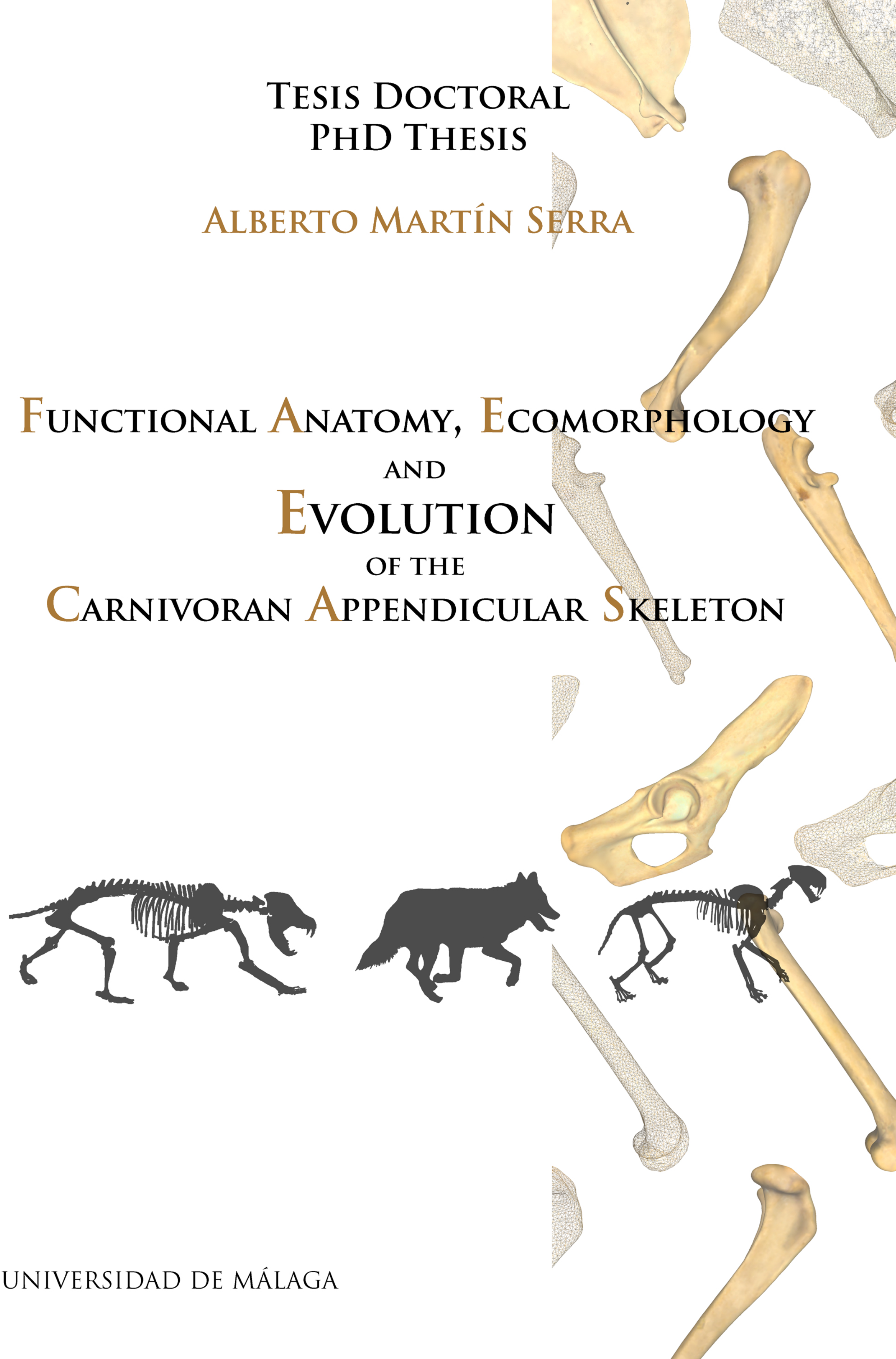
TESIS DOCTORAL
PHD THESIS

ALBERTO MARTÍN SERRA

FUNCTIONAL ANATOMY, ECOMORPHOLOGY AND EVOLUTION OF THE CARNIVORAN APPENDICULAR SKELETON



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PhD THESIS

FUNCTIONAL ANATOMY, ECOMORPHOLOGY AND EVOLUTION OF THE CARNIVORAN APPENDICULAR SKELETON

por/by Alberto Martín Serra

Dirigida por/Supervised by
Dr. Paul Palmqvist Barrena &
Dr. Francisco De Borja Figueirido Castillo

DEPARTAMENTO DE ECOLOGÍA Y GEOLOGÍA

FACULTAD DE CIENCIAS

UNIVERSIDAD DE MÁLAGA

Paul Palmqvist Barrena, Catedrático de Paleontología y perteneciente al Departamento de Ecología y Geología de la Universidad de Málaga, y Francisco de Borja Figueirido Castillo, Doctor en Biología y Contratado Doctor del Departamento de Ecología y Geología de la Universidad de Málaga, acreditan que,

Alberto Martín Serra, Licenciado en Biología, ha realizado, en el Departamento de Ecología y Geología de la Facultad de Ciencias de la Universidad de Málaga, las investigaciones contenidas en la presente memoria de Tesis Doctoral, titulada: **Functional Anatomy, Ecomorphology and Evolution of the Carnivoran Appendicular Skeleton**.

Como directores de la tesis, consideramos que la presente memoria reúne todos los requisitos para ser sometida a juicio de la Comisión correspondiente, por lo que autorizamos su exposición y defensa pública para la obtención del Grado de Doctor en Biología con la mención de Doctor Internacional.

Y para que así conste, en cumplimiento de las disposiciones vigentes, firmamos la presente acreditación en Málaga, a 11 de Diciembre de 2014.

Prof. Dr. Paul Palmqvist Barrena

Dr. Francisco de Borja Figueirido Castillo

Memoria presentada para optar al grado de Doctor en Biología

Visado en Málaga
Diciembre de 2014

El Licenciado	El Director	El Director
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Memoria presentada para optar al grado de Doctor en Biología, con mención internacional

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Dr. Sergio Almécija. Stony Brook University (Stony Brook, NY, Estados Unidos).

A mis padres

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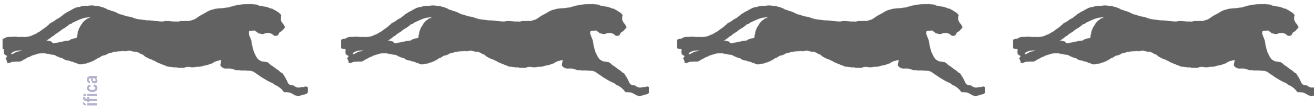
1. Introduction and objectives

"So much of motion, is so much of life, and so much of joy, and to stand still, or get on but slowly, is death and the devil"

Laurence Sterne



1.1. General aims

Locomotion is one of the most important ecological aspects of animals. It is crucial for the survival of organisms, as many biological activities depend on it, including food finding, mating or escaping from danger. Thus, the structures involved in locomotion (e.g., the appendicular skeleton) have been modified by natural selection at the same time that organisms adapt towards different modes. The relationship between modes of locomotion and structural adaptations has been largely recognized by evolutionary biologists (e.g., Maynard-Smith and Savage 1956) and can be addressed from different perspectives. Biomechanics and physiology are among the most recurrent approaches to study locomotion, they are focused on understanding how the mechanisms of locomotion function under the rule of physical laws (e.g., Alexander and Jayes 1978; Taylor et al. 1982). In contrast, paleobiologists usually follow a different approach, as they study adaptations towards different modes of locomotion in the appendicular skeleton of the living species and use them as morphological indicators to infer these paleoecological aspects in extinct taxa (e.g., Van Valkenburgh 1987). However, the appendicular skeleton can also be studied in an strict sense from an evolutionary point of view, with the focus on different aspects and processes that may have shaped its morphological evolution, such for example, the presence of functional adaptations, convergences and developmental constraints. All of these studies have contributed promising achievements during the last two decades (e.g., Wayne 1986a, 1986b; Young and Hallgrímsson 2005; Schmidt and Fischer 2009; Kelly and Sears 2010; Bennett and Goswami 2011; Bell et al. 2011).

In light of the above, in this PhD dissertation I have taken a comparative morphometric perspective for studying the functional association between locomotion and morphology in the appendicular skeleton of carnivorans. Therefore, the main aim of this work is to assess how functional adaptations for locomotor modes can influence the morphological evolution of the appendicular skeleton in order to derive paleoecological inferences for extinct species. For doing this, I have chosen a large number of living and extinct mammalian carnivores (Mammalia, Carnivora) as study case. Mammalian carnivores are a good choice for this purpose because they show a wide range of body size, habitats and locomotor adaptations (e.g., Ewer 1973; Nowak 1999). In addition, their phylogenetic relationships are mostly resolved (Nyakatura and Bininda-Emonds 2012) and they are relatively well represented in the fossil record (e.g., Janis et al. 1998).

Before going on with the specific analyses and results obtained during the elaboration of my PhD Thesis, I will expose an overview of how the appendicular skeleton of tetrapods is structured and the developmental processes that take place during limb formation, with a mention of some of the key genes involved. Afterwards, I will introduce the most important biomechanical properties of the major limb elements and the physical laws that rule their function. Locomotor adaptations in carnivorans are also briefly explained, including the locomotor modes of the living taxa and those inferred for extinct ones. Finally, I will also briefly describe the methods that I have followed to take the metric data and the logic behind the statistical and morphometric analyses that I have applied.

1.2. Evolution and development of the tetrapod appendicular skeleton

1.2.1. Limbs

Since Owen (1849), it has been recognized that the appendicular skeleton of all tetrapods shares a basic internal structure. In agreement with his own concept of homology, each appendicular element can be recognized in different species due to its position and structural relationship with other elements (Owen 1848, 1849). Furthermore, he also established that the fore- and hind limbs share a similar structure; this kind of homology within the same individual was called serial homology (Owen 1948). Although he defined these concepts in relation with the archetypical theory of the German *Naturphilosophie*, they fitted easily within the paradigm of the Darwinian evolutionary theory, as the concept of homology is now explained as resulting from shared ancestry. In this section, I will expose how the appendicular skeleton of tetrapods is structured.

The appendicular skeleton is composed by two pairs of appendages (limbs) located in both flanks of the body. One of these pairs is close to the head (pectoral limb or forelimb) and the other pair is near the base of the tail (pelvic limb or hind limb). In both cases, the most proximal structure is a girdle that connects the limb to the axial skeleton (see below). Distal to the girdle there is the stylopodium, which is composed of a single long bone, the humerus for the forelimb and the femur for the hind limb (Figure 1.1; Kardong 2006). Distal to the stylopodium there is the zeugopodium, which is composed of two bones, the radius and ulna for the forelimb, and the tibia and fibula for the hind limb (Figure 1.1; Kardong 2006). Finally, there is the autopodium, the manus for the forelimb and the pes for the hind limb (Figure 1.1; Kardong 2006). The autopodii are more complex, as they are subdivided into: (*i*) the basipodium, which includes several small podial bones (carpal or tarsal) that make the wrist or ankle for the fore- and hind limb,

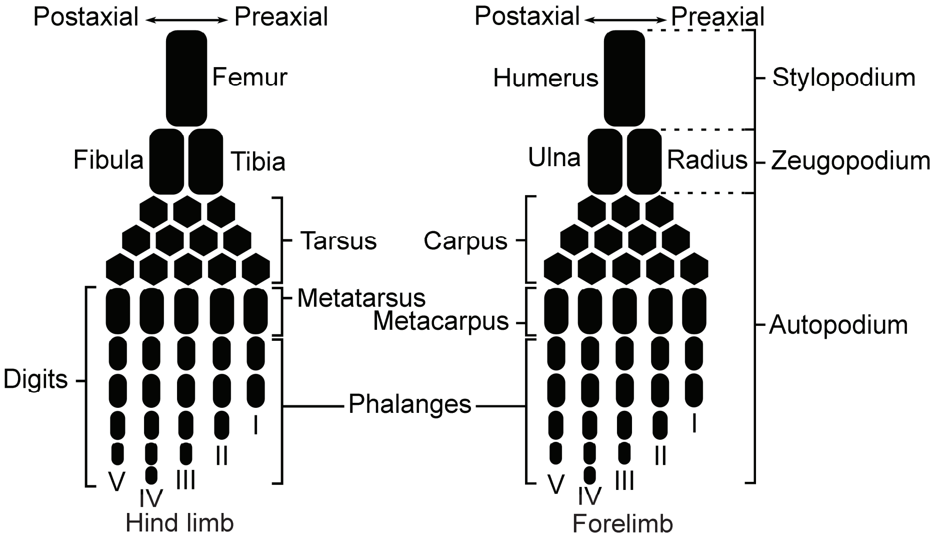


Figure 1.1. Basic structure of the fore- and hind limb in tetrapods. Modified from Kardong (2006).

respectively; (*ii*) the metapodium, which is composed of five metapodial bones (metacarpals or metatarsals); and (*iii*) the acropodium, which is composed of five series of phalanges that constitute the digits (Figure 1.1; Pescitelli and Stocum 1980; Kardong 2006).

1.2.1.1. Pectoral girdle

The pectoral girdle is composed of two kinds of bones with a different developmental origin: endoskeletal and dermal bones. In early tetrapods, the dermal bones are the cleithrum, the clavicle and interclavicles, and the endoskeletal bone is the scapulocoracoid (Figure 1.2A). In sarcopterigian fishes, the dermal bones connect the pectoral girdle with the skull, but this connection was lost in the basal tetrapods (Coates and Ruta 2007), so they can turn their head on the neck independently of limb movements (Coates and Ruta 2007). The scapulocoracoid usually shows the glenoid fossa (Figure 1.2A), which is the articular surface with the humeral head or proximal epiphysis. Dermal bones were progressively reduced through the evolution of tetrapod clades. In fact, only the clavicle is conserved in most crown groups (Hildebrand and Goslow 2001). In contrast, the scapulocoracoid increased its relevance and the scapula enlarged, becoming the most important bone of the pectoral girdle in many amniotes (Kardong 2006). The coracoid remained as an important bone in modern reptiles and birds, whereas in mammals is largely reduced and becomes a small process fused with the scapula (Figure 1.2A; Hildebrand and Goslow 2001).

1.2.1.2. Pelvic girdle

In contrast with the pectoral girdle, the pelvic girdle is only composed of endoskeletal bone. In tetrapods there are three fused bones: ilium, ischium and pubis (Figure 1.2B). Near the point of connection of these bones there is a socket (acetabulum) with the articular surface for the femoral head or proximal epiphysis. Each half of the pelvis, or innominate bone, is fused dorsally with the sacral vertebrae and with each other through the ventral pelvic symphysis (Hildebrand and Goslow 2001). The illium, ischium and pubis arrange in different fashions across the evolution of tetrapods. Early amphibians had a triangular arrangement

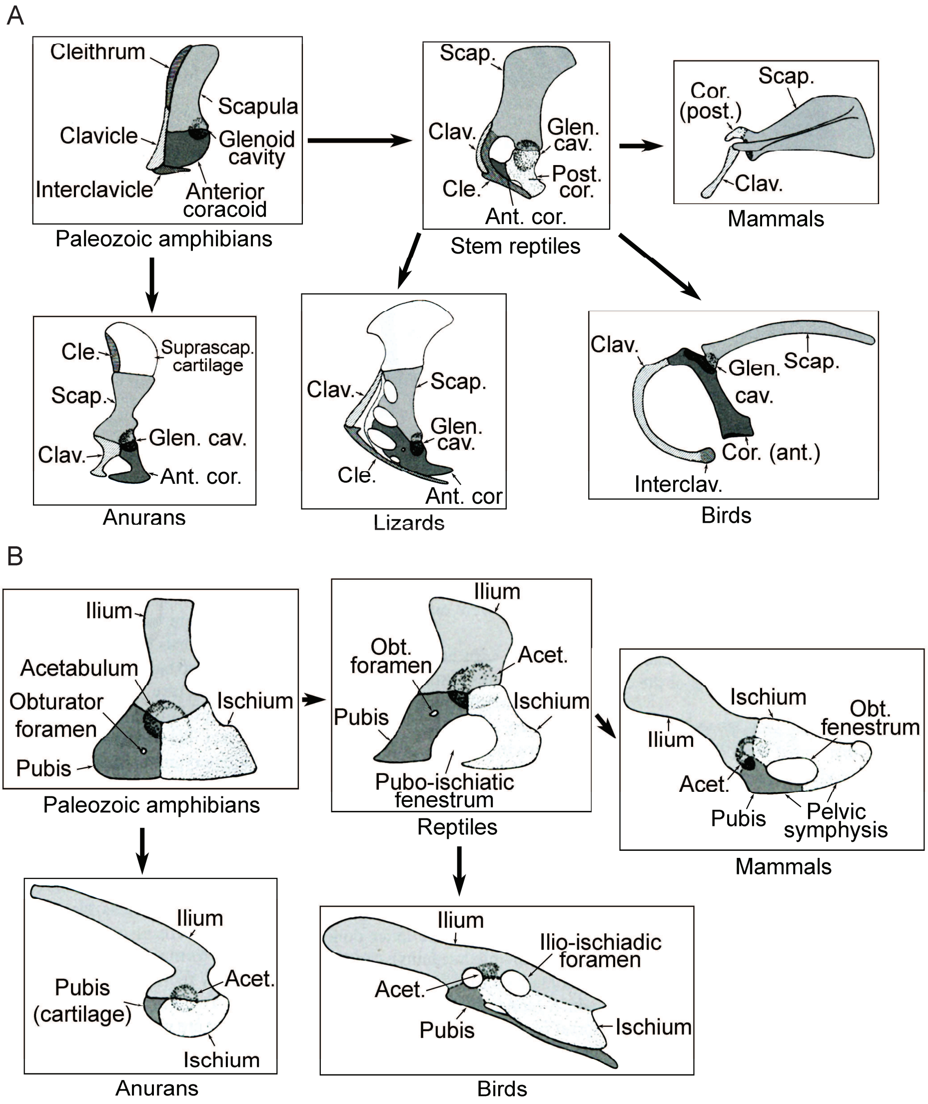


Figure 1.2. Morphological evolution of the tetrapod girdles. A, pectoral girdle; B, pelvic girdle. Modified from Hildebrand and Goslow (2001).

with the ilium as the dorsal apex (Figure 1.2B), whereas modern amphibians show the ischium and the pubis reduced while the ilium is expanded anteriorly (Figure 1.2B; Hildebrand and Goslow 2001). In reptiles, the pelvis takes a wide variety of shapes, but it usually has a pubo-ischiatic foramen (Figure 1.2B; Hildebrand and Goslow 2001). Mammals typically have an anteriorly expanded ilium and an obturator foramen between the pubis and ischium (Figure 1.2B; Hildebrand and Goslow 2001).

1.2.1.3. Stylopodium and zeugopodium

The stylopodium and zeugopodium are very conservative among tetrapods. Unless the limbs are completely lost, they are always present with the same arrangement: the humerus or femur plus the radius/ulna or the tibia/fibula complex, respectively. However, they can have very different shapes and functions, and in some cases one of the zeugopodial bones can be highly reduced, as in the case of the avian fibula (Baumel et al. 1993), or both can be fused, as in the case of the radius and ulna of some ungulate mammals (Polly 2007).

1.2.1.4. Autopodium

The autopodium is, by far, the most variable portion of the limb. There are a plethora of cases of digit reduction and carpal/tarsal fusions in all major lineages of tetrapods (e.g., Shapiro et al. 2007; Polly 2007). For example, the living birds only retain three digits in their wings and some carpal bones are fused with the metacarpals (Baumel et al. 1993). In the case of mammals, there are many cases of digit losses or metapodial fusions, for example in many ungulate groups. However, these changes, as well as the changes in the stylopodium and zeugopodium, respond to particular adaptations that are beyond the scope of this introduction.

1.2.2. Ontogeny of the appendicular skeleton

1.2.2.1. Limb bud initiation and spatial patterning

The position of the limbs along the antero-posterior axis of the body is established by the expression of *Hox* genes in the lateral plate mesoderm (Cohn et al. 1997). At the same time, their position in the dorso-ventral boundary is associated with the ventral expression of *Engrailed1* (Kimmel et al. 2000). The initiation of limb buds is related to the expression of fibroblast growth factors (Fgfs) in the mesenchyme (Cohn at al. 1995). Specifically, *Fgf10* starts the outgrowth of the lateral plate mesoderm to form the limb bud (Tanaka and Tickle 2007). Subsequently, the apical ectodermal ridge (AER) of the bud (Figure 1.3A) generates other Fgfs (e.g., *Fgf8*) to maintain the outgrowth (Tanaka and Tickle

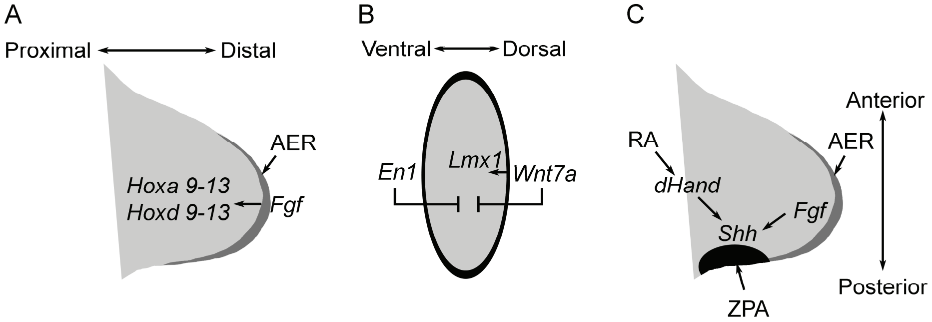


Figure 1.3. Some of the key genes involved in limb bud initiation and spatial patterning. A, proximo-distal axis; B, dorso-ventral axis; C, antero-posterior axis. AER, apical ectodermal ridge; RA, retinoic acid; ZPA, zone of polarizing activity. Modified from Tanaka and Tickle (2007).

2007). T-box transcription factors are also involved in limb initiation: *Tbx5* in the forelimb and *Tbx4* in the hind limb (Agarwal et al. 2003; Takeuchi et al. 2003). Indeed, they seem to be upstream (before in the signalling chains) of *Fgf10* (Agarwal et al. 2003). The Fgf production by the AER seems to be regulated by reciprocal signalling with the nearby mesenchyme (Tanaka and Tickle 2007). The cells located more proximally begin to differentiate as the apical ectoderm grows distally. Furthermore, it has been proposed that the proximo-distal pattern of the limb is specified by an Fgf gradient from by the AER to the more proximal parts of the limb bud (Tanaka and Tickle 2007).

Similarly to the main body axis, *Hox* genes also play an important role in the establishment of the proximo-distal patterning of limb segments (Wellik and Capecchi 2003) and they also depend on Fgf signalling (Vargesson et al. 2001). Specifically, several members of the *Hoxd* and *Hoxa* complexes are closely related to the different limb portions: *Hoxd9* and *Hoxa9* determine the development of the forelimb zeugopod, *Hox10* paralogs determines that of the hind limb zeugopodium, *Hoxd11* and *Hoxa11* establish the development of the stylopodium of both limbs, and *Hoxd13* and *Hoxa13* do the same for digits (Tanaka and Tickle 2007).

The dorso-ventral axis of the limb bud is controlled by the ectoderm through the expression of *Wnt7a* dorsally, which activates *Lmx1* in the mesenchyme (Tanaka and Tickle 2007). In the ventral side of the limb, *Wnt7a* is inhibited by the expression of *En1* in the ectoderm (Figure 1.3B; Tanaka and Tickle 2007). The antero-posterior axis of the limb is controlled by the expression of *Shh* in the polarizing region (zone of polarizing activity, ZPA), located in the posterior margin of the bud. Several morphogens control the expression of *Shh*, as they are the product of *dHand* and *Hoxb8* genes, and retinoic acid (Figure 1.3C; Riddle et al. 1993; Tanaka and Tickle 2007). Furthermore, *Shh* also interacts with the Fgfs produced by the AER with mutual induction and with *Wnt7a*, which seems to be a key mechanism for the whole spatial patterning of the limb bud (Niswander et al. 1994; Yang and Niswander 1995). One of the genes affected by *Shh* expression is *Gli3*, which is involved in digit formation and identity (Tanaka and Tickle 2007).

Finally, one aspect of particular interest in limb development is how the morphological differences between the fore- and hind limb arise during their ontogeny. The expression of *Tbx* genes seems to underlie to these shape differences, because the forelimb expresses only *Tbx5* and the hind limb expresses *Tbx4* and *Pitx1* but no *Tbx5* (Tanaka and Tickle 2007).

1.2.2.2. Morphogenesis of the limb skeleton

The first step of limb bone formation is the aggregation and proliferation of the mesenchymal cells and their differentiation into chondrocytes (Figure 1.4A; Weatherbee and Niswander 2007). This process of cell aggregation and differentiation follows a proximo-distal axis in the limbs of tetrapods. In their seminal paper on limb morphogenesis, Shubin and Alberch (1986) described this proximo-distal axis of endochondral formation as homologous with the metapterygial axis of sarcopterygian fins. Furthermore, they postulated a series of morphogenetic rules that control limb skeleton formation (condensation, bifurcation and segmentation), including a hypothetical bending of the metapterygial axis (digital arch) to form the autopodium (Shubin and Alberch 1986). Although more recent studies have challenge this model (see Cohn et al 2002; Wagner and Larsson 2007), the existence of a primary proximo-distal axis seems to be true (Wagner and Larsson 2007). Thus, this primary axis of

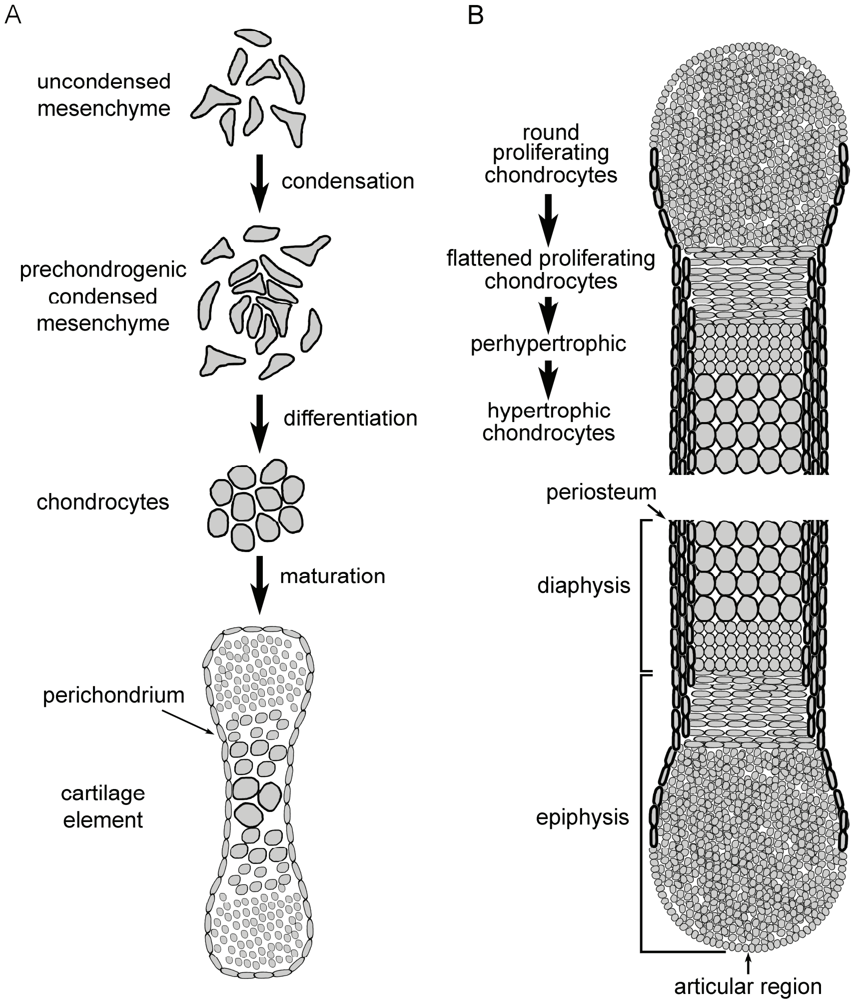


Figure 1.4. Endochondral bone formation during limb development. A, early steps of bone formation from mesenchyme. B, schematic representation of a limb bone during its development. Modified from Weatherbee and Niswander (2007).

chondrogenesis extends from the humerus/femur through the ulna/fibula to the carpal/tarsals, whereas the radius/tibia have a preaxial position.

During the chondrogenetical process, the chondroprogenitors deposite extracellular matrix, which is basically composed of different types of collagen (Weatherbee and Niswander 2007). The cells that enfold the cartilage elements begin to differentiate into a fibroblastic layer called the perichondrium (Figure 1.4A; Weatherbee and Niswander 2007). As the cartilage primordium grows, the different parts of the future bone begin to differentiate: a diaphysis can be observed in the middle part when the chondrocytes become hypertrophic and the chondroblasts of the perichondrium become flattened (Figure 1.4A); in contrast, the cells located in the ends of the bone or epiphyses remain proliferating. At this point the perichondrium becomes periosteum in the diaphysis and the osteoblasts begin the secretion of bone matrix that calcifies progressively (Weatherbee and Niswander 2007). The bony diaphysis is then separated from the cartilaginous epiphyses by growth plates, which are composed of proliferating chondrocytes in different stages of hypertrophy that are responsible of growth in bone length (Figure 1.4B; Weatherbee and Niswander 2007). After the ossification of the perichondrium, blood vessels come in to irrigate cartilage diaphysis, while osteoblasts and osteoclasts invade it to replace cartilage with bone. Osteoblasts and osteoclasts are the most important cells in bone formation and remodelling. Osteoblasts come from the periosteum and secrete the bone extracellular matrix, whereas osteoclasts come from monocytes (blood cells that originates from bone marrow) and are involved in bone resorption (Weatherbee and Niswander 2007).

The balance between chondrocyte proliferation and differentiation, as well as between bone formation and resorption, are both regulated by a complex network of morphogens. Changes in this network underlie all the morphological evolution observed in the appendicular bones of tetrapods (Weatherbee and Niswander 2007).

1.3. Biomechanics of terrestrial locomotion

1.3.1. General properties of the musculo-skeletal system

Now that I have introduced how the development of appendages evolved in tetrapods, we will explain how they work to move the organism. Thus, I will start with the main biomechanical properties of the musculo-skeletal system. This system is mainly composed of bones, muscles, joints, tendons and ligaments. Each component has particular properties, which relate to their functional role during locomotion.

1.3.1.1. Bones

Bone tissue is mainly composed of an extracellular matrix with collagen and hydroxylapatite. This dual composition, biological and mineralogical, makes this tissue rigid and, thus, resistant to compression, bending and torsion (Hildebrand and Goslow 2001).

All appendicular long bones are structured in a similar fashion: in the centre of the diaphysis there is a cavity filled with hematopoietic tissue (marrow); surrounding this cavity there is a thick layer of cancellous bone (porous bone) and a thin layer of compact bone more externally (Figure 1.5; Hildebrand and Goslow 2001). The epiphyses are composed of cancellous bone and include the articular surfaces that are in contact with other bones.

