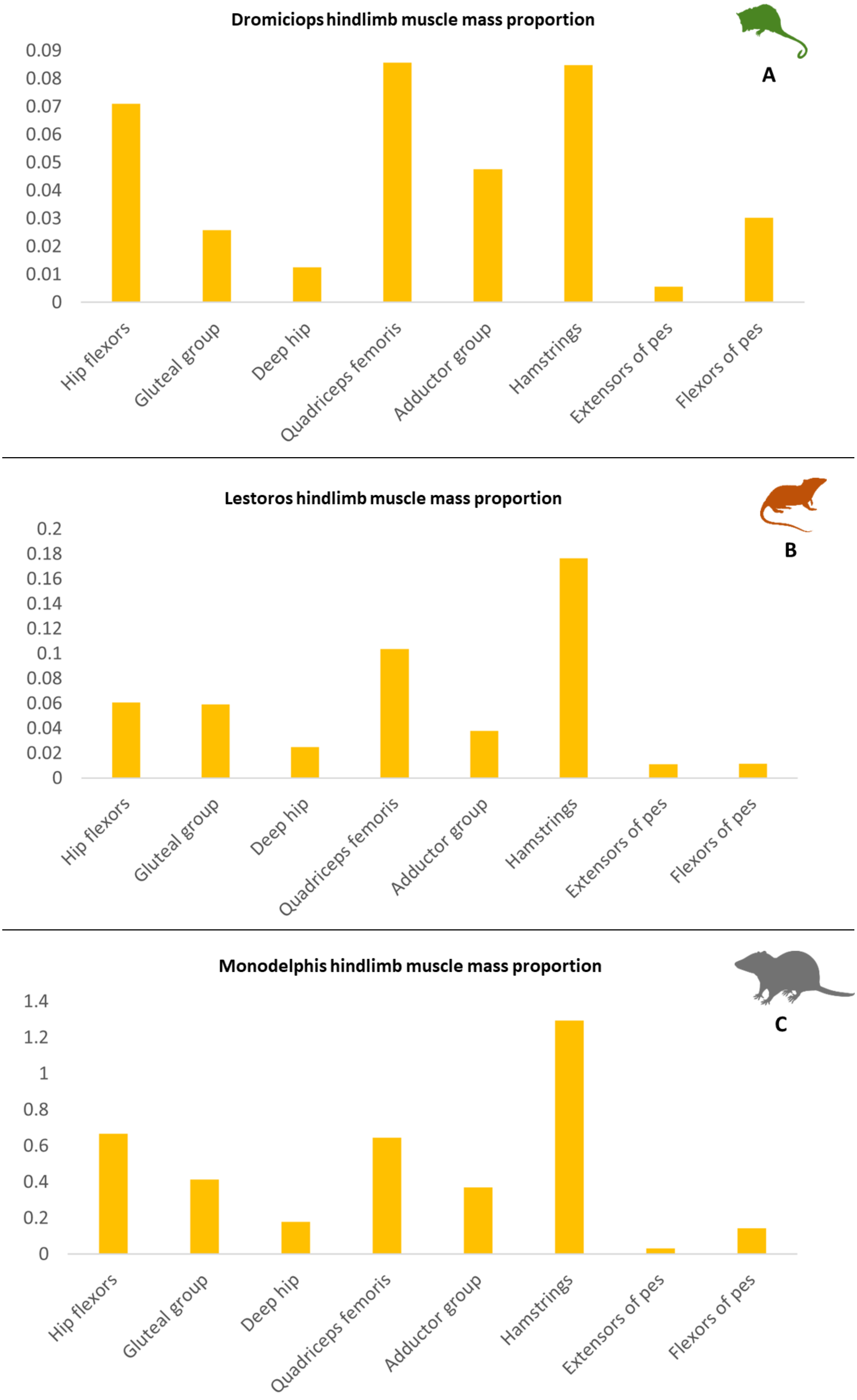

Figure 27. Hindlimb muscle mass proportion of the functional muscle groups of the three species, *Dromiciops* (A), *Lestoros* (B) and *Monodelphis* (C). The x axis contains each muscle group and the y axis the muscle mass proportion. **Muscle groups:** Hip flexors (rectus femoris + iliacus + psoas major + sartorius); gluteal group (All gluteal muscles); deep hip (piriformis + gemelli + obturators + quadratus femoris); quadriceps femoris (rectus femoris + vastus lateralis, v. intermedius and v. medialis); adductor group (all adductors' muscles + pectineus); hamstrings (biceps femoris + semitendinosus + semimembranosus); extensors of *pes* (extensor digitorums); flexors of *pes* (flexor digitorums).

DISCUSSION

During physical dissections we were able to confirm muscles identified in the digital segmentation and extra muscles there were not visible in the digital dissection. That means that, even though digital dissection might be a way to study the myology of rare specimens without resorting to destructive analysis (du Plessis 2017), physical dissections are still essential to access all the muscular anatomy due to limitations of digital dissections as inaccuracies and/or visual limitations, depending on the staining process.

Limb musculature in Marsupials

Marsupial appendicular myology has been studied in both American and Australian marsupials, in both descriptive (e.g.: Osgood 1921, Coues 1872, Argot 2001, Barbour 1963, Chapter 2) and comparative analysis (e.g.: Stein 1981, Diogo *et al.* 2016, Warburton and Marchal 2017). Some of the main variation explored in the forelimb is on *m. coracobrachialis*, *m. triceps brachii*, *m. atlantoscapularis anticus/posticus* and the muscle mass proportions of functional groups. In terms of hindlimbs, the myology mostly followed the typical marsupial arrangement with complex gluteal, adductor and hamstring groups (Warburton *et al.* 2015), with exception of very specialized forms like macropodids and moles.

