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ORIGINAL PAPER

Anatomical comparison across heads, fore- and hindlimbs in mammals using network models

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Abstract

Animal body parts evolve with variable degrees of integration that nonetheless yield functional adult phenotypes: but, how? The analysis of modularity with Anatomical Network Analysis (AnNA) is used to quantitatively determine phenotypic modules based on the physical connection among anatomical elements, an approach that is valuable to understand developmental and evolutionary constraints. We created anatomical network models of the head, forelimb, and hindlimb of two taxa considered to represent a 'generalized' eutherian (placental: mouse) and metatherian (marsupial: opossum) anatomical configuration and compared them with our species, which has a derived eutherian configuration. In these models, nodes represent anatomical units and links represent their physical connection. Here, we aimed to identify: (1) the commonalities and differences in modularity between species, (2) whether modules present a potential phylogenetic character, and (3) whether modules preferentially reflect either developmental or functional aspects of anatomy, or a mix of both. We predicted differences between networks of metatherian and eutherian mammals that would best be explained by functional constraints, versus by constraints of development and/or phylogeny. The topology of contacts between bones, muscles, and bones + muscles showed that, among all three species, skeletal networks were more similar than musculoskeletal networks. There was no clear indication that humans and mice are more alike when compared to the opossum overall, even though their musculoskeletal and skeletal networks of fore- and hindlimbs are slightly more similar. Differences were greatest among musculoskeletal networks of heads and next of forelimbs, which showed more variation than hindlimbs, supporting previous anatomical studies indicating that in general the configuration of the hindlimbs changes less across evolutionary history. Most observations regarding the anatomical networks seem to be best explained by function, but an exception is the adult opossum ear ossicles. These ear bones might form an independent module because the incus and malleus are involved in forming a functional primary jaw that enables the neonate to attach to the teat, where this newborn will complete its development. Additionally, the human data show a specialized digit 1 module (thumb/big toe) in both limb types, likely the result of functional and evolutionary pressures, as our ape ancestors had highly movable big toes and thumbs.

KEYWORDS

connectivity, human, modules, mouse, musculature, opossum

1 | INTRODUCTION

Robustness and evolvability in living organisms are promoted by the modular organization of organisms (Esteve-Altava, 2017a; Klingenberg, 2014; Melo et al., 2016). Among the three different types of modules: (1) developmental modules are defined by a common cell origin and the timing of cell differentiation (Koyabu et al., 2014); (2) variational modules are defined by size and shape covariation among body regions (Klingenberg, 2010); and (3) phenotypic modules are multiple functionally related body parts that do not necessarily develop from a common cell origin (Esteve-Altava, 2017a, 2017b). As such, modules—and the specific elements (i.e., structures like muscles or bones) that define a given module – are a source of rich data with which to identify and understand factors underpinning macroevolutionary changes within and among diverse animal groups such as mammals.

Modular organization implies that each phenotypic module can grow and function semi-independently of other modules across the body. As a consequence, modularity allows body parts to evolve semi-independently without disturbing the overall functionality of the organism (Wagner & Zhang, 2011). Thus, semi-independence of each module can support the evolution of complexity as a result of a hierarchical arrangement of components that may influence and facilitate greater evolvability of the organism (Simon, 1962, 2005; West-Eberhard, 2003). Therefore, defining the various modules and their potential covariance and hierarchy is important to understand the evolution of complex organisms.

Consequently, identifying phenotypic modules is important in anatomical sciences and in evolutionary developmental biology (EvoDevo) both to improve our understanding of phenotypic evolution more broadly (von Dassow & Munro, 1999; Raff & Raff, 2000; Rasskin-Gutman & Esteve-Altava, 2014) as well as to decode the genotype-to-phenotype map more specifically (Wagner & Zhang, 2011). Hard and soft tissues are intimately linked together—that is, integrated—due to development, growth, and/or function. If the elements of a module are strongly integrated, a minor change in one module, or even one element of a module, might be harmful for the proper form and function of an organism.

Anatomical Network Analysis (AnNA) is a relatively new method to identify and study modules. Although previously detailed by Knight and Pinney (2009) and Fortunato (2010), it is only recently that AnNA is used in anatomical and EvoDevo studies (Diogo et al., 2015a; Esteve-Altava et al., 2013a, 2013b, 2015b; Rasskin-Gutman & Esteve-Altava, 2014) and in evolutionary medical/human pathology studies (Diogo et al., 2019; Esteve-Altava & Rasskin-Gutman, 2015). In short, AnNA quantifies morphological organization without *a priori* assumptions about phylogenetic, developmental, or functional relationships among modules. For our purpose, AnNA uses computational methods (i.e., math) to define the strengths and directions of relationships among bones and cartilages as well as muscles, and their physical connections (e.g., joints, muscle attachments) as nodes (elements) and links (contacts) of a network model. In this way, AnNA identifies patterns of modularity and integration among

different body parts/modules. Here, we use AnNA to test whether shared evolutionary history (phylogeny), function, or developmental processes, or a combination of them can be used to explain variations in modules observed in the opossum and mouse, two taxa considered to represent a 'generalized' eutherian and marsupial anatomical configuration, respectively (Diogo et al., 2016), and compared them with our species, which has a derived eutherian configuration.

There are three main groups of mammals, monotremes (egg-laying mammal, e.g., echidna), metatherians (crown marsupials and their stem group), and eutherians (placentals and their stem group). The latter two groups bear live young, which differ greatly in their maturity at the time of birth. Marsupials have a very short gestation, and the highly altricial young complete their development while attached to and feeding at the teat (e.g., Smith, 2001). Marsupial pups use their robust forelimbs to crawl from the birth canal to the teat; and then to anchor their premature bodies to the teat, while their less-developed hindlimbs passively hang (Kelly & Sears, 2010). Indeed, marsupial limbs develop at different rates, associated with this strong functional need immediately after birth, such that the forelimb is significantly developmentally advanced compared to the delayed hindlimb (Kelly & Sears, 2010). Eutherians, on the other hand, are born with more equally developed fore- and hindlimbs and do not need to complete this epic crawl and anchoring to the teat.

Marsupials also have precocially developed oral structures due to the need to suckle very early in life; conversely, braincase development is delayed compared to eutherians (Jeffery et al., 2005, 2002; Smith, 2001). Numerous studies analyzed heterochronic differences between marsupial and eutherian development (e.g., Goswami et al., 2009, 2012; Nunn & Smith, 1998; Weisbecker et al., 2008a), a summary of which is beyond the scope of this study (but see review by Smith & Keyte, 2018).

Specific to humans, a recent AnNA study of upper and lower limbs, including healthy and abnormal adult and newborn human limbs, indicated that the skeletal upper and lower extremities were similar with respect to their module composition, with the exception of the limb girdles (Diogo et al., 2015a). However, when muscles were included in the AnNA, the fore- and hindlimbs were clearly different as their respective modules contained enough different elements to not closely resemble each other anymore, particularly their proximal parts. This supports the notion that fore- and hindlimbs are not serial homologues as previously thought (e.g., Diogo & Molnar, 2014; Diogo & Ziermann, 2015) because in serial homologous structures the modules are expected to resemble each other. The comparison of modularity in distantly related mammals, e.g., marsupial vs. placental, might therefore give additional insights into the evolution of mammalian including human limbs. For example, if special adaptations to precocious marsupial development, or to human bipedal walking and manual dexterity, are reflected in the phenotypic modules of the marsupial or human extremities, respectively, then the modules would clearly differ from each other even when the extremities in mammals have very similar elements.

The mammalian head is a challenging subject to study because it comprises densely packed structures with overlapping – and/or

highly specialized – functions. Furthermore, complexity and modularity often occur together in the development and evolution of the tetrapod head (e.g., Esteve-Altava et al., 2013a, 2013b, 2015a,). The soft and hard tissues facilitate functionality, stability, and evolvability and are therefore important in shaping the modular organization of the head (Diogo & Wood, 2013). Studies on skull modularity in mammals were previously undertaken by various researchers (e.g., Jamniczky & Hallgrímsson, 2011; Mitteroecker & Bookstein, 2008; Porto et al., 2009). Moss and Young (1960) already hypothesized that the head is divided into so-called functional components (i.e., modules) determined by soft tissues and cranial cavities (e.g., neural, oral). The latter are bound by surrounding skeletal units, and their development is guided by soft tissue components. For example, the muscles of mastication (temporalis, masseter, and pterygoids) are hypothesized to form a functional unit with the mandible as they help to shape the dentary bone via their effects on bone microstructure due to their action at their insertions (Moss & Salentijn, 1969). Functional components as defined by Moss and Young (1960) are, however, educated guesses based on form, function, and position, and therefore are *a priori* expectations rather than outcomes of quantitative analysis (Esteve-Altava & Rasskin-Gutman, 2014). Esteve-Altava and Rasskin-Gutman (2014) performed AnNA showing that functional matrices are vital to make a proper facial connectivity pattern. However, the cranial vault and base connectivity pattern can be sufficiently explained by their growth pattern, and was independent of the developing brain, which would influence shape changes of cranial vault and base (Esteve-Altava & Rasskin-Gutman, 2014; Richtsmeier & Flaherty, 2013).

This is, thus, the first AnNA work to compare in detail the networks of the skeletal (bone and cartilage) and muscular units of the heads, forelimbs, and hindlimbs of ‘generalized’ marsupials (opossum) and placental (mouse), and with our own species (specialized placental), as nodes (elements of a network), whose links (interactions at their contacts) determine the boundaries of a given phenotypic module of the respective body part. Both mouse and human are major biomedical model organisms: we not only dissected them ourselves and collected all data in a long process of checking, re-checking, and literature review but we also compared our data with the not as well-studied opossum. Each of the chosen species in this study has a different type of locomotion: opossum is a non-cursorial mammal (Jenkins, 1971), mouse is a classical quadruped mammal, and human is bipedal. Each of them has also quite different demands on their head-neck region (size, position to vertebrae and trunk). We not only compared the networks but we also looked at the developmental and functional theoretical networks and the observed networks, to see if development or function is the more driving force in networks. Our aims are to (1) identify the commonalities and differences in modularity between species, and (2) test whether modules present potential phylogenetic character (i.e., are modules potentially representative for a specific clade or rather for a specific lifestyle). We also test (3) whether modules reflect developmental or functional aspects, or if they are best understood by the combination of both commonalities and differences in modules between

species agree with *a priori* assumptions (H_0 -Hypothesis developed based on development or functions). The latter point is significant as differences might reflect an evolutionary trend: in this sense, we expect the eutherian mammals (mouse and human) to be more similar to each other than either of them is to the marsupial (opossum). In case function is more relevant to the connectivity, we expect the networks of mouse and opossum to be more similar to each other.

2 | MATERIAL AND METHODS

2.1 | Material and anatomical dissections

The matrices of humans (*Homo sapiens*) were generated via previous studies of the head (Esteve-Altava et al., 2015a, 2015b) and human extremities (Diogo et al., 2019). The adult opossum (*Didelphis virginiana*) matrix is based on dissections by Diogo et al. (2016). Mouse and human matrices were slightly modified to enable the comparison with opossum (e.g., extrinsic eye muscles were excluded because we were not able to code them for opossum). The adult mouse (*Mus musculus*) matrix was based on dissections (muscles, bones, and cartilages) and μ CT scans (bones). The adult mice for the dissection by JMZ were purchased from Carolina Lab (stored in Carolina's Perfect Solution[®]). For all species, we did extensive literature reviews on anatomical descriptions.

2.2 | Network modeling

Unweighted, undirected network models are built of the musculoskeletal anatomy of the head, forelimb, and hindlimb of *Didelphis virginiana* (opossum), *Mus musculus* (mouse), and *Homo sapiens* (human). Anatomical networks formalized the anatomical organization of the head and limbs as nodes connected by links. The codification of musculoskeletal networks follows that of previous works (Diogo et al., 2015a; Esteve-Altava et al., 2015b). Here, elements represent skeletal components (i.e., bones and cartilages) and muscles, while links represent physical joints among skeletal and muscular components (e.g., articulations, attachments, and blending). Between any two nodes, every contact was coded as 1 and every absence of contact was coded as 0, all logged in the adjacency matrix that ultimately codes the network model (Supplementary Tables: STables M1–M9). We also analyzed the skeletal and muscular components separately from each other, and created skeletal and muscular networks. Skeletal networks included bones and cartilages (as nodes) connected by their articulations (as links). Muscular networks included muscles (as nodes) connected by tendinous joints and fibrous fusions among them (links). Musculoskeletal networks included all elements (bones, cartilages and muscles) and all connections (attachments, joints, and fusions). Anatomical networks were coded as adjacency matrices in Excel sheets and analyzed in R (R-Core-Team, 2016) using the package *igraph* (Csardi & Nepusz, 2006). The code can be found as Supplementary File ('code Ziermann et al. Mammals').