This structure provides maximum strength with minimum material (Hildebrand and Goslow 2001). Furthermore, the shape of the bones can be adapted to withstand different kinds of loads. For example, the most common loads

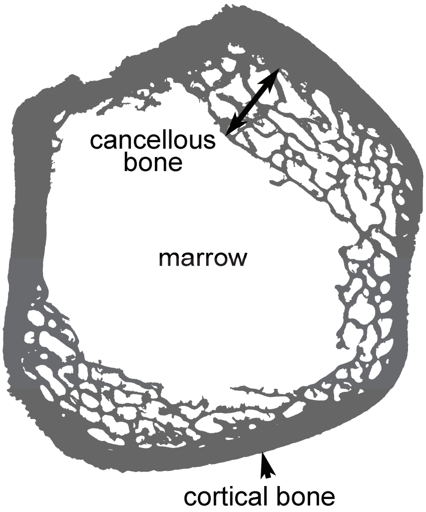


Figure 1.5. Cross section of a femoral diaphysis. The different layers of bone can be distinguished. Picture provided by A. Pérez-Ramos

that the appendicular bones have to withstand during terrestrial locomotion are axial forces (compression) and bending forces. Axial forces (i.e., forces parallel to the main shaft of the bone) are usually supported with little risk of damage for the bone (Lanyon and Rubin 1985). Bones that are subject of this kind of loads have usually straight and cylindrical shafts (Figure 1.6A; Hildebrand and Goslow 2001). In contrast to axial forces, bending forces are usually more critical for an appendicular bone. A bone can withstand bending forces in a main direction of action, or can support bending forces in different directions. In the former case, while the material located in the direction of the force is stressed, the remaining material is wasted somehow (Figure 1.6B; Hildebrand and Goslow 2001). For this reason, these bones usually loss the cylindrical shape to achieve one that resembles that of "I-beams" (Lanyon and Rubin 1985), so most of the bone material is concentrated in the same plane than the bending force (Figure 1.6B). When a bone has to withstand bending forces applied in different directions, the cylindrical shape is still the most advantageous shape (Lanyon and Rubin 1985).

Another important aspect is that bones are continuosly remodelled during the life of the organism. The mechanism of bone remodelling involves the action of osteoblasts and osteoclasts: the former produce new collagenous matrix that is

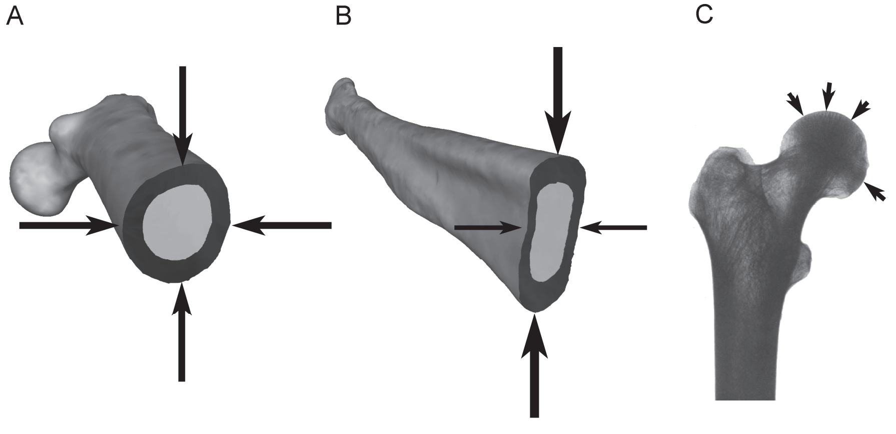


Figure 1.6. Differences in bone shape resulting from functional demands related to the type of forces exerted on them. A, round-shaped cross section of a femoral diaphysis that receives similar forces in different directions; B, elliptic-shaped cross section of an ulnar diaphysis that receives forces in one main direction; C, femoral head with bone fibres oriented parallel to the main directions of forces (modified from Carter et al. 1989).

mineralized and the latter reabsorb older extracellular matrix (Lanyon and Rubin 1985). This process is crucial to repair bone fractures produced by peak loads during abnormal locomotor activity or accidents. However, the most important benefit of this remodelling process is that it precludes fatigue failure of bone tissue (Lanyon and Rubin 1985). Fatigue failure is the result of the repetitive application of low-magnitude loads to the bone during long periods of time. During these loading cycles, bone tissue accumulates deformations and microcracks that eventually result in bone failure (Lanyon and Rubin 1985). For this reason, bone remodelling process repairs these microcracks and, hence, increases bone fatigue life (Lanyon and Rubin 1985). This process also involves the rearrangement of bone tissue in response to load distributions coming from normal activity (Hildebrand and Goslow 2001). In the different parts of bones, especially their articular surfaces and insertions for muscles and tendons, the fibres of cancellous tissue (trabeculae) align parallel to the main direction of the loads that they regularly withstand (Figure 1.6C; Lanyon and Rubin 1985). The reason is that, similarly to the bone as whole, trabeculae resist better axial loads (either compressive or tensile) than bending ones (Lanyon and Rubin 1985).

1.3.1.2. Muscles

The muscles of the appendicular skeleton are the active element of the musculo-skeletal system (in other words, they transform chemical energy into physical movement). They are in connection with the bones and with other elastic elements that move passively. I will focus on the striated muscles, as they are the responsible of voluntary movements of the appendages. Each muscle is surrounded by a layer

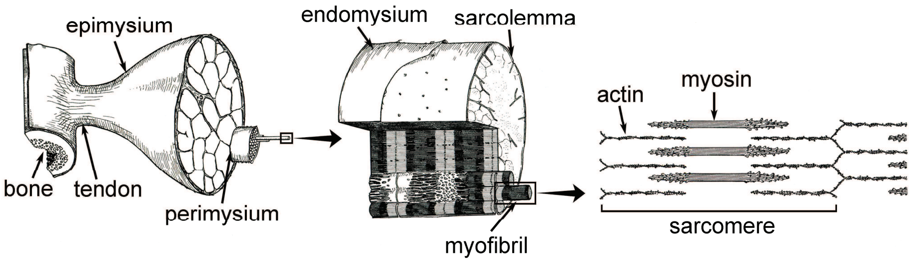


Figure 1.7. Structure of skeletal muscle. From the macroscopic (left) to the molecular level (right). Modified from Hildebrand and Goslow (2001).

of connective tissue, which is called the epimysium (Figure 1.7). Internally, this connective layer forms septa (perimysium) between the fascicles of fibres (Figure 1.7). Surrounding each muscle fibre, the connective tissue is the endomysium and there is below it a membrane called sarcolemma (Figure 1.7; Hildebrand and Goslow 2001). Each muscle fibre has a group of myofibrils that have a banding pattern. This results from the parallel, interdigitated arrangement of myosin and actin molecules (Figure 1.7). Each band is a sarcomere, the contractile unit of the muscle (Hildebrand and Goslow 2001).

There are several types of fibres according to their contractile properties, such for example, contractile speed and resistance to fatigue. Two main types are found among the muscles of the appendicular skeleton of tetrapods: tonic fibres and twitch fibres. Tonic fibres contract slowly and are highly resistant to fatigue; they are present in the limbs of all vertebrates except mammals (Hildebrand and Goslow 2001). In contrast, twitch fibres contract rapidly after stimulus. Following Hildebrand and Goslow (2001), there are several subtypes within this type of fibres:

- Slow twitch fibres: they contract and fatigue slowly, but they generate small forces. They have many mitochondria and myoglobin, and obtain energy from the oxidative process, which makes them energetically efficient. They are efficient for isometric contractions (i.e., contractions with no change in fibre length) and for slow and repetitive isotonic contractions (i.e., contractions with constant resistance).
- Fast twitch, fatigable fibres: they contract fast and also fatigue fast, but are able to generate large forces as they have large diameters. They have few mitochondria, so they obtain the energy mostly from the glycolytic process. They are used for bursts of fast activity.
- Fast twitch, fatigue-resistant fibres: they contract only moderately fast and fatigue slowly. They have many mitochondria and obtain the energy from both the oxidative and glycolytic processes. They are most common in muscles that perform strong and repetitive movements.

Most muscles combine the three types of twitch fibres, although their proportions vary depending on the activity of each specific muscle.

Another key property of muscles is the force or tension that they can generate. As this property depends on the number of fibres that are contracting, cross sectional area is directly proportional to maximum muscle force (Hildebrand and Goslow 2001). However, muscle fibres are not always perpendicular to the anatomical cross section, as in the case of pinnate muscles they are arranged obliquely. For this reason, Alexander and Vernon (1975) measured the physiological cross sectional area (calculated as the ratio muscle volume/fibre length), which takes into account these problems and is a more accurate proxy for estimating maximum muscle force.

1.3.1.3. Tendons and ligaments

The musculo-skeletal system has also other elements whose main function is to connect different elements within the system. The most important properties of these elements are their resistance to tension and their elasticity (i.e., their capacity to return to their original shape after being deformed by a load; Hildebrand and Goslow 2001). These elements are made of connective tissue with packages of collagen and elastic fibres (Hildebrand and Goslow 2001). The elements that connect the muscles with the bones are called tendons. Aponeuroses are a special type of tendon, as they are shaped as a flat sheet to distribute the tension forces over broad areas (Hildebrand and Goslow 2001). Sometimes, muscles are not connected directly to bones; instead, they are attached to other muscles. In these cases the connective tissue is called fascia. Finally, the tissues that connect two bones, usually close to bone joints, are called ligaments, which are predominantly elastic (Hildebrand and Goslow 2001).

The most important function of tendons (including aponeuroses and fascia) is to transmit the forces generated by the muscles to the bones (or to other tissues). For this reason, they play a key role during active movement. In contrast, the main functions of the ligaments are to keep together the skeleton and limit the movement of bone joints (Hildebrand and Goslow 2001).

1.3.1.4. Joints

There are several types of joints in the skeleton of vertebrates. Diarthroses are the most important in the appendicular skeleton. These joints allow the free movement of one bone with respect to other (Hildebrand and Goslow 2001). The parts of the bones in connection are the articular surfaces, which form a joint cavity filled with synovial fluid and are covered by a joint capsule (Figure 1.8). Some joints have also a meniscus, which is a pad of cartilage that facilitates the movement between the two articular surfaces (Hildebrand and Goslow 2001). All these characteristics represent adaptations in tetrapods to facilitate movement and body support under the action of gravity, in contrast with aquatic locomotion (Archer et al 2007).

Several types of diarthroses can be distinguished according to their shape. The most important of the appendicular skeleton of tetrapods are, following Hildebrand and Goslow (2001):

- Hinge joint: a more or less cylindrical head that rotates in a cylindrical socket. Thus, rotation occurs almost exclusively in one plane. Elbow joint of cursorial mammals can be placed into this category.
- Ball-and-socket joint: a spherical head rotates in a spherical socket. The range of motion is wider than in hinge joints, and rotation can occur in two or more planes. Hip joint is an example of this type of joints.

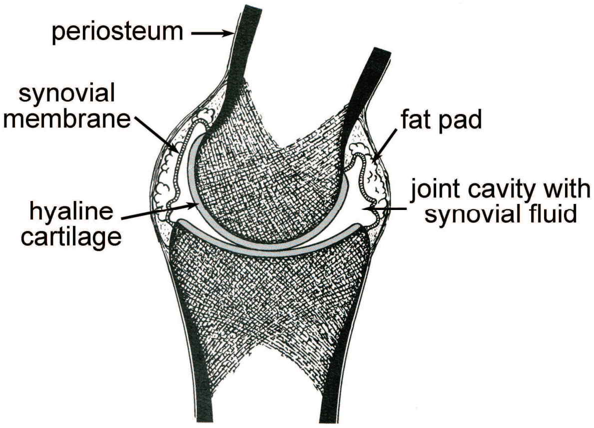


Figure 1.8. Basic structure of a diarthrosis. Modified from Hildebrand and Goslow (2001).

- Pivot joint: this kind of joint allows rotation of a bone around its own axis. The movement of the manus around the radial styloid process fits within this category.

Apart from diarthroses, some appendicular bones also have less mobile joints or amphiarthroses. This type of joints allows some movement but the bones are strongly connected by fibrous cartilage. The pelvic symphysis or the connection between the elements of the zeugopodium (radius-ulna and tibia-fibula joints) are within this category (Hildebrand and Goslow 2001).

1.3.2. Mechanics of motion

The musculo-skeletal system works as a complex mechanism of bony levers activated by muscles (Hildebrand 1985). As such, the principles of lever mechanisms can be applied to understand the function of different morphologies (bone shapes and muscle insertions) and their adaptive meaning.

A lever is a physical system composed of a solid structure fixed in a point of rotation or fulcrum, which experiences an input force (F_i) that generates a resultant output force (F_o) that overcomes a resistance (Figure 1.9A), and hence, produces movement (Hildebrand 1985). The distance between the fulcrum and the point of application of the input force is the input lever arm (L_i) and the distance between the fulcrum and the point of output force is the output lever arm (L_o) (Hildebrand and Goslow 2001).

Depending on the relative position of each element, three classes of levers can be distinguished (Hildebrand and Goslow 2001):

- Class I: the fulcrum is located between the input force and the output force (Figure 1.9A top).
- Class II: the output force is located between the fulcrum and the input force (Figure 1.9A centre).
- Class III: the input force is located between the fulcrum and the output force (Figure 1.9A bottom).

The general laws that govern the function of a lever are (Hildebrand 1985):

$$F_iL_i = F_oL_o \tag{1}$$

$$V_iL_o = V_oL_i \tag{2}$$

where V_i and V_o are the velocity of the in and out levers, respectively. The product of each force by its lever arm is the moment or torque (τ).

These laws can be used to study the mechanical properties of different traits of the musculo-skeletal system (Hildebrand 1985). As the muscles are the elements that primarily exert the forces, they always function as the input force, while the bones function as the passive elements of the lever. Bone joints are either the

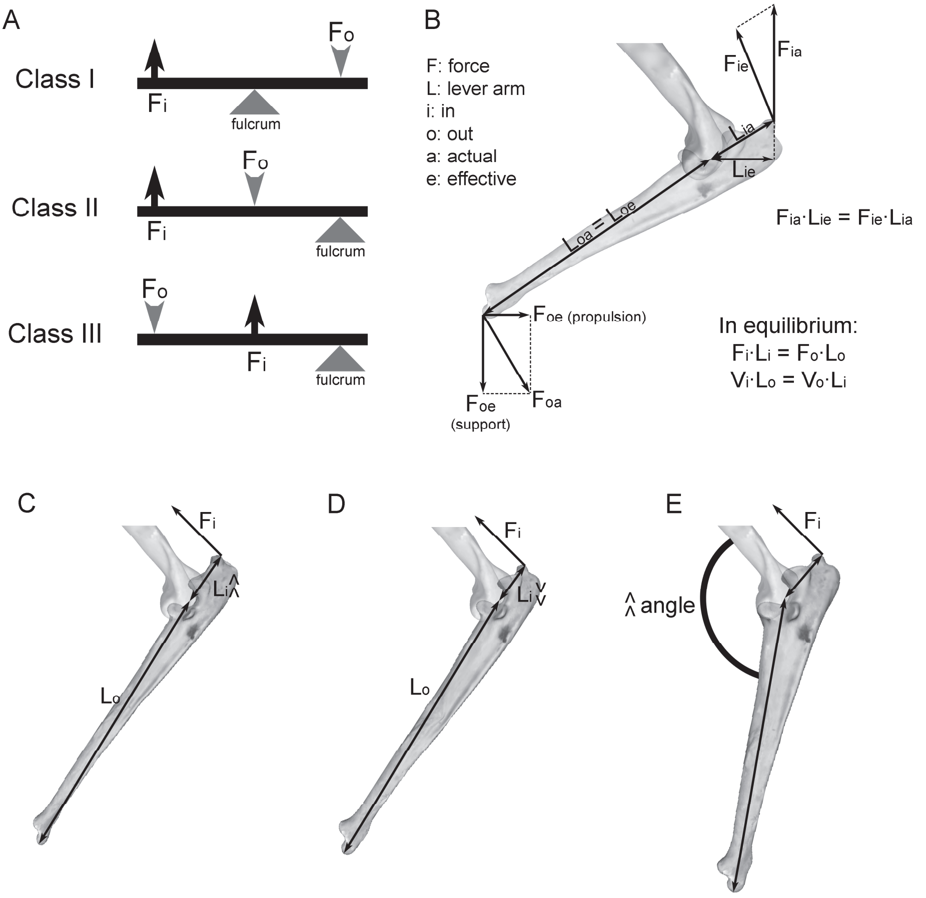


Figure 1.9. Lever mechanics. A, the three classes of levers according to the relative position of the input and output forces; B, relevant components of a biological lever and general laws that rule its function (modified from Hildebrand 1985); C, D, E, different examples of lever mechanisms with a long input arm (C), a short input arm (D), and an input arm not parallel to the output arm (E).

fulcrum or the point where the movement or outpout force is transmitted to another bone (Fig. 1.9B).

The effective mechanical advantage (EMA) can be calculated as the ratio L_i/L_o. It is one of the most important factors influencing the effectiveness of a lever (Hildebrand and Goslow 2001). If EMA > 1, it implies that the output force generated is larger than the input force applied. In contrast, if EMA < 1, this means that the output force is smaller than the input force. Therefore, at equal input force, those changes in the shape of the bone or muscle insertions that make the input lever longer (Figure 1.9C) increase the output force (equation 1) but decrease output velocity (equation 2) (Hildebrand and Goslow 2001). For this reason, those lever systems adapted to exert large forces have usually long input levers. For example, the olecranon process of diggers is relatively long because it is the input lever for the forearm extensor muscle (triceps brachii), which is the most important movement when digging (Hildebrand and Goslow 2001). In contrast, those changes that reduce the input lever increase the output velocity at the expense of a reduced output force. In addition, this change also enhances energetic efficiency, as the length of contraction of the muscle (i.e., the difference between its resting length and contracting length) can be shorter to move the output lever for a given distance. Taking the same example exposed above, a reduced olecranon process (Fig. 1.9D) implies a short input lever for the triceps brachii, which is useful for long-distance runners (e.g., cursorial carnivores such as pack-hunting canids), as they need to reach high speeds with low energy consumption (Hildebrand and Goslow 2001).

Another important factor that greatly influences the function of bone levers is the angle of application of the forces (Hildebrand 1985). In this sense, we can differentiate actual and effective forces (Figure 1.9B). The actual force is the force generated by muscle contraction and is always applied following the major axis of the muscle. In contrast, the effective force is the portion of the actual force that is perpendicular to the movement of the input lever (Hildebrand 1985). Thus, when the actual force is applied perpendicular to the input lever, both the actual and effective forces are equal, which is the most efficient situation. For this reason, many morphological changes of the appendicular bones and their muscles tend to orientate the input forces perpendicular to the movements that they generate (Hildebrand and Goslow 2001). For example, a caudally bended olecranon process (Figure 1.9E) maximizes the effective force of the triceps brachii with the forearm more extended in comparison with a straight olecranon process (Figure 1.9C). The first case is more efficient for animals with a more upright posture, whereas the second one is more effective for animals with a more crouched posture (Hildebrand and Goslow 2001).

Although the basic lever mechanisms are easy to understand, the appendicular musculo-skeletal system is a complex combination of levers, forces, and joints, which is more difficult to study (Hildebrand 1985).

One of the complications is that several muscles can act to produce the same movement (synergistic muscles), but with different angles and torques. Broadly speaking, we can differentiate “high-gear” and “low-gear” muscles. The first have short input levers (Figure 1.10) and produce comparatively small forces but with high velocity and little energy consumption. In contrast, “low-gear” muscles have long input levers (Figure 1.10) and generate large forces but at the expense of low velocity and high-energy consumption (Hildebrand 1985). Although both kinds of muscles are usually activated during normal activities, one of them can be enhanced for some activity. For example, “low-gear” muscles are more suitable during acceleration or jumping, whereas “high-gear” muscles are more suitable during long-distance running (Hildebrand and Goslow 2001). In addition to input levers differences, synergistic muscles can have also different directions of forces, so they are activated in different moments during the movement of a joint to provide higher effective forces.

Another aspect that complicates the biomechanical study of the musculo-skeletal system is the presence of multiple-joint muscles, that is, muscles that pass through two or more joints (Hildebrand 1985). Although the activation of these muscles can potentially move all the joints that they pass through, their actual effect cannot be easily determined, as it depends on the action of other muscles

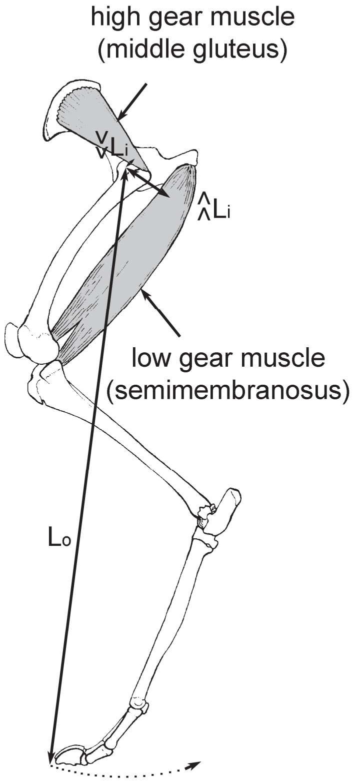


Figure 1.10. Two muscles that perform the same movement with different mechanical properties. Modified from Hildebrand and Goslow (2001).

and the degree of rotation of each joint (Hildebrand 1985). However, multiple-joint muscles provide several mechanical advantages, because they transmit the movement from proximal muscles to distal bones, thus allowing a more coordinated movement of the limbs and saving energy (Hildebrand 1985; Prilutsky and Zatsiorsky 1994).

Finally, there is a passive mechanism that also helps to move the limbs during locomotion. As we explained above, tendons are particularly elastic, so they can potentially act as springs when they are loaded (Alexander 2003). The mechanism functions as follows: in each stride, when a limb lands on the ground with the extensor muscles contracted, their tendons are stretched. In this way, they store elastic potential energy. When the limb takes off, this energy is released (i.e., transformed into kinetic energy), which contributes to the extension movement in this phase of the stride. This mechanism is very important to save energy during locomotion in cursorial mammals, such as ungulates (Hildebrand and Goslow 2001).

1.3.3. Terrestrial locomotion

In this section I will introduce the outcome of the mechanisms that generate the movement of the musculo-skeletal system during terrestrial locomotion. In this kind of behaviour, animals can move using different gaits. Following Hildebrand (1985), a gait is a "regularly repeated sequence and manner of moving the legs in walking and running". Quadrupedal mammals can adopt a wide array of gaits depending on their size, on the characteristics of their limbs (e.g., limb proportions or foot posture) and on the speed that they achieve, as some gaits are used for slow speeds and others for high speeds. Two main types of gaits can be distinguished: symmetrical and asymmetrical. In symmetrical gaits, both feet of each pair (fore- and hind limb) touch the ground evenly spaced in time (Hildebrand 1985). These gaits are used at low or moderately high speeds; pace and trot are among the most common symmetrical gaits (Figure 1.11). Asymmetrical gaits have, for both fore- and hind limbs, a leading foot and a trailing foot (not evenly spaced). The trailing foot touches the ground first in time but the leading foot achieves a more advanced position (Hildebrand 1985). Asymmetrical gaits are selected to achieve higher running speeds (Figure 1.12). The gallop is the most usual in large quadrupeds (e.g., horses and wolves) and the bound or half bound are characteristic of small animals (e.g., many rodents, rabbits and weasels).

There are several factors that influence gait selection in quadrupeds:

- Cost of transport: animals tend to use the most efficient gait at the speed selected to reduce the transport costs (Hildebrand and Goslow 2001). Thus, walking gaits, such as the pace (see Figure 1.11), are more efficient at low speeds, but as speed increases the trot and, finally, the gallop become more energetically efficient (Alexander 2003). A low cost of transport favours endurance (i.e., the ability to perform long-distance runs with low energy expenditure). Several morphological changes represent adaptations towards

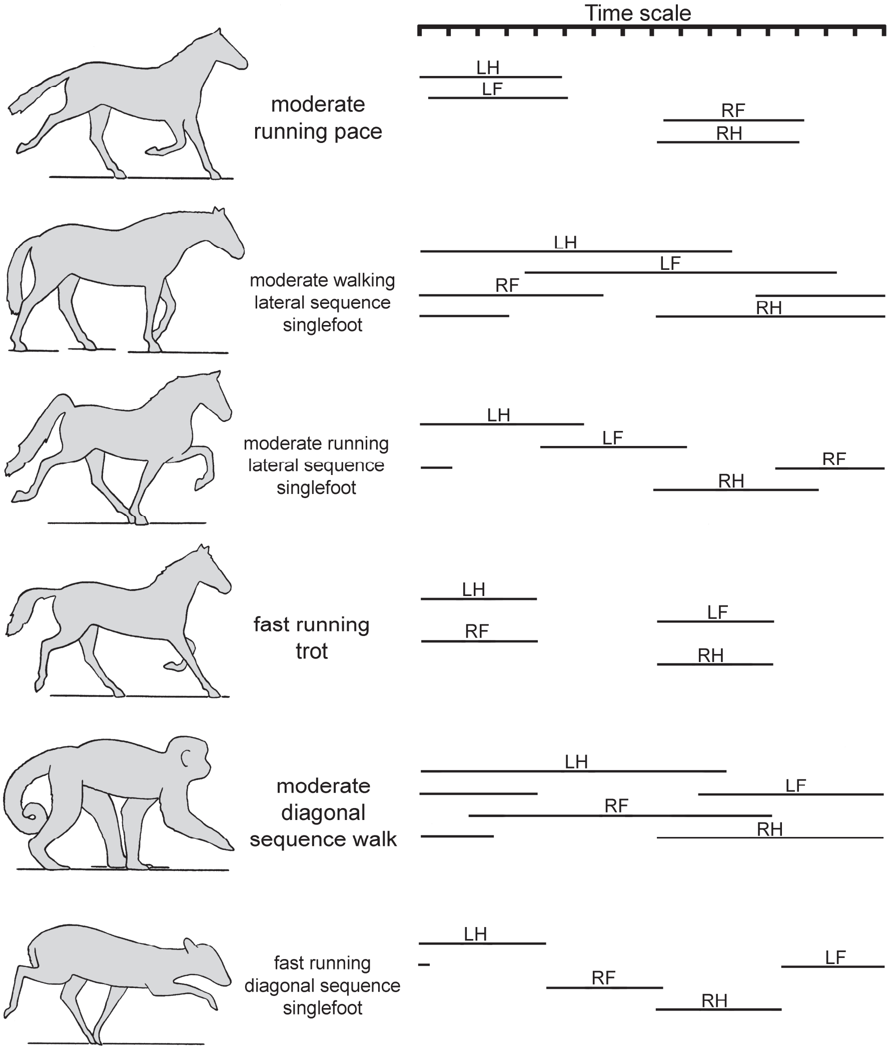


Figure 1.11. Representative symmetrical gaits. Each diagram shows one complete cycle starting when the left hind limb touches the ground. LH, left hind foot; LF, left forefoot; RF, right forefoot; RH, right hind foot. Modified from Hildebrand and Goslow (2001).

a reduced cost of locomotion and high endurance: longer and more slender limbs, which reduces the mass of the limb and increases the out-lever velocity; a more upright posture, which increases stride length; stronger "high-gear" muscles that need shorter and more efficient contractions to generate movement; and longer tendons in the distal part of the limbs to favour storage of elastic energy (Alexander 2003). Those species that present such adaptations are usually called cursorial, for example ungulates and some carnivorans (Janis and Wilhelm 1993).

- Bone loading: gaits are also selected to reduce the stresses over the limb bones in order to avoid bone failure during locomotion (Hildebrand and Goslow 2001). Therefore, the stresses generated are the lowest possible at any given speed (Alexander 2003). However, large peak loads are sometimes unavoidable. This is the case of animals that need to exert large forces with their limbs (e.g., ambush predators when grasping a prey, such as the tiger, or fossorial mammals when digging, such as the badger). Therefore, these animals need a number of skeletal adaptations to perform and withstand large peak stresses in their limbs. For example, they usually have robust bones to withstand large loads; they maximize strength by having comparatively short out-lever arms and long in-lever arms (which increases strength at the expense of velocity); and they also have "low-gear" muscles well developed (Hildebrand and Goslow 2001). As it can be deduced, these adaptions are the opposite of those necessary for reducing locomotion costs. Thus, there is a trade-off between energetic efficiency and resistance to stresses, as animals cannot improve both factors at the same time.
- Stability and manoeuvrability: these factors are also very important during locomotion. They depend on multiple aspects. First, the more time the feet spend on the ground the more stability they have. Therefore, stability decreases as speed increases (Hildebrand 1985). This can be compensated by having a centre of mass positioned as close to the ground as possible. In addition, some gaits provide more stability than others: for example, those gaits that maintain the centre of mass within the polygon created by the feet on the ground (Hildebrand 1985) are the most stable. In contrast, manoeuvrability is favoured when the centre of mass is elevated and when the areas of support are small, because they increase oblique thrust and, hence, changes of direction (Hildebrand 1985).

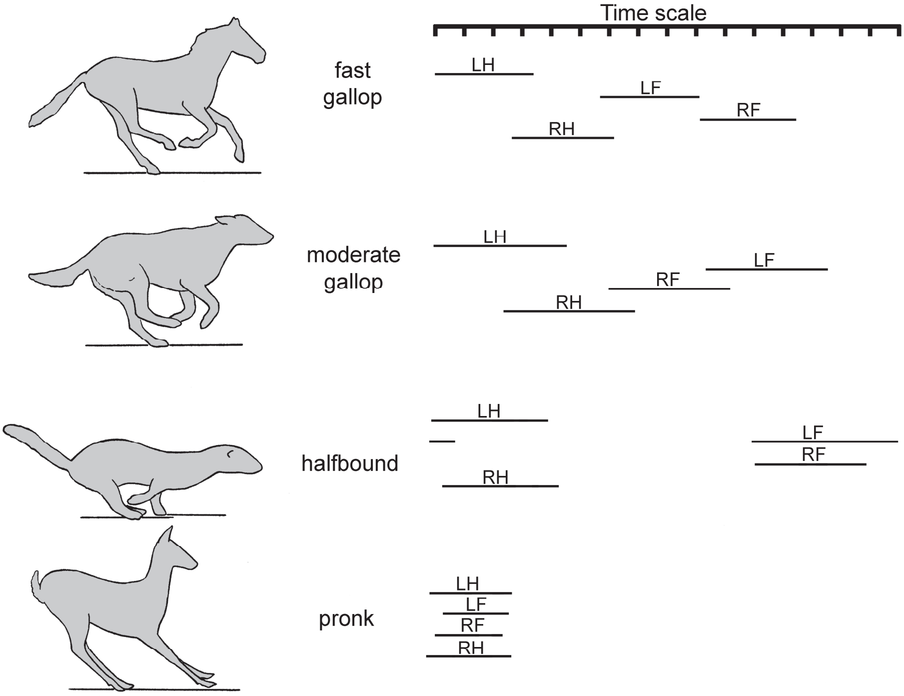


Figure 1.12. Representative asymmetrical gaits. Each diagram shows one complete cycle starting when the left hind limb touches the ground. LH, left hind foot; LF, left forefoot; RF, right forefoot; RH, right hind foot. Modified from Hildebrand and Goslow (2001).

1.3.4. Scaling and allometry

Many aspects of biomechanics and locomotion are greatly influenced by body size, including bone shape, limb posture, and gait selection. For this reason, the effects of body size on limb morphology and locomotor performance deserve special attention.

The term scaling refers to the relationship between body size and body proportions (Hildebrand and Goslow 2001). Scaling can be studied at different levels: ontogenetic scaling refers to the proportional growth of different parts of the body during the life of an individual; static scaling applies to the changes in body proportions among several organisms that belong to the same species and are at the same growth stage; and interspecific scaling refers to changes in body proportions among several species of different body size that belong to the same lineage or taxon. There are two cases of scaling regardless the level of study:

isometry (i.e., if body size changes do not imply changes in body proportions) and allometry (i.e., if body proportions change when body size increases) (Kardong 2006).