In our study, we found the long and short heads of *M. coracobrachialis* only in the forelimb of *D. gliroides*. This muscle origin is commonly described as coming from the tip of the scapular coracoid process for both heads, while the short head insertion is more proximal in the humerus and the long head is in the humerus mid shaft (Ilayperuma *et al.* 2015). This muscle will produce flexion and abduction in the glenohumeral joint, but mainly it will prevent the arm deviation from the frontal plane, acting as a stabilizer of the humerus head inside the glenoid fossa (Moore *et al.*

2013), and the presence of the long head could increase the adductive power of this muscle (Argot 2001). In marsupials, it has been being rarely recorded as absent, such as in the southern pig-footed bandicoot *Chaeropus ecaudatus* (Ogilby, 1838) (Parsons 1903), and in the marsupial mole *Notoryctes* Stirling, 1891 (Wilson 1894, Warburton 2006), the former being a terrestrial and semi-fossorial species and the latter a completely specialized fossorial species. This could raise the question of whether this absence could be an expected feature in burrowing habits, although the short head was found in semi-fossorial species such as the brown bandicoot (*Isoodon obesulus* (Shaw, 1797)), the bilby (*Macrotis lagotis* Reid, 1837) (Warburton *et al.* 2013), and the wombat, another extreme specialized digging marsupial (Richards *et al.* 2023). *Lestoros inca* is frequently considered a semi-fossorial species, despite the lack of direct evidence to support this (Patterson, 2015), and also shows a short *m. coracobrachialis* head as *M. domestica*, which has a terrestrial habit (Astúa 2015). Apparently, in marsupials the *m. coracobrachialis* frequently has only a single head (e.g. Coues 1872, Osgood 1921, Stein 1981, Diogo *et al.* 2016). The presence of the long head seems to be more consistent and deeply connected with locomotion habits: so far, it was only found in arboreal marsupials. It is present in the mostly arboreal didelphid *Caluromys philander* (Linnaeus, 1758) (Argot 2001), the brush-tailed opossum *Trichosurus vulpecula* (Kerr, 1792) (Barbour 1963), the cuscus *Spilocuscus maculatus* (E. Geoffroy, 1803) (Cunningham 1882), koala *Phascolarctos cinereus* (Goldfuss, 1817) (Richards *et al.* 2023), and in the monito-del-monte *D. gliroides* (present study, **Figure 19**). The presence of a strong *m. coracobrachialis* was already associated with the presence of a well-developed coracoid process in felids (Salesa 2010), and Flores (2009) observed this process in a “hooklike” shape in several didelphid marsupials, mostly arboreal and including *C. philander*, but some of them were dissected by Argot (2001) and she did not find the long head of *m. coracobrachialis*. These features combined must be analyzed in a larger marsupial sample and in a phylogenetic framework. More broadly, this same muscle-habit association is present in primates (e.g. Japanese macaque and rhesus monkey (Sullivan *et al.* 1933, Aversi-Ferreira *et al.* 2016)), and it might be present in all primates including modern humans (Aversi-Ferreira *et al.* 2016). In Caniformia carnivores this muscle is also present only in climbing lineages, but it might be more associated to phylogenetic inertia than to functionality in Carnivora (Ercoli *et al.* 2015).

The presence of extra heads in the *m. triceps brachii* of *M. domestica* and *L. inca* was an unexpected outcome from our analysis. This muscle seems to be variable across marsupials and other mammalian groups. It was first named from human anatomy, due to the clear presence of three distinct heads with unique origins but sharing the same insertion (Gray, 2013). All studies on the most well studied marsupial in terms of myology, *Didelphis virginiana*, point to the conventional triceps with three heads (Coues 1872, Jenkins and Weijs 1979, Stein 1981, Diogo *et al.* 2016), which also seems to be the case in other species of American (Stein 1981, Argot 2001) and Australian marsupials (Warburton and Marchal 2017), suggesting that this could be a widespread pattern in the group. However, the presence of an extra accessory head in this muscle might have been unnoticed in previous studies and may not be uncommon in marsupials, as seem in *Lutreolina crassicaudata* (see Chapter 2) and *M. domestica* (present Chapter). Still, the presence of a fifth head, recognized as "angular head", was only found on *L. inca.* This may be a compeling evidence of *L. inca* adaptation for digging which, together with its humerus singular morphology, add to the case of convergence between caenolestids and other shrew-like mammals (He *et al.* 2015). Paucituberculatans marsupials are commonly called "shrew-opossums" and are rare in mammal collections, with very few studies *in vivo* or about its natural history. Therefore, the indirect evidence presented here may have to wait further kinematics and biomechanical analyses to support or discard this scenario about its ecomorphology.

In our study, both *L. inca* and *M. domestica* presented both *m. atlantoscapularis anticus* and *m. atlantoscapularis posticus* separated, while we did not identify these muscles in *D. gliroides.* These muscles are usually located between the *m. trapezius thoracica* and *m. supraspinatus*, being hard to miss during dissection unless there is considerable damage to the specimen or if it is in an unusual arrangement. As reviewed by Warburton and Marchal (2017), the presence of the two distinct muscles instead of the presence of only one muscle known as *m. omotransversarius* is a plesiomorphic condition for both marsupials and eutherian mammals, and its reduction or absence tend to occur in those species with terrestrial and/or digging locomotion, being a derived condition. We would have expected exactly the opposite considering the habits of species studied here, but it might be consistent with their phylogenetic historyinstead. The phylogenetic position of the order Microbiotheria is still ambiguous: the order is sometimes recovered as sister group of Diprotodontia, which means a more derived position (see phylogeny of **Figure 1** on General Introduction) or as the sister group to all the Australidelphia radiation, being a less derived position (Martin 2019). On the other hand, the phylogenetic position of Paucituberculata and Didelphimorphia suggest that their members would show more primitive conditions (Archer and Kirsch 2006, Voss and Jansa 2009, May-Collado *et al.* 2015).