2.3 | Community detection

We delimited the modules of the anatomical networks using a standard random walk algorithm, using the function *cluster_walktrap* of igraph. The heuristics of this algorithm are based on the idea that short random walks—we used random walks of three steps—tend to concatenate nodes within the same module (Pons & Latapy, 2006). This algorithm can find groups of nodes (modules) that are more densely connected among themselves than they are to other nodes. The quality of the defined modules is evaluated using the optimization function modularity (Q) (Newman & Girvan, 2004) to assess whether the module identified by a community detection algorithm is better than what is expected at random. Q will be close to 0 if the number of links within modules is no better than that expected at random; Q will be closer to 1 if the modules identified deviate from what is expected for a random network. According to Newman and Girvan's (2004) observations, strongly modular networks return Q values that range between 0.3 and 0.7. The expected error of Q is calculated using a jackknife procedure, where every link is an independent observation. Details for modules are provided in STables: Sk 1–9 (skeleton modules), Mu 1–9 (musculature modules), and MuSk 1–9 (musculoskeletal modules).

Additionally, we performed a two-sample Wilcoxon rank-sum test on the internal versus external connections of every module identified to test each module's statistical significance. According to the general definition of a module as a group of nodes that are highly interconnected (internal connections) and poorly connected to nodes in other groups (external connections), we expect internal connections to be significantly higher than external connections ($H_0: K_{\text{internal}} = K_{\text{external}}; H_a: K_{\text{internal}} > K_{\text{external}}$). For $p < 0.05$, we reject the H_0 and assume the alternative hypothesis that the nodes of the module are more connected among them than to other nodes outside the module (i.e., the module is not expected by chance). Note, however, that the accuracy of this test will depend on the size of the module (i.e., the number of nodes within), and smaller modules will render unreliable p -values due to small sample size. For this reason, using statistical test to assess network modules is not a common practice; the quality of partitions is usually assessed by the value of Q (see above). For further details on modularity methods and statistical tests, see Porter et al. (2009), Fortunato (2010), and (Esteve-Altava, 2017a, 2017b).

To test the fit of the identified network models to biological modules of the head and limbs, we created null hypotheses (H_0) about the grouping of bones (bone), muscles (mm), and musculoskeletal elements (bone.mm) according to their function (fkt) and development (dev). See details in STables O1–9. We compared the fit of network modules to each hypothesis using the normalized mutual information index (Danon et al., 2005). It ranges from 0 (totally different) to 1 (totally equal) and measures how similar are the partition of modules identified with the partition of the H_0 . The closer to 1 the closer the expected modules to the found modules. This index measure the amount of information needed to go from the observed modules as identified in STables Sk 1–9 (skeletal modules), Mu 1–9

(muscular modules), and MuSk 1–9 (musculoskeletal modules) to the H_0 as indicated in STables O1–9, and is commonly used in network science to compare partitions (Fortunato, 2010). We calculated the information index using the function *compare* in igraph.

2.4 | Developmental and/or functional hypotheses

Based on previous studies we have a good understanding of which elements function together, for example, during chewing, locomoting, and manual tasks. Furthermore, the development of these various body parts (head, limbs) is well studied. That information is important to interpret modules of muscles and/or bones with respect to their development or function. We compared the modules as predicted by their development and/or function and/or the combination of both and compared them to the actual identified modules. Basically, we wanted to identify if the found modules better present a functional group or a development group, or a combination of both. Details on which body parts and their elements belong to which functional, developmental, or combined (function and development) modules are found in STables C1–7 (head) C8–11 (forelimb), and C12–15 (hindlimb). Tables reporting results for analyses of connectivity modularity versus the hypothetical modules are presented in STables O1–9. The results of the analyses comparing the hypothetical modules with the identified modules are shown in STables S7–9. The closer the value is to 1, the more similar are hypothetical and identified modules.

3 | RESULTS

Module names, if present, are indicated between apostrophes ('name') in the text and after the module number in the tables. Importantly, module names reflect the overall content of a module without necessarily being precise about the module's contents (e.g., a module 'carpals' might include only seven of eight carpals present in humans). We chose this form as it simplifies the comparisons between species. Colors in figures and skeletal and musculoskeletal Supplementary Tables (STables Sk 1–9, MuSk 1–9) correspond to easier identify modules.

3.1 | Modules

Modules with a p -value > 0.05 are no true modules per definition (highlighted yellow in all modularity tables). However, if modules with more than one element are comparable between species, we consider them to be modules. Within the musculature analyses (STables Mu 1–9), many muscles are single ($p = 1$) and obviously do not form a module. We therefore list only the module numbers and refer to 'single muscles' (e.g., in STable Mu 1 modules 23 to 52 are all single muscles). The musculoskeletal analyses have the least number of 'no true' modules, which might be expected as soft tissue elements connect hard tissue elements, enabling the recovery of phenotypic modules.

3.1.1 | Head

To compare among the three species more effectively, we combine modules with the same elements that are present on both sides of the head (STables Sk 1–3, Mu 1–3, and MuSk 1–3). Furthermore, we combine elements from both sides if they are in the same module (element.left, element.right→element.L/R); in this way, the elements are still distinguished from those that are only single elements. Elements that cross the median line (mid-sagittal plane) but are in a left or right dominant module are marked RED in the Supplementary Tables (STables Sk 1–3, Mu 1–3, and MuSk 1–3).

Skeleton

Human and mouse have a single hyoid bone “pseudo module” ($p = 1$, STables Sk 1–3), which is unsurprising as the hyoid bone does not articulate with any other bones (Figure 1). Opossum has a module containing all elements from the ‘hyoid arcade’ (orange in Figure 1B; STable Sk 1: basihyal, hypohyal.L/R, and thyrohyal.L/R). All species have a left and right module for the ‘pectoral girdle’ elements (clavicle and scapula) plus the sternum. Also present in all species is a module (‘larynx’) containing all laryngeal cartilages/bones. Mouse and human each have a module containing the ossicles on each side, while in opossum the ossicles are divided into two modules (incus + malleus and stapes + tympanic). Note that the tympanic is in a different module in mouse, and part of the temporal bone in human. The laryngeal arch cartilage is absent in human or opossum and is a single element (no module) in mouse (STable Sk 2). Finally, all species have a module ‘neurocranium’ and a module ‘viscerocranium’ that contain similar bones. The former includes the vertebrae and the mandible, and in mouse also the zygomatic bones, which in human and opossum are included in the ‘viscerocranium’.

Musculature

The largest modules ‘mid-face’ left and right in humans include many muscles of facial expression (mid and lower face) from the left/right side of the face (STable Mu 3). The human upper facial region (around the eyes, forehead, and back of the head) form one module ‘upper face’ that includes muscles from both sides. The mouse also has a left and right ‘mid-face’ module, which are similar but not identical to each other; the single ‘upper face’ module does also include the orbito-temporo-auricularis muscles, which corresponds to the frontalis, temporoparietalis, and auricularis anterior muscles in human (Diogo et al., 2018b). The opossum has a single large ‘facial’ module including muscles from the lower and middle face and an ‘upper face/neck’ module that includes different trapezius portions as well as the frontalis, occipitalis, and auricularis posterior (STable Mu 1). The latter is also included in the ‘upper face’ module of mouse. A left and a right ‘upper face’ module is present in opossum, which include muscles around the eye.

The ‘mastication’ module is present on the left and the right side of all species analyzed here; the content, however, differs. In all three species, the masseter and temporalis muscles are included, but in opossum the module also contains the pterygoideus medialis

(STable Mu 1). The pterygoid lateralis pars superior and pars inferior form left/right modules in human (STable Mu 3), while the pterygoids are not included in any of the mouse modules.

The opossum has two ‘glossus’ modules that include four muscles associated with the tongue and the geniohyoideus. This module is separated in several modules in human and mouse, both species having a left and a right module ‘glossus.2’ (hyoglossus and styloglossus) and a single module containing both geniohyoideus muscles. The mouse also has a module ‘glossus.1’ that contains both genio-glossus muscles.

The ‘pharyngeus’ module comprises the palatopharyngeus in all species included here. Besides that, the modules are different in that opossum and human have a left and a right ‘pharyngeus’ module, while the mouse has a single module containing all (bilateral) muscles ending with -pharyngeus plus the constrictor pharynges medius (STable Mu 2).

The ‘arytenoid’ module includes muscles attached to the arytenoid cartilages; in mouse and opossum, those muscles are the cricoarytenoideus and thyroarytenoideus; but in humans, those are only the arytenoideus muscles, which are also included in the opossum module.

From the presented selection of muscular modules above, it is obvious that they are hardly comparable between the three species investigated here. Still, based solely on the muscle modules, the mouse and the human are more similar to each other than either of them to the opossum.

Musculoskeletal

Opossum has the most musculoskeletal (MuSk) head modules (20) and human the least (10) (Figure 2). All three species have a left and right ‘inner ear’ module containing the incus and stapes bones as well as the stapedius muscle (STables MuSk 1–3). Opossum additionally includes the tympanic bone and the tensor tympani muscle. Mouse and opossum ‘inner ear’ modules also contain the malleus, which is in the ‘scalp’ module in human.

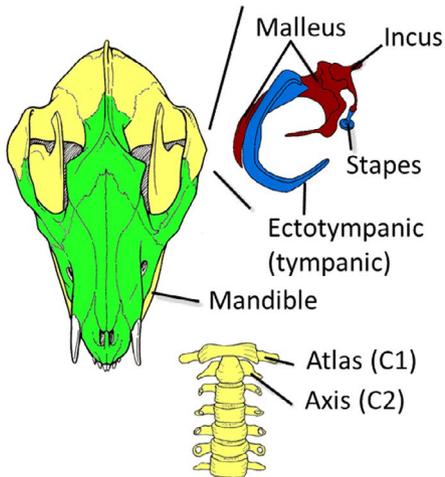
All three species have a ‘larynx & pharynx’ module with the arytenoideus, cricoarytenoideus, and thyroarytenoideus muscles. Mouse and human additionally contain the thyroid cartilage and the laryngeal alar cartilage (mouse) or cricoid cartilage (human); opossum also has one additional cartilage, the interarytenoid, in this module. The constrictor pharyngis inferior and cricothyroideus are included in mouse and human; the latter has also other muscles in this module.

Human and opossum have a module ‘pectoral girdle’ (vertebrae, both clavicles, both scapulae, trapezius: 1 or 2, and omohyoideus: 1 or 2). Opossum’s ‘pectoral girdle’ also includes the sternum and the muscles that would collectively correspond to the sternocleidomastoid in humans. A similar module is present in mouse, but it only contains both scapulae, vertebrae, and the trapezius muscles.