Although there are many cases of isometry in nature, isometric growth of bones implies that, given any increase of bone length (L), bone area will scale to L^2 and bone volume (or mass, keeping density constant) will scale to L^3 (Huxley 1932; Gould 1966). Therefore, as bone size increases the ratios length/area, length/mass and area/mass decrease, which unbalances the relationship between the biological properties that depend on them. For example, if we focus on the biomechanical aspects of locomotion, we find that bone loading depends directly

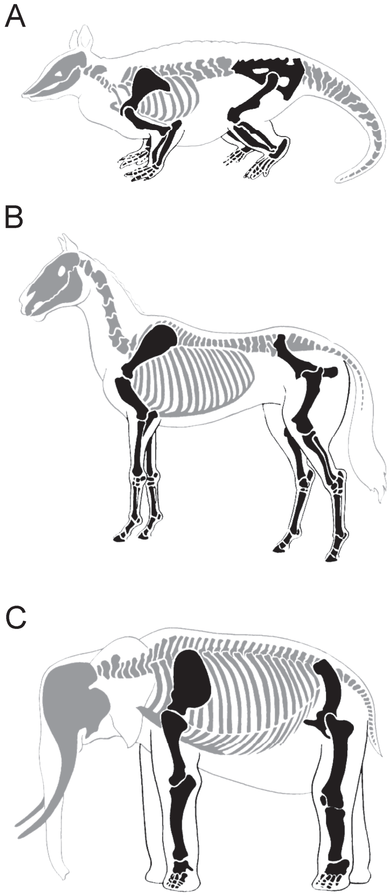


Figure 1.13. Three schematic models showing the differences in limb posture from small to large mammals. A, armadillo; B, horse; C, elephant. Modified from Radinsky (1987).

on body mass (L^3), whereas bone resistance largely depends on bone cross-sectional area (L^2). Thus, if bones scale isometrically, animals would reach a size in which their bones would not be able to withstand the stresses posed by body mass and would collapse under their own weight. For this reason, bones must scale allometrically to keep bone stresses within similar levels regardless of body mass (McMahon 1973; Alexander 1985, 2003). McMahon (1973) proposed a rule of elastic similarity, which implies that bone diameters scale differently than bone lengths ($D = L^{3/2}$) to keep constant elastic deformations as size increases. However, many studies in the last decades have demonstrated that although larger animals usually have more robust limb bones, they do not follow a single rule (Biewener 1983; Bertram and Biewener 1989; Alexander 2003). Furthermore, to increase bone robustness is not the only mechanism to reduce the stresses produced by large body size. In this sense, Biewener (1983) showed that acquiring a more upright posture is also an extended mechanism in mammals to reduce bone loading (Figure 1.13) for two reasons: *(i)* because it increases the effective mechanical advantage (EMA) without increasing muscle input forces, which reduces bone loading, as well as the muscular energy consumption required to withstand body weight (Biewener 1983); and *(ii)* given that bones can withstand better axial stresses than bending stresses, the upright posture reduces the former ones (Biewener 1983). The most extreme case of an upright posture occurs, not surprisingly, in the largest terrestrial mammals (e.g., elephants), which are called graviportal (Hildebrand and Goslow 2001). Their limb bones are almost completely aligned (joint angles near 180º), so the bending stresses they withstand are negligible.

These morphological or postural changes also produce changes in animal locomotion and gait selection. The crouched posture of small animals gives them more agility and readiness to escape quickly from predators (Biewener 1990). They usually move with a poorly efficient bound or with half-bound gaits (Hildebrand 1985). In contrast, large animals with an upright posture have less agility and readiness (Biewener 1990), but they reduce bone loading and cost of transport (Hildebrand and Goslow 2001). For example, some graviportal animals are not able to perform running gaits, instead, they use walking gaits even when they move with high speed.

1.4. Evolution of locomotor performance in carnivorans

1.4.1. Locomotor adaptations in extant carnivorans

The order Carnivora includes 286 species distributed among 16 major lineages (Figure 1.14; Nyakatura and Bininda-Emonds 2012). They encompass a wide array of locomotor modes and habitats, from fully aquatic species like pinnipeds to arboreal species like the kinkajou (*Potos flavus*). This variety of locomotor adaptations has led to a number of morphological changes in their skeleton, especially in the appendages. In what follows I provide an overview of the main locomotor modes of fissiped (terrestrial) carnivorans and the morphological changes associated with them. Fully aquatic carnivorans or pinnipeds (families Phocidae, Otariidae and Odobenidae) were excluded from this PhD dissertation.

Together with their specific locomotor modes, many carnivorans display specific hunting behaviours that are closely related with locomotion: for example, pursuit predators are well adapted to perform long runs. Therefore, the appendicular skeleton also plays an important role in this activity, which imposes additional adaptations that have also shaped its morphological evolution.

1.4.1.1. Cursorial and sprinter carnivorans

The term cursorial broadly refers to those species adapted to run. However, different authors have defined the term differently, using behavioural, ecological and/or morphological criteria. The biomechanical and morphological aspects usually found in cursorial mammals, including carnivorans, are those that enhance

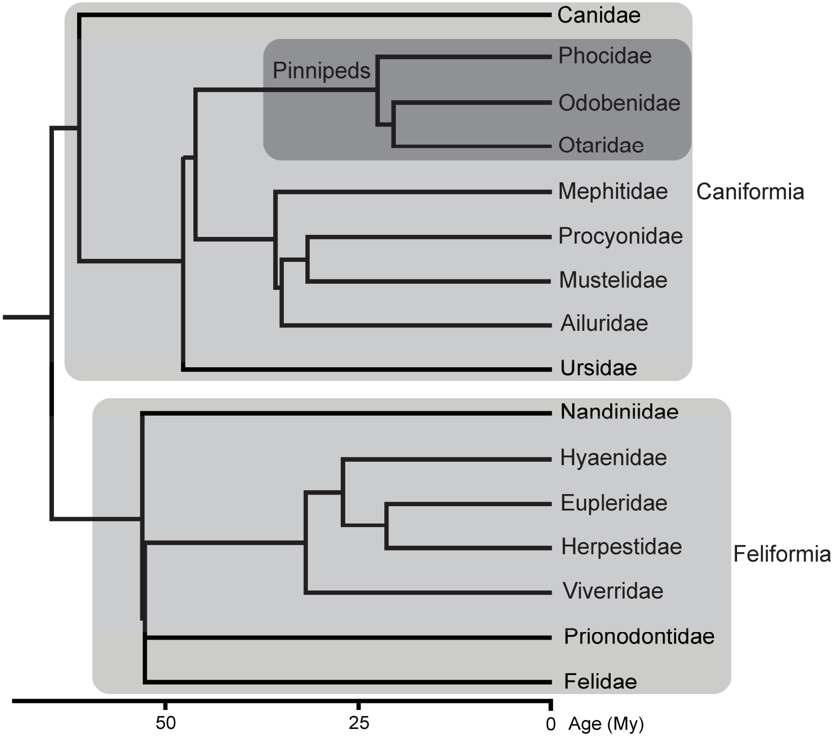


Figure 1.14. Phylogenetic tree of modern carnivoran families. Branch lengths proportional to time of divergence estimated from molecular data. Modified from Nyakatura and Bininda-Emonds (2012).

speed and endurance during running, for example the presence of gracile limbs to reduce limb weight, an upright posture to increase stride length, increased out-velocities at the expense of out-forces, “high-gear” muscles more developed to save energy, and limb movements restricted to flexion/extension in the parasagittal plane (Jenkins 1974; Jenkins and Camazine 1977; Taylor 1989; Steudel and Beattie 1993; Janis and Wilhelm 1993; Stein and Casinos 1997; Hildebrand and Goslow 2001). Among carnivorans, the living hyaenids, most canids and the cheetah all fall within this category. However, there are some discrepancies regarding felids, as they are considered cursorial by several authors (e.g., Jenkins and Camazine 1977; Taylor 1989). However with the only exception of the cheetah, large felids have powerful limbs that provide rapid acceleration for short distances (Taylor 1989), which implies opposite biomechanical necessities than those of “true” cursorial species (see also the previous section).

In addition, the modes of locomotion of carnivorans are closely related to their hunting strategies: for example, most cursorial species are either pounce or pursuit predators (sensu Van Valkenburgh 1985; Janis and Figueirido 2014) that do not use their forelimbs regularly to catch prey. In this way, they can hunt small prey stalking and pouncing over them (Van Valkenburgh 1985; Harris and Steudel 1997; Figueirido and Janis 2011; Janis and Figueirido 2014), such as most foxes do, or they can be pack hunting predators that chase large prey over long distances to exhaustion, such as the grey wolf or the African painted dog do (Van Valkenburgh 1985; Andersson 2005). In contrast, sprinting carnivorans are more likely ambushing predators, as they ambush until they are close to their prey and use a burst of high speed to reach them. This predatory behaviour implies an important use of their forelimbs to grapple and subdue their prey before the killing bite is performed (Anyonge 1996; Meachen-Samuels and VanValkenburgh 2009).

1.4.1.2. Climbing and arboreal carnivorans

This mode of locomotion includes those carnivorans that usually live on trees (i.e., arboreal species) and those that live on the ground but climb trees regularly (i.e., scansorial species) (Hildebrand and Goslow 2001). This mode of locomotion usually involves a high degree of limb mobility, which allows movements of supination/pronation and abduction/adduction, as this is necessary when walking along narrow branches (Taylor 1974). The manus, and sometimes also the pes, is usually broad, with long phalanges that allow grasping branches (Taylor 1974). Flexor muscles, such as the biceps brachii, play an important role during vertical climbing, so they are also enhanced (Taylor 1989).

Additionally, this type of locomotion is also closely related with the ability to manipulate food items, as the morphological adaptations for both activities are very similar, including enhanced pronation-supination movements of the forelimb and grasping ability (Fabre et al. 2013). Arboreal carnivorans tend to be small, as they usually belong to lineages of small-sized species as procyonids, mustelids and viverrids. However, some ursids and felids have well-developed scansorial abilities (Van Valkenburgh 1987).

1.4.1.3. Fossorial and semiaquatic carnivorans

Fossorial carnivorans (sometimes also called semi-fossorial; Van Valkenburgh 1987) are those that usually dig with their feet and excavate burrows using or not pre-existing holes (Taylor 1989). To perform this activity, fossorial carnivorans need to generate enough force with their forefeet to be able to scratch hard soils (Taylor 1989). Therefore, their forelimbs are usually powerful with large in-lever arms (e.g., a long olecranon process) and heavily muscled (Taylor 1989). Accordingly, their bones are robust and massive (Taylor 1989). Their forefeet are equipped with long and curved claws (Taylor 1989; Van Valkenburgh 1987). Their hind limbs are not as specialized as their forelimbs, because their function during digging is secondary (Taylor 1989). Most fossorial carnivorans are mustelids (e.g., European and American badgers), but some viverrids and herpestids can also excavate burrows, although their adaptations are not as extreme as the ones shown by badgers (Taylor 1989).

Semiaquatic carnivorans are those species that are adapted to move and hunt in water but also are capable to move on the ground. Otters show the highest degree of adaptation to move in water (Taylor 1989). These species can swim using an undulatory movement, in which the posteriorly extended hind limbs and tail produce the forward thrust, but they can also swim by propelling with their hind limbs and manoeuvring with the forelimbs (Taylor 1989). The morphological adaptations to this mode of locomotion involve short and mobile forelimbs to reduce turbulence and drag (Taylor 1989). The zeugopod and metapods of the hind limb are relatively longer in comparison with those of the forelimb. Of course, the digits of the hind limb are elongated and have a web of skin between them to increase the propulsive surface (Taylor 1989).

1.4.2. Ecomorphological inferences in extinct carnivorans

All the morphological features that are associated with different locomotor modes have been used as morphological indicators to infer this ecological aspect in extinct taxa. In carnivorans, the starting point is probably represented by the papers of Van Valkenburgh (1985, 1987). In these studies, Van Valkenburgh related a number of morphometric indices with the biomechanical and functional aspects of the various locomotor modes performed by the modern carnivorans and then used these indices to infer the habitat of extinct taxa (Van Valkenburgh 1985) or their locomotor behaviour (Van Valkenburgh 1987). Since then, paleobiologists have used a number of morphometric and statistical approaches to infer different aspects related to locomotion, hunting strategies or habitat use in extinct carnivorans, such for example, the metatarsal/femur ratio (Janis and Wilhelm 1993), brachial and crural indices (Palmqvist et al. 2003) or the shape of the elbow joint (Andersson and Werdelin 2003; Andersson 2005; Figueirido and Janis 2011; Janis and Figueirido 2014) among others (MacLeod and Rose 1993; Anyonge 1996; Schutz and Guralnick 2007; Meachen-Samuels and Van Valkenburgh 2009; Polly 2010; Lewis and Lague 2010; Meloro 2011; Meachen-Samuels 2012; Meloro et al. 2013; Samuels et al. 2013).

One of the most recurrent issues that have been investigated by some of these authors is the adaptation towards a cursorial mode of locomotion and the appearance of pursuit predators during the evolution of carnivoran groups. With this purpose, Janis and Wilhelm (1993) used the metatarsal/femur ratio as a proxy for cursoriality in Cenozoic ungulates and carnivores. They found that cursorial ungulates appeared much earlier than pursuit carnivorans, which does not support the hypothesized “arms race” between prey and their predators (Janis and Wilhelm 1993). Indeed, according to their results, long-legged pursuit predators, such as wolves, are a very recent (Pleistocene) phenomenon. Andersson and Werdelin (2003) reached similar conclusions by analyzing the shape of the anterior surface of the humerus distal epiphysis (the elbow-joint): box-like articular elbows, typical of cursorial carnivorans, have lost the ability to supinate and are present only in recent canids, hyaenids and the cheetah. In contrast, most Cenozoic predators retain the supination ability, which indicates that they were not adapted to a cursorial mode of locomotion. In spite of this, other authors have suggested that some amphicyonids and hemicionine ursids would have been partially or totally cursorial (Hunt 1998a, 1998b, 2009; Van Valkenburgh 1999).

Another issue that has also received considerable attention is the adaptation to grapple and subdue prey with the forelimbs, a behaviour that is typical of ambushing predators (e.g., Anyonge, 1996; Antón et al. 2004; Meachen-Samuels and Van Valkenburgh 2009; Figueirido and Janis 2011; Meachen-Samuels 2012; Janis and Figueirido 2014). Several lineages developed ambushing strategies, although with some differences (Van Valkenburgh 1999). One remarkable case is the saber-tooth ecomorph, which has appeared at least three times among carnivorans (Morlo et al. 2004; Van Valkenburgh 2007): nimravids (family Nimravidae), barbourofelids (family Barbourofelidae) and machairodontine felids (subfamily Machairodontinae). Their enlarged upper canines allowed them to perform an almost instantaneously killing bite in the prey's throat, but their shape made them vulnerable if they contacted with bone (Van Valkenburgh and Ruff 1987; Slater and Van Valkenburgh 2009). For this reason, many saber-toothed cats had strikingly robust forelimbs to immobilize their prey before performing the killing bite (Van Valkenburgh and Ruff 1987; Anyonge 1996; Turner and Anton 1997; Meachen-Samuels and Van Valkenburgh 2010; Meachen-Samuels 2012). In contrast, conical-toothed ambushers, such as modern felids, do not need extremely powerful limbs, because their canines are not so fragile and they can subdue their prey combining the action of their forelimbs with a bite in the throat or muzzle to produce suffocation (Salesa et al. 2005). Other extinct carnivorans that have been hypothesized to be ambusher or "cat-like" predators are some hesperocyonine (Hesperocyoninae, Canidae) and borophagine (Borophaginae, Canidae) species (Wang and Tedford 1996; Munthe 1998; Van Valkenburgh et al. 2003; Andersson 2005). The case of borophagines is of special interest, as they reached an important morphological and body size diversity during the Cenozoic, with a number of species developing skull adaptations for bone-cracking, as in the living hyaenids (Werdelin 1989).

1.5. Objectives

In this section I will enumerate the specific objectives addressed during the development of this PhD thesis.

- To characterize the shape of the girdles and the major limb bones of the appendicular skeleton of living and extinct fissiped carnivorans, three-dimensional landmarks and geometric morphometric methods were used. The bones selected for these analyses were the scapula, humerus, radius and ulna for the forelimb, and the pelvic bones, femur and tibia for the hind limb.
- To explore the allometric effects (i.e., size-related shape changes) on the shape of the appendicular bones.
- To assess if bone shape variation is influenced by the phylogenetic relationships among the taxa studied.
- To test the influences of locomotor performance and behaviour on the shape of the appendicular bones. In order to address this objective, maximum running speed and daily movement distance were used as proxies for locomotor performance and their correlations with bone shape variation were quantified. Locomotor behaviour was tested using a categorical classification and the morphological differences between the categories compared were explored.
- To explore the morphological variability of appendicular bones, paying special attention to the evolutionary pathways followed by the different carnivoran families and how their shape variability is influenced by phylogenetic inheritance or by other constraints.
- To investigate the pattern and degree of morphological integration between the appendicular bones, both within limbs and between limbs.
- To test the influence of functional specialization on the degree of morphological integration between the appendicular bones or if those

species with both limbs specialized towards similar functions show also a higher degree of integration between limbs.

- To explore how variation in limb bone shape is influenced by different ecological aspects, such as modes of locomotion, hunting strategies or habitat. For this purpose, the forelimb was selected given that it is more involved than the hind limb in different locomotor activities, such as manipulating food or subduing prey.
- To select those ecological aspects that are better reflected in bone morphology, in order to assess the functional and biomechanical use of these features as ecomorphological indicators.
- To use borophagine canids (subfamily Borophaginae, Canidae) as a potential example of paleobiological studies in which these morphological indicators can provide inferences on life styles and habitat predictions for extinct carnivorans. This taxon is a good choice for this purpose because it is completely extinct and achieves a high taxonomic diversity and a high disparity of sizes and morphologies during the Cenozoic.

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2. Material and methods

2.1. Morphometric data acquisition

In this section I will offer a general overview of the criteria used of data collection, including the institutions where the specimens studied came from, a specific description of the number of appendicular elements collected, as well as the morphometric and statistical procedures used in all the articles. Therefore, the main aim of this section is to provide to the readers a very general and intuitive idea on the methods and procedures followed to test my hypotheses, because a detailed mathematical description of the methods used is far beyond the scope of this dissertation.

2.1.1. Sample

The major appendicular bones are the scapula, humerus, radius and ulna for the forelimb, and the pelvis (half-pelvis or innominate bone), femur and tibia for the hind limb. The autopodium was not included in this study because it is a complex structure with multiple small bones (podial bones, metapods, and phalanges) whose study would have involved an oversized number of analyses. Indeed, this structure deserves in the future an in-depth study.

I collected 134 living specimens distributed over 46 species (Figure 2.1A, B; Table 2.1). The species studied belong to seven families (Figure 2.1A, B): one ailurid (Ailuridae), 17 canids (Canidae), three mustelids (Mustelidae), four procyonids (Procyonidae), eight ursids (Ursidae), 10 felids (Felidae), and three hyaenids (Hyaenidae). My main focus of interest were those Carnivoran families that today include large representatives (i.e., canids, ursids, felids, and hyaenids), although I tried to cover a higher spectrum of morphological and ecological variability by analysing some mustelids, ailurids and procyonids, as their members include several arboreal, semiaquatic and semifossorial species.

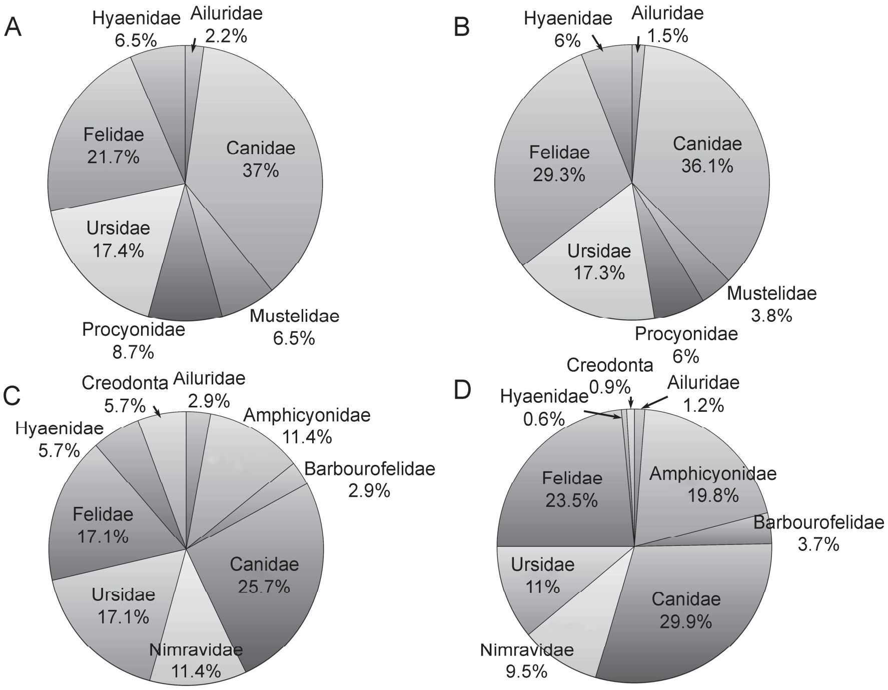

Figure 2.1 Sample of living and extinct carnivorans (plus creodonts) included in this thesis. Percentages of extant species (A) and specimens (B) among carnivoran families, and percentages of extinct taxa (C) and fossil specimens (D) among carnivoran families and the order Creodonta.

Each bone was considered separately for the extinct taxa studied. The reason is that it is not frequent to find anatomically connected skeletons in the fossil record, which implies that most of the bones could not be assigned to the same individual: four scapulae, 51 humeri, 97 radii, 52 ulnae, seven pelvises, 58 femora, and 61 tibiae (Table 2.2). In some cases, the fossil specimens were not identified at the species level; instead, they were only classified at the genus-level. Therefore, in order to include the highest number of specimens as possible, I grouped together them averaging by genus. Although this procedure may reduce the accuracy of the results, in my opinion this does not have a significant effect because I looked for general patterns in carnivorans and not for minor differences within species or genera.

Table 2.1. List of specimens analysed of the living species included in this thesis. Host institution and identity number (ID) are indicated. AMNH, American Museum of Natural History (New York); NHM, Natural History Museum (London). * Indicates a specimen in which a bone was absent (S, scapula; P, pelvis; T, tibia).

Species	ID	Host Institution
Acinonyx jubatus	119654	AMNH
Acinonyx jubatus	119655	AMNH
Acinonyx jubatus	119656	AMNH
Acinonyx jubatus	1940.1.20.17	NHM
Acinonyx jubatus	1962.7.6.15	NHM
Ailuropoda melanoleuca	147746	AMNH
Ailuropoda melanoleuca	110454	AMNH
Ailuropoda melanoleuca	89028	AMNH
Ailuropoda melanoleuca (S*)	89030	AMNH
Ailurus fulgens	185346	AMNH
Ailurus fulgens	80164	AMNH
Bassariscus astutus	182560	AMNH
Canis adustus	114174	AMNH
Canis aureus	187714	AMNH
Canis aureus	54516	AMNH
Canis latrans	123183	AMNH
Canis latrans	1317	AMNH
Canis latrans	1316	AMNH
Canis latrans	141170	AMNH
Canis latrans	141153	AMNH
Canis lupus	98226	AMNH
Canis lupus	98227	AMNH
Canis lupus	98225	AMNH
Canis lupus	134941	AMNH
Canis lupus	134942	AMNH
Canis mesomelas	34734	AMNH
Canis mesomelas	187712	AMNH
Canis mesomelas	187713	AMNH
Canis mesomelas	114228	AMNH
Canis simensis	81001	AMNH
Cerdocyon thous	134049	AMNH
Cerdocyon thous	214709	AMNH
Cerdocyon thous	214703	AMNH
Cerdocyon thous	209123	AMNH
Cerdocyon thous	209128	AMNH
Chrysocyon brachyurus	133941	AMNH
Chrysocyon brachyurus	133940	AMNH
Crocuta crocuta	35358	AMNH
Crocuta crocuta	83593	AMNH
Crocuta crocuta	52097	AMNH
Crocuta crocuta	187769	AMNH
Crocuta crocuta	187776	AMNH
Cuon alpinus	102083	AMNH
Cuon alpinus	54976	AMNH
Cuon alpinus	54984	AMNH
Cuon alpinus	54842	AMNH
Eira barbara	214736	AMNH
Eira barbara	23487	AMNH

Helarctos malayanus	35364	AMNH
Hyaena brunnea	1962-7.23.1	NHM
Hyaena hyaena	244436	AMNH
Hyaena hyaena	54512	AMNH
Leptailurus serval (P*)	119207	AMNH
Leptailurus serval	27837	AMNH
Lutra canadensis	165762	AMNH
Lutra canadensis	182561	AMNH
Lycaon pictus	82085	AMNH
Lycaon pictus	85154	AMNH
Lynx rufus	119206	AMNH
Lynx rufus	208417	AMNH
Lynx rufus	128527	AMNH
Lynx rufus	171361	AMNH
Meles meles	70604	AMNH
Melursus ursinus	150205	AMNH
Melursus ursinus	54465	AMNH
Melursus ursinus	54464	AMNH
Nasua nasua	214722	AMNH
Neofelis nebulosa	238650	AMNH
Nyctereutes procyonoides	249766	AMNH
Nyctereutes procyonoides	249767	AMNH
Otocyon megalotis	233011	AMNH
Otocyon megalotis	63993	AMNH
Panthera leo	1952.10.20.13	NHM
Panthera leo	112.a	NHM
Panthera leo	1857.2.24.1	NHM
Panthera leo	75.1998	NHM
Panthera leo	75.945	NHM
Panthera onca	35571	AMNH
Panthera onca	139959	AMNH
Panthera onca (P*)	135928	AMNH
Panthera onca	1858.5.26.9	NHM
Panthera pardus	209087	AMNH
Panthera pardus	1940.1.20.18	NHM
Panthera pardus (P*)	115p	NHM
Panthera pardus	1851.2.17.3	NHM
Panthera pardus	1940.1.20.20	NHM
Panthera pardus	1849.6.20.2	NHM
Panthera tigris	113743	AMNH
Panthera tigris	113748	AMNH
Panthera tigris	135846	AMNH
Panthera tigris	85404	AMNH
Potos flavus	266597	AMNH
Potos flavus	265959	AMNH
Potos flavus	266599	AMNH
Procyon lotor	173897	AMNH
Procyon lotor	147436	AMNH
Procyon lotor	237438	AMNH
Puma concolor	1335	AMNH
Puma concolor	90213	AMNH
Puma concolor	14026	AMNH
Puma concolor	135341	AMNH
Speothos venaticus	52.1086	NHM

Speothos venaticus (T*)	1966.1.24.1	NHM
Tremarctos ornatus	81.784	NHM
Uncia uncia	207704	AMNH
Uncia uncia	266952	AMNH
Uncia uncia	119662	AMNH
Uncia uncia	100110	AMNH
Urocyon cinereoargenteus	35695	AMNH
Urocyon cinereoargenteus	90134	AMNH
Urocyon cinereoargenteus	148799	AMNH
Urocyon cinereoargenteus	137028	AMNH
Ursus americanus	128521	AMNH
Ursus americanus	98950	AMNH
Ursus americanus	45149	AMNH
Ursus arctos	14054	AMNH
Ursus arctos	135502	AMNH
Ursus arctos	45150	AMNH
Ursus arctos	70254	AMNH
Ursus maritimus	35065	AMNH
Ursus maritimus	31573	AMNH
Ursus maritimus (P*)	215283	AMNH
Ursus maritimus (P*)	75244	AMNH
Ursus thibetanus	70320	AMNH
Ursus thibetanus	80248	AMNH
Ursus thibetanus	23086	AMNH
Vulpes lagopus	28117	AMNH
Vulpes lagopus	28116	AMNH
Vulpes velox	35392	AMNH
Vulpes velox	100215	AMNH
Vulpes vulpes	69550	AMNH
Vulpes vulpes	128487	AMNH
Vulpes vulpes	128486	AMNH

Table 2.2. List of fossil specimens included in this thesis. Host institution and identity number (ID) are indicated. AMNH, American Museum of Natural History (New York); NHM, Natural History Museum (London); NMB, Naturhistorisches Museum (Basel); MNCN, Museo Nacional de Ciencias Naturales (Madrid); MSN, Museo di Storia Naturale (Firenze); SNM, Staten Naturhistoriske Museum (Copenhagen); MCNV, Museo de Ciencias Naturales de Valencia (Valencia).