In the hindlimbs, a qualitative feature that seems to be influenced by locomotion is the proximity or distance between tibia and fibula that, consequently, influence in the presence and development of the interosseous membrane and the m. flexor digitorum longus (Argot 2002). We found the interosseous membrane only in *D. gliroides,* being noticeable enough to be weighted and segmented (**Figure 21**, **C**) but absent (or not perceptible) in *L. inca* and *M. domestica* (Wright *et al.* 2022). Even though being formed by strong and dense aponeurotic fibers, it can not really pronate the limb, but it could be homologous with *m. pronator quadratus* (forelimb) (Coues 1872) and might be an important character to future evolutionary analysis. The presence or absence of this membrane is definitely associated with distance between tibia and fibula, since *L. inca* presented some degree of fusion in the distal half of the leg, being extremely hard to recognize any kind of membrane, while well separated bones like *D. gliroides* (present study), the Virginia opossum (Coues 1872), bandicoots and bilbies (Warburton *et al.* 2015) have this membrane. Regarding the distance between tibia and fibula, Argot (2002) associated their broad separation with a great development of *m. flexor digitorum fibularis* (here treated as *m. flexor digitorum longus*), usually seen in arboreal forms as the didelphid *Caluromys philander*, while less arboreal species like *Marmosa murina* would have these bones fused at some level.

PCSA values, muscle mass proportion and implications for limb performance

Superficially, forelimbs and hindlimbs of the three species seem to have a harmonious and similar distribution, but some subtle differences associated PCSA analysis might suggest some degree of functional morphology adaptations. In the forelimb muscle mass proportion, *D. gliroides* presented a very balanced muscle mass (except elbow extensors due to *m. triceps brachii* mass), with greater elbow flexors and digit flexors muscle mass than *L. inca* and *M. domestica*, comparatively. This is compatible with the flexed arboreal archetype, keeping a low position of the center of gravity allowing them to increase their stability and control their movements (Taylor 1970, 1974, Argot 2001). The *m. triceps brachii* long (and biggest) head will act together with *m. teres major* and *m. deltoideus* to flex both the humerus and scapula and forming a “z shape” in the forelimb, helping this flexed body position. Besides, *D. gliroides* digit flexors are much bigger (proportionally) than *L. inca* and *M. domestica*, showing its adaptation for grasping habits, essential for the arboreal locomotion. The same proportion was observed in the specialized arboreal didelphid *Caluromys philander*, with twice the weight of forearm muscles than the terrestrial *Metachirus myosurus* (Argot 2001). Another feature related to the arboreal habit is the presence of an incredibly strong *m. flexor digitorum profundus* in *D. gliroides* (**Figure 24**, **A**), which is the muscle responsible to flex all the five digits, vital for the species fast locomotion between vines and branches. As mentioned in Chapter 1, this muscle also helps the prehensility function in the manus and originates from the radius. In the very specialized arboreal didelphid *C. lanatus*, the radius is slightly concave medially and with a craniocaudal convexity, which increases the space for these muscles, that it is particularly well-developed in arboreal forms, especially when compared with terrestrial forms (Argot 2001). The forelimb muscle mass proportion of *L. inca* was similar with *Monodelphis domestica*, with the exception of *m. triceps brachii* discussed before. Also, the former has a bigger muscle mass in the flexors of the carpus than the latter. This might be evidence of an adaptation for digging habits, something regularly proposed for the shrew-opossums, but with no behavioral studies to confirm *in vivo*. Finally, in *M. domestica*, the more distinctive feature was a very powerful *m. trapezius cervicis* (**Figure 24**, **C**) than the other species, followed by *L. inca*. This feature might influence the limb posture to be more flexed and crouched, as suggested for cursorial small mammals to increase manoeuvrability and acceleration capacity (Biewener 1989), which is the case of *M. domestica* posture during a fast terrestrial locomotion.

In the hindlimbs, *D. gliroides* presented a striking different muscle mass distribution than *L. inca* and *M. domestica*, with hip flexors, quadriceps femoris group, adductor group and flexors of *pes* in a considerable bigger proportion than the other two species. The first three groups will help to keep the flexed/crouched position discussed above, and the quadriceps muscle group will act during the second part of the propulsive phase to push the body forward (Argot 2002), very necessary in *D. gliroides* and its bounced locomotion with its hindlimbs. The flexor of *pes* it is similar to the muscle mass proportion of the digit flexors of the species – it needs digits from both limbs with an increased flexor capacity to perform a fast and agile arboreal locomotion. This is once again consistent with the arboreal didelphid *Caluromys philander*, with 9% of the hindlimb musculature represented by *m. flexor digitorum longus* compared to only 4% in *Metachrus myosurus*, a terrestrial/cursorial species (Argot 2002). Adding to the group of flexors, the *m. peroneus longus* (**Figure 25**, **A** "PEL") is a powerful muscle only in *D. gliroides*, as a flexor of the whole tarsus during locomotion. In *L. inca* hindlimb, the *m. biceps femoris* is (proportionally) slightly bigger than *M. domestica*, and this muscle is responsible for the hip and tarsal joint extension (Miller *et al.* 2013), which is critical for terrestrial/cursorial locomotion. Once again, Argot (2002) observed similar muscle mass proportion, with the *m. biceps femoris* of the terrestrial *M. myosurus* representing 11% of the hindlimb musculature, compared to 4% with the arboreal *C. philander*. Warburton *et al.* (2015) also recorded the hamstrings group (which includes the *m. biceps femoris*) as the greatest muscle mass in the terrestrial bandicoot *Isoodon obesulus*. The morphology of this muscle is definitely an important feature when comparing the functional morphology of species with different locomotion habits. More distally in the leg, the tibia and fibula are partially fused in *L. inca*, similar to *Marmosa* analyzed by Argot (2002). The author suggested that this could provide some saltatorial ability to the species, which could be the case for *L. inca* as well, but further behavior or *in vivo* observations are needed to confirm this hypothesis.

Considering both limbs in the three species, we observed that the general muscle mass proportion is more balanced in *D. gliroides* due to the need of balance and to keep its gravity center near the ground, commonly seen in arboreal species. Furthermore, even though *L. inca* might be considered as semi-fossorial, its myology is very similar with the terrestrial *M. domestica*, with feel morphological remarks that might indicate some semi-fossorial adaptation.