The largest modules (‘face’) in human are a left and a right module containing the midfacial bones (zygomatic, nasal, maxilla, and lacrimal) and 17 muscles (STable MuSk 3). The right module also

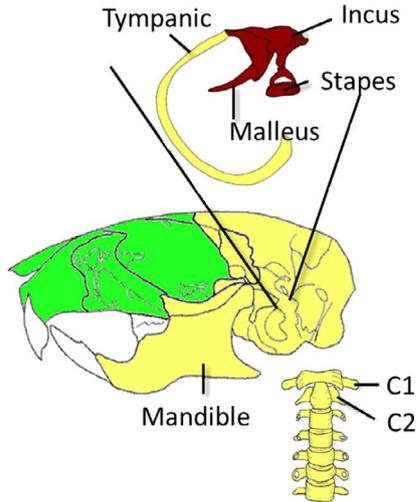
(a) **Opossum**

Dorsal view



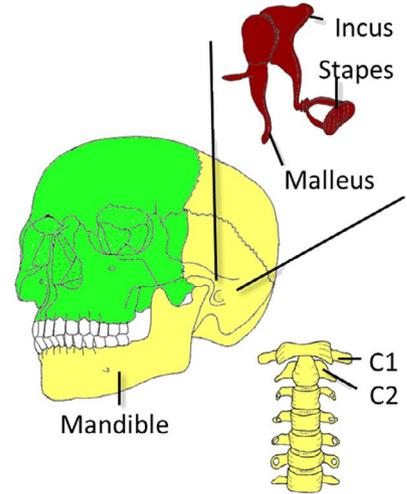
Mouse

Left lateral view

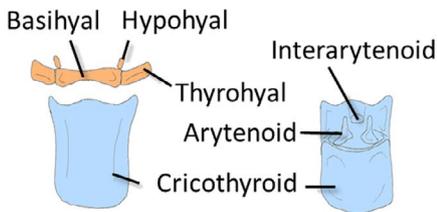


Human

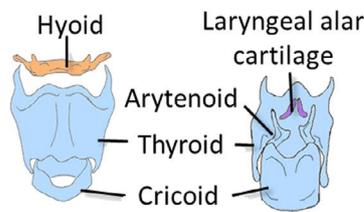
Antero-lateral view



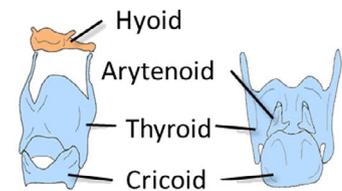
Ventral view



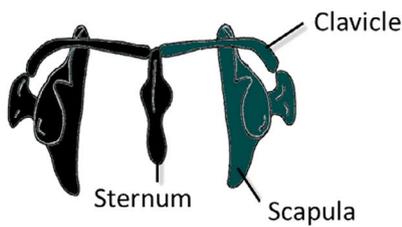
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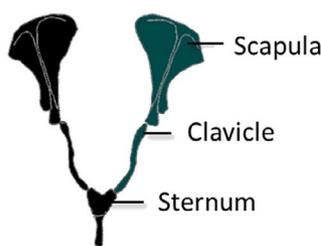
Anterior view



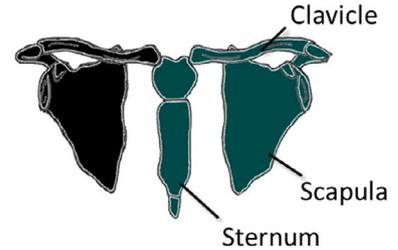
Ventral view



Dorsal view



Anterior view



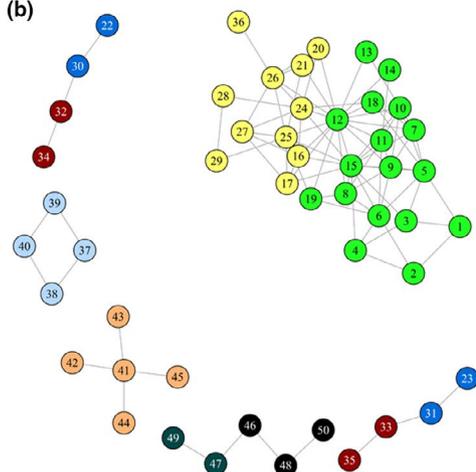
dark red	ossicles
dark blue	ossicles

light blue	larynx
purple	larynx

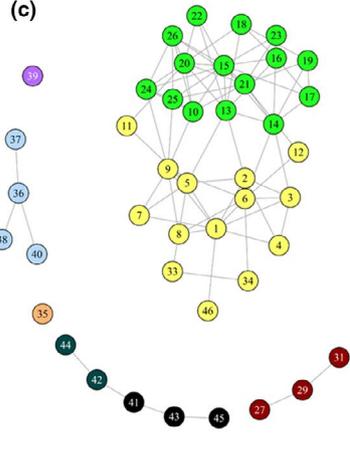
dark green	left pectoral girdle
black	right pectoral girdle

yellow	neurocranium & vertebrae
green	viscerocranium
orange	hyoid bone / hyoid arcade

(b)



(c)



(d)

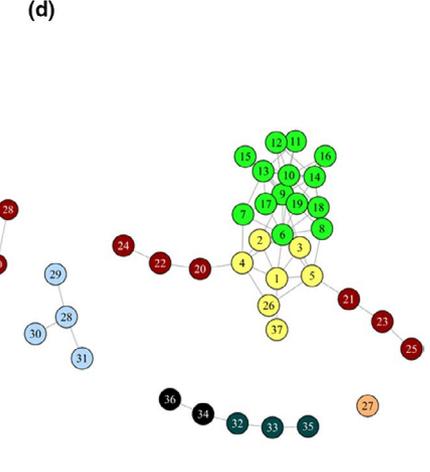


FIGURE 1 Head and neck in opossum, mouse, and human. (a) Schematic drawings of skeletal modules of the head and neck, which are overall very similar to each other. Same colors indicate identical or similar modules. Module names are indicated in the legend on the left and correspond to the names in the Supplementary Tables (STables) Sk 1–3. Note there is a left and right ossicle module in all three species, the left and right tympanic bones in mouse are both included in one medial module (yellow). Not to scale. Skull of opossum by Julia Molnar, modified from Diogo et al. (2016). Skulls of mouse and human modified from Achilleos and Trainor (2015). Ossicles of opossum modified from Rich et al. (2005). Anatomical network of skeletal elements in (b) opossum, (c) mouse, and (d) human; numbers in circles (elements) are listed below; lines represent connections. (b) *Opossum head*: 1 premaxilla left, 2 premaxilla right, 3 nasal left, 4 nasal right, 5 maxilla left, 6 maxilla right, 7 lacrimal left, 8 lacrimal right, 9 ethmoid vomer, 10 palatine left, 11 palatine right, 12 sphenoid, 13 pterygoid left, 14 pterygoid right, 15 frontal, 16 parietal left, 17 parietal right, 18 zygomatic left, 19 zygomatic right, 20 petromastoid left, 21 petromastoid right, 22 tympanic left, 23 tympanic right, 24 squamosal left, 25 squamosal right, 26 occipital, 27 supraoccipital, 28 mandible left, 29 mandible right, 30 stapes left, 31 stapes right, 32 incus left, 33 incus right, 34 malleus left, 35 malleus right, 36 vertebrae, 37 cricothyroid cartilage, 38 arytenoid cartilage left, 39 arytenoid cartilage right, 40 interarytenoid cartilage, 41 basihyal, 42 hypohyal left, 43 hypohyal right, 44 thyrohyal left, 45 thyrohyal right, 46 sternum, 47 clavicle left, 48 clavicle right, 49 scapula left, 50 scapula right. (c) *Mouse head*: 1 occipital, 2 parietal left, 3 parietal right, 4 interparietal, 5 squamosal left, 6 squamosal right, 7 tympanic left, 8 tympanic right, 9 basisphenoid, 10 presphenoid, 11 zygomatic left, 12 zygomatic right, 13 frontal left, 14 frontal right, 15 ethmoid, 16 nasal left, 17 nasal right, 18 premaxilla left, 19 premaxilla right, 20 maxilla left, 21 maxilla right, 22 lacrimal left, 23 lacrimal right, 24 palatine left, 25 palatine right, 26 vomer, 27 malleus left, 28 malleus right, 29 incus left, 30 incus right, 31 stapes left, 32 stapes right, 33 mandible left, 34 mandible right, 35 hyoid bone, 36 thyroid cartilage, 37 arytenoid cartilage left, 38 arytenoid cartilage right, 39 laryngeal alar cartilage, 40 cricoid cartilage, 41 sternum, 42 clavicle left, 43 clavicle right, 44 scapula left, 45 scapula right, 46 vertebrae. (d) *Human head*: 1 occipital, 2 parietal left, 3 parietal right, 4 temporal left, 5 temporal right, 6 sphenoid, 7 zygomatic left, 8 zygomatic right, 9 frontal, 10 ethmoid, 11 nasal left, 12 nasal right, 13 maxilla left, 14 maxilla right, 15 lacrimal left, 16 lacrimal right, 17 palatine left, 18 palatine right, 19 vomer, 20 malleus left, 21 malleus right, 22 incus left, 23 incus right, 24 stapes left, 25 stapes right, 26 mandible, 27 hyoid bone, 28 thyroid cartilage, 29 arytenoid left, 30 arytenoid right, 31 cricoid cartilage, 32 sternum, 33 clavicle left, 34 clavicle right, 35 scapula left, 36 scapula right, 37 vertebrae

includes the frontal bone, both frontalis muscles and both corrugator supercilii muscles. Neither mouse nor opossum has such large left or right module and their large modules include mostly the bilateral structures.

The 'tongue, supra- & infrahyoid' module is similar in all three species. Most of the muscles in the human 'tongue, supra- & infrahyoid' module are included in the opossum module 'tongue, supra- & infrahyoid' which also includes the basihyal and thyrohyal among others (STable MuSk 1). The mouse and human 'tongue, supra- & infrahyoid' modules are very similar; all muscles except the sternothyroideus (left and right) muscles in the human module are included in the mouse module, which has an additional 12 other muscles included [those 12 muscles are bilateral muscles, i.e., 6 muscles x2: left + right; 4 (8 considering both sides) of them are absent in humans].

3.1.2 | Forelimb

Skeleton

The skeletal modules of the forelimbs are similar in all three species (STables Sk 4–6; Figure 3). Several modules are identical for all three species: 'occipital, vertebrae' (not shown in sketches in Figure 3, but included in the networks depicted in the same figure), 'pectoral girdle', 'digit II', and 'digit IV'. Other modules are identical only in two of the species and similar to the third: 'digit III' and 'digit V' (mouse, human) and 'digit I' (opossum, human = thumb) with the difference that opossum included the metacarpal in the 'digit III' and 'digit V' modules and mouse did not include the metacarpal 1 in the 'digit I' modules. Another example is a module 'forelimb' that includes all arm and forearm bones and two carpal bones in the quadruped species (mouse and opossum), but human has the

humerus, ulna, and radius in one module and all carpal bones and metacarpals 2–5 in another. This is also where the three species differ the most: in opossum most carpals (6/8) and two metacarpals are in one module, and in mouse most carpals (6/9) and metacarpals 1 and 2 are in one module and two carpals and metacarpals 3–5 are in another.

Musculature

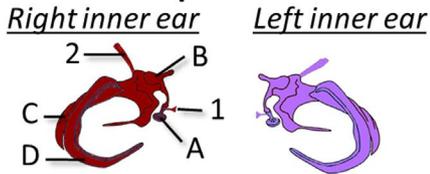
The forelimb musculature modules (STables Mu 4–6) are different in all three species and most of the muscles were recovered as single elements. A module 'FDS' (includes flexor digitorum superficialis = FDS, one or two palmaris longus = PL, and flexor carpi ulnaris) is found in opossum and human but includes in humans also the flexor carpi radialis and the pronator teres; the latter is the only muscle in the module that is not superficial. In mouse, the FDS and PL are in the 'FDP' module (flexor digitorum profundus = FDP, 4 lumbricals); the 'FDP' module is present in opossum and human (note that lumbricals 2–5 in other mammals correspond to lumbricals 1–4 in human).

The 'LD' module includes the latissimus dorsi (LD) and dorsoepitrochlearis and one (opossum) or four (mouse) other muscles; it is not found in human. The 'triceps' module includes the triceps brachii and the anconeus muscle in human; it also includes the biceps brachii and coracobrachialis in mouse, but it is not identified in opossum, where the triceps is included in the 'LD' module. The biceps and the coracobrachialis form a module in human.

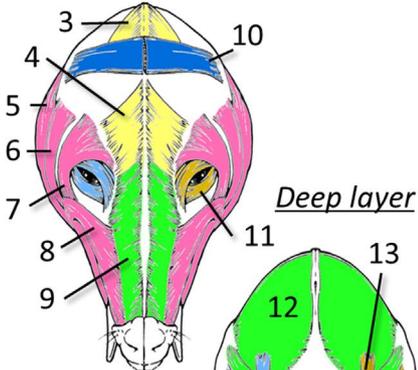
The 'PeM' was found in all three species and included the pectoralis major (PeM) and deltoid in human, PeM and deltoideus acromialis et clavicularis in opossum, and PeM and panniculus carnosus in mouse.

Finally, a module containing the serratus anterior and levator scapulae was found in mouse and opossum, but not in human.