Species	**Id**	**Host institution**
Scapula		
Amphicyon sp.	Cast	AMNH
Barbourofelis lovei	125115	AMNH
Dinictis sp.	38805 R	AMNH
Dinictis sp.	38805 L	AMNH
Machairodus aphanistus	B-6043	MNCN
Promegantereon ogygia	BAT-1-'05-E3-41	MNCN
Humerus		
Aelurodon ferox	27479	AMNH
Aelurodon taxoides	67481	AMNH
Aelurodon taxoides	30902	AMNH
Aelurodon taxoides	67442	AMNH
Amphicyon sp.	Cast	AMNH
Amphicyon ingens	68118-B	AMNH

Amphicyon sp.	617-27298	AMNH
Amphicyon sp.	68100	AMNH
Amphicyon sp.	68108	AMNH
Amphicyon sp.	68124	AMNH
Barbourofelis lovei	125115	AMNH
Daphoenus sp.	11857	AMNH
Dinictis sp.	125652 L	AMNH
Dinictis sp.	125652 R	AMNH
Dinictis sp.	1396	AMNH
Epicyon haydeni	67403	AMNH
Epicyon haydeni	67603	AMNH
Epicyon saevus	67489	AMNH
Hemicyon urisnus	21101	AMNH
Homotherium crenatidens	7570V	MSN
Ischyrocyon sp.	54220-B	AMNH
Ischyrocyon sp.	68158-A	AMNH
Machairodus sp.	M8960	NHM
Megantereon cultridens	Se311 L	NMB
Megantereon cultridens	Se311 R	NMB
Nimravus sp.	62151	AMNH
Paratomarctus euthos	67536	AMNH
Paratomarctus temerarius	105340	AMNH
Promegantereon ogygia	B-2465	MNCN
Promegantereon ogygia	BAT-1-'03-D4-361	MNCN
Promegantereon ogygia	BAT-3-'08-70	MNCN
Pseudaelurus lorteti	GA5727	NMB
Pseudaelurus sp.	62202	AMNH
Pseudaelurus transitorius	GA5728	NMB
Simocyon batalleri	B-2390	MNCN
Simocyon batalleri	BAT-1'05-C8-22	MNCN
Smilodon ensenadensis	61	MCNV
Smilodon fatalis	LB41	NMB
Smilodon populator	54	SNM
Smilodon populator	2	SNM
Tomarctus sp.	67775	AMNH
Tomarctus sp.	67527	AMNH
Tomarctus sp.	67547	AMNH
Tomarctus sp.	67714	AMNH
Tomarctus sp.	67715	AMNH
Tomarctus sp.	67716	AMNH
Tomarctus sp.	67737	AMNH
Tomarctus sp.	67740	AMNH
Tomarctus sp.	67746	AMNH
Tomarctus sp.	67747	AMNH
Ursus spelaeus	Jf771	NMB
Radius		
Aelurodon ferox	27479 L	AMNH
Aelurodon ferox	27479 R	AMNH
Aelurodon ferox	61746	AMNH
Aelurodon ferox	67459	AMNH
Aelurodon ferox	70624	AMNH
Aelurodon taxoides	67428	AMNH
Aelurodon taxoides	67445	AMNH
Amphicyon sp.	Cast	AMNH

Amphicyon ingens	68119	AMNH
Amphicyon ingens	68193	AMNH
Amphicyon major	10428	AMNH
Amphicyon major	29617	AMNH
Amphicyon sp.	23391	AMNH
Amphicyon sp.	25422	AMNH
Amphicyon sp.	617-27298	AMNH
Amphicyon sp.	68103-A	AMNH
Amphicyon sp.	68109-A	AMNH
Amphicyon sp.	68126	AMNH
Amphicyon sp.	68167	AMNH
Amphicyon sp.	68212	AMNH
Amphicyon sp.	68221	AMNH
Arctodus simus	225-2626	AMNH
Arctodus simus	850	AMNH
Barbourofelis fricki	2672	AMNH
Barbourofelis fricki	61998	AMNH
Barbourofelis fricki	61999	AMNH
Barbourofelis lovei	125115	AMNH
Barbourofelis morrisi	61898	AMNH
Carpocyon tagarctus	67565	AMNH
Dinictis sp.	125652	AMNH
Dinictis sp.	62074	AMNH
Dinictis sp.	62147	AMNH
Epicyon haydeni	67404	AMNH
Epicyon haydeni	67406	AMNH
Epicyon haydeni	67407	AMNH
Epicyon haydeni	67607	AMNH
Epicyon saevus	67490	AMNH
Epicyon saevus	67508	AMNH
Epicyon saevus	8305	AMNH
Hemicyon sp.	68176	AMNH
Hemicyon ursinus	21101	AMNH
Homotherium serum	1798V	MSN
Hoplophoneus insolens	655	AMNH
Hoplophoneus primaevus	38980	AMNH
Hoplophoneus sp.	62077	AMNH
Ischyrocyon sp.	54220-B	AMNH
Ischyrocyon sp.	68152-A	AMNH
Ischyrocyon sp.	68217	AMNH
Machairodus aphanistus	B-1482	MNCN
Machairodus aphanistus	B-2621	MNCN
Machairodus giganteus	M8963	NHM
Machairodus sp.	104725	AMNH
Megantereon cultridens	Se311	NMB
Megantereon cultridens	StV774.1953	NMB
Megantereon cultridens	VA1201	NMB
Borophagus sp.	61664	AMNH
Borophagus sp.	67918-A	AMNH
Borophagus sp.	67918	AMNH
Pachycrocuta brevirostris	12823	MSN
Pliocrocuta perrieri	107777	AMNH
Paratomarctus temerarius	105347	AMNH
Patriofelis ferox	1507-A	AMNH

Pogonodon sp.	1399	AMNH
Promegantereon ogygia	B-2207A	MNCN
Promegantereon ogygia	B-4413	MNCN
Promegantereon ogygia	B-4566	MNCN
Promegantereon ogygia	BAT-1-'05-D8-30	MNCN
Promegantereon ogygia	BAT-3-9-'01	MNCN
Pseudaelurus sp.	61942-A	AMNH
Pseudaelurus sp.	62209	AMNH
Pseudaelurus sp.	62225	AMNH
Simocyon batalleri	B-3680	MNCN
Simocyon batalleri	B-430	MNCN
Smilodon ensenadensis	61	MCNV
Smilodon fatalis	LB41	NMB
Smilodon populator	1.7.85-2	SNM
Smilodon sp.	MPCB64-624	MCNV
Smilodon sp.	SN	AMNH
Tomarctus sp.	67749	AMNH
Tomarctus sp.	67751	AMNH
Tomarctus sp.	67752	AMNH
Tomarctus sp.	67754	AMNH
Tomarctus sp.	67756	AMNH
Tomarctus sp.	67757	AMNH
Tomarctus sp.	67758	AMNH
Tomarctus sp.	67528	AMNH
Tomarctus sp.	67529	AMNH
Tomarctus sp.	67548	AMNH
Tomarctus sp.	67550	AMNH
Tomarctus sp.	67552	AMNH
Tomarctus sp.	67717	AMNH
Ursus spelaeus	17484	NHM
Ursus spelaeus	1953	NMB
Ursus spelaeus	1954	NMB
Ursus spelaeus	Jf771	NMB
Ursus spelaeus	M324	NHM
Ursus spelaeus	M462	NHM
Ulna		
Aelurodon ferox	27479 L	AMNH
Aelurodon ferox	27479 R	AMNH
Aelurodon taxoides	67943	AMNH
Aelurodon taxoides	67980	AMNH
Amphicyon sp.	Cast	AMNH
Amphicyon ingens	68117	AMNH
Amphicyon ingens	68169	AMNH
Amphicyon major	TD1158	NMB
Amphicyon sp.	68103-H	AMNH
Amphicyon sp.	68110-B	AMNH
Amphicyon sp.	68110-C	AMNH
Amphicyon sp.	68110-D	AMNH
Amphicyon sp.	68127	AMNH
Amphicyon sp.	68221	AMNH
Amphicyon sp.	68266	AMNH
Arctodus simus	1077	AMNH
Arctodus simus	2625	AMNH
Barbourofelis lovei	125115	AMNH

Barbourofelis morrisi	61976	AMNH
Cephalogale sp.	54464	AMNH
Dinictis sp.	62147	AMNH
Epicyon haydeni	67611	AMNH
Epicyon saevus	67492	AMNH
Epicyon saevus	8305	AMNH
Hoplophoneus primaevus	38980	AMNH
Hoplophoneus sp.	62077	AMNH
Indarctos sp.	99236	AMNH
Ischyrocyon sp.	54220-B	AMNH
Ischyrocyon sp.	68181-B	AMNH
Machairodus aphanistus	B-2365	MNCN
Machairodus aphanistus	B-2624	MNCN
Machairodus aphanistus	B-2720	MNCN
Megantereon cultridens	Se311	NMB
Megantereon cultridens	VA1201	NMB
Paratomarctus temerarius	61071	AMNH
Promegantereon ogygia	BAT-1-'02-E7-72	MNCN
Promegantereon ogygia	BAT-3-5-38	MNCN
Smilodon ensenadensis	61	MCNV
Smilodon fatalis	LB41	NMB
Smilodon populator	2	SNM
Tomarctus sp.	67759	AMNH
Tomarctus sp.	67760	AMNH
Tomarctus sp.	67761	AMNH
Tomarctus sp.	67720	AMNH
Tomarctus sp.	67721	AMNH
Tomarctus sp.	67722	AMNH
Tomarctus sp.	67725-D	AMNH
Ursus spelaeus	1953a	NMB
Ursus spelaeus	1953b	NMB
Ursus spelaeus	1953c	NMB
Ursus spelaeus	43752	NHM
Ursus spelaeus	Jf771	NMB
Pelvis		
Amphicyon sp.	Cast	AMNH
Arctodus simus	12392	AMNH
Hoplophoneus primaevus	38980	AMNH
Machairodus aphanistus	BAT-1-'06-F6-58	MNCN
Promegantereon ogygia	B-466	MNCN
Pseudaelurus sp.	62210-B	AMNH
Smilodon fatalis	LB41	NMB
Femur		
Aelurodon ferox	27479 L	AMNH
Aelurodon ferox	27479 R	AMNH
Aelurodon ferox	67467	AMNH
Aelurodon taxoides	67446	AMNH
Aelurodon taxoides	67447	AMNH
Amphicyon sp.	Cast	AMNH
Amphicyon ingens	68117	AMNH
Amphicyon ingens	68147	AMNH
Amphicyon sp.	68100-A	AMNH
Amphicyon sp.	68104-A	AMNH

Amphicyon sp.	68111-C	AMNH
Arctodus simus	12392 L	AMNH
Arctodus simus	12392 R	AMNH
Arctodus simus	25531	AMNH
Barbourofelis fricki	61986	AMNH
Carpocyon tagarctus	67559	AMNH
Carpocyon tagarctus	67560	AMNH
Daphoenus minimus	63343	AMNH
Dinictis sp.	125652	AMNH
Dinictis sp.	62074	AMNH
Dinictis sp.	62122	AMNH
Dinictis sp.	69425	AMNH
Dinictis sp.	69426	AMNH
Epicyon haydeni	67613	AMNH
Epicyon saevus	67505	AMNH
Epicyon saevus	8305	AMNH
Hemicyon sp.	68176	AMNH
Hoplophoneus primaevus	38980	AMNH
Hoplophoneus sp.	38981	AMNH
Hoplophoneus sp.	62077	AMNH
Hoplophoneus sp.	62090	AMNH
Ischyrocyon sp.	68153-A	AMNH
Ischyrocyon sp.	68153	AMNH
Machairodus aphanistus	B-199	MNCN
Machairodus sp.	104727	AMNH
Megantereon cultridens	Se311	NMB
Nimravus brachyops	6935	AMNH
Paratomarctus temerarius	105338	AMNH
Paratomarctus temerarius	61071	AMNH
Patriofelis ulta	17505	AMNH
Pogonodon platycopis	6953	AMNH
Promegantereon ogygia	B-3-2561	MNCN
Promegantereon ogygia	BAT-1-'01-D6-115	MNCN
Promegantereon ogygia	BAT-1-'08-D3-4	MNCN
Promegantereon ogygia	BAT-1-'08-E3-26	MNCN
Pseudaelurus sp.	62167	AMNH
Smilodon ensenadensis	61	MCNV
Smilodon fatalis	LB41	NMB
Smilodon gracilis	69229	AMNH
Smilodon gracilis	69230	AMNH
Tomarctus sp.	67781	AMNH
Tomarctus sp.	67783	AMNH
Tomarctus sp.	67726	AMNH
Tomarctus sp.	67763	AMNH
Tomarctus sp.	67764	AMNH
Tomarctus sp.	67766	AMNH
Ursus spelaeus	Jf 1119	NMB
Ursus spelaeus	Jf 1120	NMB
Tibia		
Aelurodon ferox	27479	AMNH
Aelurodon ferox	67459	AMNH

Amphicyon sp.	Cast	AMNH
Amphicyon ingens	68117-A	AMNH
Amphicyon ingens	68117	AMNH
Amphicyon ingens	68122	AMNH
Amphicyon major	10428	AMNH
Amphicyon major	29619	NHM
Amphicyon sp.	18848	AMNH
Amphicyon sp.	26872	AMNH
Amphicyon sp.	617-27298	AMNH
Amphicyon sp.	68104-E	AMNH
Amphicyon sp.	68131	AMNH
Arctodus simus	12392	AMNH
Arctodus simus	217-2297	AMNH
Arctodus simus	25531 L	AMNH
Arctodus simus	25531 R	AMNH
Barbourofelis fricki	61994	AMNH
Barbourofelis fricki	61995	AMNH
Borophagus sp.	23366	AMNH
Borophagus sp.	67637	AMNH
Borophagus sp.	67950-A	AMNH
Borophagus sp.	67950	AMNH
Daphoenodon sp.	68276	AMNH
Daphoenus sp.	11857	AMNH
Epicyon haydeni	67414	AMNH
Epicyon haydeni	67418	AMNH
Epicyon haydeni	67616 L	AMNH
Epicyon haydeni	67616 R	AMNH
Epicyon saevus	67688	AMNH
Hemicyon sp.	68176	AMNH
Hoplophoneus insolens	655	AMNH
Hyaenodon pervagus	19002	AMNH
Ischyrocyon sp.	68153-B	AMNH
Ischyrocyon sp.	68157	AMNH
Machairodus aphanistus	B-398	MNCN
Machairodus sp.	104726	AMNH
Machairodus sp.	M8964	NHM
Megantereon cultridens	Se311	NMB
Paratomarctus euthos	61088	AMNH
Paratomarctus euthos	67539	AMNH
Pogonodon sp.	1399 L	AMNH
Pogonodon sp.	1399 R	AMNH
Promegantereon ogygia	BAT-1-'05-F6-42	MNCN
Promegantereon ogygia	BAT-1-'06-F4-232	MNCN
Pseudaelurus sp.	62163	AMNH
Pseudaelurus sp.	62173	AMNH
Smilodon ensenadensis	61	MCNV
Smilodon fatalis	LB41	NMB
Smilodon populator	2	SNM
Smilodon sp.	MRCB-260a	MCNV
Tomarctus sp.	67767	AMNH
Tomarctus sp.	67771	AMNH

Tomarctus sp.	67773	AMNH
Tomarctus sp.	67731	AMNH
Tomarctus sp.	67871	AMNH
Ursus etruscus	7531V	MSN
Ursus etruscus	VA870	NMB
Ursus spelaeus	43822	NHM
Ursus spelaeus	Jf1021	NMB
Ursus spelaeus	Jf372	NMB

Extinct taxa are distributed among eight carnivoran families (Figure 2.1C, D): one ailurid (Ailuridae), four amphicyonids (Amphicyonidae), one barbourofelid (Barbourofelidae), nine canids (Canidae), six felids (Felidae), two hyaenids (Hyaenidae), four nimravids (Nimravidae), and six ursids (Ursidae). I also included two taxa of carnivorous mammals of the order Creodonta, in order to test if they share similar morphological patterns than the members of the order Carnivora. Specifically, these taxa are *Patriofelis* sp. and *Hyaenodon pervagus*.

Morphometric data were obtained from specimens housed in the following institutions:

- American Museum of Natural History (AMNH, New York): modern species data were obtained from the collections of the Department of Mammalogy (Division of Vertebrate Zoology). Extinct species data were obtained from the fossil collection at the Division of Paleontology.
- Natural History Museum (NHM, London): modern species data were taken from the mammalian collection of the Vertebrate Division (Department of Life Sciences). Extinct species data were obtained from the vertebrate fossil collection of the Department of Earth Sciences.
- Naturhistorisches Museum Basel (NMB): extinct species data were obtained from the Vertebrate Paleontology collections (Geosciences).
- Museo Nacional de Ciencias Naturales (MNCN, Madrid): fossil specimens data obtained come from the collections of site of Batallones (Madrid) housed at this museum.

- Museo di Storia Naturale (MSN, Firenze): fossil specimen data were recovered from the collections housed at the Museo di Geologia e Paleontologia.
- Statens Naturhistoriske Museum (SNM, Copenhagen): extinct species data were obtained from the collections of the Zoologisk Museum.
- Museo de Ciencias Naturales de Valencia (MCNV): data of some fossil specimens were obtained from the Botet collection housed at this museum.

2.1.2. Landmarks

Bone shape was captured using homologous landmarks (points of anatomical equivalence with functional relevance, which are located on the bone surface and can be found in all the taxa analysed) in three-dimensions. Landmark coordinates were gathered using a Microscribe G2X and the software Immersion Inc. to recover the data into a spreadsheet of Excel. The microscribe device is composed of an articulated arm fixed into a basement. The arm has a pointer at the end and, using the articulated arm, this pointer can be moved freely in the space around the machine. The software of the microscribe generates a system of three-dimensional coordinates (x, y and z axes) and detects the position of the pointer within this system, providing point coordinates at any time. Therefore, the pointer must be located on each selected landmark, so the coordinates exported into the spreadsheet are its coordinates. This procedure is repeated sequentially and in the same order for all landmarks while keeping the bone immobile with respect to the microscribe.

Anatomical and geometrical criteria (e.g., tips of processes and tuberosities) were used for selecting landmarks. The advantages of this type of criteria are that landmarks can be easily recognized in both modern and fossil bones, and that many of them have clear biomechanical implications, as they usually coincide with lever arms for muscles, or allow to capture the shape of a given articular surface. Furthermore, given that the diaphyseal cross section of bones has clear biomechanical consequences (see introduction), landmarks located at the middle of the diaphysis of the major long bones were also taken to complete shape information. The anatomical keys of the bones analysed here, as well as the origins and insertions of the most important muscles that originate or anchor in them are shown in Figures 3.1 and 4.6. These Figures and Figure 5.2 show the position of the landmarks selected in different views. Here, Table 2.3 describes the specific criteria used for each landmark.

Table 2.3. Detailed description of the anatomical position of each landmark used in this thesis.

	Scapula:
1	Most dorsal point of the posterior border.
2	Ventral boundary of the teres major process.
3	Distal tip of the acromion process.
4	Most posterior point of the metacromion.
5	Point of maximum curvature between the proximal end of the spine and the scapular body.
6	Point of maximum curvature at the posterior border of the neck.
7	Most posterior point of the border of the glenoid fossa.
8	Midpoint of the lateral border of the glenoid fossa.
9	Midpoint of the medial border of the glenoid fossa.
10	Most proximal point of the border of the glenoid fossa at the anterior side.
11	Most anterior point of the glenoid tuberosity.
12	Point of maximum curvature at the anterior border of the neck.
	Humerus:
1	Most proximal point of the lesser tuberosity.
2	Most proximal point of the greater tuberosity.
3	Most anterior point of the greater tuberosity.
4	Midpoint of the infraspinatus insertion fossa.
5	Most lateral point of the lateral epicondyle.
6	Lateral-proximal corner of the capitulum at the anterior side.
7	Point of maximum curvature of the articular surface at the anterior side of its proximal border.
8	Medial-proximal corner of the trochlea at the anterior side.
9	Most medial point of the medial epicondyle.
10	Most lateral point at the middle of the shaft.
11	Most anterior point at the middle of the shaft.
12	Most medial point at the middle of the shaft.
13	Most distal point of the humeral head.
14	Medial-proximal corner of the trochlea at the posterior side
15	Lateral-proximal corner of the capitulum at the posterior side.
16	Most distal point of the trochlear ridge.
17	Most posterior point at the middle of the shaft.

	Radius:
1	Most proximal point of the bicipital tuberosity.
2	Point of maximum curvature of the head border of the radius at the medial side.
3	Point of maximum curvature of the head border of the radius at the posterior side.
4	Most proximal point in the radial coronoid process.
5	Most medial point at the middle of the shaft.
6	Most posterior point at the middle of the shaft.
7	Most lateral point at the middle of the shaft.
8	Most medial point of the distal epiphysis.
9	Most distal point of the radial styloid process.
10	Most proximal point of the anterior border of the distal articular surface.
11	Most lateral point of the anterior border of the distal articular surface.
12	Most posterior point of the distal epiphysis.
13	Most lateral point of the distal epiphysis.
14	Point of maximum curvature of the radius head border at the lateral side.
15	Most anterior point at the middle of the shaft.
16	Most proximal point of the radial styloid process at the anterior side.

	Ulna:
1	Most proximal point of the olecranon process.
2	Most anterior point of the lateral edge of the olecranon process.
3	Most anterior point of the medial edge of the olecranon process.
4	Most anterior point of the anconeus process.
5	Most distal point of the trochlear groove at the lateral side.
6	Midpoint of the lateral border of the trochelar groove.
7	Point of maximum curvature between the border of the trochlear groove and the beginning of the lateral coronoid process.
8	Most lateral point of the lateral coronoid process.
9	Midpoint of the distal border of the radial groove.
10	Most anterior point of the medial coronoid process.
11	Most medial point of the trochlear groove border.
12	Point of maximum curvature of the medial border of the trochlear groove, where the anconeus process begins.
13	Most lateral point at the middle of the shaft.
14	Most anterior point at the middle of the shaft.
15	Most medial point at the middle of the shaft.
16	Most anterior point of the distal epiphysis.
17	Point of maximum curvature at the beginning of the ulnar styloid process.
18	Most distal point of the ulnar styloid process.
19	Most posterior point at the middle of the shaft.
20	Most posterior point of the distal epiphysis.

	Pelvis:
1	Most anterior point of the ventral border of the illium.
2	Most posterior point of the antero-ventral spine of the ilium.

3	Point of maximum curvature in the anterior border of the pubis.
4	Most anterior point of the pubic symphysis.
5	Most anterior point of the obturator foramen edge.
6	Most dorsal point of the obturator foramen edge.
7	Most ventral point of the obturator foramen edge.
8	Most posterior point of the obturator foramen edge.
9	Most posterior point of the ischial tuberosity.
10	Most posterior point of the acetabular incisure.
11	Point of maximum curvature in the anterior extreme of the acetabular articular surface.
12	Point of maximum curvature of the internal border of the acetabular articular surface.
13	Point of maximum curvature in the posterior extreme of the acetabular articular surface.
14	Most anterior point of the antero-dorsal spine of the ilium.
15	Most posterior point of the postero-dorsal spine of the ilium.
16	Most dorsal point of the ischial spine.
17	Most posterior point of the pubic symphysis.
	Femur:
1	Midpoint of the fovea capitis.
2	Most posterior point of the lesser trochanter.
3	Most proximal point of the greater trochanter.
4	Point of maximum curvature in the proximal edge between the femoral head and the greater trochanter.
5	Most medial point at the middle of the shaft.
6	Most posterior point at the middle of the shaft.
7	Most lateral point at the middle of the shaft.
8	Proximo-medial corner of the medial condyle.
9	Proximo-lateral corner of the medial condyle.
10	Proximo-medial corner of the lateral condyle.
11	Proximo-lateral corner of the lateral condyle.
12	Most distal point of the intercondylar fossa.
13	Most anterior point at the middle of the shaft.
14	Point of maximum curvature of the lateral edge between the condyle and the trochlea.
15	Point of maximum curvature of the medial edge between the condyle and the trochlea.
16	Most proximal point of the lateral edge of the trochlea.
17	Most proximal point of the medial edge of the trochlea.
	Tibia:
1	Point of maximum curvature of the posterior edge of the medial condyle.
2	Point of maximum curvature of the posterior intercondylar edge.
3	Point of maximum curvature of the posterior edge of the lateral condyle.
4	Most proximal point of the medial condyle.
5	Point of maximum curvature in the intercondylar eminence.
6	Most proximal point of the lateral condyle.
7	Most medial point of the proximal epiphysis.

8	Most lateral point of the proximal epiphysis.
9	Most medial point at the middle of the shaft.
10	Most posterior point at the middle of the shaft.
11	Most lateral point at the middle of the shaft.
12	Postero-distal corner of the medial malleolus.
13	Most distal point of the posterior edge of the distal articular surface.
14	Most posterior point of the internal edge of the distal articular surface.
15	Most proximal point of the lateral edge of the distal articular surface.
16	Midpoint of the medial half of the distal articular surface.
17	Point of maximum curvature of the lateral edge of the tibial tuberosity.
18	Point of maximum curvature of the medial edge of the tibial tuberosity.
19	Most anterior point at the middle of the shaft.
20	Most distal point of the anterior edge of the distal articular surface.
21	Point of maximum curvature in the notch of the anterior edge of the distal articular surface.
22	Antero-distal corner of the medial malleolus.

In addition to landmark information, we obtained also a three-dimensional surface model of each appendicular bone using a Nextengine Hd surface scanner and the software ScanStudio Pro, kindly provided by Dr. S. Almécija (Stony Brook University). More specifically, we chose the scapula, humerus, radius, ulna, femur and tibia of one specimen of *Panthera onca* (AMNH 139959) and the pelvis of one skeleton of *Uncia uncia* (AMNH 100110). Although these 3D models were not analysed statistically, they were very helpful for visualizing the morphological changes associated to the morphometric analyses performed over the landmark data.

2.2. Phylogenetic tree reconstruction

A phylogenetic tree of the carnivoran species analysed in this dissertation was assembled for assessing the effects of phylogenetic signal in subsequent morphometric analyses. Mesquite software (Maddison and Maddison 2011) was used for doing this. Although the phylogenetic information used to assemble the composite tree comes from different sources (i.e., molecular and morphological phylogenies for living and extinct taxa, respectively), we were able to combine them and weight branch lengths in million years before present (My). The supertree obtained by Nyakatura and Bininda-Emonds (2012) with molecular data was used for the phylogenetic information on most living taxa, including their phylogenetic position and branch lengths. However, given that the super-tree of Nyakatura and Bininda-Emonds (2012) presents polytomies for some procyonid species, we used the well-resolved phylogeny provided by Koepfli et al. (2007) for this family, which was also obtained from molecular data. Within this composite tree of living species, extinct taxa were included according to their inferred phylogenetic position, and branch lengths were weighted using their stratigraphic ranges (i.e., first and last appearance data, FAD and LAD, respectively). Phylogenetic data of extinct species and the specific published sources used are shown in Table 2.4. In those nodes were molecular and stratigraphic data resulted in conflicting age estimates, the estimation that provided the older age was chosen. In those cases in which two or more successive nodes were located at the same age, an arbitrary difference of 0.1 My was established between them.

Table 2.4. Stratigraphic ranges and time of divergence for the extinct taxa included in the composite tree used in this thesis. The source references for phylogenetic position and stratigraphic range are indicated. The time of divergence of two extinct species (*Arctodus simus* and *Ursus spelaeus*) have been obtained from molecular data (MD). PBDB: Paleobiology Database: www.fossilworks.org; NOW: New and Old Worlds database: http://www.helsinki.fi/science/now.

Taxa	**Strat. Range (My)**	**Ref. phylogenetic position**	**Ref. strat. range**
A. ferox	15 – 12	Wang et al. 1999	Wang et al. 1999
A. taxoides	12 – 9	Wang et al. 1999	Wang et al. 1999
Amphicyon	23 - 7.2	Hunt 1998	PBDB
A. simus	MD 5.66	Krause et al. 2008	Krause et al. 2008
Barbourofelis	11 – 6	Morlo et al. 2004	Martin 1998, Janis et al. 2008
Borophagus	12 – 2	Wang et al. 1999	Wang et al. 1999
Carpocyon	16 – 5	Wang et al. 1999	Wang et al. 1999
Cephalogale	33.9 – 16	McLellan and Reiner 1994	PBDB
Daphoenodon	23 - 17.5	Hunt 1998	Hunt 1998, Janis et al. 2008
Daphoenus	39.5 – 27	Hunt 1998	Hunt 1998, Janis et al. 2008
Dinictis	37 – 26	Peigné 2003	Martin 1998, Peigné 2003, Janis et al. 2008
E. haydeni	10 – 5	Wang et al. 1999	Wang et al. 1999
E. saevus	7 – 12	Wang et al. 1999	Wang et al. 1999
Hemicyon	16 - 13.6	McLellan and Reiner 1994	PBDB
Homotherium	3 - 0.5	Anton et al. 2004	Turner and Anton 1997
Hoplophoneus	37 – 28	Peigné 2003	Martin 1998, Peigné 2003, Janis et al. 2008

Indarctos	11 - 5.3	McLellan and Reiner 1994	PBDB
Ischyrocyon	14 – 8	Hunt 1998	Hunt 1998, Janis et al. 2008
Machairodus	15 – 2	Anton et al. 2004	Turner and Anton 1997
Megantereon	5.3 - 0.78	Anton et al. 2004	PBDB
Nimravus	34 – 24	Peigné 2003	Peigné 2003, Martin 1998, Janis et al. 2008
P. brevirostris	3.4 - 0.3	Turner et al. 2008	NOW database
P. euthos	13 – 9	Wang et al. 1999	Wang et al. 1999
P. temerarius	16 – 13	Wang et al. 1999	Wang et al. 1999
Patriofelis	50.3 - 40.4	Gunnell 1998	PBDB
P. perrieri	4.2 - 1.2	Turner et al. 2008	NOW database
Pogonodon	34 – 23	Peigné 2003	Peigné 2003, Martin 1998, Janis et al. 2008
P. ogygia	11 - 8.2	Salesa et al. 2010	NOW database
Pseudaelurus	20.4 - 4.9	Rothwell 2003	PBDB
S. batalleri	11.1 - 8.7	Salesa et al. 2011	Salesa et al. 2011
Smilodon	4.9 - 0.01	Anton et al. 2004	PBDB
Tomarctus	16 – 14	Wang et al. 1999	Wang et al. 1999
U. etruscus	2.6 - 1.3	McLellan and Reiner 1994	NOW database
U. spelaeus	MD 2.75	Krause et al. 2008	Krause et al. 2008

	Time of divergence (My)	**Ref. phylogenetic position**	**Ref. time of divergence**
Creodonta	65.1	Gunnell 1998	Nyakatura and Bininda-Emonds 2012
Amphicyonidae	61.5	Finarelli and Flynn 2006	Nyakatura and Bininda-Emonds 2012
Barbourofelidae	20	Morlo et al. 2004	PBDB
Nimravidae	37	Peigné 2003	Martin 1998, Peigné 2003, Janis et al. 2008
Borophaginae	34	Wang et al. 1999	Wang et al. 1999
Machairondontinae	15	Anton et al. 2004	Turner and Anton 1997
Simocyoninae	17	Salesa et al. 2011	Salesa et al. 2011, Wallace 2011

The resulting phylogenetic tree was adapted with the species available for the fore- and hind limb, because some species were available for forelimb bones and not for hind limb bones, and vice versa. For this reason, the composite tree for the forelimb (Figure 3.2) is slightly different from the one for the hind limb (Figure 4.7). Furthermore, given that only living species were included in Article III, this phylogenetic tree (Figure 5.3) was only based on the molecular trees of Nyakatura and Bininda-Emonds (2012) and Koepfli et al. (2007), as explained above.

2.3. Morphometric analyses

2.3.1. Procrustes superimposition

The Procrustes superimposition method was initially developed to compare the shape of two or more objects defined by landmark configurations (Rohlf and Slice 1990). When only shape differences (i.e., differences in the relative position of landmarks) are to be analysed, Procrustes superimposition of landmarks removes the effects of size, translation and rotation of raw coordinates. The first step is to calculate the centroid of each object as the average coordinates of all landmarks. Afterwards, these centroids are centred in the Cartesian origin (0,0) and all landmarks are expressed as deviations from the origin (Rohlf and Slice 1990), which removes the differences in position between the objects. The next step is to eliminate size differences by dividing each landmark coordinate by the square root of the sum of squared distances between each landmark and the centroid (i.e., centroid size). As a result, the squared distances of each object after removing size effects are equal to one (Rohlf and Slice 1990). To eliminate the orientation effect, each object is rotated a given angle to reach the best-fit orientation according to an object of reference (Rohlf and Slice 1990). After this first round of rotation, a consensus configuration of landmarks is calculated as the average configuration of all objects, and subsequent iterative rounds of rotation and recalculation of consensus shapes are performed to find the distribution of landmarks that only takes into account shape differences; the method used to find this orientation is a least-squared approach (Rohlf and Slice 1990). This procedure also takes into account the possibility of reflections in the case of mirrored configurations (Rohlf and Slice 1990). The variables obtained are called Procrustes coordinates and are the basic shape information to be analysed in subsequent steps.

2.3.2. Multivariate regression

Multivariate regression was developed by Monteiro (1999) for geometric morphometric data. This kind of regression allows testing the effects of one or more independent variables on multiple dependent variables. This is the opposite of usual multiple regressions, which incorporate multiple independent variables and only one dependent variable. Multivariate regression is the most appropriate method to explore the influence of a given biological variable (e.g., size, age, or any ecological aspect) on shape, because shape is defined by a group of variables, the Procrustes coordinates (Monteiro 1999). Therefore, following this method, hypothetical shapes can be predicted using the independent variable and its predictive power can be measured using a coefficient of determination (Monteiro 1999).

2.3.3. Phylogenetic comparative methods

Phylogenetic comparative methods are useful for assessing the effects of phylogenetic inheritance on the distribution of any phenotypic variable among several species (Felsenstein 1985). During the last decades, different comparative methods have been developed, including phylogenetic independent contrast (Felsenstein 1985), phylogenetic generalized least squares (Martins and Hansen 1997) and phylogenetic principal components analysis (Polly et al. 2013), among others. A detailed description of these methods is beyond the scope of this section. For this reason, I will briefly expose independent contrast, which is the method used in this thesis.