Limitations

We suggest that the use of muscle mass proportion in functional muscle groups for comparative analysis are not the most appropriate method to access the myological variation in relation to locomotion habits in mammals. This is due to the "normal and expected" weight distribution for the muscles included in the groups, in which we would need a massive difference between them to result in changes to their normal distribution. Besides, it is important to take into account that not all muscles were identified in all specimens, causing some missing data in the functional groups. Finally, the combined use of muscle mass proportion plus PCSA analysis are helpful to reduce the effect of these limitations, added to the minimal use of two limbs for each region (anterior and posterior), whenever possible.

CONCLUSIONS

We found out that each species has specific myological features that can be associated in various levels with its habits. In the monito del monte (*Dromiciops gliroides*), the forelimb presented a m. coracobrachialis longus head and a well-developed m. flexor digitorum profundus, while the hindlimb presented a bigger muscle mass in the adductor group and a substantial bigger muscle mass of flexors of *pes* when compared with the other species. These characteristics were previously documented as important adaptations for climbing or the arboreal habit. In the shrew-opossum (*Lestoros inca*), the forelimb presented a m. triceps with five heads never documented before in marsupials, a feature that were previously documented as important adaptations for digging movements, as in placentals as shrews and armadillos. In both species, this is the first time that their functional morphology is being documented and associated with their locomotion. In the grey short-tailed opossum (*Monodelphis domestica*), the forelimb presented a very powerful m. trapezius cervicis and a balanced forearm muscle mass proportion while in its hindlimb we observed an intermediate hip flexors proportion between *L. inca* (less) and *D. gliroides* (more), besides a great hamstrings muscle mass when compared with other muscle groups if the species. This could both represent a generalized or more subtle adaptations of *M. domestica*, that it is a terrestrial species that might be able to perform some saltatorial habits according to its hindlimb muscles proportion.

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GENERAL THESIS CONCLUSION AND FUTURE PERSPECTIVES

We briefly presented the state of art of the Neotropical marsupial myology and, in a lesser degree, also the Australian marsupials. The broad understanding of the myology evolution in marsupials is hindered by the absence of basic descriptive and comparative studies, especially in some of the least specialized species. We have almost 20 studies of American marsupial myology, including (some or most) data about 23 species in a group of ~135 species (and increasing, Burgin *et al.* 2018, Voss 2022). We recongize that the physical dissection of small (<150g) species it is a very arduous process, especially considering rare and/or damaged material that we are usually unable to analyze more than one or two specimens. This might explain the lack of studies with small species, which include the greatest portion of American marsupials, but also a significant number of Australian ones.

It is also very challenging to summarize all the nuances of muscle morphology, similarities and differences between all appendicular muscles and several species in a descriptive or comparative method. For this reason, besides the first chapter review of Neotropical marsupials and the data gathered for this thesis, we are also building a data set of all the marsupial myological characters available both in literature and collected manually, for evolutionary analysis. We intend is to develop, in the future, a continuation of this thesis establishing ancestral state and muscle map analysis, to better understand the functional morphology variation and evolution of marsupial muscles through the Marsupialia infraclass/group. This might also shed a light on which species would be the key for next dissections that would give us a better understanding about how these morphotypes might be changing through "Ameridelphia" and the most morphogically diverse, Australidelphia.

Hopefully with this thesis we can change the perspective towards the Neotropical marsupial "unspecialized" morphological *bauplan*: there is many things hidden in the soft tissue that has been unexplored for so long. There is a potential to discover new myological characters that might be leading to functional morphological adaptation, that, added to previous and future osteological research, it will considerably increase data for total evidence phylogenetic analysis in the group.

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APPENDIX I

Table 6. Muscle architecture properties for all muscles in the forelimb and hindlimb of the monito del monte *Dromiciops gliroides* (FMNH22677). Values listed are the average of five measurements for each architecture property based on dissections of four limbs (3 forelimbs, one hindlimb); body mass 25.4 (g).

Muscle	**MM (g)**	**Log MBL (mm^1/3)**	**PA (º)**	**Log FL (mm^1/3)**	**PCSA***
Forelimb					
Abductor Pollicis Longus (APL)	0.0037	0.79	5,22	0.36	0.0037
Epitrochleoanconeus (EPI)	0.0017	0.80	7,64	0.73	0.0017
Biceps Brachii (BIB)	0.0191	0.78	23,11	0.74	0.0176
Brachialis (BRA)	0.0089	0.76	19,31	0.80	0.0084
Brachiocephalicus (BRAC)	0.0061	0.78	19,65	0.60	0.0057
Brachioradialis (BRAR)	0.0098	0.67	6,78	0.79	0.0097
Coracobrachialis Longus (CORL)	0.0016	0.82	7,65	0.60	0.0016
Coracobrachialis Short (CORS)	0.0013	0.64	13,86	0.61	0.0013
Deltoideus Acrom+Clav (DCA+DCL)	0.0103	0.72	11,14	0.61	0.0101
Deltoideus Scapularis (DSCA)	0.0056	0.73	14,99	0.75	0.0054
Extensor Carpi Radialis Brevis (ECRB)	0.0013	0.88	8,42	0.84	0.0013
Extensor Carpi Radialis Longus (ECRL)	0.0123	0.63	3,86	0.67	0.0123
Extensor Carpi Ulnaris (ECU)	0.0036	0.73	9,6	0.57	0.0035
Extensor Digitorum Communis (EDC)	0.0032	0.65	13,77	0.69	0.0031
Flexor Carpi Radialis (FCR)	0.0036	0.64	15,56	0.64	0.0035
Flexor Carpi Ulnaris (FCU)	0.0085	0.70	17,11	0.64	0.0081
Flexor Digitorum Profundus (3heads) (FDP)	0.0240	0.69	21,82	0.82	0.0223
Flexor Digitorum Superficialis (FDS)	0.0065	0.68	8,45	0.50	0.0064
Infraspinatus (INF)	0.0129	0.89	15,73	0.67	0.0124
Latissimus (LAT)	0.0343	0.96	18,25	0.82	0.0326
Palmaris Longus (PAL)	0.0008	0.66	3,82	0.61	0.0008
Pectoralis Major (PCMA)	0.031	0.82	13,15	0.82	0.0302