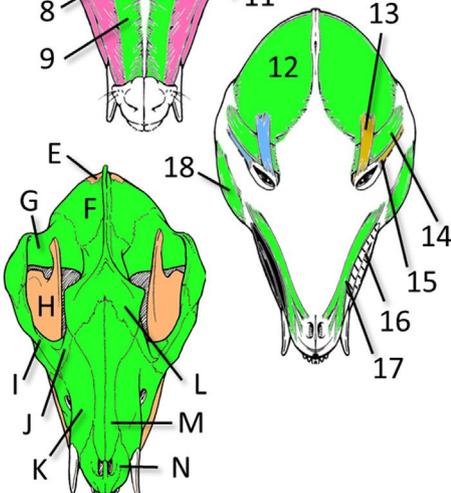
Opossum



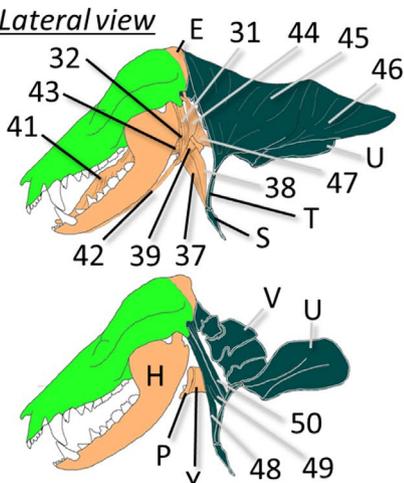
Superficial layer



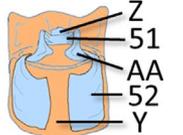
Deep layer



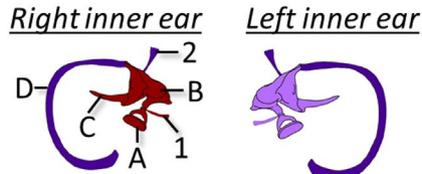
Lateral view



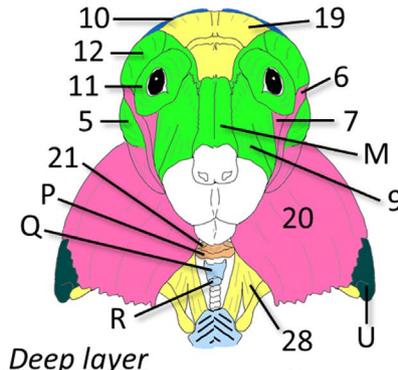
Posterior view



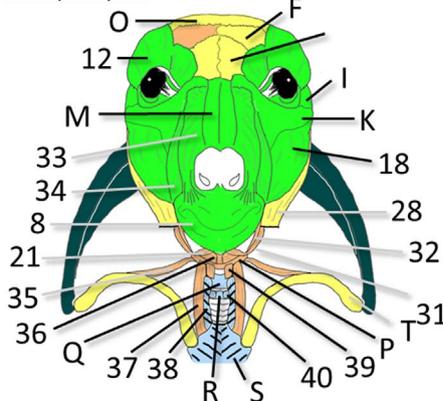
Mouse



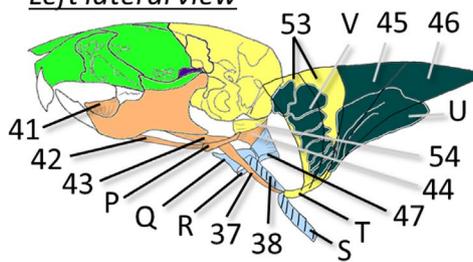
Superficial layer



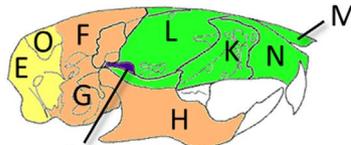
Deep layer



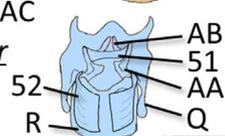
Left lateral view



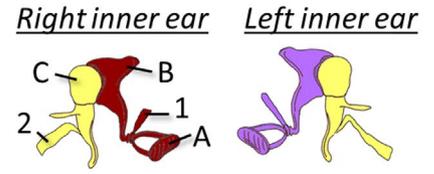
Right lateral view



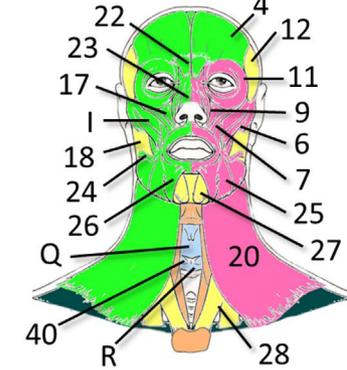
Posterior view



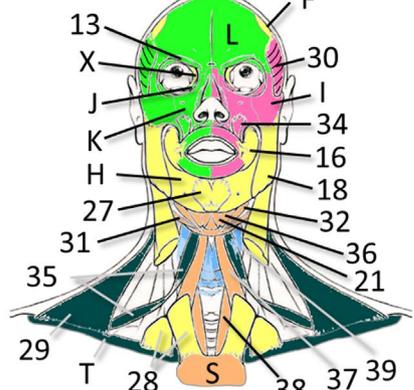
Human



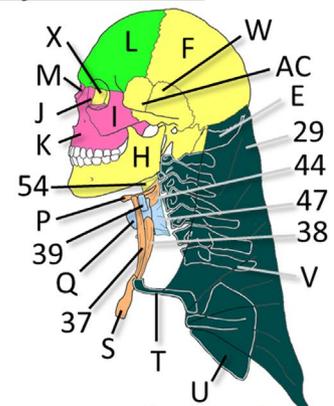
Superficial layer



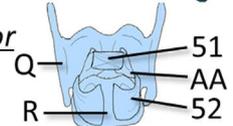
Deep layer



Left lateral view



Posterior view



Color bilateral		Color bilateral		Right	Left	
dark blue	external ear	orange	tongue, supra- & infrahyoid	blue	mustard	pericocular
yellow	scalp	light blue	larynx & pharynx	dark red	purple	inner ear
pink	face	light blue w/ black stripes	pectoral/neck	green w/ black stripes	pink w/ black stripes	external ear
green	face	dark green	pectoral girdle	black	white w/ black stripes	cheek
dark purple	mid-skull					

FIGURE 2 Schematic drawings of musculoskeletal modules of the head and neck in opossum, mouse, and human. Same colors indicate identical or similar modules. Module names are indicated in the legend on the bottom and correspond to the names in the Supplementary Tables (STables) MuSk 1–3. Opossum has the most modules (20) and human the least (10). For details see text. Letters indicate bones, numbers indicate muscles. Opossum – superficial and deep layer and skull from dorsal by Julia Molnar, modified from Diogo et al. (2016); lateral view modified from Crompton et al. (1977). Mouse – superficial and deep layer by Vance Powell, modified from Powell et al. (2018); skulls modified from Achilleos and Trainor (2015). Human – superficial and deep layer by Julia Molnar, modified from Diogo et al. (2015). Not to scale. Bones/cartilages: A, stapes; AA, arytenoid cartilage; AB, laryngeal alar cartilage; AC, sphenoid; B, incus; C, malleus; D, ectotympanic/tympanic; E, occipital; F, parietal; G, squamosal; H, mandible; I, zygomatic (aka jugal in mouse and opossum); J, lacrimal; K, maxilla; L, frontal; M, nasal; N, premaxilla; O, interparietal; P, hyoid bone/hyoid arcade (opossum); Q, thyroid cartilage; R, cricoid cartilage; S, sternum; T, clavicle; U, scapula; V, vertebrae; W, temporal; X, ethmoid; Y, cricothyroid cartilage; Z, interarytenoid cartilage. Muscles: 1, stapedius; 2, tensor tympani; 3, occipitalis; 4, frontalis; 5, platysma cervicale; 6, zygomaticus major; 7, zygomaticus minor; 8, orbicularis oris; 9, levator labii superioris alaeque nasi; 10, interscutularis; 11, orbicularis oculi; 12, temporalis; 13, corrugator supercilii; 14, auriculo-orbitalis; 15, retractor anguli oculi lateralis; 16, buccinatorius; 17, levator labii superioris; 18, masseter; 19, orbito-temporo-auricularis and galea aponeurotica; 20, platysma myoides; 21, digastricus anterior; 22, procerus; 23, nasalis; 24, risorius; 25, depressor anguli oris; 26, depressor labii inferioris; 27, mentalis; 28, sternocleidomastoid; 29, trapezius; 30, temporoparietalis; 31, digastric posterior; 32, stylohyoideus; 33, nasalis-nasolabialis profundus; 34, levator anguli oris (human)/levator anguli oris facialis (mouse); 35, omohyoideus; 36, mylohyoideus; 37, sternohyoideus; 38, sternothyroideus; 39, thyrohyoideus; 40, cricothyroideus; 41, tongue (glossal) muscles; 42, geniohyoideus; 43, hyoglossus; 44, constrictor pharyngis medius; 45, acromiotrapezius; 46, spinotrapezius; 47, constrictor pharyngis inferior; 48, sternomastoideus; 49, cleidomastoideus; 50, cleidooccipitalis; 51, arytenoideus; 52, cricoarytenoideus posterior; 53, cleidooccipitalis-clavotrapezius; 54, constrictor pharyngis superior

Opossum has one more module (subclavius + cleidoacromialis) and human has four more modules, which all contain two muscles. If strictly counting modules by similarity, then opossum–human have three similar modules ('FDS', 'FDP', and 'PeM'), opossum–mouse two ('LD', 'serratus anterior'), and mouse–human one ('triceps').

Musculoskeletal

The musculoskeletal (MuSk) modules of the forelimbs (STables MuSk 4–6) revealed only one module that is similar in all three species (Figure 4). This 'forelimb' module includes for all species: scapula, humerus, radius, ulna, and 14 identical muscles (**bold** in STables MuSk 4–6). However, each species additionally includes four or more other muscles of which most (5: deltoideus scapularis, latissimus dorsi, dorsoepitrochlearis, flexor carpi ulnaris, and epitrochleoanconeus) are the same between mouse and opossum, some (2: abductor pollicis longus and palmaris longus) are the same between opossum and human (but the palmaris longus in opossum has an externus and an internus portion), and the rest (one for opossum, one for mouse, and two for human) are muscles included in the module in only one of the species. Three of the five muscles present in this module in mouse and opossum are absent in human.

The proximal part of digit I (thumb) is found in similar modules 'digit I-carpus' across the analyzed species. All three include the trapezium, metacarpal 1, and proximal phalanx 1, as well as flexor pollicis brevis, flexor brevis profundus 2, and abductor pollicis brevis. The adductor pollicis is present in this module for mouse and human and the adductor pollicis accessories in opossum and human. Each species has one additional muscle that is not present in the other species' module. Mouse and human include other carpal bones, but mouse 'digit I-carpus' module contains additional ones like the distal phalanx 1, central bone, and falciform. Human has a 'digit I' module with the distal phalanx 1 and the long extensor and long flexor of the thumb.

Opossum has a large 'hand' module including middle phalanges 2–4 and distal phalanges 1–5, as well as the superficial and deep

flexor digitorum, lumbricals 2–4, and the extensors of the digits (digitum, indicis, and minimi). Mouse has an almost identical 'hand' module which excludes the distal phalanges 1 and 5 and includes the palmaris longus. In another module, 'digit V-carpus' found in opossum includes seven carpal bones and the digit 5-associated bones (metacarpal, proximal and middle phalanges), and associated muscles. Mouse has a similar module with only three carpal bones and all digit 5-associated bones and muscles, excluding the extensor digiti minimi. Human has also a 'digit V-carpus' module but with less elements than either of the other species.

The 'midhand' modules of opossum and mouse are similar to each other; both containing metacarpals 2 and 3, proximal phalanges 2 and 3, and 10 identical muscles. The 'midhand' module of opossum additionally includes the metacarpal 4 and proximal phalanx 4 as well as the associated muscles and the mouse module includes the intermetacarpal 1. Those elements (metacarpal 4, proximal phalanx 4, and three muscles) are in a separate module 'digit IV' in mouse. Human has a module for each digit, with the 'digit III' module resembling the closest the prox. digit modules of the other species.

The quadruped species contain a module 'pectoral girdle' with identical bone elements (5) and very similar muscles (six identical, three extra opossum, one extra mouse, and rhomboideus in opossum =rhomboideus major, minor, and occipitalis in mouse). Human has divided this module into two, one 'axial' (occipital bone, vertebrae, levator scapulae, and rhomboid minor and major) and one 'pectoral girdle' that also includes the pectoralis muscles, the serratus anterior, and latissimus dorsi.