Given that species are related through phylogeny, they cannot be treated as independent data in any statistical analysis (Felsenstein 1985). For this reason, I performed phylogenetic independent contrast analysis on the ecological variables used in some articles (i.e., shape, size, maximum running speed and daily movement distance) for taking into account the phylogenetic inheritance of the variables and exploring the relationships among independent variables.

The phylogenetic independent contrast method was first developed by Felsenstein (1985) and assumes a model of evolution based on Brownian motion (i.e., a constant and uniform rate of shape change throughout the phylogeny). Based on a resolved phylogenetic tree and on the distribution of the variables among the tips of the phylogeny, the differences between sister taxa are calculated and scaled by their standard deviations (a proxy for phylogenetic distance, see Felsenstein [1985] for a detailed mathematical description). Additionally, the values of the internal nodes are estimated from the tip values and their contrasts are also calculated. This method provides *N*-1 (*N:* number of species or tips) scaled differences or contrasts, each of them independent from the remaining ones because contrasts only depend on their own phylogenetic distance (Felsenstein 1985). With this new independent contrast data of the original variables, any statistical analysis can be performed.

One of the most important problems of performing phylogenetic independent contrasts is to assume a Brownian motion mode of evolution (Felsenstein 1985). The validity of this assumption can be confirmed if there is an absence of correlation between the contrast values and their standard deviations (Diaz-Uriarte and Garland 1998). Thus, I checked this correlation to ensure that phylogenetic independent contrast could be applied to my data.

In addition to this comparative method, there are other approaches that can be used to estimate internal node values for any given variable. For this purpose, I used the squared-change parsimony method, developed by Maddison (1991). This method calculates the minimum sum of squared changes for each ancestor-descendant pair. The minimum sum of squared changes of the root of the phylogenetic tree is the minimum possible length of the tree (Maddison 1991). Minimum tree length is also useful to test if there is a phylogenetic signal in the variable under study. The reasoning behind this test is as follows: if closely related taxa have similar values for the variable under study (which implies a strong phylogenetic signal), the minimum tree length estimate obtained will be relatively small (Klingenberg and Gidaszewski 2010). In contrast, if the values of the variable distribute randomly across terminal taxa (which means an absence of phylogenetic signal), the minimum tree length estimated will be higher (Klingenberg and Gidaszewski 2010). To test this statistically, we used a permutation test developed by Laurin (2004) and extended for multivariate shape data by Klingenberg and Gidaszewski (2010). This method simulates the null hypothesis of absence of phylogenetic signal by reshuffling randomly tip values across the tree and recalculating minimum tree length for each permutation (10,000 in our case). If there is a phylogenetic signal in the data, the original tree length would be considerably smaller than the ones obtained randomly. Therefore, the proportion of random trees with equal or smaller lengths than the original one can be used as an empirical *p* value to assess if the null hypothesis of no phylogenetic signal can be rejected.

2.3.4. Principal components analysis

Principal components analysis (PCA) is a statistical method that can be used to reduce the number of dimensions in multivariate datasets (Zelditch et al. 2004), for example the geometric shape coordinates used in this thesis. Given that the original variables are usually numerous and many of them tend to be intercorrelated, PCA reduces the number of variables by finding another set of new variables (i.e., components) that are linear combinations of the original variables (Zelditch et al. 2004). These new components are independent of each other (i.e., they are orthogonal) and coincide with the axes of maximum variance in the dataset analysed. Therefore, the first component accounts for the highest portion of the original variance in the dataset, the second explains the highest portion of the remaining variance, and so on. In this way, the variance accounted for by each component decreases sequentially (Zelditch et al. 2004). This method concentrates the original variance in the first components, which means that the most important fraction of the original variation can be usually studied using only a few components (Zelditch et al. 2004).

In addition to the use of PCA for studying the morphological variation of our sample, I also performed a different kind of analysis: between-group PCA (Mitteroecker and Bookstein 2011). The aim of this method is to find differences between groups (ecological groups in my case) within the sample. It involves two steps: first, to perform a PCA over group averages to find the PCs that better separate these averages; and, second, to apply the PCs obtained before to the entire sample (Mitteroecker and Bookstein 2011), which provides a complete view of sample variation within this morphospace. This method is comparable to canonical variate analysis (CVA), but it lacks many of the statistical assumptions that arise with CVA (see below; Mitteroecker and Bookstein 2011).

2.3.5. Two-block partial least squares

The two-block partial least squares (2B-PLS) is a method developed to study the covariation between two blocks of variables. The method is very similar to PCA (Zelditch et al. 2004), but the major difference is that it finds the axes of maximum covariance between two blocks of variables instead of the axes of maximum variance within one block. The new variables generated by 2B-PLS analysis are called singular axes or PLS axes (Zelditch et al. 2004) and are represented by two morphological axes, one for each block. Similarly to principal components, each PLS axis is independent from the remaining ones and the highest amount of covariance explained is concentrated in the first PLS axes. The main difference between PLS and regression models is that 2B-PLS does not assume any dependence between the two blocks of variables; instead, they are treated symmetrically (Zelditch et al. 2004).

In addition to the 2B-PLS analyses, we also calculated the RV coefficient (Escoufier 1973; Klingenberg 2009) to estimate the strength of covariation between two blocks in relation to the total amount of variance within each block. This coefficient is calculated as:

$$\mathrm{RV}= tr(S_{12}S_{21})/[tr(S_1S_1)tr(S_2S_2)]^{1/2}$$

where *tr* is the trace (i.e., the sum of the diagonal elements of a squared matrix), S_{12} is the matrix of covariance between the two blocks (S_{21} is its transpose), and S_1 and S_2 are the matrices of covariance within each of the two blocks, respectively (Klingenberg 2009).

2.3.6. Multivariate analysis of variance

The multivariate analysis of variance (MANOVA) is a statistical method used to test for between group differences in a multivariate dependent variable. This method takes into account the within-group variance and the between-group variance in the dependent variables for assessing if the between groups differences are significant (Zelditch et al. 2004). If there are more than two groups, the most usual method to test the significance of their differences is Wilk's λ, which can be converted to an F-distribution (Zelditch et al. 2004). Together with the MANOVA, η^2 (eta squared), a proxy for the effect size, was also calculated. This parameter indicates the proportion of the total variance in the dependent variables (Total Sum of Squares) that is accounted for by variation in the independent variable (Between-groups Sum of Squares) and can be calculated as $SS_{between}/SS_{total}$ (Levine and Hullett 2002). Therefore, η^2 values indicate the degree of separation between the different groups according to the dependent variables.

In addition, Levene's tests were performed to check if within-group variances were homogeneous. When within-group variances are homogeneous, the F-tests can be used. In contrast, when variances are not homogeneous the non-parametric Welch's test should be preferred, as this test is more robust in such cases (Quinn and Keough 2002).

A series of *post hoc* tests were also performed to identify which groups were better differentiated by each dependent variable. When within-group variances were homogeneous, parametric Bonferroni's test were carried out, whereas non-parametric Dunnett T3 was preferred when variances were not homogeneous (Quinn and Keough 2002).

2.3.7. Canonical variate analysis

The canonical variate analysis (CVA) is a multivariate statistical method commonly used to differentiate with a set of variables among several groups previously defined (Zelditch et al. 2004). This method is designed to find the combination of the original variables that better separates the groups defined. More specifically, it tends to maximize between-group differences and reduce within-group variances. For doing this, the method generates a number of new variables (i.e., the canonical functions) that maximize the separation between the groups compared. This procedure involves three main steps (Zelditch et al. 2004): (*i*) a PCA of the group variances is performed, which generates the set of new variables that better account for the within-group variation; (*ii*) these axes are rescaled to standardize the within-group variances in different directions of the morphospace by calculating the Mahalanobis distance (i.e., an Euclidean distance proportional to the variance in each direction); and (*iii*) a second PCA is performed on the group centroids to better separate them (in a similar way to the between-groups PCA explained above).

Although this method is the one that best maximizes between groups differences, it has several statistical problems that make it not recommendable in some cases. Its two main difficulties are the following ones: (*i*) in contrast with PCA, canonical functions generate a distorted morphospace, which means that different functions can be correlated and sometimes they cannot be easily interpreted; (*ii*) CVA is very sensitive to the proportion of cases and variables; if there are more variables than cases per group, it can produce misleading results (this is further discussed by Mitteroecker and Bookstein 2011).

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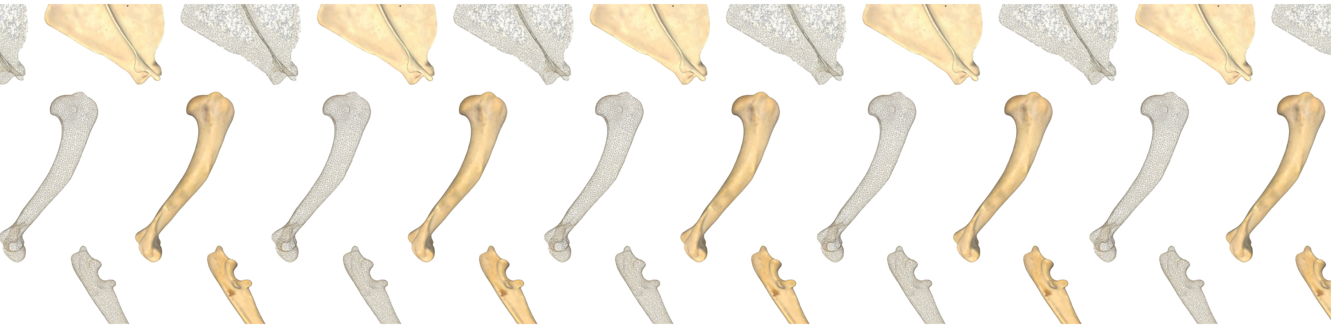
3. Article I: carnivoran forelimb evolution



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Abstract

In this study, three-dimensional landmark-based methods of geometric morphometrics are used for estimating the influence of phylogeny, allometry and locomotor performance on forelimb shape in living and extinct carnivorans (Mammalia, Carnivora). The main objective is to investigate morphological convergences towards similar locomotor strategies in the shape of the major forelimb bones. Results indicate that both size and phylogeny have strong effects on the anatomy of all forelimb bones. In contrast, bone shape does not correlate in the living taxa with maximum running speed or daily movement distance, two proxies closely related to locomotor performance. A phylomorphospace approach showed that shape variation in forelimb bones mainly relates to changes in bone robustness. This indicates the presence of biomechanical constraints resulting from opposite demands for energetic efficiency in locomotion –which would require a slender forelimb– and resistance to stress –which would be satisfied by a robust forelimb–. Thus, we interpret that the need of maintaining a trade-off between both functional demands would limit shape variability in forelimb bones. Given that different situations can lead to one or another morphological solution, depending on the specific ecology of taxa, the evolution of forelimb morphology represents a remarkable "one-to-many mapping" case between anatomy and ecology.

4. Article II: carnivoran hind limb evolution

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Abstract

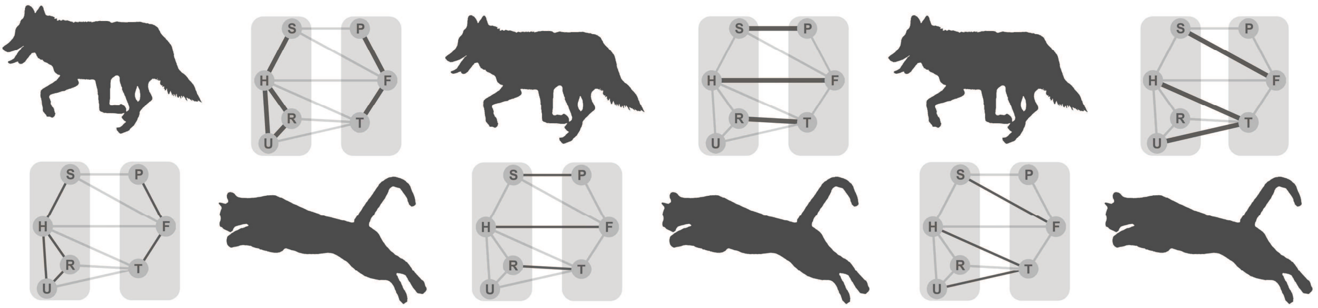
Background: The shape of the appendicular bones in mammals usually reflects adaptations towards different locomotor abilities. However, other aspects such as body size and phylogeny also play an important role in shaping bone design.

We used 3D landmark-based geometric morphometrics to analyse the shape of the hind limb bones (i.e., femur, tibia, and pelvic girdle bones) of living and extinct terrestrial carnivorans (Mammalia, Carnivora) to quantitatively investigate the influence of body size, phylogeny, and locomotor behaviour in shaping the morphology of these bones. We also investigated the main patterns of morphological variation within a phylogenetic context.

Results: Size and phylogeny strongly influence the shape of the hind limb bones. In contrast, adaptations towards different modes of locomotion seem to have little influence. Principal Components Analysis and the study of phylomorphospaces suggest that the main source of variation in bone shape is a gradient of slenderness-robustness.

Conclusion: The shape of the hind limb bones is strongly influenced by body size and phylogeny, but not to a similar degree by locomotor behaviour. The slender-robust "morphological bipolarity" found in bone shape variability is probably related to a trade-off between maintaining energetic efficiency and withstanding resistance to stresses. The balance involved in this trade-off impedes the evolution of high phenotypic variability. In fact, both morphological extremes (slender/robust) are adaptive in different selective contexts and lead to a convergence in shape among taxa with extremely different ecologies but with similar biomechanical demands. Strikingly, this "one-to-many mapping" pattern of evolution between morphology and ecology in hind limb bones is in complete contrast to the "many-to-one mapping" pattern found in the evolution of carnivoran skull shape. The results suggest that there are more constraints in the evolution of the shape of the appendicular skeleton than in that of skull shape because of the strong biomechanical constraints imposed by terrestrial locomotion.

5. Article III: integration in carnivoran appendicular skeleton



This article has been published as: Martín-Serra, A., Figueirido, B., Pérez-Claros, J.A., Palmqvist, P. 2014. Patterns of morphological integration in the appendicular skeleton of mammalian carnivores. Evolution. http://onlinelibrary.wiley.com/doi/10.1111/evo.12566/abstract

Abstract

We investigated patterns of evolutionary integration in the appendicular skeleton of mammalian carnivores. The findings are discussed in relation to performance selection in terms of organismal function as a potential mechanism underlying integration. Interspecific shape covariation was quantified by 2B-PLS analysis of 3D landmark data within a phylogenetic context. Specifically, we compared pairs of anatomically connected bones (within-limbs) and pairs of both serially homologous and functional equivalent bones (between-limbs). The statistical results of all the comparisons suggest that the carnivoran appendicular skeleton is highly integrated. Strikingly, the main shape covariation relates to bone robustness in all cases. A bootstrap test was used to compare the degree of integration between specialized cursorial taxa (i.e., those whose forelimbs are primarily involved in locomotion) and non-cursorial species (i.e., those whose forelimbs are involved in more functions than their hind limb) showed that cursors have a more integrated appendicular skeleton than non-cursors. The findings demonstrate that natural selection can influence the pattern and degree of morphological integration by increasing the degree of bone shape covariation in parallel to ecological specialization.

6. Article IV:

ecomorphology of the carnivoran forelimb

This article will be published as: Martín-Serra, A., Figueirido, B., Palmqvist, P. In the pursuit of carnivoran forelimb adaptations: paleoecological inferences in Borophaginae (Mammalia, Carnivora, Canidae). In prep.

6.1 Abstract

We have performed an ecomorphological study of anatomy of the major bones (humerus, radius and ulna) of the carnivoran forelimb using landmarks-based methods of geometric morphometrics. We explored the effects of three ecological aspects that presumably have functional implications on forelimb morphology: (*i*) predatory behaviour; (*ii*) locomotor modes; and (*iii*) habitat preferences. Our results suggest that the morphology of the major forelimb bones of carnivorans is more closely associated with their predatory behaviour than with their locomotion mode or habitat preferences. The main morphological adaptions towards different predatory behaviours relate to: (*i*) the capacity to perform long and efficient runs in pounce/pursuit and pursuit predators; (*ii*) the ability to manoeuvre in occasional predators; and (iii) the capacity to exert and resist large forces in ambushers. Given their controversy on the predatory behaviour of borophagine canids (Mammalia, Carnivora, Canidae), we chose this carnivoran group for exemplifying the potential use of these adaptive traits as ecomorphological indicators of the predatory behaviour of extinct taxa. Our results indicate that in general, borophagines display a limited set of adaptions towards efficient running, including a reduced joint mobility in both the elbow and the wrist. Furthermore, they possess forelimbs as powerful as those of the living ambushers. This suggests that borophagines had a unique predatory behaviour among carnivorans, which was not fully equivalent to any of the living species.

6.2 Introduction

One recurrent topic in mammalian ecomorphology is the search for an association between the shape of the major appendicular bones and the ecology of locomotion. As a result, several researchers have proposed that different skeletal traits of the living mammals that can be interpreted as ecomorphological indicators of their locomotor behaviour (e.g., Maynard-Smith and Savage 1955; Taylor 1974, 1976, 1989; Garland and Janis 1993; Harris and Steudel 1997; Iwaniuk et al. 1999; Salton and Sargis 2008; Walmsley et al. 2012; Fabre et al. 2013). These morphological correlates have been in turn applied to investigating the paleoautoecology of extinct species and deriving paleosinecological inferences for past communities (e.g., Van Valkenburgh 1985, 1987; Munthe 1989; Janis and Wilhelm 1993; MacLeod and Rose 1993; Anyonge 1996; Argot 2001, 2002, 2004; Palmqvist et al. 2003; Andersson and Werdelin 2003; Andersson 2005; Schutz and Guralnick 2007; Samuels and Van Valkenburgh 2008; Meachen-Samuels and Van Valkenburgh 2009; Figueirido and Janis 2011; Polly 2010; Lewis and Lague 2011; Meloro et al. 2011; Janis et al. 2012; Ercoli et al. 2012; Meachen-Samuels 2012; Samuels et al. 2013; Janis and Figueirido 2014).

Mammalian carnivorans are particularly interesting subjects of study because their predatory behaviour, together with other activities (e.g., climbing, manipulating food, or agonistic fighting), has deeply influenced the morphological evolution of their appendicular bones. For this reason, a number of researchers have searched for ecomorphological indicators in the appendicular skeleton of carnivorans related to: (*i*) locomotion modes (e.g., Van Valkenburgh 1987; Samuels et al., 2013); (*ii*) habitat occupation (e.g., Polly 2010; Meloro et al., 2013); and (*iii*) predatory strategies and prey size preferences (e.g., Van Valkenburgh 1985; Harris and Steudel 1997; Figueirido and Janis 2011; Meachen-Samuels and Van Valkenburgh 2009; Janis and Figueirido 2014). These studies have shown interesting associations between such aspects of carnivoran ecology and the morphology of the appendicular bones. However, none of them have tested which of these aspects account more for the changes in shape and adaptations experienced through the evolution of the major limb bones (i.e., humerus, radius and ulna) of carnivorans. Even more interestingly, it is not clear if the effects of carnivoran ecology are uniformly reflected in all bones or, alternatively, each limb element is more influenced by a different ecological aspect. The latter is the main goal of this paper: to test which ecological adaptations are better reflected in the shape of the major forelimb bones of carnivorans. This will allow us to explore the adaptive meaning of the shape changes undergone by the forelimb of carnivorans and if there are differences between the bones in these adaptations. Given that we have included in our sample several species that can be classified as "occasional predators", as they are not specialized in hunting vertebrate prey of a particular body size (e.g., most bears or the badger), we did not explore those morphological aspects related to prey size preference. Therefore, our study is focused on the first three ecological aspects described above: habitat occupation, predatory behaviour and locomotion mode, with two different classifications for the latter aspect (see the methods section). In addition, we also included a phylogenetic classification at family level to compare the effects of ecological adaptations and historical legacy. We focused the study on the forelimb anatomy –instead of analyzing the anatomy of both limbs– because previous studies have shown that the forelimb is especially informative of carnivoran locomotor behaviour (see Anderson and Werdelin 2003; Anderson 2004; Figueirido and Janis 2011; Samuels et al. 2012; Fabre et al. 2013; Janis and Figueirido 2014).

Once identified which of these three ecological aspects is best correlated with the shape of the forelimb bones, its biomechanical implications and functional consequences will be explored. Finally, this information will be used to derive paleoecological inferences for the extinct borophagines, as a number of aspects of the paleoecology and behaviour of this subfamily are still controversial, including if they were pack hunters, as some of their modern relatives or, in contrast, were solitaire predators, as many modern ambushing carnivores (Munthe 1989; Van Valkenburgh et al. 2003; Andersson 2005). Up to the moment, the comparisons performed with morphological data have not provided conclusive results on this matter (Munthe 1989; Van Valkenburgh et al. 2003).

Therefore, our specific objectives are: *(i)* to test which ecological aspect is better discriminated by the shape of forelimb bones; *(ii)* to explore if all long bones of the forelimb reflect the same ecological aspect or, alternatively, if the shape of each bone reflects different ecological aspects; *(iii)* to interpret functionally those adaptive shape changes towards a specific ecology; and *(iv)* to derive ecological inferences for borophagines.

Table 6.1. Living species included in this study (N: number of individuals for each species). The ecological category in which each species is grouped is also indicated (see the main text for the specific bibliographic sources of each ecological grouping). See also Table 2.1 for the specific museum numbers of the fossils collected.

Species	**N**	**Family**	**Pred. beh.**	**Locomotion 1**	**Locomotion 2**	**Habitat**
Acinonyx jubatus	5	Felidae	Pursuit	Terrestrial	Cursorial	Open
Ailuropoda melanoleuca	4	Ursidae	Occasional	Terrestrial	Terrestrial	Closed
Canis adustus	1	Canidae	Pounce/pursuit	Terrestrial	Cursorial	Mixed
Canis aureus	2	Canidae	Pounce/pursuit	Terrestrial	Cursorial	Mixed
Canis latrans	5	Canidae	Pounce/pursuit	Terrestrial	Cursorial	Mixed
Canis lupus	5	Canidae	Pursuit	Terrestrial	Cursorial	Mixed
Canis mesomelas	4	Canidae	Pounce/pursuit	Terrestrial	Cursorial	Open
Canis simensis	1	Canidae	Pounce/pursuit	Terrestrial	Cursorial	Open
Cerdocyon thous	5	Canidae	Pounce/pursuit	Terrestrial	Terrestrial	Mixed
Chrysocyon brachyurus	2	Canidae	Pounce/pursuit	Terrestrial	Terrestrial	Open
Crocuta crocuta	5	Hyaenidae	Pursuit	Terrestrial	Cursorial	Mixed
Cuon alpinus	4	Canidae	Pursuit	Terrestrial	Cursorial	Closed
Helarctos malayanus	1	Ursidae	Occasional	Scansorial	Scansorial	Closed
Hyaena brunnea	1	Hyaenidae	Occasional	Terrestrial	Cursorial	Open
Hyaena hyaena	2	Hyaenidae	Pounce/pursuit	Terrestrial	Cursorial	Open
Leptailurus serval	2	Felidae	Ambush	Terrestrial	Terrestrial	Open
Lycaon pictus	2	Canidae	Pursuit	Terrestrial	Cursorial	Open
Lynx rufus	4	Felidae	Ambush	Scansorial	Scansorial	Open
Melursus ursinus	3	Ursidae	Occasional	Terrestrial	Terrestrial	Mixed
Nasua nasua	1	Procyonidae	Occasional	Scansorial	Scansorial	Closed
Nyctereutes procyonoides	2	Canidae	Pounce/pursuit	Terrestrial	Terrestrial	Mixed
Panthera leo	5	Felidae	Ambush	Terrestrial	Terrestrial	Open
Panthera onca	4	Felidae	Ambush	Scansorial	Scansorial	Closed
Panthera pardus	6	Felidae	Ambush	Scansorial	Scansorial	Mixed
Panthera tigris	4	Felidae	Ambush	Terrestrial	Terrestrial	Closed
Procyon lotor	3	Procyonidae	Occasional	Scansorial	Scansorial	Mixed
Puma concolor	4	Felidae	Ambush	Scansorial	Scansorial	Mixed
Speothos venaticus	2	Canidae	Pounce/pursuit	Terrestrial	Terrestrial	Closed
Tremarctos ornatus	1	Ursidae	Occasional	Scansorial	Scansorial	Closed
Uncia uncia	4	Felidae	Ambush	Scansorial	Scansorial	Closed
Urocyon cinereoargenteus	4	Canidae	Pounce/pursuit	Scansorial	Scansorial	Closed
Ursus americanus	3	Ursidae	Occasional	Scansorial	Scansorial	Closed
Ursus arctos	4	Ursidae	Occasional	Terrestrial	Terrestrial	Mixed
Ursus thibetanus	3	Ursidae	Occasional	Scansorial	Scansorial	Closed
Vulpes lagopus	2	Canidae	Pounce/pursuit	Terrestrial	Terrestrial	Open
Vulpes velox	2	Canidae	Pounce/pursuit	Terrestrial	Cursorial	Open
Vulpes vulpes	3	Canidae	Pounce/pursuit	Terrestrial	Cursorial	Mixed

6.3. Material and methods

6.3.1. Sample

The sample of living carnivorans analysed comprises the humerus, radius and ulna of 115 individuals distributed among 37 species (Table 6.1). The sample of extinct species includes 66 fossil specimens –sum of humeri, radii and ulnae– from 10 species of borophagines (Table 6.2). The sample of living species was selected in order to cover the highest variability as possible in both body size and ecology. However, we paid special attention to those families which show a range of body size comparable to the extinct species analysed. The effect of ontogenetic variation was avoided by selecting only adult specimens, as indicated by complete ossification of the epiphyseal growth plates. All specimens analysed are housed in the following institutions: American Museum of Natural History (AMNH, New York) and Natural History Museum (NHM, London). Museum numbers and specific locations of the specimens are provided in Table 2.1 for the living species and in Table S6.1 for the extinct ones.

Table 6.2. List of extinct taxa of the subfamily Borophaginae studied. The number of specimens for each taxon is indicated for humerus, radius and ulna. See also Table S6.1 for specific museum numbers of the fossils collected.

Species (abbreviation)	**Humerus**	**Radius**	**Ulna**
Aelurodon ferox (*Afe*)	1	5	2
Aelurodon taxoides (*Ata*)	3	2	2
Borophagus pugnator (*Bpu*)	0	1	0
Borophagus secundus (*Bse*)	0	2	0
Carpocyon tagarctus (*Cta*)	0	1	0
Epicyon haydeni (*Eha*)	2	4	1
Epicyon saevus (*Esa*)	1	3	2
Paratomarctus euthos (*Peu*)	1	0	0
Paratomarctus temerarius (*Pte*)	1	1	1
Tomarctus (*Tom*)	10	13	7

6.3.2. Morphometrics

The shape of the forelimb bones was recorded by digitizing a set of three-dimensional homologous landmarks (LK) using a Microscribe G2X. LK coordinates (x, y, z) were collected with software Inmersion Inc. We digitized the same LK's used in a previous article (see Figure 3.1) and the criteria for homology and the specific locations of the selected LK's are further explained in Table 2.3. In addition, we scanned the bone surfaces of the humerus, radius and ulna of one individual of *Panthera onca* (AMNH-139959) with a 3D-mobile surface scanner (Nextengine HD) and using the software ScanStudio Pro. The same LK's digitized on the bones of the specimens analysed were also digitized on these 3D-scannings with software Landmark from the Institute of Data Analysis and Visualization (IDAV 2002-2006). In this way, the three-dimensional surface of the shape changes accounted for by the statistical analyses could be modeled by morphing (Wiley et al. 2005; Martín-Serra et al. 2014a; Martín-Serra et al. 2014b; Martín-Serra et al. in press), which facilitated their morphological interpretation.

Scaling, translation and rotation effects were discarded from the LK's coordinates by performing a Procrustes fit for each bone (Rohlf and Marcus 1993; Dryden and Mardia 1998) using software MorphoJ (Klingenberg 2011). Once the specimens were aligned, a Principal Component Analysis (PCA) of the Procrustes coordinates (Pco) was performed separately on the covariance matrix for each limb bone using MorphoJ (Klingenberg 2011). The morphological changes accounted for by each PC were discussed independently.

6.3.3. Grouping taxa according to different ecological criteria

In this study, the first step was to explore which ecological aspect is better reflected in the shape of the forelimb bones. For doing this, the living taxa were classified into different ecological categories. Categories of "predatory behaviour", were based on the criteria of Van Valkenburgh (1985): (*i*) ambushers (e.g., all felids except the cheetah, *Acinonyx jubatus*): predators that usually stalk and chase their prey for short distances, using the forelimbs to grapple with it; (*ii*) occasional predators (e.g., most ursids): species that hunt only occasionally and are not specialized for a particular predatory behaviour; (*iii*) pounce/pursuit predators (e.g., most canids): species that usually pounce or chase their prey for short distances but do not use their forelimbs to grapple with it; and (*iv*) pursuers (e.g., pack hunting canids and hyaenids): predators adapted to endurance running that chase their prey for relatively long distances and do not use their forelimbs to grapple with it. See Table 6.1 for the classification of each species. The only exception to the criteria used by Van Valkenburgh (1985) was the cheetah, a sprinter not adapted to endurance running, which was also classified as a pursuit predator following Figueirido and Janis (2011) and Janis and Figueirido (2014). In addition, the brown hyena, not included in Van Valkenburgh (1985), was considered as an occasional predator following the ecological description of Wilson and Mittermeier (2009).

Two locomotor categories were used following the criteria established by Van Valkenburgh (1987) and modified by Meachen-Samuels and VanValkenburgh (2009): (*i*) terrestrial: species that rarely or never climb, performing almost all of their activities on the ground; and (*ii*) scansorial: species that frequently climb but do not spend much time on the trees, as they usually forage on the ground (see Table 6.1). However, given that the terrestrial category of Van Valkenburgh (1987) encompasses a wide range of ecomorphologies within the order Carnivora, we further subdivided it into terrestrial and cursorial taxa (Table 6.1) following the criteria of Samuels et al. (2013), as an alternative classification for locomotor behaviour. In this case, the difference between terrestrial and cursorial taxa is that the latter regularly display rapid locomotion (e.g., gallop).

For the “habitat occupation” categories, we used the criteria established by Meloro et al. (2013) for felids and Wilson and Mittermeier (2009) and references therein for the remaining species (Table 6.1): (*i*) open habitat: species that are present mainly in grassland, artic or desert biomes; (*ii*) closed habitat: species mainly present in temperate or tropical forests; and (*iii*) mixed habitats: species with similar presence in open and closed habitats.

The phylogenetic classification was also included as an additional test. For doing so, each species was classified into its family (Table 6.1).

6.3.4. Comparing the ecological classifications

The first method selected to test for differences between ecological groups was a multivariate analysis of variance (MANOVA). Canonical variate analysis (CVA) was used in a subsequent step. The reason is that CVA can yield misleading results when the number of morphometric variables (three-dimensional coordinates of LK's in this study) is higher than the number of cases (i.e., specimens) per group compared (Mitteroecker and Bookstein 2011), as happens in our case. For each bone, the MANOVA tests were applied to the four ecological classifications, which provided a total of twelve separate analyses, in order to explore if any of these classifications is reflected in the morphology of each bone. The scores on the principal components (PCs) of each bone were used as dependent variables and the ecological classifications as the independent factor. Of course, each of these MANOVA's was performed excluding the extinct species. An additional MANOVA per bone (i.e., three in total) was also computed using the PC's as the dependent variables and the family to which each taxa belongs (Table 6.1) as the independent factor. The objective of the latter analysis was to evaluate the role of phylogeny in sorting limb bone shape variation in all PC's. Those PC's that jointly accounted for at least 90% of the original variance were included in the MANOVA's. These PC's were selected for avoiding the inflation of variables in the analysis, as all PCA's yielded more than 48 eigenvectors for each limb bone (i.e., the number of LK's digitized on each). All MANOVA's and associated analyses (see below) were performed using IBM SPSS Statistics v.15.