Pectoralis Minor (PCMI)	0.0114	0.65	30,41	0.58	0.0098
Pronator Quadratus (PRQ)	0.0012	0.76	7,86	0.75	0.0012
Pronator Teres (PRT)	0.01	0.76	7,87	0.49	0.0099
Rhomboideus (RHO)	0.004	0.88	18,67	0.85	0.0038
Subscapularis (SUBS)	0.0297	0.86	31,13	0.80	0.0254
Supraspinatus (SUPR)	0.0206	0.91	12,19	0.62	0.0201
TeresMajor (TMA)	0.0119	0.84	4,68	0.79	0.0119
Trapezius Cervicis (TZC)	0.0045	0.74	17,13	0.43	0.0043
Trapezius Thoracic (TZT)	0.0154	0.77	28,24	0.61	0.0136
Triceps Lateral (TRLA)	0.0147	0.93	13,27	0.80	0.0143
Triceps Long (TRLO)	0.0391	0.86	6,33	0.53	0.0389
Triceps Medial (TRM)	0.0091	0.92	3,87	0.68	0.0091
	Hindlimb				
Adductor Brevis (ADB)	0,0112	0.94	27.459	0.87	0.010
Adductor Longus (ADL)	0,0112	1.02	15.147	0.93	0.011
Adductor Magnus (ADM)	0,0216	0.95	16.409	0.73	0.021
Biceps Femoris (BF)	0,0179	0.94	16.000	0.74	0.017
Extensor Digitorum Longus (EDL)	0,0056	0.81	12.333	0.71	0.005
Femorococcygeus (FMC)	0,0234	1.00	24.527	1.01	0.021
Flexor Digitorum Longus (FDL)	0,0208	0.88	9.952	0.89	0.020
Flexor Hallucis Longus (FHL)	0,0094	0.83	17.704	0.80	0.009
Gastrocnemius Lateralis + Plantaris (GASL+PLN)	0,0202	0.83	12.458	0.97	0.020
Gastrocnemius Medialis (GASM)	0,0077	0.75	13.414	0.81	0.007
Gemmellus (GMM)	0,0043	1.04	24.203	0.99	0.004
Gluteus Maximus (GMX)	0,0182	1.06	26.053	0.99	0.016
Gluteus Medius (GMD)	0,0076	1.00	16.784	0.91	0.007
Gracilis (GR)	0,0238	0.95	21.249	0.87	0.022

Iliacus (ILI)	0,0083	1.06	19.940	0.95	0.008
Interosseous Cruris (IC)	0,0027	0.77	36.464	0.57	0.002
Obturator Externus (OE)	0,0046	0.65	16.858	0.67	0.004
Obturator Internus (OI)	0,0037	0.54	20.463	0.50	0.003
Pectineus (PEC)	0,0037	0.95	11.254	0.78	0.004
Peroneus Brevis (PEB)	0,0089	1.03	8.474	0.78	0.009
Peroneus Longus (PEL)	0,0173	0.93	14.141	0.66	0.017
Psoas Major (PMA)	0,0074	0.96	13.423	0.93	0.007
Rectus Femoris (RF)	0,044	0.96	26.567	0.75	0.039
Sartorius (SA)	0,0114	0.93	15.856	0.84	0.011
Semimembranosus (SM)	0,0301	0.97	23.311	0.98	0.028
Semitendinosus (ST)	0,0368	0.93	13.531	0.83	0.036
Soleus (SO)	0,0146	0.90	18.681	0.87	0.014
Tensor Fascia Latae (TFL)	0,0066	0.80	26.689	0.83	0.006
Tibialis Caudalis (TCA)	0,0032	0.94	15.141	0.71	0.003
Tibialis Cranialis (TCR)	0,0056	0.91	9.975	0.75	0.006
Vastus Intermedius (VI)	0,0088	1.00	16.432	0.91	0.008
Vastus Lateralis (VL)	0,0190	1.01	13.116	1.00	0.019
Vastus Medialis (VM)	0,0139	0.94	20.485	0.91	0.013

Obs.: 2. *PCSA is muscle mass x (cosPA), where PA is the Pennation Angle.

Table 7. Muscle architecture properties for all muscles in the forelimb and hindlimb of the shrew-opossum *Lestoros inca* (FMNH172050). Values listed are the average of five measurements for each architecture property based on dissections of one forelimb and one hindlimb; body mass 35 (g).

Muscle	**MM (g)**	**Log MBL (mm^1/3)**	**PA (º)**	**Log FL (mm^1/3)**	**PCSA***
Forelimb					
Abductor Pollicis Longus (APL)	0,0028	0.66	27.667	0.19	0.002
Atlantoscapularis Anticus (AA)	0,0038	0.78	15.534	0.54	0.004
Atlantoscapularis Posticus (AP)	0,0022	0.79	15.694	0.54	0.002
Biceps Brachii (BIB)	0,0162	0.74	17.615	0.50	0.015
Brachialis (BRA)	0,0115	0.76	12.620	0.47	0.011
Brachioradialis (BRAR)	0,0217	0.77	9.554	0.49	0.021
Coracobrachialis Short (CORS)	0,0014	0.34	0	0	0.001
Deltoideus Acromialis (DAC)	0,0048	0.66	21.941	0.40	0.004
Deltoideus Clavicularis (DCL)	0,0046	0.63	23.456	0.48	0.004
Deltoideus Scapularis (DSC)	0,0128	0.96	19.784	0.59	0.012
Dorsoepitrochleris (DOR)	0,0037	0.70	6.966	0.65	0.004
Epitrochleoanconeus (EPI)	0,0016	0.48	18.229	0.33	0.002
Extensor Carpi Radialis Brevis (ECRB)	0,0003	0.41	7.001	0.40	0.000
Extensor Carpi Radialis Longus (ECRL)	0,0026	0.67	16.687	0.31	0.002
Extensor Carpi Ulnaris (ECU)	0,0027	0.63	8.199	0.44	0.003
Extensor Digitorum Communis (EDC)	0,0063	0.73	18.403	0.43	0.006
Extensor Digitorum Profundus (EDP)	0,0013	0.60	17.818	0.21	0.001
Flexor Carpi Radialis (FCR)	0,0054	0.68	5.086	0.47	0.005
Flexor Carpi Ulnaris (FCU)	0,0125	0.73	25.540	0.50	0.011
Flexor Digitorum Profundus (3heads) (FDP)	0,0269	0.80	26.001	0.46	0.024
Flexor Digitorum Superficialis (FDS)	0,0064	0.62	23.693	0.42	0.006
Infraspinatus (INF)	0,0139	0.85	21.156	0.37	0.013
Latissimus (LAT)	0,061	1.10	21.245	0.87	0.057