3.1.3 | Hindlimb

Skeleton

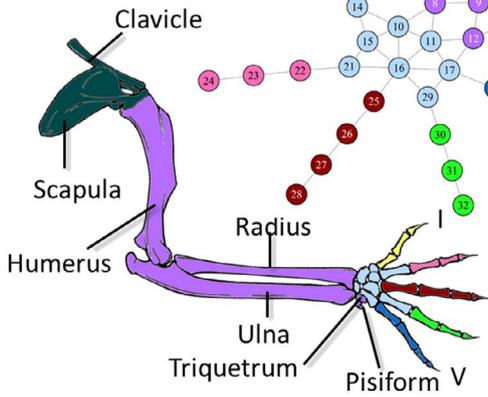
The skeletal modules of the hindlimbs are similar in all three species (STables Sk 7–9), but mouse and human are more similar to each

FORELIMBS

dark green	pectoral girdle
purple	forelimb
light blue	carpals
orange	metacarpals

Opossum

Postero-lateral view

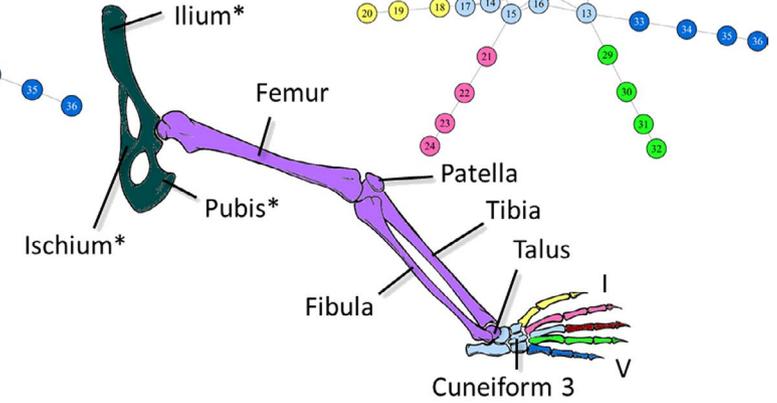


HINDLIMBS

yellow	digit I
pink	digit II
dark red	digit III
green	digit IV
dark blue	digit V

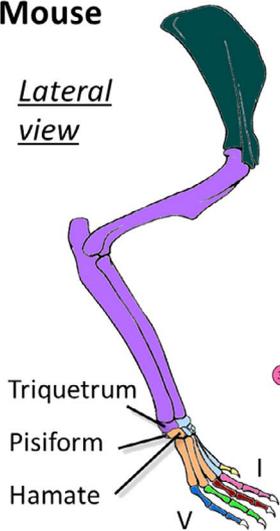
dark green	pelvic girdle
blue	thigh
purple	leg
light blue	tarsals, metatarsals
black	

Posterior view

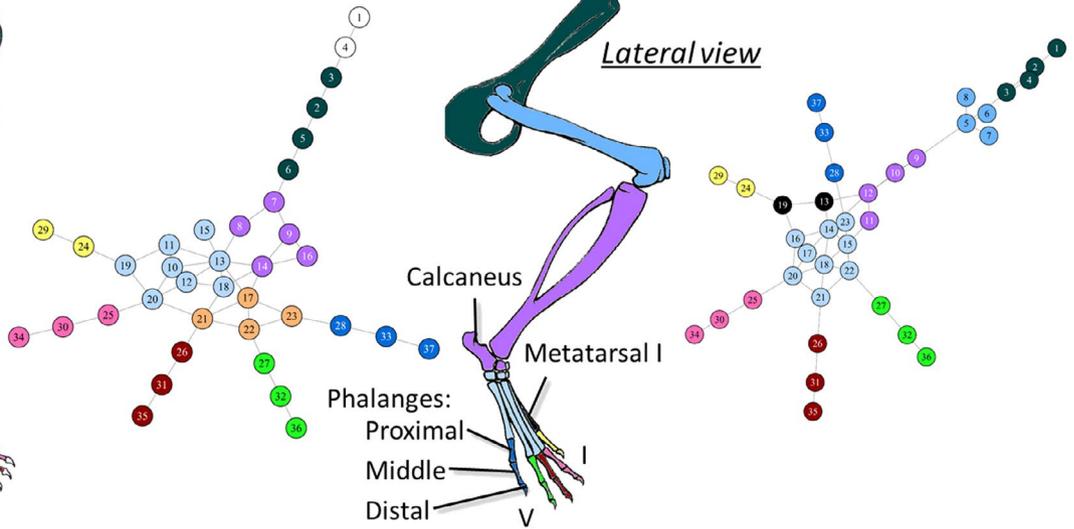


Mouse

Lateral view

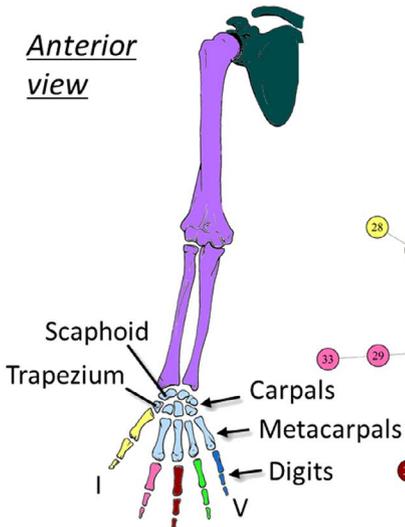


Lateral view



Human

Anterior view



Anterior view

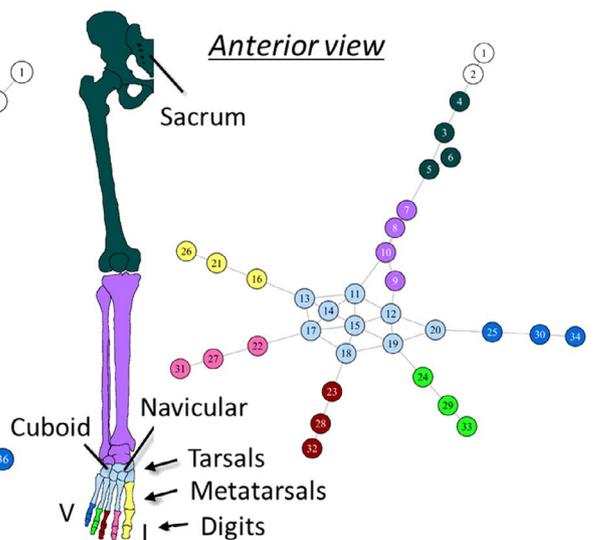


FIGURE 3 Schematic drawings (by Julia Molnar) and anatomical networks of limb skeletons of opossum, mouse, and human. Colorations indicate skeletal modules of forelimbs (left) and hindlimbs (right) identified using AnNA. All limbs displayed in natural positions. Module terminology with corresponding colors displayed as in Supplementary Tables (STables) Sk 4–6 (forelimbs) and Sk 7–9 (hindlimbs). *All three parts are fused in adult specimens as the pelvis. Cuneiform 1, 2, 3 = medial, intermediate, lateral cuneiform in human. Forelimbs: The carpals 'triquetrum' and 'pisiform' are part of the forelimb module in opossum and mouse. The carpal bones 'hamate' is part of a metacarpal module in mouse. In human, all carpals and metacarpals 2–5 form one module. Most digit modules contain only the three phalanges (two for digit 1/thumb). Hindlimbs: Each species analyzed here has the 'femur' placed in a different module. The tarsal bone 'talus' is part of the leg module in all species analyzed here; mouse and human also include the tarsal bone 'calcaneus'. Metatarsal I and tibiale (not shown) form a separate module in mouse. Most digit modules contain only the three phalanges (two for digit 1/big toe). Limbs are not to scale. Numbers in circles (elements) are listed below; lines represent connections. *Opossum forelimb*: 1 occipital, 2 vertebrae, 3 ribs, 4 sternum, 5 scapula, 6 clavicle, 7 humerus, 8 radius, 9 ulna, 10 scaphoid, 11 lunate, 12 triquetrum, 13 pisiform, 14 trapezium, 15 trapezoid, 16 capitate, 17 hamate, 18 metacarpal 1, 19 proximal phalanx 1, 20 distal phalanx 1, 21 metacarpal 2, 22 proximal phalanx 2, 23 middle phalanx 2, 24 distal phalanx 2, 25 metacarpal 3, 26 proximal phalanx 3, 27 middle phalanx 3, 28 distal phalanx 3, 29 metacarpal 4, 30 proximal phalanx 4, 31 middle phalanx 4, 32 distal phalanx 4, 33 metacarpal 5, 34 proximal phalanx 5, 35 middle phalanx 5, 36 distal phalanx 5. *Mouse forelimb*: 1 occipital, 2 sternum, 3 ribs, 4 vertebrae, 5 clavicle, 6 scapula, 7 humerus, 8 radius, 9 ulna, 10 trapezoid, 11 trapezium, 12 central bone, 13 scapholunate, 14 triquetrum, 15 falciform, 16 pisiform, 17 hamate, 18 capitate, 19 metacarpal 1, 20 metacarpal 2, 21 metacarpal 3, 22 metacarpal 4, 23 metacarpal 5, 24 proximal phalanx 1, 25 proximal phalanx 2, 26 proximal phalanx 3, 27 proximal phalanx 4, 28 proximal phalanx 5, 29 distal phalanx 1, 30 middle phalanx 2, 31 middle phalanx 3, 32 middle phalanx 4, 33 middle phalanx 5, 34 distal phalanx 2, 35 distal phalanx 3, 36 distal phalanx 4, 37 distal phalanx 5. *Human forelimb*: 1 Occipital, 2 Sternum, 3 Ribs, 4 Vertebrae, 5 Clavicle, 6 Scapula, 7 Humerus, 8 Radius, 9 Ulna, 10 Trapezoid, 11 Trapezium, 12 Scaphoid, 13 Lunate, 14 Triquetrum, 15 Pisiform, 16 Hamate, 17 Capitate, 18 Metacarpal 1, 19 Metacarpal 2, 20 Metacarpal 3, 21 Metacarpal 4, 22 Metacarpal 5, 23 Proximal Phalanx 1, 24 Proximal Phalanx 2, 25 Proximal Phalanx 3, 26 Proximal Phalanx 4, 27 Proximal Phalanx 5, 28 Distal Phalanx 1, 29 Middle Phalanx 2, 30 Middle Phalanx 3, 31 Middle Phalanx 4, 32 Middle Phalanx 5, 33 Distal Phalanx 2, 34 Distal Phalanx 3, 35 Distal Phalanx 4, 36 Distal Phalanx 5. *Opossum hindlimb*: 1 ribs, 2 vertebrae, 3 hip bone, 4 sacrum, 5 epipubis, 6 femur, 7 patella, 8 tibia, 9 fibula, 10 talus, 11 calcaneus, 12 navicular, 13 cuboid, 14 cuneiform 1, 15 cuneiform 2, 16 cuneiform 3, 17 accessory ossicle, 18 metatarsal 1, 19 proximal phalanx 1, 20 distal phalanx 1, 21 metatarsal 2, 22 proximal phalanx 2, 23 middle phalanx 2, 24 distal phalanx 2, 25 metatarsal 3, 26 proximal phalanx 3, 27 middle phalanx 3, 28 distal phalanx 3, 29 metatarsal 4, 30 proximal phalanx 4, 31 middle phalanx 4, 32 distal phalanx 4, 33 metatarsal 5, 34 proximal phalanx 5, 35 middle phalanx 5, 36 distal phalanx 5. *Mouse hindlimb*: 1 ribs, 2 vertebrae, 3 hip bone, 4 sacrum, 5 femur, 6 patella, 7 medial fabella, 8 lateral fabella, 9 tibia, 10 fibula, 11 calcaneus, 12 talus, 13 tibiale, 14 navicular, 15 cuboid, 16 cuneiform 1, 17 cuneiform 2, 18 cuneiform 3, 19 metatarsal 1, 20 metatarsal 2, 21 metatarsal 3, 22 metatarsal 4, 23 metatarsal 5, 24 proximal phalanx 1, 25 proximal phalanx 2, 26 proximal phalanx 3, 27 proximal phalanx 4, 28 proximal phalanx 5, 29 distal phalanx 1, 30 middle phalanx 2, 31 middle phalanx 3, 32 middle phalanx 4, 33 middle phalanx 5, 34 distal phalanx 2, 35 distal phalanx 3, 36 distal phalanx 4, 37 distal phalanx 5. *Human hindlimb*: 1 Ribs, 2 Vertebrae, 3 Hip Bone, 4 Sacrum, 5 Femur, 6 Patella, 7 Tibia, 8 Fibula, 9 Calcaneus, 10 Talus, 11 Navicular, 12 Cuboid, 13 Medial Cuneiform, 14 Intermediate Cuneiform, 15 Lateral Cuneiform, 16 Metatarsal 1, 17 Metatarsal 2, 18 Metatarsal 3, 19 Metatarsal 4, 20 Metatarsal 5, 21 Proximal Phalanx 1, 22 Proximal Phalanx 2, 23 Proximal Phalanx 3, 24 Proximal Phalanx 4, 25 Proximal Phalanx 5, 26 Distal Phalanx 1, 27 Middle Phalanx 2, 28 Middle Phalanx 3, 29 Middle Phalanx 4, 30 Middle Phalanx 5, 31 Distal Phalanx 2, 32 Distal Phalanx 3, 33 Distal Phalanx 4, 34 Distal Phalanx 5

other than to opossum (Figure 3). The digital modules 'digit II', 'digit III', 'digit IV', and 'digit V' are identical between mouse and human, 'digit III' is also the same in opossum, but all other digital modules in opossum also include the corresponding metatarsal (compare also the networks depicted in Figure 3). The 'leg' module that includes the tibia, fibula, and other bones, is identical in mouse and human, but differs with opossum which also includes the thigh bone (femur) and the patella. The latter two bones are in a module with two fabellas in mouse and with the hip bone in human.