In order to investigate which of the four ecological aspects are better reflected in the shape of each forelimb bone, we compared the MANOVA tests using the F statistic and eta squared (η^2). The first is the usual parametric method to test the significance of this kind of analyses (Quinn and Keough 2002). The second is a proxy for the effect size and indicates the proportion of the total variance of the dependent variables (Total Sum of Squares; SS_{total}) that is accounted for by the variation of the independent variable (Between-groups Sum of Squares; $SS_{between}$), and it is calculated as $SS_{between}/SS_{total}$ (Levine and Hullett 2002). Therefore, high η^2 values (i.e., close to one) indicate a good separation in shape among the groups compared and low η^2 values (i.e., close to zero) denote poor differences.

Once the MANOVA tests were compared by the statistical parameters reported above, the ecological classification (based on η^2 and F statistics) better reflected in each bone was chosen.

6.3.5. Shape variation and ecological differences

The next step was to explore those morphological aspects accounted for by the PC's that better characterize each ecological group. For doing this, we first performed the Levene’s tests for each PC to explore if the within-group variances were homogeneous, as the MANOVA F-test is very sensitive to differences in within-group variances (Quinn and Keough 2002). For those PC's that showed homogeneity of variances, the F-statistic was used to test if they discriminated the ecological groups or not. In contrast, when heterogenetity of variances was noted, a single-factor robust ANOVA computing Welch’s test for the level of significance (Quinn and Keough 2002) was performed. The latter is a non-parametric test for ANOVA, which does not require to assume homogeneity of variance. Subsequently, the partial eta squared (η^2) was calculated for each PC, as this parameter measures the proportion of the total variance of each dependent variable (PC) that is accounted for by between-groups differences. Therefore, those PC's that yielded significant results of the F-statistic (or Welch’s test) and higher values for the partial eta squared were selected for subsequent interpretations.

Together with the MANOVA's, we also performed post hoc analyses, in order to identify the ecological groups that were significantly separated by each

PC. We used either Bonferroni’s test (parametric) or Dunnett’s T3 (non-parametric) for those PC's that showed homogeneous within-group variances or not, respectively (see Quinn and Keough 2002 and references therein). Using the information provided by these tests and the morphological changes associated with each PC, we made the ecomorphological interpretations of the differences among ecological groups for each bone.

6.3.6. Ecomorphological inferences for borophagine canids

Although the fossil specimens were not included into the MANOVA's, they were previously included in the PCA's. Therefore, after deciphering the functional and ecological meaning of each PC, we paid particular attention to the scores of borophagine specimens along these eigenvectors. The main objective was to extend to the extinct taxa the same functional interpretations obtained from the living ones, which allows to infer the aspects of their paleoecology associated with these interpretations.

A series CVA's was also performed with the Procrustes coordinates. However, as explained above, this analysis can provide misleading results if there are more variables than cases per group (Mitteroecker and Bookstein 2011). For this reason, it was necessary to reduce dimensionality using a stepwise method, which precludes the morphological interpretation of the functions obtained. For this reason, these CVA's were performed only to confirm the results obtained with the MANOVA tests and not to make new functional interpretations. The stepwise procedure was performed using the model available in software IBM SPSS Statistics v15. The variables were selected according to F-probability values of < 0.05 to be included and > 0.1 to be discarded.

6.4. Results

6.4.1. Comparison between ecological classifications

The PC's selected for using them in the MANOVA's were the first 15 in the case of the humerus and the radius, and the first 11 in the case of the ulna (Table S6.2).

The three forelimb bones showed F-statistics of the MANOVA's that were significant for the four ecological classifications and also for the phylogenetic classification (Table 6.3). Although the η^2 values obtained for phylogeny were the highest in all classifications, the η^2 values for the groups of predatory behaviour were also higher than for other ecological classifications (Table 6.3). Therefore, apart from phylogeny, differences among groups of predatory behaviour are better reflected in the shape variation of the three bones than those among locomotor or habitat groups. For this reason, the following analyses were made using only this ecological criterion.

Table 6.3. Results obtained in the MANOVA's performed for each classification and bone. *P*-values for F statistics and η^2 values are indicated.

		Phylogeny	**Pred. Beh.**	**Locomotion 1**	**Locomotion 2**	**Habitat**
Humerus	*P*-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	η^2	0.54	0.396	0.045	0.145	0.100
Radius	*P*-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	η^2	0.458	0.388	0.061	0.189	0.128
Ulna	*P*-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	η^2	0.516	0.466	0.111	0.261	0.158

6.4.2. Shape variation and group separation

Once established that predatory behaviour was the ecological aspect most closely related to forelimb bone shape variation, the morphological features associated with this ecological criterion were explored in detail for each bone.

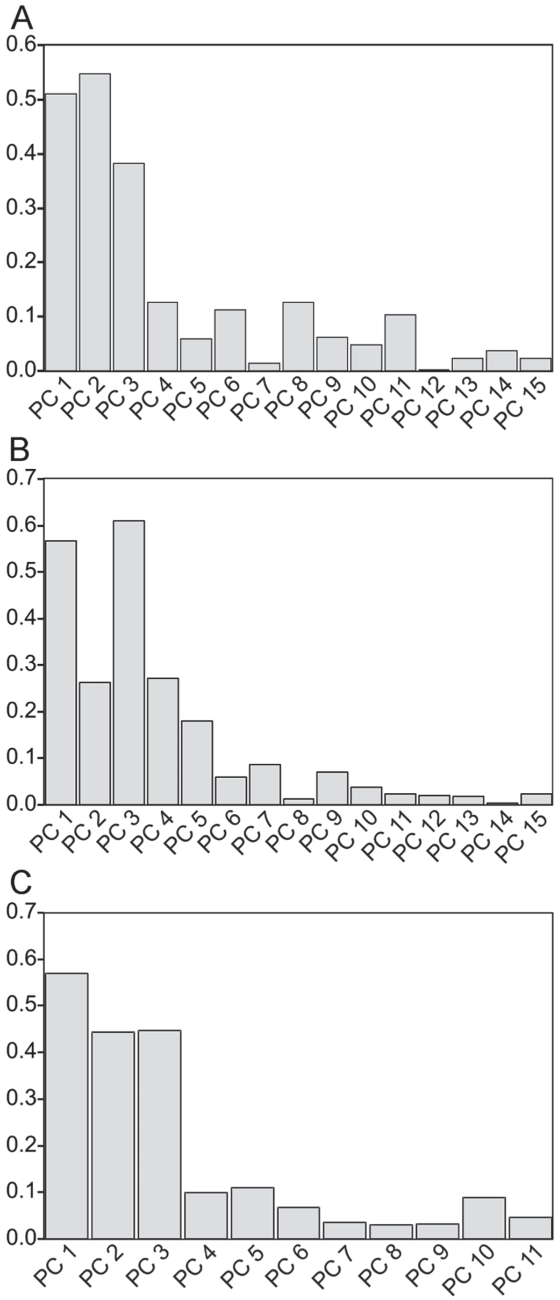


Figure 6.1. Histograms showing partial η^2 values for each PC included in the MANOVA performed with the predatory groups. Humerus (A), radius (B) and ulna (C).

6.4.2.1. Humerus

The *P*-values and partial η^2 values of each PC obtained for humeral morphology (Table S6.2) indicate that the first three components account for the majority of the between-group separation in this bone (Figure 6.1A). Therefore, these three PC's were selected for the following functional interpretations. Ambushers and occasional predators take positive scores on the first component (Figure 6.2A), which is associated with a robust humerus (Figure 6.2B), and both groups do not differ statistically from each other according to a Bonferroni's test. Pursuit predators take intermediate scores (Figure 6.2A) and, hence, show an intermediate degree of robustness (Figure 6.2B). Finally, pounce/pursuit predators show the most negative scores (Figure 6.2A), which are associated with a slender humerus (Figure 6.2B). Both pursuit and pounce/pursuit predators are statistically different from each other and also from ambushers and occasional predators (Table S6.3).

Occasional predators take the highest scores on the second PC (Figure 6.2C), as their humeri show reduced greater tuberosities and shallow and wide trochleas (Figure 6.2D). In contrast, pursuit predators take the lowest scores on this axis (Figure 6.2C), as their humeri have an expanded greater tuberosity and a shaft that is compressed mediolaterally and expanded anteroposteriorly, showing a narrow and deep trochlea (Figure 6.2D). Both ambushers and pounce/pursuit predators take intermediate scores on this axis (Figure 6.2C) and, thus, show an intermediate morphology. In fact, these two groups are not statistically different from each other (according to Dunnett's T3), but both differ from occasional and pursuit predators (Table S6.3).

Ambushers take positive scores on the third PC (Figure 6.2E) and are separated from the remaining groups (Table S6.3). Their humerus shows a caudally oriented head, a curved shaft and an expanded medial epicondyle (Figure 6.2F). Occasional, pounce/pursuit and pursuit predators take all negative scores (Figure 6.2E), with a humerus that shows a straight shaft, an expanded lateral epicondyle and a proximally expanded trochlea (Figure 6.2F). The projections of the three predatory groups on this PC are not statistically different (Table S6.3).

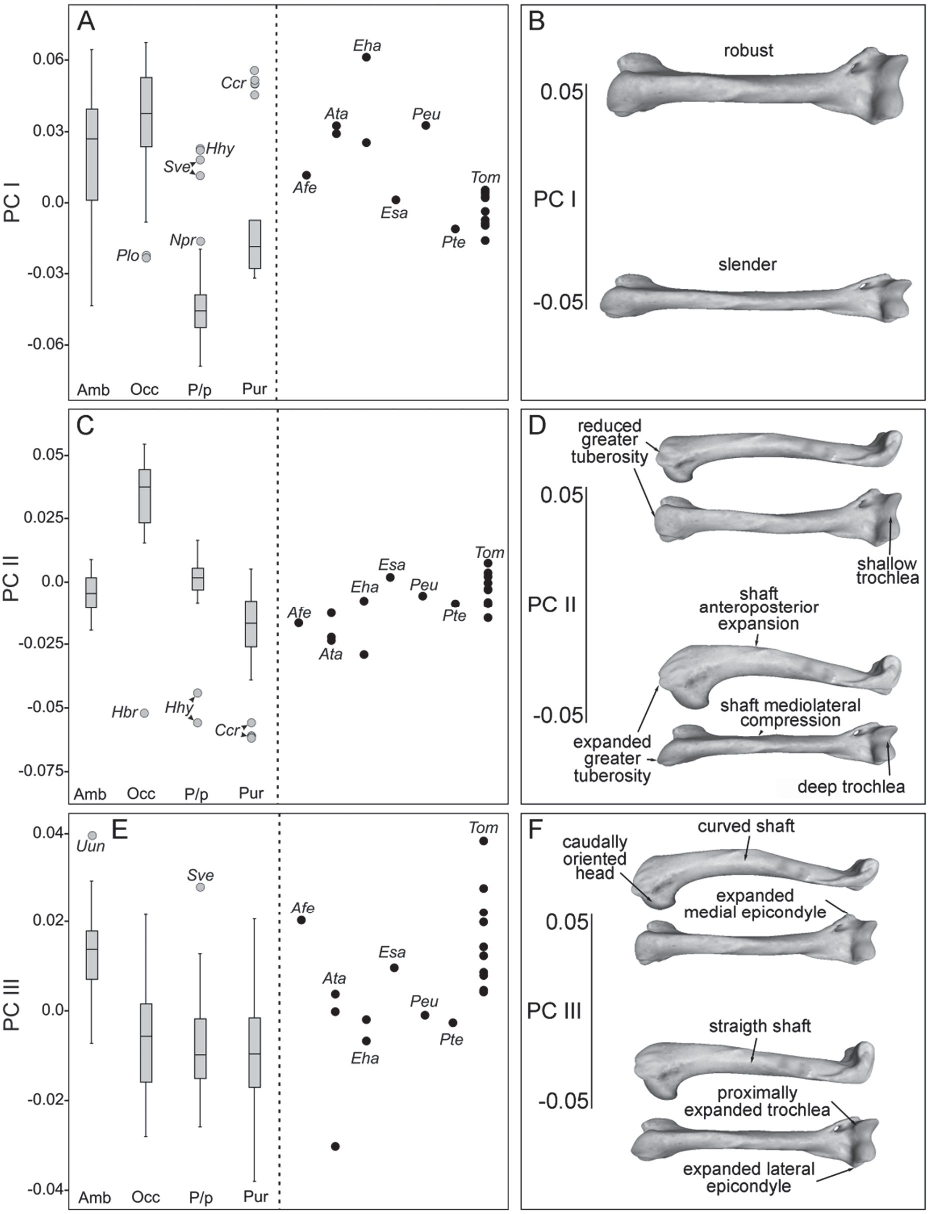


Figure 6.2. Scores and shape changes for the first three PCs for the humerus. Box plots (A, C, E) are accompanied by their associated shape changes (B, D, F) for each PC. Humerus models are shown in cranial and lateral views. For each predatory group: bar displays the median, the box shows 25-75 % of the distribution and the whisker represents 5-95 % of cases (grey circles indicate outliers). Amb, ambushers; Occ, occasional predators; P/p, pounce/pursuit predators; and Pur, pursuers. *Plo*, *Procyon lotor*; *Npr*, *Nyctereutes procyonoides*; *Sve*, *Speothos venaticus*; *Hhy*, *Hyaena hyaena*; *Ccr*, *Crocuta crocuta*; *Hbr*, *Hyaena brunnea*; *Uun*, *Uncia uncia*. Black circles represent fossil specimens of Borophaginae species (see Table 6.2 for species labels).

6.4.2.2. Radius

The P-values and partial η^2 values for radius shape (Table S6.2) indicate that the first and third components account for a large proportion of the between-group separation (Figure 6.1B). For the first PC, pounce/pursuit predators have the most positive scores (Figure 6.3A) and this portion of the morphospace is associated with a slender radius with an oval-shaped radial head whose major axis is parallel to the mediolateral plane (Figure 6.3B). Pursuers take slightly positive values on this eigenvector (Figure 6.3A), but they are statistically different from

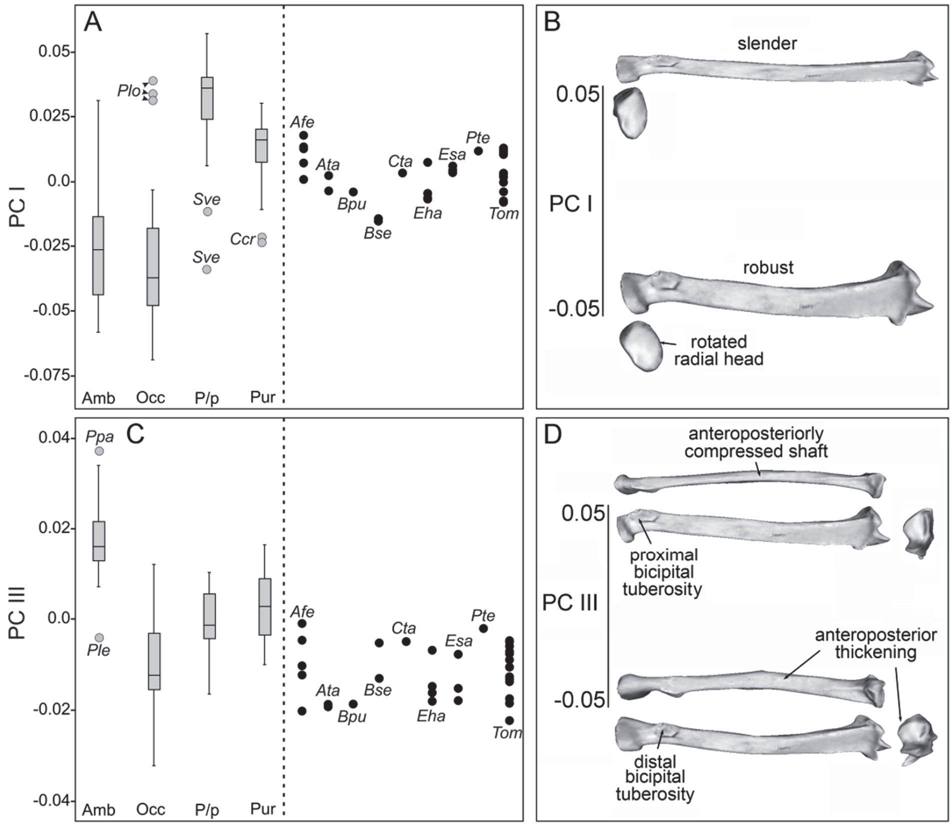


Figure 6.3. Scores and shape changes for the first and third PC's for the radius. Box plots (A, C) are accompanied by their associated shape changes (B, D) for each PC. Radius models are shown in caudal, lateral, proximal and distal views. For each predatory group: the bar displays the median, the box shows the 25-75 % quartiles of the distribution and the whisker represents 5-95 % of cases (grey circles indicate outliers). Amb, ambushers; Occ, occasional predators; P/p, pounce/pursuit predators; Pur, pursuers. *Ple*, *Panthera leo*; *Ppa*, *Panthera pardus* (see Figure 6.2 for more labels). Black circles represent fossil specimens of Borophaginae species (see Table 6.2 for species labels).

pounce/pursuit predators (Table S6.3). In contrast, both ambushers and occasional predators show similar scores, which do not differ significantly, on the negative portion of the morphospace (Figure 6.3A; Table S6.3) and this is associated with a robust radius with an oval-shaped radial head whose major axis is rotated with respect to the mediolateral plane (Figure 6.3B).

Ambushers differ statistically from the remaining groups on the third PC, on which they take the most positive scores (Figure 6.3C), and this is related to the presence of a radial shaft anteroposteriorly compressed and a bicipital tuberosity proximally shifted (Figure 6.3D). In contrast, occasional predators take the most negative scores (Figure 6.3C), as their radii show an anteroposterior thickening in the shaft and distal epiphysis, and a distally shifted bicipital tuberosity (Figure 6.3D). Both pursuit and pounce/pursuit predators have similar intermediate scores and morphology (Figure 6.3C; Table S6.3) and are statistically different from ambushers and occasional predators (Table S6.3).

6.4.2.3. Ulna

As indicated by the partial η^2 and *P*-values for ulna shape (Figure 6.1C; Table S6.2), the first three PCs account for the most important fraction of the between-group variances. For the first PC, both ambushers and occasional predators occupy the positive portion of the morphospace (Figure 6.4A), which characterized by the possession of a robust ulna (Figure 6.4B). These two groups do not differ statistically on this eigenvector (Table S6.3). Pounce/pursuit predators take the most negative scores (Figure 6.4A), as reflected in their extremely slender ulnae (Figure 6.4B). In contrast, pursuers show slightly negative scores (Figure 6.4A) and, accordingly, their ulnae are not as slender as those of pouncers. The scores on this eigenvector of both pursuit and pounce/pursuit predators differ statistically from each other and also from the remaining groups (Table S6.3).

Ambush predators take the most positive scores on the second PC (Figure 6.4C), which reflects that their ulnae have a long olecranon process and a straight shaft (Figure 6.4D). This group is statistically different from the others. Occasional predators occupy the opposite extreme on this axis (Figure 6.4C), because their ulnae show a short olecranon processes and a caudally curved shaft (Figure 6.4D). Pounce/pursuit predators have intermediate scores and morphologies (Figure 6.4C) along this second eigenvector. Although the scores of occasional and pounce/pursuit predators on this eigenvectors are significantly different, pursuers score between these two groups and cannot be distinguished from any of them (Figure 6.4C; Table S6.3).

Pursuit predators take the highest scores on the third component (Figure 6.4E) and are characterized by an ulna with a long olecranon process and a caudally curved shaft (Figure 6.4F). In contrast, occasional predators take the lowest ones (Figure 6.4E), which indicates that their ulna has a short olecranon process and a straight shaft (Figure 6.4F). Both groups differ statistically from each other and also from the remaining groups. However, ambushers and pounce/pursuit predators take intermediate scores along this eigenvector (Figure 6.4E) and do not differ between them (Table S6.3).

6.4.3. Paleobiological inferences for borophagines: PCA and CVA

The distribution of borophagine taxa along these PC's varies among the three bones. For example, for the first PC of humeral shape, *Tomarctus*, *Paratomarctus temerarius* and *Epicyon saevus* take all intermediate scores, which overlap to a great extent with those of ambush predators (Figure 6.2A), but also with some occasional predators, such as *Epicyon haydeni*, which shows extreme positive values (Figure 6.2A). In contrast, they group on the second PC near the average shape of carnivorans (Figure 6.2C), occupying the same area of pursuit and ambush predators. *Aelurodon ferox* and several specimens of *Tomarctus* take the most positive scores on the third PC, which is also the case of ambush predators (Figure 6.2E). In the opposite direction, *E. haydeni*, *Paratomarctus euthos* and *P. temerarius* take intermediate scores, similar to those of occasional, pursuit and pounce/pursuit predators (Figure 6.2E).

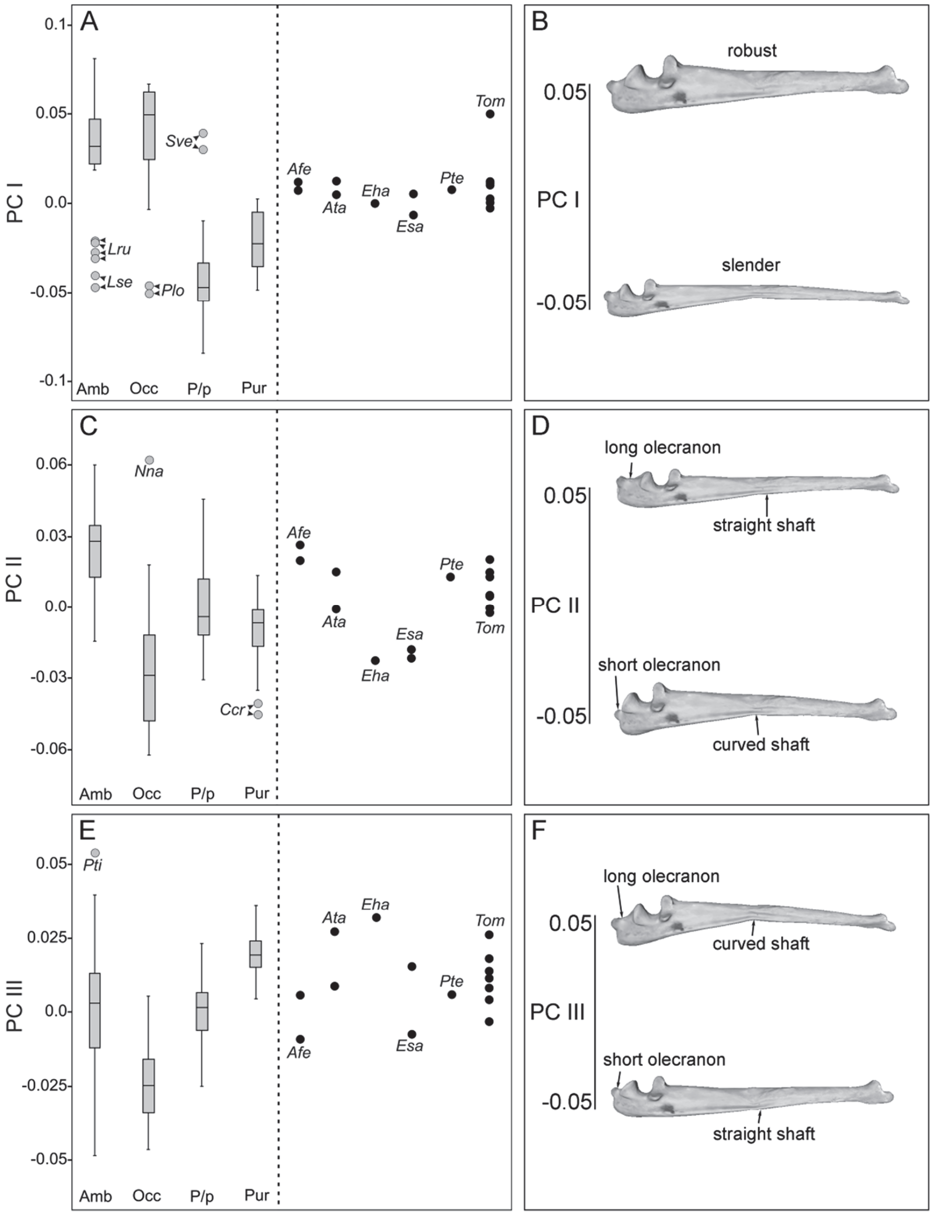


Figure 6.4. Scores and shape changes for the first three PCs for the ulna. Box plots (A, C, E) are accompanied by their associated shape changes (B, D, F) for each PC. Ulna models are shown in lateral view. For each predatory group: bar displays the median, the box shows 25-75 % of the distribution and the whisker represents 5-95 % of cases (grey circles indicate outliers). Amb, ambushers; Occ, occasional predators; P/p, pounce/pursuit predators; and Pur, pursuers. *Lru*, *Lynx rufus*; *Lse*, *Leptailurus serval*; *Nna*, *Nasua nasua*; *Pti*, *Panthera tigris* (see Figure 6.2 for more labels). Black circles represent fossil specimens of Borophaginae species (see Table 6.2 for species labels).

In the case of the first PC of radius shape, most borophagines overlap with pursuit predators (Figure 6.3A), although *Borophagus secundus* is closer to ambushers and occasional predators (Figure 6.3A). For the third PC, borophagines tend to overlap with occasional predators (Figure 6.3C), with the exception of *Carpocyon tagarctus*, *Paratomarctus temerarius* and some specimens of *A. ferox*, which are closer to pursuers or pounce/pursuit predators (Figure 6.3C).

The PCA performed for ulna shape shows that most borophagines cluster around the average shape on the first PC (Figure 6.4A), although they are not clearly associated with any of the predatory groups (Figure 6.4A). They also take intermediate scores on the second PC (Figure 6.4C), but the specimens of *A. ferox* show the most positive scores and are closer to ambushers, while *E. saevus* and *E. haydeni* have the most negative ones and are closer on this axis to occasional and pursuit predators (Figure 6.4C). In the case of the third PC, borophagines take, in general, positive scores (Figure 6.4E). The most positive projections are those of *Aelurodon taxoides*, *E. haydeni* and some specimens of *Tomarctus*, which overlap mainly with pursuers (Figure 6.4E). In contrast, *A. ferox* and one specimen of *E. saevus* take the most negative scores and coincide with ambushers and pounce/pursuit predators (Figure 6.4E).

The stepwise CVA performed for humeral shape yielded highly significant results for the discrimination of predatory groups (Table 6.4). The percentage of specimens that were correctly classified after cross-validation was 94.8 %. According to the probability of belonging to a given predatory group, borophagines are classified within the following groups (Figure 6.5A): *Aelurodon ferox* is mainly classified as a pursuer, while *E. haydeni*, *E. saevus*, *P. euthos* and *P. temerarius* are all classified as ambushers (Figure 6.5A). However, *A. taxoides* and *Tomarctus* are not clearly assigned to a single group, as they have similar probabilities of belonging to ambush and pursuit predators (Figure 6.5A).

The CVA for the radius showed also significant results (Table 6.4), with 85.2 % of correct classifications after cross-validation. The probabilities for borophagines of belonging to one of these groups are shown in Figure 6.5B.

Aelurodon ferox, *Borophagus pugnator*, *E. saevus*, *P. temerarius* and *Tomarctus* are mostly classified as pounce/pursuit predators, while *A. taxoides* and *E. haydeni* are classified as pursuers (Figure 6.5B). In contrast, both *B. secundus* and

Table 6.4. Results obtained in the stepwise CVA for the three bones. The Pco included in the analysis and their standardized coefficients for each CV are shown. The eigenvalue, percentage of variance explained, Wilks' λ, χ^2 and *P*-value for each CV are also shown.

Humerus	**CV 1**	**CV 2**	**CV 3**
Pco 2	0.8896	0.6895	0.1277
Pco 5	-0.0305	-0.6629	-0.0871
Pco 7	-0.6504	0.5260	-0.4494
Pco 10	0.0168	-0.7805	-0.3172
Pco 12	0.7193	-0.2277	0.5938
Pco 14	-0.6169	0.0163	-0.6292
Pco 15	-0.0115	-0.5308	1.3856
Pco 24	0.2039	0.3837	0.8079
Pco 27	1.3979	-0.4143	0.1771
Pco 38	1.2487	0.0308	-0.5046
Pco 40	-0.1995	0.7509	0.1908
Pco 41	0.3796	0.1788	-0.6404
Pco 42	0.5640	0.3026	0.1102
Pco 43	0.2788	-0.3595	-0.3189
Pco 45	0.3468	0.5845	-0.1909
Pco 46	-0.0780	0.4478	-0.5265
Pco 51	0.5357	-0.0937	0.4970
Eigenvalue	*16.53*	*5.89*	*1.1*
% variance	*70.3*	*25*	*4.7*
Wilks' λ	*0.004*	*0.069*	*0.477*
X^2	*572.84*	*276.42*	*76.68*
P-value	*< 0.001*	*< 0.001*	*< 0.001*
Radius	**CV 1**	**CV 2**	**CV 3**
Pco 8	0.0717	-0.4498	0.6046
Pco 14	0.0193	-0.4660	0.6512
Pco 19	0.5208	-0.2767	0.2315
Pco 21	-0.2918	0.5928	-0.0259
Pco 22	0.5024	0.3388	0.1559
Pco 23	0.3278	-1.0574	-0.4041

% variance	*60*	*33.9*	*6.1*
Wilks' λ	*0.016*	*0.125*	*0.596*
X^2	*434.51*	*219.55*	*54.61*
P-value	*< 0.001*	*< 0.001*	*< 0.001*
Ulna	**CV 1**	**CV 2**	**CV 3**
Pco 3	1.0090	0.2711	0.4172
Pco 16	0.0418	0.6672	-0.2442
Pco 20	-0.1876	0.0174	0.5407
Pco 28	0.0890	0.6930	0.3473
Pco 29	-0.1076	0.3906	-1.1632
Pco 30	0.7520	0.4878	0.1543
Pco 39	-0.3293	-0.4404	0.3471
Pco 40	0.5464	0.0396	0.1133
Pco 47	-1.0632	0.6670	0.7506
Pco 52	0.6444	-0.1168	0.4177
Pco 56	0.6549	0.3823	-0.0215
Pco 58	0.7527	-0.1411	0.4316
Pco 60	-0.7647	-0.3087	-0.1243
Eigenvalue	*11.75*	*3.42*	*0.539*
% variance	*74.8*	*21.8*	*3.4*
Wilks' λ	*0.012*	*0.147*	*0.65*
X^2	*470.9*	*202.35*	*45.48*
P-value	*< 0.001*	*< 0.001*	*< 0.001*

Carpocyon tagarctus have uncertain classifications and are grouped with ambushers and as occasional predators or pounce/pursuit predators, respectively (Figure 6.5B).