Muscle	**MM (g)**	**Log MBL (mm^1/3)**	**PA (º)**	**Log FL (mm^1/3)**	**PCSA***
Palmaris Longus (PAL)	0,0016	0.61	11.283	0.48	0.002
Pectoralis Transversus (PCMI)	0,0137	0.83	17.547	0.82	0.013
Pectoralis Abdominis (quartus) (PCDE)	0,0149	0.99	11.519	0.85	0.015
Pectoralis Major (PCMA)	0,0393	0.88	38.771	0.65	0.031
Pectoralis Minor (PCMI)	0,0235	0.98	5.185	0.83	0.023
Pronator Quadratus (PRQ)	0,0001	0.47	5.816	0.03	0.000
Pronator Teres (PRT)	0,0080	0.68	15.029	0.52	0.008
Rhomboideus Major (RHMA)	0,0121	0.65	10.532	0.53	0.012
Rhomboideus Minor (RHMI)	0,0022	0.91	18.606	0.66	0.002
Serratus (SER)	0,0702	0.77	28.940	0.83	0.061
Subclavius (SUBC)	0,0030	0.74	17.109	0.37	0.003
Subscapularis (SUBS)	0,0471	0.93	26.088	0.58	0.042
Supinator+Flexor Digitorum Communis (SUP+FDC)	0,0062	0.68	41.095	0.45	0.005
Supraspinatus (SUPR)	0,0424	0.94	18.926	0.59	0.040
Teres Major (TMA)	0,0220	0.90	18.200	0.69	0.021
Teres Minor (TMI)	0,0019	0.65	20.998	0.39	0.002
Trapezius Cervicis (TZC)	0,0241	0.85	11.523	0.79	0.024
Trapezius Thoracic (TZT)	0,0141	0.96	24.087	0.62	0.013
Triceps Accessory (TRA)	0,0048	0.80	12.062	0.68	0.005
Triceps Extra (TRE)	0,0126	0.74	26.824	0.65	0.011
Triceps Lateral (TRLA)	0,0246	0.83	12.077	0.74	0.024
Triceps Long (TRLO)	0,0641	0.81	33.357	0.54	0.054
Triceps Medial (TRM)	0,0139	0.70	27.466	0.55	0.012
Hindlimb					
Adductor Brevis (ADB)	0,0053	0.62	11.072	0.45	0.005

Muscle	MM (g)	Log MBL (mm^1/3)	PA (º)	Log FL (mm^1/3)	PCSA*
Adductor Longus (ADL)	0,0048	0.64	11.592	0.56	0.005
Adductor Magnus (ADM)	0,0222	0.73	12.602	0.70	0.022
Biceps Femoris (BF)	0,0847	0.88	14.916	0.68	0.082
Extensor Digitorum Longus (EDL)	0,0112	0.76	16.555	0.32	0.011
Femorococcygeus (FMC)	0,0155	0.86	11.752	0.69	0.015
Flexor Digitorum Longus (FDL)	0,0045	0.69	17.584	0.21	0.004
Flexor Hallucis Longus (FHL)	0,0072	0.78	8.786	0.32	0.007
Gastrocnemius Lateralis (GASL)	0,0263	0.67	16.518	0.42	0.025
Gastrocnemius Medialis (GASM)	0,0247	0.73	31.035	0.42	0.021
Gemmellus (GEM)	0,0029	0.46	25.865	0.28	0.003
Gluteus Maximus (GMX)	0,0364	0.74	29.686	0.52	0.032
Gluteus Medius (GMD)	0,0186	0.74	25.495	0.40	0.017
Gluteus Minimus (GMI)	0,0041	0.60	14.403	0.43	0.004
Gracilis (GRA)	0,0197	0.85	13.441	0.81	0.019
Iliacus (ILI)	0,0221	0.81	20.908	0.52	0.021
Popliteus (POP)	0,0042	0.55	0	0.12	0.004
Obturator Externus (OE)	0,0095	0.52	23.122	0.38	0.009
Obturator Internus (OI)	0,0086	0.50	37.275	0.38	0.007
Pectineus (PEC)	0,0056	0.55	16.220	0.43	0.005
Peroneus Brevis (PB)	0,0015	0.61	0	0.05	0.002
Peroneus Longus (PL)	0,0073	0.71	24.755	0.35	0.007
Plantaris (PLN)	0,0036	0.60	18.991	0.30	0.003
Psoas Major (PMA)	0,0070	0.74	10.079	0.54	0.007
Pyramidalis (PYR)	0,0025	0.58	17.260	0.40	0.002
Quadratus Femoris (QF)	0,0040	0.48	18.783	0.38	0.004
Rectus Femoris (RF)	0,0317	0.87	20.518	0.50	0.030

Muscle	MM (g)	Log MBL (mm^1/3)	PA (º)	Log FL (mm^1/3)	PCSA*
Semimembranosus (SM)	0,0694	0.84	18.012	0.81	0.066
Semitendinosus (ST)	0,0226	0.90	9.133	0.63	0.022
Soleus (SOL)	0,0146	0.79	20.587	0.40	0.014
Tensor Fascia Latae (TFL)	0,0174	0.66	47.600	0.57	0.012
Tibialis Caudalis (TCA)	0,0231	0.81	13.964	0.39	0.022
Tibialis Cranialis (TCR)	0,0254	0.74	7.892	0.57	0.025
Vastus Lateralis (VL)	0,0603	0.84	22.768	0.53	0.056
Vastus Medialis (VM)	0,0117	0.82	14.253	0.49	0.011

Obs.: 3. *PCSA is muscle mass x (cosPA), where PA is the Pennation Angle.