The pelvic girdle bones are in one module with the vertebrae and ribs in opossum and mouse (ribs and vertebrae not shown in Figure 3), but the vertebrae and ribs form an independent module in human. The 'digit I' module includes the metatarsal 1 and both phalanges in human and opossum but not in mouse, where the metatarsal 1 forms a module with the tibiale. All tarsals, except the talus, are in one module in opossum (with metatarsal 3). In mouse and human, the calcaneus and talus are in the 'leg' module and all other tarsals are together with the metatarsals 2–5.

With respect to the skeletal modules, the forelimb versus hindlimb comparison shows that they are more similar to each other in

mouse and humans than in opossum (see sheet in STables Sk 1–9 'Skeletal modules comparison'). However, in opossum, the proximal elements are more similar to each other and, in mouse and humans, the distal elements (carpals/tarsals and digits) are more similar between forelimb and hindlimb. Furthermore, there are less false skeletal modules in the hindlimb results than in the forelimb results.

Musculature

The hindlimb musculature modules (STables Mu 7–9) are more similar between mouse and opossum than each of them to human. Most of the muscles in all three species were recovered as single elements. The human only has two modules: the 'FDL' and 'EDL'. The former includes the flexor digitorum longus (FDL), quadratus plantae, and all lumbricals and is also found in mouse and in opossum; however, in opossum, it additionally includes three additional muscles (STable Mu 7). The human 'EDL' module includes the extensor digitorum longus (EDL) and the fibularis tertius and is not recovered in mouse and includes the fibularis brevis and fibularis longus instead of fibularis tertius in opossum. Comparing mouse and opossum revealed more similar modules with two identical modules

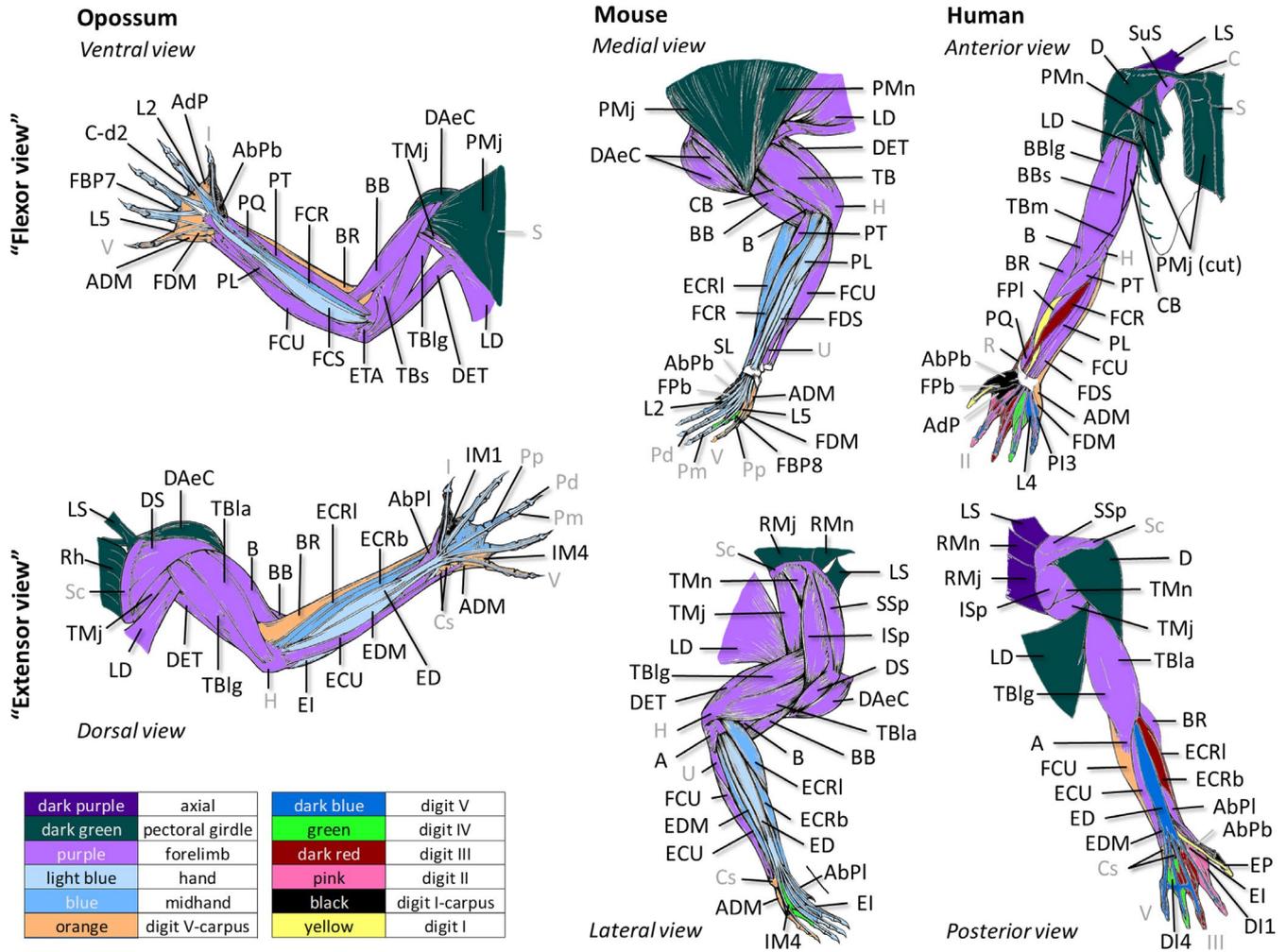


FIGURE 4 Schematic drawings (by Julia Molnar) of forelimbs of opossum, mouse, and human. Colorations indicate musculoskeletal modules identified using AnNA. Flexor and extensor views; detailed list of elements in each module are found in Supplementary Tables (STables) MuSk 4–6. Mouse – medial view with paw lateral rotated to visualize the flexor tendons. Extremities are not to scale. Colors whenever possible same as in skeletal modules. Mouse: brachioradialis absent. Human anterior view: pectoralis major cut. Black letters = muscles; gray letters = bones. A, anconeus; ADM, abductor digiti minimi; AbPb/AbPI, abductor pollicis brevis/longus; AdP, adductor pollicis; B, brachialis; BB, biceps brachii; BBlg/BBs, biceps brachii long/short head; BR, brachioradialis; C, clavicle; C-d2, contrahens to digit II; CB, coracobrachialis; Cs, carpal bones; D, deltoid; DAeC, deltoideus acromialis et clavicularis; DI1–4, dorsal interosseus 1–4; DET, dorsoepitrochlearis; DS, deltoideus scapularis; ECRb/ECRI, extensor carpi radialis brevis/longus; ECU, extensor carpi ulnaris; ED, extensor digitorum; EDM, extensor digiti minimi; EI, extensor indicis; EP, extensor pollicis; ETA, epitrochleoanconeus; FBP7, flexor brevis profundus 7; FCR, flexor carpi radialis; FCU, flexor carpi ulnaris; FDS, flexor digitorum superficialis; FDM, flexor digiti minimi; FPb/FPI, flexor pollicis brevis/longus; H, humerus; I, II, III, V, digit I, II, III, V; IM1–4, intermetacarpales 1–4; Isp, Infraspinatus; L1–5, lumbrical 1–5 (L1–4 in human = L2–5 in mouse and opossum); LD, latissimus dorsi; LS, levator scapulae; Pd/Pm/Pp, distal/medial/proximal phalanx; PI1–3, palmar interosseus 1–3; PL, palmaris longus; Pm, middle phalanx; PMj/PMn, pectoralis major/minor; Pp, proximal phalanx; PQ, pronator quadratus; PT, pronator teres; Rh, rhomboideus; RMj/RMn, rhomboideus major/minor; S, sternum; Sc, Scapula; SL, scapholunate; SSp, supraspinatus; SuS, subscapularis; TB, triceps brachii; TBla/TBlg/TBm/TBs, triceps brachii lateral/long/medial/short head; TMj/TMn, teres major/minor; U, ulna; V, digit V

(obturator internus + both gemelli; iliacus + psoas major) and three similar modules (one containing the gluteus maximus, one the biceps femoris, and one the plantaris; STables Mu 7–8).

Forelimb and hindlimb musculature modules are not similar to each other in any of the species (see sheet in STables Mu 1–9 Musculature modules comparison). The only similarity in all limbs in all species is the connection of all lumbricals with the muscle they originate from.

Musculoskeletal

The hindlimb musculoskeletal (MuSk) modules (STables MuSk 7–9) revealed only few similarities between the species, although the figure implies that mouse and human are more similar than each of them to opossum (Figure 5). This perceived similarity is largely caused by the large 'hindlimb' module that includes the same five bones and 25 muscles in mouse (STable MuSk 8) and human (STable MuSk 9). These elements are distributed over two modules in