As in the case of the analysis performed from the humerus and radius, the CVA computed from ulna shape separates the predatory groups fairly well (Table 6.4), with an 86.1 % of correct classifications after cross-validation. Figure 6.5C shows the probabilities obtained for borophagine species of belonging to each predatory group. Accordingly, *E. haydeni* and *P. temerarius* are unambiguously classified as pounce/pursuit predators (Figure 6.5C). However, this analysis is characterized by classifying many species with similar probabilities for several predatory groups. For example, *A. taxoides* may be interpreted as an ambusher or as a pursuer, and *E. saevus* as a pounce/pursuit or as an occasional predator (Figure 6.5C). The cases of *A. ferox* and *Tomarctus* are the most extremes, as they have similar probabilities to belong to even three predatory groups (Figure 6.5C).

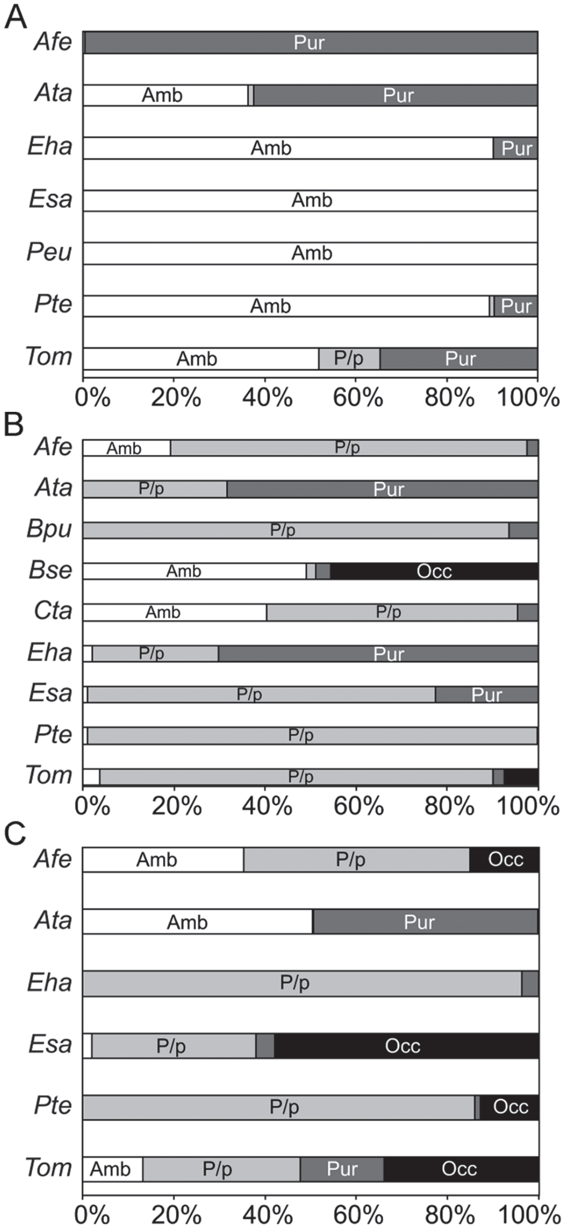


Figure 6.5. Probabilities of pertinence to predatory categories obtained in the CVA's for borophagine species. Humerus (A), radius (B) and ulna (C). Amb, ambushers (white); P/p, pounce/pursuit predators (light grey); Pur, pursuers (dark grey); Occ, occasional predators (black). See Table 6.2 for species labels.

6.5. Discussion

6.5.1. Predatory behaviour as the ecological aspect most closely related to forelimb morphology

According to our comparative analyses, forelimb morphology primarily reflects the effects of phylogenetic legacy (i.e., shape differences among carnivoran families) and secondarily the ecological adaptations towards predatory behaviour (Table 6.3). This result agrees with the study of Harris and Steudel (1997) of hind limb morphology, as they found that predatory behaviour (instead of prey size, home range or daily movement distance) was the ecological variable that better correlates with hind limb length. However, phylogenetic patterning does not prevent to make functional inferences on the morphological changes. In fact, Van Valkenburgh (1985) proposed that each carnivoran clade has developed specific trophic or locomotor adaptations for exploiting particular adaptive zones. Our results indicate that, for a sample that comprises members from several families of the order Carnivora, the main ecological adaptions reflected in the shape of the forelimb bones relate to predatory behaviour and not to locomotor modes or habitat types. It is also worth noting that the three bones analysed yielded similar results, which indicates that the forelimb responds as a whole to the same ecological adaptations. This suggests that the forelimb, at least for the bones analysed here, is a single functional module (Fabre et al. 2014), although this finding will probably be extended for the whole appendicular skeleton in future research.

Nevertheless, our results have been obtained from a sample that encompasses several carnivoran families, which does not preclude that both locomotor mode and habitat type may influence forelimb morphology at other taxonomic levels. This has been demonstrated for felids (Meachen-Samuels and Van Valkenburgh 2009; Walmsley et al. 2012; Meloro et al. 2013) and musteloids (Schutz and Guralnick 2007; Fabre et al. 2013). Therefore, we propose that the morphological evolution of the carnivoran forelimb bones could reflect a hierarchical structure for ecological adaptations, with predatory behaviour accounting for the higher taxonomic level within the order Carnivora and other minor ecological aspects, such as locomotor mode or habitat type, accounting for the within-families differences. However, this question is beyond the scope of this paper, as it is not possible to answer it with the data at hand. Therefore, we deserve its study for future research.

6.5.2. Morphological variation and function

The morphological changes accounted for the first eigenvector are essentially the same in the three bones analysed. These morphologies involve a change in the degree of bone robustness (Figures 6.2A, B; 6.3A, B and 6.4A, B), which is probably associated with a trade-off between maintaining resistance to stresses and keeping energetic efficiency during locomotion (Pasi and Carrier 2003; Kemp et al. 2005; Martín-Serra et al. 2014a, 2014b). As such, the robust bones of ambush predators and occasional predators indicate that locomotor efficiency is not a key factor for these groups. While occasional predators do not usually perform long or fast runs (Van Valkenburgh 1985), in which energetic efficiency is crucial, ambushers are adapted to perform short bursts of speed when hunting, although they do not chase their prey for long distances (Van Valkenburgh 1985; Anyonge 1996). Therefore, both predatory strategies require the possession of robust bones for resisting the high peak stresses generated during acceleration or deceleration, and also when grappling with prey (Anyonge 1996; Kemp et al. 2005). As a result, the radii of ambushers and occasional predators show a round-shaped and rotated head (Figure 6.3B), which gives more freedom to the rotational movements of this element with respect to the ulna (Taylor 1974; MacLeod and Rose 1993; Argot 2001; Fabre et al. 2013). This trait is advantageous for both predatory groups as they use their forelimbs to grapple with prey or manipulate food (Van Valkenburgh 1985; Anyonge 1996; Anton et al. 2004; Fabre et al. 2013). In contrast, pounce/pursuit and pursuit predators, which are adapted to perform long travels for foraging or long chases after prey, increase their energetic efficiency during locomotion by having slender forelimb bones (Van Valkenburgh 1985; Taylor

1989). The difference observed between these two groups could be caused solely by size differences: a pounce/pursuit predator tends to be smaller than a pursuit predator and thus, their forelimb bones are comparatively slender due to allometric effects (see Martín-Serra et al. 2014a).

The shape changes associated with the second PC of humeral shape mainly account for differences in mobility and efficiency for movements in the parasagittal plane. In this sense, the shallow trochlea of occasional predators (Figures 6.2C, D) indicates a high degree of freedom for the rotation of the elbow joint (Andersson 2004; Figueirido and Janis 2011; Janis and Figueirido 2014). In this way, they increase their ability to supinate the forearm. This is accompanied by a round-shaped cross-section of the shaft and a reduced greater tuberosity (Figure 6.2D), which suggest that this predatory group does not require a restriction of limb movements to the parasagittal plane (Anyonge 1996; Figueirido and Janis 2011; Janis and Figueirido 2014; Martín-Serra et al. 2014a). Accordingly, occasional predators can use their forelimbs for different tasks, including manipulating food, climbing or grappling with prey (Fabre et al. 2013), rather for specializing them to perform long and/or fast runs. In contrast, the deep and narrow trochlea of pursuers (Figures 6.2C, D) decreases their ability to rotate the elbow joint and thus, improves efficiency for parasagittal movements (Andersson 2004; Figueirido and Janis 2011; Janis and Figueirido 2014). In addition, the greater tuberosity is expanded, increasing the mechanical advantage of the supraspinatus muscle for humeral protraction (Spoor and Badoux 1986), and the shaft is expanded anteroposteriorly and compressed mediolaterally (Figure 6.2D). These traits suggest that the main direction of movement of the humerus is in the anteroposterior (parasagittal) plane (Anyonge 1996), which is advantageous for animals that perform fast runs pursuing their prey (Janis and Wilhelm 1993).

The third PC for humeral shape separates ambush predators from other groups (Figure 6.2E). The morphology associated to this axis reflects the ability of this group to subdue their prey with the forelimbs. For this reason, the humeral head is oriented caudally and the shaft is curved anteroposteriorly (Figure 6.2F), which suggest that the humerus functions in a partially flexed position when the prey is grappled with the forelimb. In addition, the medial epicondyle is expanded, which provides a large surface for the origin of the flexor muscles of the manus (Taylor 1974). This indicates the presence of well-developed flexor muscles to grasp with prey using the forepaw.

The third PC for radius shape allows to identify the anatomy typical of ambushers, which show a proximal bicipital tuberosity (Figure 6.3D) that indicates a lower mechanical advantage for the biceps brachii (Homberger and Walker 2004), because it exerts less power to flex the forearm with a quick flexion (Taylor 1974). However, the position and development of the bicipital tuberosity is not always consistent with other indicators of flexion capability (Argot 2001). In any case, this trait is associated with a distal epiphysis anteroposteriorly compressed and concave (Figure 6.3D), which reflects a more restricted movement of the manus (Taylor 1974). This trait helps to prevent dislocation at this joint, which could be advantageous for ambushers when hunting, as they have to exert large forces to subdue their prey. In contrast, the bicipital tuberosity of occasional predators, which is more distally located (Figure 6.3D), seems to increase the power of biceps brachii to flex the forearm. However, this trait is not always reliable, as discussed above. In addition, the square-shaped and less concave distal epiphysis of occasional predators (Figure 6.3D) may allow them to develop less restricted movements with the wrist, which relates to manipulating abilities (Taylor 1974).

The morphological traits accounted for by the second and third PC's obtained for ulna shape are striking, as they account for similar, although inverted, changes (Figures 6.4D, F). The long olecranon process and the straight ulnar shaft of ambushers (Figures 6.4C, D) may be associated with their need to exert large forces with the forelimbs when grappling with prey, as this ulnar shape provides a high mechanical advantage for the triceps brachii when the forearm is partially flexed (Van Valkenburgh 1987; Argot 2001; Martín-Serra et al., 2014a). In contrast, the short olecranon process of occasional predators (Figures 6.4C-F), which decreases the mechanical advantage for the triceps brachii in comparison with the anatomical condition of ambushers, probably relates to the fact that they do not usually need to exert large forces with their forelimb when manipulating food items, or even when hunting small prey. The curved ulnar shaft of pursuit predators (Figures 6.4E, F) is clearly associated with a more upright posture (Martín-Serra et al., 2014a). This increases functional limb length and, hence, stride length (Janis and Wilhelm, 1993), which is advantageous for reducing the costs of locomotion during a long chase after prey (Heglund et al. 1974; Strang and Steudel 1990; Janis and Wilhelm 1993).

6.5.3. Paleobiological inferences for Borophaginae

Depending on the bone analysed, forelimb morphology combines features of different predatory categories in borophagines. In general, they have a robust humerus (Figures 6.2A, B), which is not as mobile as in occasional predators and many ambushers (Figures 6.2C, D). However, *A. ferox* and *Tomarctus* seem to have been able of some degree of interaction with their prey using their forelimbs (Figures 6.2E, F). These results agree in general with those obtained from CVA's, as they also classified borophagines either as ambush or pursuit predators, or placed them between both types (Figure 6.5A), which evidences that the forelimb of borophagines is somewhat restricted to parasagittal movements (i.e., those species classified as pursuers), but with a certain ability to interact with their prey using the forelimbs in some cases (i.e., those species classified as ambushers).

The radius of borophagines is relatively slender, similarly to those of the living pursuers, and the rotational movement with respect to the ulna is partially restricted (Figures 6.3A, B). However, the morphology of the radial distal epiphysis (Figures 6.3C, D) indicates a certain degree of movement for the wrist. This combination of features is not shared with any living carnivoran, which suggests that, whatever was the predatory behaviour of borophagines, the function of the radius was not completely comparable with any of the living groups. This result is corroborated by the results of both PCA and CVA, as both multivariate analyses give uncertain classifications for the borophagines.

The ulna has an intermediate degree of robustness in all borophagines (Figures 6.4A, B), which suggests they were not as extremely adapted for energetic efficiency as modern pursuit or pounce/pursuit predators. However, the length of the olecranon process and the curvature of the shaft show both a high variability. For example, those species with a straight shaft and a long olecranon process (e.g., *A. ferox*; Figures 6.4C, D) have a high mechanical advantage for the triceps brachii and the ability to develop a more flexed posture. This indicates that these species could have used their forelimbs to grapple with prey. In contrast, those species with a curved shaft and a more upright posture (e.g., *A. taxoides*, *E. saevus* and *E. haydeni*; Figures 6.4C, D) were not adapted to grapple with prey. In contrast, they could develop a more efficient locomotion (e.g., *A. taxoides* and *E. haydeni*; Figures 6.4E, F) or perhaps were not secondarily adapted to grapple with prey if a cursorial condition was basal to these groups (e.g., *E. saevus*; Figures 6.4E, F). The CVA classifications obtained for *E haydeni* point to the same direction, but in the case of *E. saevus* and *A. taxoides* they are more ambiguous (Figure 6.5C). Other taxa, such as *P. temerarius* and *Tomarctus,* have a more generalized morphology, as they do not show adaptations for manipulating prey running fast and efficiently (Figure 6.5C). This is confirmed by the classification obtained for *Tomarctus*, but not for *P. temerarius*, as the former is assigned clearly to the pounce/pursuit predatory category (Figure 6.5C).

These results agree with the conclusions achieved in previous studies (Munthe 1989), which showed that borophagines had a certain ability to manipulate prey according to the morphology of several forelimb structures (e.g., scapulohumeral and elbow joints, muscle insertion scars, etc.) and other traits of the postcranial skeleton (i.e., hind limb morphology and vertebral column; Munthe 1989). In summary, these studies concluded that borophagines did not optimize endurance running, as do modern canids (Munthe 1989). However, they also recognized differences among borophagine taxa: for example, *Epicyon* had more slender limbs, and hence was more “cursorial” than *Aelurodon*, *Tomarctus* and *Borophagus*, which showed more robust limbs (Munthe 1989). Although our findings broadly match with these interpretations, we have shown some adaptive differences between *E. haydeni* and *E. saevus*. However, Van Valkenburgh et al. (2003) concluded that although the body size and craniodental morphology of some borophagines suggest that they fed on large prey, they probably did not have the ability to grapple and subdue it as the living felids do. In addition, compared to the living strict scavengers (e.g., the brown hyena), borophagines seem to have been more abundant in their paleocommunities. For this reason, Van Valkenburgh et al. (2003) they proposed that such specialized trophic behaviour was unlikely for borophagines. Instead, they concluded that most borophagine species were pack-hunting predators, not scavengers as proposed previously by other authors (e.g., Werdelin 1989; Munthe 1998). Finally, Andersson (2005) pointed to the opposite direction, as he found that borophagines retained the ability to supinate the forearm, which suggests that they could grapple with prey like the living felids, which led him to propose that pack-hunting predation was unlikely. In summary, the study of the morphological traits of the postcranial skeleton can hardly be conclusive on this aspect. This study is not an exception, but the differences among the three forelimb bones related to functional adaptions and ecological classifications suggest that the predatory behaviour of most borophagines is not adequately represented in any of the living categories. For this reason, the speculation about pack-hunting in borophagines should not only restrict their comparisons to the living pack-hunting canids, as other predatory strategies may also have been possible for this extinct group.

6.6. Conclusions

We have shown in this study that the morphology of the carnivoran forelimb, represented by the shape of the humerus, radius and ulna, reflects adaptions to different predatory behaviours rather than to different modes of locomotion or habitat. This indicates that the forelimb may work as an integrated functional module. In addition, the main variations of shape associated with a given predatory strategy are: (*i*) a change in the degree of robustness, which relates to a trade-off between energetic efficiency and resistance to stresses (Martín-Serra et al. 2014a); (*ii*) a change in the degree of freedom for rotational movements in the humerus and radius; (*iii*) a change in the length of the olecranon process and, hence, in the mechanical advantage for the triceps brachii; and (*iv*) a change in posture (flexed or upright), which is reflected in the humerus and ulna.

The borophagines included in these analyses showed a particular combination of these traits, which suggests that their predatory behaviour cannot be unequivocally assigned to any of the modern categories. They were not as well adapted to grapple with their prey as are modern ambushers, but they neither had the cursorial adaptions of modern pounce/pursuit and pursuit predators. Therefore, our hypothesis is that their predatory behaviour was not fully comparable to any of those shown by the living carnivorans. However, in this study emerged some differences among borophagine species, which can be functionally interpreted as indicating that some taxa had cursorial adaptions (e.g., *E. haydeni*) while others behaved more as an ambusher (e.g., *B. secundus*).

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6.8. Supporting information

Table S6.1. List of fossil specimens included in this study. The reference number (ID) and hosting institution is indicated. AMNH, American Museum of Natural History.

Species	ID	Host Institution
Humerus		
Aelurodon ferox	27479	AMNH
Aelurodon taxoides	67481	AMNH
Aelurodon taxoides	30902	AMNH
Aelurodon taxoides	67442	AMNH
Epicyon haydeni	67403	AMNH
Epicyon haydeni	67603	AMNH
Epicyon saevus	67489	AMNH
Paratomarctus euthos	67536	AMNH
Paratomarctus temerarius	105340	AMNH
Tomarctus sp.	67775	AMNH
Tomarctus sp.	67527	AMNH
Tomarctus sp.	67547	AMNH
Tomarctus sp.	67714	AMNH
Tomarctus sp.	67715	AMNH
Tomarctus sp.	67716	AMNH
Tomarctus sp.	67737	AMNH
Tomarctus sp.	67740	AMNH
Tomarctus sp.	67746	AMNH
Tomarctus sp.	67747	AMNH
Radius		
Aelurodon ferox	27479 L	AMNH
Aelurodon ferox	27479 R	AMNH
Aelurodon ferox	61746	AMNH
Aelurodon ferox	67459	AMNH
Aelurodon ferox	70624	AMNH
Aelurodon taxoides	67428	AMNH
Aelurodon taxoides	67445	AMNH
Borophagus pugnator	61664	AMNH
Borophagus secundus	67918-A	AMNH
Borophagus secundus	67918	AMNH
Carpocyon tagarctus	67565	AMNH
Epicyon haydeni	67404	AMNH
Epicyon haydeni	67406	AMNH
Epicyon haydeni	67407	AMNH
Epicyon haydeni	67607	AMNH
Epicyon saevus	67490	AMNH
Epicyon saevus	67508	AMNH
Epicyon saevus	8305	AMNH
Paratomarctus temerarius	105347	AMNH
Tomarctus sp.	67749	AMNH
Tomarctus sp.	67751	AMNH
Tomarctus sp.	67752	AMNH
Tomarctus sp.	67754	AMNH
Tomarctus sp.	67756	AMNH
Tomarctus sp.	67757	AMNH

Tomarctus sp.	67758	AMNH
Tomarctus sp.	67528	AMNH
Tomarctus sp.	67529	AMNH
Tomarctus sp.	67548	AMNH
Tomarctus sp.	67550	AMNH
Tomarctus sp.	67552	AMNH
Tomarctus sp.	67717	AMNH
Ulna		
Aelurodon ferox	27479 L	AMNH
Aelurodon ferox	27479 R	AMNH
Aelurodon taxoides	67943	AMNH
Aelurodon taxoides	67980	AMNH
Epicyon haydeni	67611	AMNH
Epicyon saevus	67492	AMNH
Epicyon saevus	8305	AMNH
Paratomarctus temerarius	61071	AMNH
Tomarctus sp.	67759	AMNH
Tomarctus sp.	67760	AMNH
Tomarctus sp.	67761	AMNH
Tomarctus sp.	67720	AMNH
Tomarctus sp.	67721	AMNH
Tomarctus sp.	67722	AMNH
Tomarctus sp.	67725-D	AMNH

Table S6.2. Principal components used for the MANOVA and tests associated to each bone. For each PC: percentage of variance explained, *P*-value for Levene's test, *P*-value for the F statistic or Welch's test (*) and partial η^2 values.

		% Variance	*Levene's test*	*P-value*	*Partial* η^2
Humerus	*PC 1*	43.269	0.056	< 0.001	0.511
	PC 2	14.664	0.003	< 0.001*	0.548
	PC 3	7.052	0.142	< 0.001	0.383
	PC 4	4.589	0.008	0.002*	0.127
	PC 5	3.751	0.281	0.079	0.059
	PC 6	3.300	0.002	< 0.001*	0.113
	PC 7	2.447	0.414	0.656	0.014
	PC 8	2.324	0.002	0.001*	0.126
	PC 9	1.953	0.337	0.069	0.062
	PC 10	1.661	< 0.001	0.133*	0.048
	PC 11	1.477	0.909	0.007	0.104
	PC 12	1.200	0.120	0.980	0.002
	PC 13	1.163	0.244	0.454	0.023
	PC 14	1.009	0.083	0.246	0.036
	PC 15	0.922	0.552	0.449	0.023
Radius	*PC 1*	39.792	0.039	< 0.001*	0.567
	PC 2	8.380	0.064	< 0.001	0.263
	PC 3	7.889	0.126	< 0.001	0.610
	PC 4	7.535	0.553	< 0.001	0.271
	PC 5	5.228	0.307	< 0.001	0.180
	PC 6	4.046	0.002	0.123*	0.059
	PC 7	3.303	0.186	0.019	0.086
	PC 8	2.956	0.596	0.724	0.012
	PC 9	2.431	0.006	0.041*	0.071
	PC 10	2.136	0.061	0.227	0.038
	PC 11	1.832	0.053	0.458	0.023
	PC 12	1.518	0.711	0.513	0.020
	PC 13	1.497	0.963	0.564	0.018
	PC 14	1.183	0.015	0.942*	0.003
	PC 15	1.039	0.060	0.439	0.024
Ulna	*PC 1*	47.353	0.037	< 0.001*	0.575
	PC 2	17.466	0.117	< 0.001	0.448
	PC 3	10.624	0.000	< 0.001*	0.452
	PC 4	3.321	0.071	0.008	0.101
	PC 5	2.214	0.304	0.004	0.111
	PC 6	1.973	0.018	0.067*	0.067
	PC 7	1.741	0.598	0.256	0.036
	PC 8	1.567	0.001	0.196*	0.030
	PC 9	1.485	0.233	0.305	0.032
	PC 10	1.294	0.157	0.015	0.089
	PC 11	1.183	0.186	0.155	0.046

Table S6.3. Results from the post hoc analyses for the PC's selected. *P*-values for the differences between pairs of predatory groups are shown, either from Bonferroni's test or from Dunnett's T3 (*).

Humerus		Ambush	Occasional	Pounce/pursuit
PC1	Occasional	0.186		
	Pounce/pursuit	< 0.001	< 0.001	
	Pursuit	0.047	< 0.001	< 0.001
*PC2**	Occasional	< 0.001		
	Pounce/pursuit	0.567	< 0.001	
	Pursuit	0.011	< 0.001	0.002
PC3	Occasional	< 0.001		
	Pounce/pursuit	< 0.001	1	
	Pursuit	< 0.001	1	1
Radius		Ambush	Occasional	Pounce/pursuit
*PC1**	Occasional	0.990		
	Pounce/pursuit	< 0.001	< 0.001	
	Pursuit	< 0.001	< 0.001	0.001
PC3	Occasional	< 0.001		
	Pounce/pursuit	< 0.001	< 0.001	
	Pursuit	< 0.001	< 0.001	0.834
Ulna		Ambush	Occasional	Pounce/pursuit
*PC1**	Occasional	0.995		
	Pounce/pursuit	< 0.001	< 0.001	
	Pursuit	< 0.001	< 0.001	0.003
PC2	Occasional	< 0.001		
	Pounce/pursuit	< 0.001	< 0.001	
	Pursuit	< 0.001	0.101	0.511
*PC3**	Occasional	< 0.001		
	Pounce/pursuit	1	< 0.001	
	Pursuit	< 0.001	< 0.001	< 0.001

7. Synthesis and conclusions

"My business is to teach my aspirations to confirm themselves to fact, not to try and make facts harmonize with my aspirations"

Thomas H. Huxley (letter to C. Kingsley, 1860)

7.1. Synthesis

Through this section, I will explain the findings obtained in the different articles published during the development of my PhD Thesis, in order to provide a broader picture on how I have contributed to the “state of the art” of the evolution of the postcranial skeleton in mammalian carnivores, together with the new tools for its analysis and its contribution to the field of Paleobiology.

In the articles I and II, I explored the effects of allometry, phylogenetic legacy and function (estimated using different methods) in the carnivoran fore- and hind limb bones, respectively. In addition, I also explored their morphological variability. Not surprisingly, the results obtained for the fore- and the hindlimb were very similar. In the case of allometric changes, the adaptation towards larger sizes is achieved through the acquisition of more robust limb bones (mainly the scapula, humerus, radius and femur) and a more upright posture (inferred by the changes in the ulna and tibia). In addition, for both limbs, bone morphology is greatly influenced by the phylogenetic legacy, especially the pectoral and pelvic girdles. With respect to the influence of the functional aspect, both articles explored this question in different ways. For the forelimb, we correlated bone shape with two proxies of locomotor performance: maximum running speed and daily movement distance; in the case of the hind limb, we explored those shape changes associated with different modes of locomotion. Both kinds of analyses yielded similar results: the association between limb bone shape and locomotion is not as evident as for allometry or phylogenetic legacy. Another important similarity between the fore- and the hind limb of carnivorans is that the morphological variability of the major limb bones is highly concentrated within an axis of slenderness-robustness, which I hypothesize that is a result of a biomechanical trade-off between resistance to stresses and energetic efficiency during locomotion. According to this hypothesis, it can be deduced that both limbs are affected by this trade-off in a quite similar fashion, because carnivorans are quadrupeds (i.e., they use their four limbs to move). In contrast to the long bones, the pectoral and pelvic girdles show a higher morphological variability, which could indicate that the biomechanical constraints that affect the long bones do not apply to the girdles.

The next step in the study of the morphological variability of the carnivoran appendicular skeleton is represented by article III. Here, I explored the degree of morphological covariation between the limb bones taking into account allometric effects and the phylogenetic legacy. As expected in the light of previous results, the long bones were strongly integrated between them in both within- and between-limb comparisons. In addition, this integrated pattern is also represented by a morphological axis of slenderness-robustness, which emphasizes the highly constrained morphological evolution of these bones. However, the pectoral and pelvic girdles are poorly integrated both with other bones and between them, which indicates again that they do not share the constraints (either developmental or biomechanical) that affect the long bones.

Additionally, the functional signal on the patterns of limb integration was tested by comparing two different functional groups: those species with a similar function for the fore- and hind limbs (i.e., primarily locomotion, as in cursorial carnivorans) show a higher between-limb integration that those species with different functions for the fore- and hind limbs (i.e., the forelimbs of non cursorial carnivorans, which are employed for manipulating food items or to grapple with prey, while their hind limbs are only used for locomotion). This demonstrates that, although other aspects sometimes mask the influence of locomotor adaptations, natural selection seems to have been able to modify the patterns of morphological integration across the evolution of the carnivoran skeleton.

Finally, in article IV, I explored in more detail the association between forelimb morphology and function. In contrast with the previous articles, I took here an ecomorphological perspective, instead of a purely evolutionary one. As such, I paid more attention to some morphological features that, although secondary from a quantitative point of view (as most of them account for a relatively low percentage of shape variance), have important biomechanical consequences. As a result, I suggest that these features can be used as ecomorphological indicators of different modes of locomotion or hunting strategies, because they represent functional adaptations. As a consequence, they are potentially useful for paleobiologists for making inferences on the locomotor abilities of extinct taxa. In order to show this, I used as case study several taxa belonging to the canid subfamily Borophaginae. The results obtained provided some clues on their adaptations towards different hunting strategies. Most borophagine taxa show a combination of adaptations that is different from those shown by modern carnivorans, which suggests that they most probably had a unique hunting strategy. However, their morphological features also allowed me to deliver a number of functional interpretations, including if they were able to grapple with their prey or if they were good runners.

7.2. Conclusions

The most important conclusions reached in this PhD dissertation are:

- Allometric shape changes of the appendicular long bones of carnivorans are associated in both the fore- and hind limb with two mechanisms that decrease the risk of bone failure under the loads generated by large sizes: (*i*) to increase bone robustness with larger sizes, as it also increases their capacity of resisting bending loads; and (*ii*) to acquire a more upright posture, as it increases the effective mechanical advantage for muscles and reduces bending stresses.
- The correlation between performance for locomotor behaviour and appendicular bone morphology is not as evident as expected, because other aspects as phylogenetic legacy or biomechanical constraints can mask this association.
- Most of the morphological variability of all long bones is highly concentrated within an axis of slenderness-robustness. I hypothesize that this axis could be associated with the need to maintain a trade-off between

resistance to stresses (achieved with robust bones) and energetic efficiency during locomotion (achieved with slender bones). One consequence of this constrained variation is that species with different ecological adaptations can have similar bone morphologies. This is because both stress resistance and energetic efficiency can be adaptive for different ecological scenarios. Therefore, the long bones of the carnivoran appendicular skeleton represent a remarkable case of "one-to-many" mapping, in which one morphological solution applies for different ecological problems. Furthermore, this fact is most probably one of the causes of the poor correlation observed between morphology and ecology.

- The pectoral and pelvic girdles are both characterized by a different morphological pattern compared with the other appendicular bones. The girdles show a more variable morphology than the long bones. In fact, they are clearly more influenced by the phylogenetic legacy than by other aspects, especially the pelvis, whose morphological variation is more complex, probably due to its particular structure, as it is composed of three fused bones and is connected with the axial skeleton.
- The appendicular skeleton of carnivorans is highly integrated as a whole, both within and between limbs. The degree of morphological covariation between the long bones is high and is associated with an axis of slenderness-robustness. I hypothesize that this morphological axis, which coincides with the most important axis of morphological variation, can be caused by the biomechanical trade-off mentioned before (i.e., resistance to stresses *vs.* locomotor efficiency). This trade-off affects both limbs, because they function in a coordinate fashion during quadrupedal locomotion. Therefore, quadrupedal locomotion imposes similar biomechanical demands on the fore- and hind limbs, which result in a higher level of integration under the action of natural selection.
- The degree of integration within and between limbs increases from the proximal bones to the distal ones. This fact points to a functional cause for

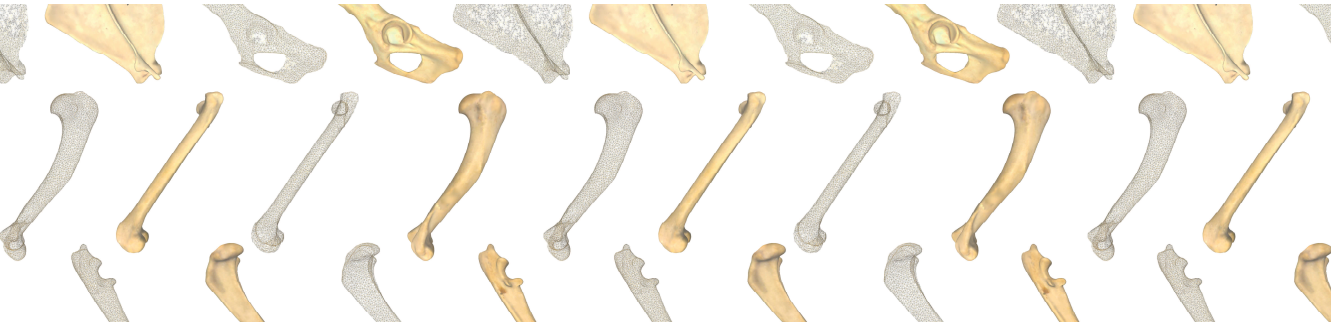
this pattern, as the distal bones are usually more influenced by functional modifications than the proximal ones.