Table 8. Muscle architecture properties for all muscles in the forelimb and hindlimb of the grey short-tailed opossum *Monodelphis domestica*. Values listed are the average of five measurements for each architecture property based on dissections of one forelimb (present study) and four hindlimbs (Wright *et al.* 2022); body mass 112,18 (g).

Muscle	MM (g)	Log MBL (mm^1/3)	PA (º)	Log FL (mm^1/3)	PCSA*
Forelimb					
Abductor Pollicis Longus (APL)	0,0196	0.74	30.766	0.25	0.017
Atlantoscapularis Anticus (AA)	0,0414	1.06	15.929	0.72	0.040
Atlantoscapularis Posticus (AP)	0,0344	1.02	15.304	0.73	0.033
Biceps Brachii (BIB)	0,0952	0.83	7.826	0.49	0.094
Brachialis (BRA)	0,0470	0.93	6.660	0.70	0.047
Brachiocephalicus (BRAC)	0,0798	1.16	13.165	1.05	0.078
Brachioradialis (BRAR)	0,0334	0.83	8.994	0.71	0.033
Coracobrachialis Short (CORS)	0,0041	0.51	27.526	0.26	0.004
Deltoideus Acromialis (DAC)	0,0405	0.86	22.323	0.64	0.037
Deltoideus Clavicularis (DCL)	0,0378	0.81	7.790	0.80	0.037
Deltoideus Scapularis (DSC)	0,1021	1.00	16.078	0.80	0.098
Dorsoepitrochlearis (DOR)	0,0242	0.87	3.077	0.74	0.024

Muscle	MM (g)	Log MBL (mm^1/3)	PA (º)	Log FL (mm^1/3)	PCSA*
Extensor Carpi Radialis Brevis (ECRB)	0,012	0.83	13.900	0.44	0.012
Extensor Carpi Radialis Longus (ECRL)	0,0296	1.02	9.062	0.92	0.029
Extensor Carpi Ulnaris (ECU)	0,0144	0.71	11.747	0.23	0.014
Extensor Digiti Minimi (EDM)	0,0135	0.70	6.507	0.33	0.013
Extensor Digitorum Profundus (EDP)	0,0427	0.77	16.845	0.46	0.041
Extensor Digitorum Superficialis (EDS)	0,0189	0.78	0	0.38	0.019
Flexor Carpi Radialis (FCR)	0,0158	0.81	14.585	0.39	0.015
Flexor Carpi Ulnaris (FCU)	0,0348	0.74	35.022	0.24	0.028
Flexor Digitorum IV&V (FD4&5)	0,0037	0.65	19.704	0.31	0.003
Flexor Digitorum Profundus (humeral) (FDPH)	0,0134	0.82	21.306	0.37	0.012
Flexor Digitorum Profundus (radial) (FDPR)	0,0540	0.87	18.131	0.50	0.051
Flexor Digitorum Profundus (ulnar) (FDPU)	0,0290	0.81	15.867	0.44	0.028
Flexor Digitorum Superficialis (FDS)	0,0312	0.95	19.941	0.57	0.029
Flexor Digitorum V (FD5)	0,0069	0.67	15.922	0.37	0.007
Infraspinatus (INF)	0,0835	1.02	25.356	0.55	0.075
Latissimus (LAT)	0,3925	1.21	29.522	1.07	0.342
Omohyoideus (OM)	0,0380	1.08	14.825	0.73	0.037
Palmaris Longus (PAL)	0,0092	0.57	15.307	0.41	0.009
Pectoralis Major (PCMA)	0,2815	1.11	29.983	0.79	0.244
Pectoralis Minor (+desc.+transv.) (PCMI)	0,1509	1.10	4.596	0.96	0.150
Pronator Quadratus (PRQ)	0,0044	0.54	0	0.01	0.004
Pronator Teres (PRT)	0,0310	0.90	22.666	0.53	0.029
Rhomboideus (total) (RHO)	0,0857	1.06	15.305	0.93	0.083
Rhomboideus Major (RHMA)	0,0220	0.75	27.350	0.68	0.020
Rhomboideus Minor (RHMI)	0,0604	0.73	48.163	0.68	0.040

Muscle	**MM (g)**	**Log MBL (mm^1/3)**	**PA (º)**	**Log FL (mm^1/3)**	**PCSA***
Serratus (SER)	0,3386	1.03	7.258	0.72	0.336
Subclavius (SUBC)	0,0122	0.80	22.371	0.65	0.011
Subscapularis (SUBS)	0,1997	0.98	36.018	0.76	0.162
Supinator (SUP)	0,0071	0.54	0	0.42	0.007
Supraspinatus (SUPR)	0,1813	1.02	41.885	0.66	0.135
Teres Major (TMA)	0,0997	1.04	22.342	0.74	0.092
Trapezius Cervicis (TZC)	0,2026	1.12	15.880	1.07	0.195
Trapezius Thoracica (TZT)	0,0791	1.08	38.818	0.83	0.062
Triceps Accessory (TRA)	0,0261	0.89	18.498	0.75	0.025
Triceps Lateral (TRLA)	0,0931	0.99	23.682	0.64	0.085
Triceps Long (TRLO)	0,2931	0.98	22.772	0.60	0.270
Triceps Medial (TRM)	0,0850	0.95	16.344	0.69	0.082
Hindlimb (data from Wright *et al.* (2022))					
Adductor brevis (AB)	0.219	1.11	13.6	1.01	0.213
Adductor longus (AL)	0.035	1.11		1.07	0.035
Adductor magnus (AM)	0.083	0.97		0.94	0.083
Biceps femoris anterior (BFA)	0.205	1.15		1.08	0.205
Biceps femoris posterior (BFP)	0.318	1.08	14.1	1.00	0.308
Caudofemoralis (CF)	0.046	1.08		1.05	0.046
Extensor digitorum longus (EDL)	0.021	1.02		1.08	0.021
Extensor hallucis longus (EHL)	0.01	0.90	12.1	0.86	0.010
Flexor digitorum longus (FDL)	0.143	1.08	9.87	1.03	0.141
Gastrocnemius lateralis (GASL)	0.105	0.99	16.7	0.92	0.101
Gastrocnemius medialis (GASM)	0.040	0.94		0.84	0.040
Gemellus inferior (GEMI)	0.046	0.66		0.62	0.046
Gemellus superior (GEMS)	0.027	0.78		0.69	0.027