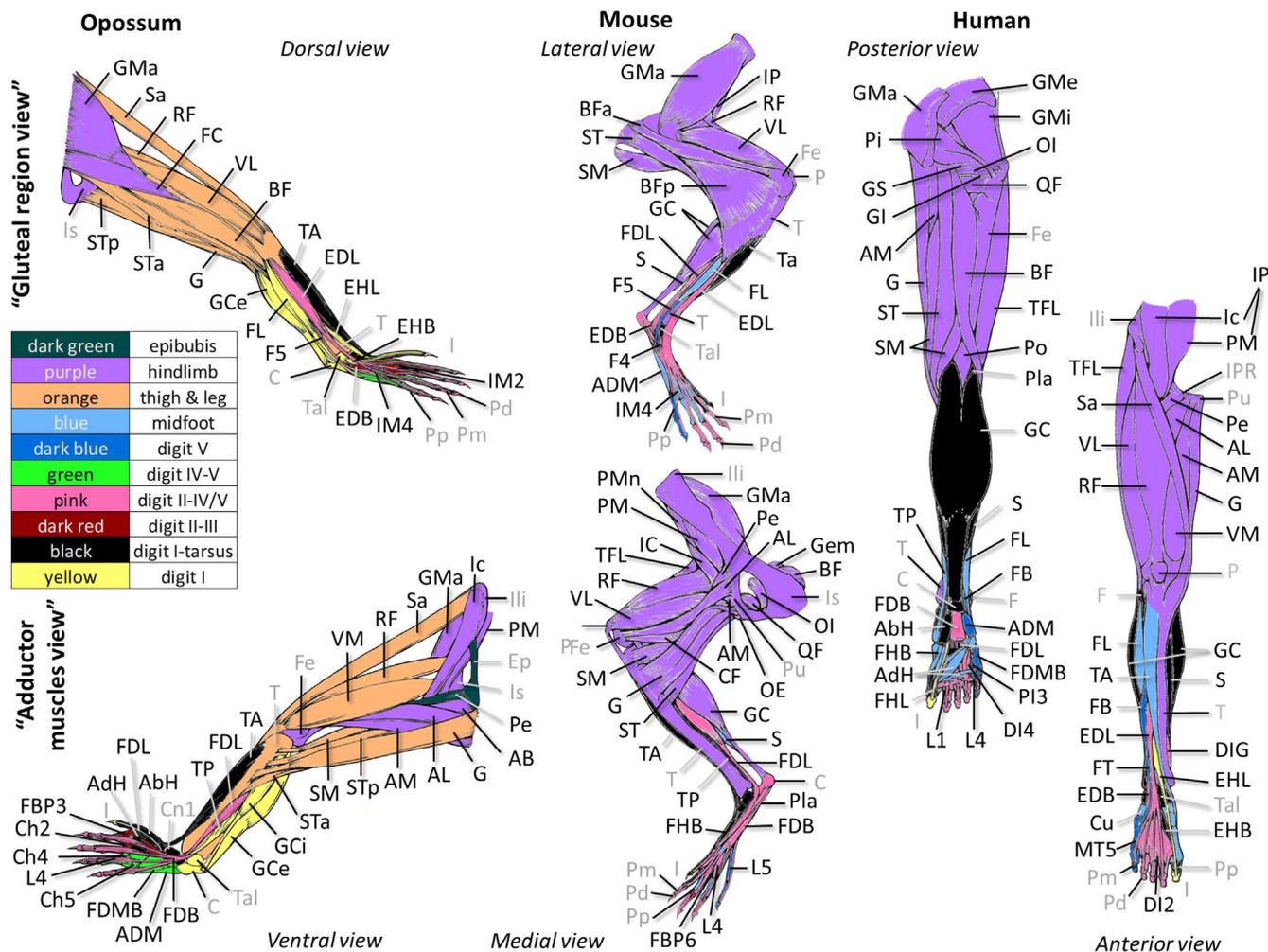


FIGURE 5 Schematic drawings (by Julia Molnar) of hindlimbs of opossum, mouse, and human. Colorations indicate musculoskeletal modules identified using AnNA. Flexor and extensor views; detailed list of elements in each module are found in Supplementary Tables (STables) MuSk 7–9. contrahens pedis 1 = adductor hallucis; lumbricals 2–5 = lumbricals 1–4 in human. AB, adductor brevis; AbH, adductor hallucis; AdH, adductor hallucis; ADM, abductor digiti minimi; AL, adductor longus; AM, adductor magnus; BF, biceps femoris; BFa/BFp, anterior/posterior portion of biceps femoris; C, calcaneus; CF, caudofemoralis; Cu, cuboid; Ch2–5, contrahens to digit II–V; Cn1, cuneiform 1; DIG, flexor digitorum longus; DI1–DI4, dorsal interosseus 1–4; EDB, extensor digitorum brevis; EDL, extensor digitorum longus; EHB, extensor hallucis brevis; Ep, epibubis; F, fibula; F4/F5, fibularis digiti quarti/quinti; FB, fibularis brevis; FBP3/6, flexor brevis profundi 3/6; FC, femorococcygeus; FDB, flexor digitorum brevis; FDL, flexor digitorum longus; FDMB, flexor digiti minimi brevis; Fe, femur; FHB, flexor hallucis brevis; FHL, flexor hallucis longus; FL, fibularis longus; FT, fibularis tertius; G, gracilis; GC, gastrocnemius; GCe/GCi, external/internal head of gastrocnemius; Gem, gemellus; GI, gemellus inferior; GMa, gluteus maximus; GMe, gluteus medius; GMi, gluteus minimus; GS, gemellus superior; I, digit I; Ic, iliacus; Ili, ilium; IM2–4, intermetatarsal 2–4; IP, iliopsoas; IPR, ischiopubic ramus; Is, ischium; L1–4/L2–5, lumbricals 1–5; MT5, metatarsal 5; OE, obturator externus; OI, obturator internus; P, patella; Pd/Pm/Pp, distal/middle/proximal phalanx; Pe, pectineus; Pi, piriformis; PI3, plantar interosseus 3; Pla, plantaris; PM, psoas major; Pm, middle phalanx; PMn, psoas minor; Po, popliteus; Pp, proximal phalanx; Pu, pubis; QF, quadratus femoris; RF, rectus femoris; S, soleus; Sa, sartorius; SM, semimembranosus; ST, semitendinosus; Sta/STp, anterior/posterior head of semitendinosus; T, tibia; TA, tibialis anterior; Tal, talus; TFL, tensor fasciae latae; TP, tibialis posterior; VL, vastus lateralis; VM, vastus medialis

opossum (STable MuSk 7): three bones and 14 muscles are present in the 'hindlimb' module and two bones and seven muscles in the 'thigh & leg' module. The axial elements (ribs, vertebrae, and quadratus lumborum) are in the 'hindlimb' module in mouse and opossum and in a separate 'axial' module in human.

The module 'distal digits II–V' of opossum and 'digits II–IV' of mouse and human are very similar to each other (six identical bones: middle and distal phalanges of digits 2–4; seven identical muscles: extensor digitorum longus and brevis, flexor digitorum longus and

brevis, and lumbricals 2–4) and each of them includes few extra bones (1–3) and extra muscles (2–6).

In all species, a module contained the navicular, cuneiform 1 (or medial cuneiform for human), metatarsal 1, tibialis anterior, and flexor hallucis brevis. In mouse and opossum, this 'digit I-tarsus' module also includes the proximal phalanx 1, the flexor brevis profundus 2, and the intermetatarsal 1. In this way, these mouse and opossum modules are more similar to each other than to the human module. The human 'digit I-tarsus' module also contains

the proximal phalanx 1 but no other elements of the same named elements module in the quadruped species. All those elements are in a larger 'midfoot' module in human, which overlaps largely with the elements found in the 'midfoot' module in mouse. Even the similar modules in mouse and opossum contain additional elements that are not included in the modules of the other species.

The modules containing digit 5 elements, such as the proximal phalanx 5, are slightly more similar between mouse and human than between those two species and opossum; however, those 'digit V' modules have only one muscle in common (flexor digiti minimi brevis) and all other muscles differ among all species. Both opossum ('digit I') and human ('digit I–tarsus') have a module containing the fibula, talus, and calcaneus and four identical muscles. Also, opossum ('digit II–III'), mouse ('midfoot'), and human ('midfoot') have a module that includes the cuneiform 2 and 3, among other bones and muscles; however, looking into the single elements it is that the opossum and mouse module are more similar to each other with respect to their element content than to the human module.

Forelimb and hindlimb MuSk modules are not similar in any of the analyzed species (see sheet in STables MuSk 1–9 Musculoskeletal modules comparison).

3.2 | Developmental and/or functional hypotheses

Details on which body parts and their elements belong to which functional, developmental, or combined (function & development) modules are found in STables C1–7 (head), C8–11 (forelimb), and C12–15 (hindlimb). Tables reporting results for analyses of connectivity modularity versus the hypothetical modules based on STables C1–15 are presented in STables O1–9. The results of the analyses comparing the hypothetical modules with the identified modules are shown in STables S7–9. The closer the value is to 1 the more similar are hypothetical and identified modules, values are displayed in brackets.

3.2.1 | Head

For the head, we originally posed nine hypotheses based on: (1) bone function and (2) development; (3) muscle function and (4) development (based on one side only), versus (5) development (based on both sides); (6) pharyngeal arch muscle development (based exclusively on pharyngeal arch muscle, cartilages, and bone development—i.e., only derivatives of pharyngeal arches); (7) bone–muscle function (includes all musculoskeletal (MuSk) elements); (8) bone–muscle development 1 (includes all MuSk elements of one side), and (9) bone–muscle development 2 (as 8) but both sides separately coded. Hypotheses 1 & 2 were compared to the connectivity modules identified in the skeletal network analyses, hypotheses 3–5 to the musculature network modules, and hypotheses 6–9 to the MuSk modules.

The head skeletal modules are best explained by function for mouse (0.75) and human (0.83), but for opossum the head skeletal modules are slightly better explained by development (0.75 development vs. 0.74 for function). However, for all three species, all values are high, and

the difference between function and development is +0.01, –0.08, and –0.06 for opossum, mouse, and human, respectively. That means that for the head skeletal modules, function and development are almost equally strong in explaining the observed modules. With respect to the muscular modules, function is also a strong explanation (all values >0.7). Compared to the unilateral coded muscle development, the bilateral coded muscle development is a slightly better match for the modules identified. For mouse, the function and bilateral developmental hypotheses are equally strong explanations, and for human the bilateral developmental hypothesis is even stronger (by 0.02) than the functional hypothesis. The MuSk hypotheses values are all lower than for bone only or muscle only (all values below 0.64, all but bone–muscle function values below 0.5). Here, the highest values are found in the hypothetical modules combining bone and muscle function. The lowest values among all hypotheses are for the pharyngeal arch developmental hypothetical modules; the lowest values show that these modules least resemble the other modules in this network analyses.

3.2.2 | Forelimb and hindlimb

We tested four different hypotheses for the limbs based on: (1) bones function & development, (2) muscle function, (3) muscle development, and (4) bone–muscle function. For the skeletal modules, the hypothetical modules have overall high values (>0.74) for both fore- and hindlimb, but for the hindlimb the values are slightly better (>0.77).

In the forelimb musculature, compared to the functional hypotheses, the developmental hypotheses explain with slightly more power mouse (0.71) and human limb (0.75) networks. In contrast, the opossum limbs return a value of 0 for the functional hypothesis; that is, the hypothetical functional modules do not at all reflect the identified modules in opossum forelimb. However, none of the three species had more than three modules identified for the forelimb musculature. The MuSk functional comparative values are lower than the bone-only or muscle-only values, but still above 0.55 (highest in mouse with 0.67).

For the hindlimb, the connectivity modules are best supported by the bone function–development hypothetical modules for the skeleton (>0.77), followed by muscle function (>0.73), then by muscle development (>0.65), and last by MuSk function (>0.54, human has highest value with 0.67).

Almost all values are above 0.5, indicating that function, development, or a combination of both factors accurately explains the modules identified by this analysis.

4 | DISCUSSION

4.1 | Present study: modules and connectivity hypothesis

Here, we compared modules (network of connected elements) of head, and fore- and hindlimbs in three species: opossum, mouse, and human. Skeletal networks included bones and cartilages (as nodes)

connected by their articulations (as links). Muscular networks included muscles (as nodes) connected by tendinous joints and fibrous fusions among them (links). Muscular networks contained the least information as they contain more single-element modules, and not true modules (p -value >0.05), than actual modules. This outcome is mainly related to the need of muscles to attach to skeletal elements in order to function, and an analysis of muscles without considering their attachments (bones and cartilages), therefore provides more pseudo-networks. Musculoskeletal networks included all elements (bones, cartilages, and muscles) and all connections (attachments, joints, and fusions). Simply comparing numbers of modules between species does not lead to a clear indication of a closer phylogenetic relationship or higher similarity between two of them compared to the third one.

Overall, the head network analyses indicate that the human head is more integrated and complex than the other two species, an observation in line with Lieberman's ideas about human head evolution (Lieberman, 2011). Mouse seems to be intermediate between human and opossum. In a previous study, the heads but also the forelimbs showed greater complexity than the hindlimbs in both normal and pathological human specimens (Diogo et al., 2019). Other studies also indicate a link between number of elements and complexity of the network in the evolution of the skull in tetrapods. In this instance, a decrease in number of bones (random loss of bones with fewer contacts and fusion of bones with more contacts to each other) led to an increase in network complexity and anisomerism (Esteve-Altava et al., 2013a, 2013b, 2014). In other words, less connected nodes (bones) are more likely to be lost over evolutionary time, while more connected elements tend to fuse with each other. However, this finding seems to be true for the skeleton; we could not identify such a trend for the muscles and musculoskeletal elements in the present study.

The comparison of fore- and hindlimbs revealed that with respect to the skeleton they are more similar to each other in mouse and human than in opossum. However, in opossum, the proximal elements are more alike, and in mouse and humans, the distal elements (carpals/tarsals and digits) are more similar between forelimb and hindlimb. Furthermore, the hindlimb returned fewer false skeletal modules than did the forelimb and fore- and hindlimb musculature modules are not alike in any of the species. The only similarity in all limbs for all three species is the network connection of all lumbricals with the muscles they originate from. Forelimb and hindlimb MuSk (musculoskeletal) modules are not similar in any of the analyzed species, except for the digit 1 (see below).

We also compared the network modules found with our biological hypotheses of modules, that is, we compared theoretical modules based on function, development, or both with the found modules. Our analyses of connectivity modules versus our hypothetical modules revealed that function seemed to be the best explanation for most of the observed anatomical networks. Exceptions are the head skeletal network for the opossum, which is better explained by development. The functional head musculature hypotheses also strongly support the identified networks; however, the bilateral

coded head muscle development is a slightly better match for the modules identified for mouse and human head muscles. The MuSk hypotheses values are all lower than for only bone or only muscle for all regions analyzed. In the head, the lowest values among all MuSk hypotheses are the ones for the pharyngeal arch hypothetical modules, which shows that they have the least resemblance with the modules as found in the network analyses.

In the limbs, the hypothetical bone development-function modules resemble the closest the identified modules in our analyses. The human and mouse, forelimb musculature networks are slightly better explained by the developmental hypothetical modules, and in the opossum, the hypothetical functional modules do not at all reflect the identified modules in the opossum forelimb. However, none of the analyzed species had more than three modules identified for the forelimb musculature. The forelimb's MuSk functional values are lower than the bone-only or muscle-only values.