Muscle	MM (g)	Log MBL (mm^1/3)	PA (º)	Log FL (mm^1/3)	PCSA*
Gluteal muscles (GMM)	0.415	1.03	21.7	0.84	0.385
Gracilis (GR)	0.168	1.15	0	1.13	0.168
Iliacus (ILI)	0.143	1.00	16.2	0.94	0.137
Obturator externos (OE)	0.094	0.80		0.76	0.094
Pectineus (PEC)	0.034	1.00		0.79	0.034
Peroneus brevis (PB)	0.022	0.90	8.04	0.90	0.022
Peroneus digiti quinti (PD5)	0.003	0.91		0.69	0.003
Peroneus longus (PL)	0.025	0.77	20.6	0.64	0.023
Peroneus tertius (PT)	0.006	0.75		0.58	0.006
Plantaris (PLN)	0.03	0.97		0.94	0.030
Psoas major (PMA)	0.127	1.02	21.5	0.75	0.118
Psoas minor (PMI)	0.032	0.94		0.93	0.032
Quadratus femoris (QF)	0.012	0.79		0.72	0.012
Rectus femoris (RC)	0.229	1.04	17	0.96	0.219
Sartorius (AS)	0.169	1.05		1.03	0.169
Semimembranosus (SM)	0.514	1.14	21.2	1.08	0.479
Semitendinosus (ST)	0.258	1.14		1.10	0.258
Tibialis anterior (TA)	0.089	0.95	24.1	0.93	0.081
Tibialis posterior (TP)	0.066	0.95	5.48	0.96	0.066
Vastus lateralis (VL)	0.321	1.05	18.8	0.77	0.304
Vastus medialis (VM)	0.097	1.01		0.96	0.097

Obs.: 4. *PCSA is muscle mass x (cosPA), where PA is the Pennation Angle.

Table 9. Actions of the main muscles identified according to Miller *et al.* (2013) and Souza-Júnior *et al.* (2018).

Forelimb	Action
Abductor pollicis longus	Extension and abduction of digit I

Anconeus lateralis	Extension of elbow
Atlantoscapularis	Scapula rotation
Biceps brachii	Flexion of elbow and extension of shoulder
Brachialis	Flexion of elbow
Brachioradialis	Supination
Coracobrachialis	Extension of shoulder
Deltoideus acromialis	Flexion of shoulder
Deltoideus clavicularis	Flexion of shoulder
Deltoideus scapularis	Flexion of shoulder
Dorsoepitrochlearis	Abduct the limb
Extensor carpi radialis	Extension of carpal joint
Extensor carpi ulnaris	Extension of lateral hand
Extensor digiti minimi	Extension of digits IV and V
Extensor digitorum communis	Extension of all digits
Extensor digitorum profundus	Extension of all digits
Flexor carpi radialis	Flexion of carpal joint
Flexor carpi ulnaris	Flexion of carpus
Flexor digitorum profundus	Flexion of all digits
Flexor digitorum superficialis	Flexion of the digits II–V
Infraspinatus	Flexion of shoulder
Latissimus dorsi	Retract the limb and flex the shoulder joint
Omohyoideus	Depresses the hyoid
Pectoralis major	Adduct the limb, stability
Pectoralis minor	Adduct the limb, retract the limb caudally, flex the shoulder joint
Pronator quadratus	Pronation
Pronator teres	Pronation
Rhomboideus	Elevate the limb
Serratus	Support of the trunk

Subclavius	Stabilize the clavicle
Subscapularis	Adduction of shoulder
Supinator	Supination
Supraspinatus	Extension of shoulder
Teres major	Flexion of shoulder
Teres minor	Flexion of shoulder
Trapezius	Elevate, protract and abduct the limb
Triceps brachii (accessory head)	Extension of elbow
Triceps brachii (lateral head)	Extension of elbow
Triceps brachii (long head)	Extension of elbow and flexion of shoulder
Triceps brachii (medial head)	Extension of elbow

Hindlimb	**Function**
Abductor cruris caudalis/tenuissimus	Tarsus flexion
Adductor caudal	Adductor and extension of the hip joint
Adductor cranial	Adductor and extension of the hip joint
Adductor intermedius	Adductor and extension of the hip joint
Biceps femoris	Hip + tarsal joint extensor
Extensor digitorum lateralis	Extension of the digits
Extensor digitorum longus	Extension of the digits, flexion of the tarsus
Flexor digitorum profundus	Flexion of the digits
Flexor digitorum superficialis	Flexion of the digits
Gemelli	Lateral rotation of hip joint
Gluteus medius	Medial rotation of hip
Gluteus profundus	Extension of hip joint
Gluteus superficialis	Medial rotation of hip
Gracilis	Adduction of the limb
Iliopsoas	Flexion of hip joint
Obturator externus	Lateral rotation of hip joint

Obturator internus	Lateral rotation of hip joint
Pectineus	Adduction of the thigh
Peroneus (fibularis)	Flexor of tarsus
Peroneus digiti quinti	Extension of the 5th digit
Popliteus	Medial rotation of femur
Quadratus femoris	Extension and lateral rotation
Rectus femoris*	Extensor of stifle joint + Flexion of the hip
Sartorius	Flex and stifle the hip
Semimembranosus	Hip extensor
Semitendinosus	Extension of hip + tarsal joints
Tibialis caudalis	Extension of tarsus
Tibialis cranialis	Lateral paw rotation
Tríceps surae	Extends the tarsal joint
Vastus intermedius*	Extensor of stifle joint + Flexion of the hip
Vastus lateralis*	Extensor of stifle joint + Flexion of the hip
Vastus medialis*	Extensor of stifle joint + Flexion of the hip

Obs.: 5. *Quadriceps femoris muscle bundle.