The overall stronger support of functional hypotheses for our observed anatomical networks is reflected in the modules, where structures from different developmental origins are often found in the same module. This result might reflect a reorganization of structures during development to reach a functional adult head, and fore- and hindlimb. However, development and function are codependent, and topological relationships often capture the presence of this co-dependence among elements (Esteve-Altava, 2017a, 2017b). The co-dependence can change during evolution by variations in element shape, loss, or fusion of them. This variation will ultimately also lead to changes in the network modules.

4.2 | Comparison with other studies

4.2.1 | Heads

In the highly altricial newborns of marsupials, the oral apparatus must be functional to enable the attachment to the teat and suckling. At birth, the marsupial head shows several specializations, including a large chondrocranium (particularly the nasal region), precociously developed bones around the oral region, a large tongue, and well-developed craniofacial muscles (e.g., Smith, 2001). However, brain and calvaria development are delayed. Also, the jaw joint presents a unique configuration, formed by cartilaginous anlagen of the malleus and incus, resembling the evolutionary primary joint in vertebrates, albeit with several derived features, too (Filan, 1991). The necessity of having a functional primary jaw joint at precocious birth might explain our finding that incus and malleus are in a separate module than the stapes in opossum, but all ossicles are in the same modules in both eutherians (note that the jaw joint in neonate eutherian is between the mandible and the temporal bone, and the primary jaw joint is already in the ear between the ossicles).

In general, the craniofacial region is anatomical very complex, and this region forms the largest modules in our network analyses. In all skeletal network analyses, we found two larger modules, 'neurocranium' and 'viscerocranium', that resemble the classic division

of the mammalian skull (Moore, 1981), and were identified in previous studies (e.g., Esteve-Altava et al., 2013a, 2013b; Jamniczky & Hallgrímsson, 2011; Mitteroecker & Bookstein, 2008; Porto et al., 2009). A division of the facial skeletal module (viscerocranium) into subdivisions as described for primates (Esteve-Altava et al., 2015a) was not found by us.

Developmentally, many structures seem to interact with each other and form in a highly coordinated manner, for example, brain and calvaria development, eye and orbit development, and tongue and palate development. In marsupials, parts of the head must become functional much earlier than other parts, and this developmental heterochrony, with specific attention to head and specifically bone morphology, are well studied (Goswami, 2007; Smith, 1994, 1996, 1997, 2001, 2006). More recently, the molecular basis of these heterochronic changes during marsupial head development are being explained (Wakamatsu et al., 2014). While these studies demonstrate that several structures have together accelerated their development, only three modules in our study could be associated with those heterochronic shifts. The ossicles (incus and malleus) are already mentioned above. The other two modules pertain to tongue muscles, which are in a left and a right module in the opossum muscular network (STable Mu1) but are spread over several modules in mouse and human.

The MuSk networks reveal yet another picture with the tongue muscles all in the same modules in all species, but the modules differ in their skeletal components as well as in the inclusion of other muscles in those modules. While in human, all the tongue muscles are in a module with the hyoid bone and sternum only (STable MuSk 6), mouse and opossum do not include the sternum, but do include the mandible and other cranial bones. Furthermore, mouse and opossum include other non-tongue muscles that also differ between both species. Therefore, we interpret those 'tongue, supra- & infrahyoid' modules as functional modules, related to the muscles attached to the hyoid, and the differences between human and the other two species might be related to the adaptations required for speech evolution.

It is without doubt that the head development occurs at different rates and in different sequences in marsupials and eutherians, but that also means that apparently integrated structures in adults may develop (e.g., appearance of first anlage) at different times and in a different sequence. Goswami et al. (2016) showed that marsupials have a high integration of the oral apparatus in early postnatal development and suggest that this is likely related to functional constraints related to suckling during a premature developmental state throughout their evolutionary history. Therefore, the adult head anatomical networks and their modules as found here, are likely best explained by function rather than development, with perhaps the exception of the ossicles.

4.2.2 | Limbs

Marsupials give birth to precocious offspring with well-developed orofacial and forelimb structures but with less developed limbs and brain. This observation led to the hypothesis that

the marsupial forelimb structures show higher phenotypic covariance, and therefore higher morphological integration, than the hindlimbs. Conversely, this hypothesis posits that eutherian fore- and hindlimbs have similar degrees of morphological integration. Previous studies of marsupials found strong integration within each limb, and very weak or no integration across limbs (Bennett & Goswami, 2011; Kelly & Sears, 2010). However, a recent study in marsupials demonstrated that fore- and hindlimbs have similar disparities without differences in integration within or between limbs (Martin-Serra & Benson, 2020). Eutherians, on the other hand, show strong within-limb and between-limb integration, which was interpreted as reflecting functional association and serial homology (Young & Hallgrímsson, 2005). However, the fore- and hindlimbs are not serial homologous, as shown by more recent developmental, comparative anatomical, and evolutionary developmental studies (see, e.g., Diogo et al., 2015a; Diogo & Molnar, 2014; and references therein). Still, those older studies provide insight into developmental mechanisms that might explain differences in the adult phenotypic covariance, including heterochronic shifts in both ossification (Goswami et al., 2009) and gene expression patterns (Keyte & Smith, 2010; Sears et al., 2012). Another study of evolutionary integration in the mammalian shoulder girdle, comparing marsupials, eutherians, and non-mammalian synapsids, revealed a weak scapular integration in eutherians and synapsids, but an increased within-scapular integration, perhaps in conjunction with increased within-forelimb integration in the early evolution of marsupials (Sears et al., 2013).

With respect to our skeletal network analyzes of the limbs, we also found that overall, the fore- and hindlimbs are more alike in eutherians (mouse and human) than in the marsupial (opossum). However, comparing proximal elements (e.g., girdles) with distal elements (hand and foot), we found that the former elements are more similar among opossum limbs, and the latter between mouse and human limbs. This outcome is unexpected in that the human hand and foot are highly specialized structures with markedly different functions in general. However, one needs to keep in mind that humans descended from apes, which are often called 'quadrumanal', in the sense that their hands and feet have not only strikingly similar functions but also morphology, so our results might reflect this evolutionary fact. Overall, there are also fewer not-true skeletal modules (i.e., modules with p values >0.05) in the hindlimb than in the forelimb, supporting the idea that the forelimb is more variable than the hindlimb, and therefore can evolve more freely, what is reinforced by the finding that heterochronic and heterotopic changes during early marsupial forelimb development contribute to their developmental acceleration (Chew et al., 2014; Keyte & Smith, 2010). Interestingly, in marsupials, the hindlimb field is also early specified and patterned, but the outgrowth of the limb bud is delayed (Keyte & Smith, 2010; Weisbecker et al., 2008b). It is currently not clear if the early developmental differences have an impact on the integration of forelimbs versus hindlimbs.

Comparing the musculature or the MuSk systems between fore- and hindlimb does not show any similarities between the structures, except digit 1 (see below). That leads to the question of whether limb evolution is driven more by adaptation of the skeletal system development, and less by adaptation in the muscular system. An indication that this might be the case is found in the study of pathology in human hands in a trisomic fetus, where one hand had six digits (2 thumbs) and the other hand had no thumb (Diogo et al., 2015c). Based on this study, it was hypothesized that muscle's attachment to skeletal structures is mainly related to the topological position of the latter structures, rather than to the developmental primordium or homeotic genetic identify of the digits. Therefore, changes in the skeletal system can lead to changes in muscle attachment sites, which in turn can lead to new functional opportunities, which again can in a feedback loop continue to influence a particular shape and/or perhaps a particular position of a bone during the evolution of a species.

Another idea that we explored is that, due to the specialist function of digit 1 in humans (thumb and big toe), the elements of those digits are in different modules than in the quadruped specimens. Only comparing the skeletal modules showed that digit 1 in both fore- and hindlimb form the same modules in opossum and human, with mouse having excluded the metacarpal 1 but included in the metatarsal 1 in the respective modules. Comparing the MuSk networks, differences between digits 1 become apparent. The human and opossum network analyses revealed the digit 1 elements in two modules with the distal and proximal phalanges being in separate ones. The forelimb modules that contain the proximal phalanx 1 also include in all specimens the trapezium, but five other carpal bones in mouse and three in human. In the same module are all short thumb muscles included, and in mouse and human additional one forearm muscle (mouse: abductor pollicis longus, human: extensor pollicis brevis). The distal phalanx 1 (thumb) is included in a large module in the opossum that contains the other phalanges and most intrinsic muscles of the hand; in mouse, the distal phalanx is included in the same module as the proximal one, but in humans we identified a 'distal digit 1' module (distal phalanx 1, flexor pollicis longus, and extensor pollicis longus; note both muscles are absent in mouse and opossum). This triplet is interesting as it was suggested that those muscles played a major role in the evolution of the human hand (Diogo et al., 2012). However, we also identified a corresponding 'digit 1' module in the hindlimb and overall those modules containing phalanges of digit 1 are strikingly similar to the modules in the forelimb. Therefore, there seems to be an evolutionary pressure in human to have a specialized digit 1 module in both limbs, but with the distal phalanx instead of more proximal elements involved. Functionally, the thumb is important for fine grip, and the big toe is requisite for weight bearing during the end of the gait cycle (Rodgers, 1988). Evolutionary, our findings in human could also be related to the fact that our ape ancestors as well as extant apes have a highly mobile big toe (Bello-Hellegouarch et al., 2013; Diogo et al., 2015a).

In an AnNA of limbs in human, chimp, and bonobo, the same 'digit 1' module was only found in the human hindlimb but not forelimb (Diogo et al., 2018a). However, comparing the coding for fore- and hindlimbs, we found that the matrix underlying the former analyses did not include the insertion of the lumbricals in the human hindlimb and missed in both limbs the insertion into the extensor expansions (connections to middle and distal phalanges). Those minor discrepancies had sufficient effect on the analyses that the modules in fore- and hindlimb musculoskeletal network differ for human in the two studies.

Like all studies, ours come with several methodological limitations that must be taken into account to interpret the results appropriately. Regarding our use of AnNA, first note that we chose to model musculoskeletal systems as unweighted networks, in which nodes and muscles (anatomical parts) were equally modeled as nodes, and the articulation, attachment, and bending among them (anatomical relations) were equally modeled as links. The reason to take this simpler approach is that it unlocks most of the algorithms required to accurately analyze anatomical networks. More complex realizations, for example, with bones and muscles as separate layers of a multi-level network, would prevent us to analyze the same topological properties because of limitations of available algorithms. In turn, weighing the connections among nodes, for example, measuring the actual area of contact, would be very time consuming and there is no evidence suggesting this would significantly change the outputs compared. Finally, although we modeled the most common (conserved) anatomical parts and relations of each species, there is always some level of intraspecific variation. However, given the large divergence time between the species compared, one can safely assume that interspecific differences overwhelm any intraspecific differences: thus, making our comparisons sound. Although statistical significance adds confidence to a module, we cannot discard non-significant modules *a priori* without a closer examination because their identification was not based on statistics, but on a network-based optimization algorithm. This study with three species is a first step. Future work in this area will want to incorporate other members of placental and marsupial groups that also represent different modes of locomotion and/or feeding types, to more broadly and deeply explain the patterns that we have reported here.

5 | CONCLUSION

We can make five main claims that can be tested in future studies.

1. Function seems to best explain the observed anatomical networks: in the modules, structures from different developmental origin are often found in the same module, implying that ontogeny is not a strong signal.
2. The opossum incus and malleus are in a separate module than the stapes, but all ossicles are in the same modules in both eutherians,

which we interpret as due to the necessity of having a functional primary jaw joint in the precocious offspring in marsupials.

3. Differences between human and mouse/opossum 'tongue, supra- & infrahyoid' musculoskeletal module might be related to the adaptations required for speech, again a functional driver of modularity and network structure.
4. Overall, the fore- and hindlimbs skeletal networks are more similar to each other in eutherians (mouse and human) than in the marsupial (opossum).
5. Humans have a specialized digit 1 module in both limbs that is likely a functional adaptation (fine grip and walking over the big toe) and an evolutionary consequence as our direct ape ancestors also had highly movable big toes and thumbs.

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CONFLICT OF INTEREST

The authors declare no competing interests.

AUTHOR CONTRIBUTIONS

JMZ, JCB, and RD performed dissections, literature review, and filled matrices with anatomical data. BEA performed the network analyses. JMZ analyzed the data and wrote the first draft of the MS. All authors reviewed the manuscript and contributed to finalization of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the supplementary material of this article.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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