- In the case of the between limbs comparisons, the hypotheses of functional equivalence have a similar level of integration than for serial homologues. Thus, the integration due to ancestral sharing of developmental mechanisms does not seem to be the only cause behind this pattern of integration.
- The pectoral and pelvis girdles do not follow the same pattern than the long bones, as they are poorly integrated with the remaining bones and also between each other. This may be due to their different developmental and evolutionary origin, or may even owe to different biomechanical and functional implications.
- Functional specialization towards performing similar functions in the fore- and hind limb increases the degree of morphological integration between the limbs. This has been demonstrated by comparing the degree of covariation within and between limbs of two functional categories: cursors (those species that use their fore- and hind limbs primarily for locomotion) have more integrated limbs than non-cursors (those species that use their forelimbs for other activities apart from locomotion, such as manipulating food items or catching their prey). This finding represents a remarkable case of matching between a strong functional interaction and a strong morphological integration due to the action of natural selection on complex morphological structures.
- Of both limbs, the morphology of the forelimb bones is highly influenced by their adaptation towards different hunting strategies, at least in mammalian carnivores. In contrast, other ecological attributes, such as locomotion modes or habitat use, seem to have less influence on forelimb bone morphology.
- The most important morphological features of the forelimb that are associated with different hunting strategies reflect adaptations towards several biomechanical demands, such as resistance to stresses, energetic

efficiency during locomotion, degree of forearm mobility, mechanical advantage for some muscles, and limb posture. Each hunting category is characterized by a particular combination of these adaptations.

- Borophagine taxa show a unique combination of morphological features in their forelimbs, which is not present in any of the living carnivorans. As a consequence, it is deduced that their predatory behaviour was not fully comparable to any of those shown by the living carnivorous mammals. However, some biomechanical and functional implications of these morphological features can be tentatively interpreted, for example the adaptations to exert large forces with the forelimb of *Aelurodon ferox,* which indicates that this species could have some capacity to manipulate prey, whereas the adaptations towards an upright posture of *Epicyon haydeni* suggest that it was a better runner.

8. Resumen



8.1. Introducción

La locomoción es uno de los aspectos ecológicos más importantes de los animales, pues resulta clave con vistas a la búsqueda de alimento, el apareamiento o la protección frente al peligro. Por lo tanto, las estructuras corporales implicadas en la locomoción, ya sea el esqueleto axial y la cola en los peces o el esqueleto apendicular en los vertebrados terrestres (tetrápodos), se han visto sujetas a modificación por parte de la selección natural en la adaptación de los organismos a estrategias locomotrices específicas. La relación entre el modo de locomoción y las adaptaciones morfológicas del aparato locomotor se puede estudiar desde diferentes perspectivas. Algunos de los enfoques más frecuentes en este tipo de estudios provienen de la biomecánica y la fisiología, centrándose en la comprensión de los mecanismos implicados en la locomoción desde una perspectiva puramente física. A diferencia de los neontólogos, los paleobiólogos han estudiado la locomoción con un enfoque diferente, establecer las adaptaciones osteológicas hacia diferentes modos de locomoción y tipos de hábitat en las especies actuales, con el objetivo de retrodecir aspectos de esta índole en taxa extintos. Por último, el esqueleto apendicular también se puede estudiar desde un punto de vista estrictamente evolutivo, centrándose entonces en los diferentes procesos que condicionan su evolución morfológica, como la presencia de adaptaciones funcionales, convergencias o limitaciones del desarrollo embrionario.

En vista de estos antecedentes, en esta tesis doctoral he utilizado métodos de morfometría comparada para analizar la asociación entre las adaptaciones funcionales del esqueleto apendicular y su evolución morfológica. Por lo tanto, el objetivo principal del trabajo será buscar aquellas características osteológicas del esqueleto postcraneal que se relacionan con las diferentes estrategias locomotrices, para evaluar los efectos potenciales de la selección natural en su evolución morfológica. De este modo, se aprovechará esta información para inferir aspectos paleoecológicos en especies extintas. Por lo tanto, el enfoque seguido para el estudio del esqueleto apendicular será de naturaleza dual, abarcando tanto aspectos estrictamente evolutivos como puramente paleobiológicos. Con este propósito, he utilizado los mamíferos carnívoros actuales y extintos (orden Carnivora) como objeto de estudio, pues representan una opción excelente a estos propósitos, ya que muestran en la actualidad una amplia variedad de tamaños, hábitats y adaptaciones del aparato locomotor. Además, sus relaciones filogenéticas están bien resueltas y se encuentran relativamente bien representados en el registro fósil. Sin embargo, cualquier conocedor del registro mastozoológico se puede plantear por qué esta tesis doctoral no se ha centrado en el estudio de los ungulados, ya que por normal general, la completitud de su registro es sustancialmente mayor que la de los carnívoros, debido a su mayor abundancia en las comunidades. No obstante, la especialización que presentan la mayoría de las familias de ungulados hacia una estrategia locomotriz hípercorredora (del inglés hypercursorial) limita comparativamente la variabilidad morfológica de su esqueleto apendicular.

8.2. Objetivos

A continuación se enumeran los objetivos específicos abordados durante el desarrollo de la tesis:

- Caracterizar la forma de las cinturas pectoral y pélvica, así como de los huesos largos del esqueleto apendicular de los carnívoros fisípedos actuales y extintos, mediante la localización de puntos homólogos o de equivalencia anatómica (*landmarks*) en tres dimensiones. Por tanto, se han utilizado diversos métodos, todos ellos basados en la morfometría geométrica, para analizar la forma así capturada. Los huesos seleccionados son la escápula, el húmero, el radio y la ulna en el caso de la extremidad anterior, y los huesos pélvicos, el fémur y la tibia en el de la posterior.
- Explorar los efectos alométricos de los huesos del esqueleto apendicular, es decir, si los cambios de tamaño se traducen en cambios de forma y si es así, describir y cuantificar tales cambios.

- Evaluar si la variación en la forma de los huesos se ve influida por las relaciones filogenéticas entre los taxones, así como sus efectos sobre los distintos elementos óseos y sus causas.
- Someter a prueba la hipótesis de si la adaptación a distintos modos de locomoción influye en la forma de los huesos de las extremidades. Para ello se han seguido dos enfoques diferentes: en primer lugar, se han utilizado la velocidad máxima y la distancia de movimiento diario como indicadores de la función locomotriz en las especies actuales; en segundo, se han clasificado estas especies en categorías discretas de locomoción, relacionables con las variaciones en la forma de los elementos apendiculares.
- Explorar la variabilidad morfológica de los huesos de las extremidades, prestando especial atención a los caminos evolutivos seguidos por los diferentes taxones y a cómo su variabilidad se ve influida por la herencia filogenética u otro tipo de limitaciones.
- Investigar el patrón y el grado de integración morfológica entre los huesos de una misma extremidad y entre los de ambas extremidades. Las comparaciones realizadas entre distintos elementos incluyen aquellos que se encuentran conectados anatómicamente dentro de la misma extremidad, los que son homólogos seriales entre las dos extremidades y los que se han propuesto como equivalentes funcionales, también entre ambas extremidades.
- Contrastar la influencia de la especialización funcional en el grado de integración morfológica entre los huesos del esqueleto apendicular, es decir, si aquellas especies con ambas extremidades especializadas hacia una misma función en la locomoción muestran también un mayor grado de integración.
- Explorar cómo la morfología de la extremidad anterior se ve influida por diferentes aspectos ecológicos, tales como el modo de locomoción, la estrategia de caza o el tipo de hábitat. Este análisis se realiza únicamente en la extremidad anterior porque participa de manera más directa que la

posterior en muchas actividades, como por ejemplo manipular objetos o manejar las presas.

- Seleccionar aquellos aspectos ecológicos que se reflejan mejor en la forma de los huesos de la extremidad anterior y evaluar las implicaciones funcionales y biomecánicas de estas características fenotípicas.
- Utilizar estas características morfológicas para hacer inferencias paleoecológicas sobre taxones extintos, tales como los representantes de la subfamilia Borophaginae (Mammalia, Carnivora, Canidae).
- Los resultados derivados de alcanzar los objetivos anteriores se han repartido en cuatro artículos (tres de ellos publicados en revistas internacionales), cuyo resumen de contenidos se presenta en la siguiente sección.

8.3. Artículo I: evolución de la extremidad anterior

En este trabajo se evaluó la influencia de la herencia filogenética, la alometría y la actividad locomotriz en la forma de los huesos de la extremidad anterior en carnívoros actuales y extintos (Mammalia, Carnivora). Además, se exploró la presencia de convergencias morfológicas hacia estrategias locomotrices similares en la forma de los huesos largos de dicha extremidad. Para ello se realizaron una serie de análisis morfométricos a partir de la forma de los elementos de esta extremidad (escápula, húmero, radio y ulna), capturada mediante puntos homólogos en tres dimensiones. En primer lugar se comparó la forma de cada hueso con un estimador del tamaño del mismo (tamaño del centroide, calculado como la suma de las distancias al cuadrado de todos los puntos al centroide o centro geométrico), para comprobar los cambios de forma asociados al cambio de tamaño (efectos alométricos). Posteriormente, se realizaron una serie de regresiones multivariantes entre la forma de los huesos y dos variables indicativas de la actividad locomotriz en los carnívoros, la velocidad máxima y la distancia de movimiento diario, con el objetivo de apreciar si existe una relación entre la forma de la extremidad anterior y la ecología. Finalmente, se llevaron a cabo una serie de análisis de componentes principales para estudiar la variabilidad fenotípica de estos huesos, así como poner de manifiesto la existencia de limitaciones evolutivas de diferente índole o de convergencias morfológicas. En todo ello he tenido muy en cuenta el efecto de la herencia filogenética, mediante el uso de diversos análisis para estimar su señal en los cambios de forma o para reconstruir la forma hipotética de los ancestros.

Los resultados obtenidos indican que el tamaño ejerce una gran influencia sobre la morfología de todos los huesos de las extremidades anteriores. Los cambios alométricos asociados al aumento de tamaño indican un aumento de la robustez de la escápula, el húmero y el radio, probablemente dirigido a incrementar su resistencia a los esfuerzos producidos por el aumento de la masa corporal. Asimismo, el cambio alométrico experimentado por la ulna parece indicar la tendencia hacia una postura más erguida en los taxones de mayor tamaño. Este cambio de postura ayudaría también a compensar el incremento en el estrés producido al soportar una mayor masa corporal, ya que reduce los esfuerzos de torsión e incrementa la ventaja mecánica de los huesos. Aunque existe una fuerte influencia de la herencia filogenética sobre la forma de los huesos, no parece afectar significativamente a los cambios alométricos descritos anteriormente.

A diferencia de lo que ocurre con el tamaño corporal, la forma de los huesos no se correlaciona de forma significativa con las dos variables estimadoras de la actividad locomotriz (velocidad máxima o distancia de movimiento diario), al menos cuando se tiene en cuenta el efecto de la filogenia. El estudio sobre la variabilidad morfológica ha dado algunas pistas sobre esta ausencia de relación directa entre la morfología de la extremidad anterior y ambas variables, indicadoras de la ecología de las especies. Estos análisis han mostrado que la variación en la forma de los huesos se concentra básicamente en un cambio relativo al grado de robustez, lo que sugiere que existen limitaciones biomecánicas resultantes del papel que desempeña la extremidad anterior en el soporte del peso corporal y en la locomoción. Tales limitaciones se pueden deber a la presencia de dos requerimientos biomecánicos opuestos: por un lado mantener la eficiencia energética en la locomoción, facilitada por una extremidad grácil, y por otro la resistencia al estrés generado por las actividades que realiza esta extremidad, que aumenta con una configuración robusta. De este modo, se emite la hipótesis de que es la imposibilidad de mejorar simultáneamente ambos aspectos lo que restringe la morfología de los huesos a meras variaciones asociadas con su grado de robustez. Dado que existen diferentes situaciones ecológicas que pueden favorecer tanto una mayor eficiencia energética como una mayor resistencia al estrés, diversas especies con ecologías muy dispares pueden mostrar morfologías similares. A modo de conclusión, se afirma que la evolución morfológica de la extremidad anterior constituye un caso interesante en el que una misma morfología está asociada a varios contextos ecológicos, dando lugar a un patrón evolutivo que se conoce como "uno para muchos" (del inglés *one-to-many mapping*).

8.4. Artículo II: evolución de la extremidad posterior

Del mismo modo que en el artículo anterior, en este artículo se utilizaron distintos métodos de morfometría geométrica basada en puntos homólogos en tres dimensiones para analizar la forma de los huesos de la extremidad posterior (fémur, tibia y cintura pélvica), con el objetivo último de cuantificar la influencia del tamaño corporal, la filogenia y el comportamiento locomotriz en el diseño morfológico de esta extremidad. Además, se realizó una exploración hipotética, basada en el principio de la parsimonia, sobre los principales patrones evolutivos de variación fenotípica de estos huesos largos en los carnívoros, tanto actuales como extintos.

En primer lugar, se efectuaron una serie de regresiones múltiples de la forma de los huesos frente a su tamaño, estimado una vez más mediante el tamaño del centroide, con objeto de cuantificar los cambios alométricos. Además, mediante el uso del contraste independiente se comprobó si tales cambios se ven influenciados por la herencia filogenética. Posteriormente, se efectuaron una serie de análisis de componentes principales, para obtener los principales ejes de variación en la forma de los huesos de la extremidad posterior. También se llevó a cabo la reconstrucción de la forma hipotética de los estados ancestrales, para observar los cambios morfológicos ocurridos en el curso de la evolución de los distintos linajes. Finalmente, se realizaron una serie de análisis para separar las especies con diferentes modos de locomoción, usando distintas categorías, como corredores, escaladores, terrestres, arborícolas, semiacuáticos y excavadores. Para ello se efectuó un análisis de componentes principales entre grupos, buscando la máxima varianza entre las morfologías medias de los distintos grupos ecológicos y generando, con los componentes obtenidos, un morfoespacio en el que se puede explorar tanto la varianza entre grupos (ecológica) como dentro de los grupos (calculando para cada especie los valores dentro de cada componente).

Los resultados muestran que la variación en tamaño influye fuertemente en la forma de los huesos de las extremidades traseras. En el caso del fémur, el incremento de tamaño va asociado a una mayor robustez, apropiada para resistir el incremento de estrés generado por el aumento en la masa corporal. Por el contrario, el cambio alométrico más importante en la tibia se asocia a la curvatura de su diáfisis, de modo que ésta adquiere una mayor resistencia a los esfuerzos axiales con el aumento de tamaño, probablemente debido a que los animales de mayor porte adoptan una postura más erguida. En el caso de la cintura pélvica, los cambios alométricos parecen estar relacionados con una mayor superficie de origen para los músculos proximales en las especies de mayor tamaño (vg., una cresta iliaca más amplia para el origen de los glúteos), lo cual apunta hacia un aumento de la potencia muscular acorde con el aumento de tamaño y robustez de los huesos. Pese a que la herencia filogenética influye en la variación de forma y tamaño de los elementos de la extremidad posterior, los efectos alométricos no se ven alterados cuando se tiene en cuenta el efecto de la herencia filogenética.

8. Resumen

Los análisis de componentes principales y el estudio de los filomorfoespacios indican que la principal fuente de variación en la forma de los huesos largos (fémur y tibia) se debe, como en el caso de la extremidad anterior, al grado de robustez de los mismos. Asimismo, el que la variación se concentre en un solo aspecto morfológico, un gradiente de gracilidad a robustez, se puede relacionar con la necesidad de mantener un equilibrio entre la energía consumida durante la locomoción y la resistencia al estrés generado durante las distintas actividades locomotrices. De hecho, esta limitación puede ser una de las causas, junto a la fuerte señal filogenética, de la ausencia de una clara separación entre las distintas categorías de locomoción a partir de la forma de los huesos, ya que ambos extremos morfológicos (grácil/robusto) son adaptativos en distintos contextos ecológicos y conducen a una similitud de morfologías entre taxones con ecologías muy diferentes. En este sentido, la extremidad posterior se comporta de un modo muy similar al de la anterior, descrita en el artículo precedente. Por tanto, el estudio de la anatomía funcional y la evolución de la extremidad posterior revela un patrón consistente en la totalidad del esqueleto apendicular de los carnívoros, según el cual una misma morfología puede ser adecuada para distintas ecologías, lo que contrasta con el patrón evidenciado en el cráneo de los carnívoros, en el que se observan convergencias morfológicas asociadas a adaptaciones hacia una ecología trófica similar en diferentes especies (vg., hacia una dieta hipercarnívora). Esto sugiere la posibilidad de que la evolución de la forma del esqueleto apendicular esté sujeta a mayores limitaciones que la del cráneo, debido a las exigencias de origen biomecánico derivadas de su participación en el soporte de la masa corporal y en la locomoción terrestre.

8.5. Artículo III: integración en el esqueleto apendicular

En este artículo se investigan los patrones de integración morfológica en el esqueleto apendicular de los mamíferos carnívoros. El objetivo fue observar los patrones de covariación (como estimadores de la integración) entre los diferentes elementos óseos, teniendo en cuenta los efectos del tamaño, la herencia filogenética y la adaptación sobre su grado de integración morfológica. El método usado para capturar la forma de los huesos apendiculares es el mismo que el de los artículos precedentes (esto es, usando landmarks en 3D). Se han efectuado una serie de análisis de mínimos cuadrados parciales de dos bloques (*two block partial least squares, 2B-PLS*) para cuantificar la covariación morfológica interespecífica. Esta metodología permite encontrar los ejes de variación morfológica que maximizan la covarianza entre dos bloques de variables. Las comparaciones entre huesos seleccionados se han ajustado a distintos criterios: (*i*) comparando pares de elementos conectados anatómicamente dentro de cada extremidad, como las parejas escápula-húmero, húmero-radio, húmero-ulna, radio-ulna, pelvis-fémur y fémur-tibia; (*ii*) comparando elementos que se consideran homólogos seriales entre la extremidad anterior y la posterior, como las parejas escápula-pelvis, húmero-fémur y radio-tibia; y (*iii*) comparando elementos que se proponen como equivalentes funcionales, por un lado la pareja ulna-tibia (pues la reducción de la fíbula puede haber llevado a una convergencia funcional entre ambos elementos) y, por otro, las comparaciones escápula-fémur y húmero-tibia (ya que se pueden considerar como el primer y el segundo elemento móvil de las extremidades de los mamíferos cuadrúpedos, respectivamente). Finalmente se ha realizado un test para comprobar si las especies de carnívoros que tienen ambas extremidades especializadas para la locomoción terrestre muestran un grado de integración morfológica mayor que aquellas que usan también la extremidad anterior en otras actividades, como manipular objetos o abatir a sus presas.

8. Resumen

Los resultados obtenidos sugieren que el esqueleto apendicular de los carnívoros está altamente integrado. Además, el principal cambio morfológico asociado a esta integración es el grado de robustez de los huesos, excepto en el caso de la pelvis. De hecho, tanto la pelvis como la escápula tienen un bajo grado de integración tanto con el resto de elementos como entre ellas mismas. Asimismo, el grado de integración dentro de cada extremidad y entre las extremidades aumenta distalmente a lo largo del eje proximodistal de las extremidades, lo cual se podría relacionar con la mayor influencia del componente adaptativo en los elementos distales, más implicados en las variaciones funcionales relacionadas con el tipo de locomoción, en comparación con los proximales, cuya forma es más conservativa por razones puramente biomecánicas, como soportar la masa corporal. Por otro lado, la integración observada entre los posibles equivalentes funcionales es alta en el caso de las comparaciones húmero-tibia y ulna-tibia, pero no en la comparación escápula-fémur. Estos patrones parecen indicar también que las adaptaciones funcionales, y no solo un origen embrionario compartido, ejercen un efecto en la integración morfológica del esqueleto apendicular.

Para confirmar la influencia de las adaptaciones en el grado de integración de las extremidades, las especies actuales se clasificaron en dos categorías funcionales: (*i*) corredoras especializadas, es decir, aquellas en las que las extremidades anteriores sólo están implicadas en la locomoción terrestre; y (*ii*) especies que no están especializadas hacia la carrera, al encontrase involucradas en diversas funciones no locomotoras, a diferencia de las posteriores. El objetivo último es comprobar si la similitud funcional en las extremidades de las especies especializadas conlleva una mayor integración. Los resultados han mostrado que, efectivamente, las especies cuyas extremidades anteriores y posteriores se ven envueltas sólo en la locomoción tienen un mayor grado de integración, lo que se manifiesta especialmente a nivel de sus elementos distales. Por lo tanto, este resultado sugiere que la selección natural puede modificar no sólo el patrón, sino también el grado de integración morfológica en aquellas estructuras que funcionan de manera coordinada, de modo que sus cambios morfológicos discurran en paralelo. Esto quiere decir que la selección natural modifica los patrones del desarrollo embrionario no solo a nivel de las diferencias observadas entre los grandes clados, como mamíferos marsupiales respecto a placentados (los marsupiales desacoplan el desarrollo de la extremidad anterior de la posterior debido a su función durante el recorrido hasta el marsupio de la madre al nacer), sino que también actúa al nivel de diferencias funcionales más sutiles entre especies próximas filogenéticamente.

8.6. Artículo IV: ecomorfología de la extremidad anterior

En este artículo se efectuó un estudio ecomorfológico de los huesos de la extremidad anterior de los carnívoros (húmero, radio y ulna). Más específicamente, se compararon diferentes aspectos ecológicos que potencialmente pueden influir en la variación morfológica de la extremidad anterior, como el modo de locomoción, la estrategia de caza o la ocupación del hábitat. Se utilizó la extremidad anterior y no la posterior debido a que es la que posee una mayor variabilidad de funciones dependiendo de la ecología de las especies y, además, participa más directamente en diversas actividades no locomotoras, como la manipulación de objetos o la lucha con las presas. En primer lugar, se analizó la variación en la forma de los huesos mediante el uso del análisis de componentes principales. Posteriormente, para calcular el efecto de cada aspecto ecológico en los componentes obtenidos, se realizaron una serie de análisis multivariantes de la varianza (MANOVA), considerando como factor independiente las distintas clasificaciones ecológicas. El parámetro η^2 ha servido para cuantificar el efecto del factor independiente sobre las varianza de las variables dependientes. De este modo se seleccionaron los aspectos ecológicos que se reflejan mejor en la forma de los huesos de la extremidad anterior, lo que ha permitido explorar las implicaciones funcionales de las características morfológicas asociadas. Por último, estas adaptaciones se usaron para inferir la paleoecología de algunas especies de la subfamilia Borophaginae (Mammalia, Carnivora, Canidae). Asimismo, se realizaron una serie de análisis discriminantes para confirmar las interpretaciones obtenidas a partir de los componentes principales, a efectos de clasificar los casos desconocidos en las categorías utilizadas.

Los resultados obtenidos muestran que la morfología de los huesos de la extremidad anterior se correlaciona mejor con las estrategias de caza (vg., emboscadores, cazadores no especializados, abalanzadores-corredores y corredores) que con el modo de locomoción (vg., terrestres, corredores, y escaladores) o la preferencia de hábitat (vg., hábitat cerrado, mixto o abierto). Por lo tanto, este aspecto ecológico es en el que se ha profundizado posteriormente. Las principales adaptaciones morfológicas hacia diferentes tipos de caza se relacionan con: (*i*) el grado de robustez de los huesos, siendo en general mayor en cazadores no especializados (ocasionales) y emboscadores que en los más especializados (abalanzadores-corredores y corredores), lo que se relaciona probablemente con el hecho de que los últimos maximizan la eficiencia energética reduciendo la masa de la extremidad; (*ii*) la movilidad de la extremidad para movimientos de rotación y abducción, potenciada en los cazadores ocasionales y, en menor medida, en los emboscadores, ya que suelen tener una mayor habilidad manipulando objetos, mientras que los carnívoros corredores restringen tales movimientos al plano parasagital y bloquean los de rotación para hacer más eficiente la carrera; (*iii*) la capacidad de la extremidad anterior de ejercer y resistir fuerzas durante la locomoción o la caza, maximizada en los emboscadores pues suelen usar las extremidades anteriores para abatir a sus presas; (*iv*) la ventaja mecánica para los músculos extensores del antebrazo (tríceps), deducida a partir de la longitud de olécranon, que es también mayor en los emboscadores, probablemente debido a la necesidad de manejar a sus presas con las extremidades anteriores; y (*v*) la curvatura de la ulna y la orientación en dirección posterior del olecranon en las especies corredoras, lo cual se puede relacionar con su postura más erguida, que les proporciona mayor eficiencia durante la locomoción.

Por último, la aplicación de estas inferencias a los cánidos borofaginos ha mostrado que estas formas presentaban una combinación de adaptaciones morfológicas única, que no coincide con ninguna de las estrategias adoptadas por los carnívoros actuales. De hecho, las interpretaciones obtenidas varían en función del elemento que se considere. Por ejemplo, el húmero de los borofaginos es relativamente robusto, como el de los emboscadores o los cazadores no especializados, mientras que el radio es más grácil, como el de los corredores. Por lo tanto, algunas de estas características parecen representar una adaptación hacia una locomoción más eficiente (el radio es grácil, el húmero tiene una epífisis distal que restringe los movimientos no parasagitales y, en algunos casos, la ulna se curva hacia atrás), mientras que otras sugieren adaptaciones hacia el manejo de presas u objetos (el húmero y la ulna son relativamente robustos, mostrando en muchos casos un olecranon largo, lo que les permitiría ejercer una gran fuerza con el tríceps). Además, las clasificaciones según estrategias de caza de las especies de borofaginos, obtenidas a partir de los análisis discriminantes, confirman esta combinación en mosaico de los caracteres, ya que la probabilidad de pertenencia a cada categoría varía en función del elemento utilizado. En vista de estos resultados, se propone que la estrategia de caza que probablemente utilizaban los borofaginos no coincidía en muchos casos de manera exacta con ninguna de las presentes en la actualidad. Pese a ello, es posible detectar diferencias entre algunas especies. Así, *Epicyon haydeni* muestra adaptaciones para aumentar la eficiencia durante la locomoción, mientras que *Aelurodon ferox* se orienta en la dirección de los emboscadores actuales.

8.7. Síntesis y conclusiones

En esta sección se exponen, de un modo general, los aspectos que relacionan los diferentes artículos presentados en la tesis, con el fin de dar una visión global sobre los objetivos alcanzados durante su desarrollo.

8. Resumen

Los artículos I y II se dirigen a evaluar el efecto de la alometría, la herencia filogenética y la función (estimada utilizando diferentes métodos) en los huesos de las extremidades anteriores y posteriores de los carnívoros, así como a explorar su variabilidad morfológica. Los resultados obtenidos para la extremidad anterior y la posterior son muy similares. En el caso de los cambios alométricos, el aumento de tamaño conlleva la adquisición de unos huesos más robustos (principalmente en el caso de la escápula, el húmero, el radio y el fémur) y una postura más erguida (inferida a partir de los cambios alométricos de la ulna y la tibia). Además, la morfología de los elementos de ambas extremidades se encuentra muy influenciada por la herencia filogenética, especialmente a nivel de las cinturas pectoral y pélvica. Respecto a la influencia del aspecto funcional, ambos artículos exploran esta cuestión de diferente manera. Para la extremidad anterior, se buscó correlacionar la forma de los huesos con dos variables relacionadas con la actividad del aparato locomotor, la velocidad máxima de carrera y la distancia de movimiento diario; en el caso de la posterior, se relacionaron los cambios de forma con los diferentes modos de locomoción. Ambos tipos de análisis suministraron resultados similares, evidenciando que la asociación entre la forma de los huesos de las extremidades y el tipo de locomoción no es tan evidente como en el caso de la alometría o del legado filogenético. Otra similitud importante entre ambas extremidades es que la variabilidad morfológica de los huesos largos de los carnívoros se concentra en un eje de esbeltez-robustez, lo que podría resultar de una limitación biomecánica debida a la contraposición entre resistencia al estrés y eficiencia energética durante la locomoción. Según esta hipótesis, se puede deducir que las dos extremidades se verían afectadas por esta limitación de manera similar pues, como cuadrúpedos, los carnívoros utilizan sus cuatro extremidades de manera coordinada para moverse. A diferencia de los huesos largos, las cinturas pectoral y pélvica muestran mayor variabilidad morfológica, lo que podría indicar que las limitaciones biomecánicas que afectan a los huesos largos no se aplican a estas estructuras.

El siguiente paso en el estudio de la variabilidad morfológica del esqueleto apendicular de los carnívoros se llevó a cabo en el artículo III. En este caso se investigó el patrón y el grado de integración morfológica entre los huesos de las extremidades, teniendo en cuenta los efectos del tamaño y la herencia filogenética. Los resultados mostraron que los huesos largos están muy integrados entre sí, tanto en las comparaciones dentro de cada extremidad como entre ambas extremidades. De hecho, al igual que en los trabajos previos, este patrón de integración se manifiesta en cambios en el grado de robustez. Sin embargo, las cinturas pectoral y pélvica están poco integradas entre sí y con el resto de los elementos óseos, lo que una vez más indica que no comparten las limitaciones que afectan a los huesos largos, ya sean éstas de origen embrionario o biomecánico. Además, el efecto de la adaptación funcional en la integración de las extremidades se puso a prueba mediante la comparación de dos categorías diferentes. Las especies con ambas extremidades especializadas para la locomoción terrestre (corredores) muestran mayor integración que las que realizan diferentes funciones con la extremidad anterior, como manipular objetos o presas, mientras usan la extremidad posterior solo en la locomoción. Esto sugiere que la selección natural es capaz de modificar los patrones de integración morfológica del esqueleto apendicular de los carnívoros.

Por último, en el artículo IV se exploró con más detalle la relación entre morfología y función en la extremidad anterior. A diferencia de los artículos anteriores, en éste se adoptó una perspectiva ecomorfológica, en lugar de una estrictamente evolutiva. El foco de interés fueron las características fenotípicas que, pese a explicar un porcentaje relativamente bajo de la varianza morfológica, tienen consecuencias biomecánicas y funcionales importantes. Tales características se pueden usar como indicadores ecomorfológicos relacionados con las adaptaciones hacia diferentes modos de locomoción o estrategias de caza. En consecuencia, son potencialmente útiles en paleobiología para inferir las capacidades del aparato locomotor de los taxones extintos. Para mostrar esto, se han utilizado como caso de estudio varios taxones pertenecientes a la subfamilia Borophaginae (Mammalia, Carnivora, Canidae). Los resultados obtenidos para este taxón extinto proporcionan pistas sobre sus adaptaciones hacia diferentes estrategias de caza. La mayoría de las especies muestran una combinación de adaptaciones diferente de las que tienen los carnívoros modernos, lo cual sugiere que podrían tener una estrategia de caza y/o modo de locomoción únicos. Sin embargo, sus características morfológicas también permiten realizar interpretaciones funcionales, como si podían abatir a sus presas con las extremidades anteriores o eran buenos corredores.

Las conclusiones más importantes alcanzadas en esta tesis doctoral son:

- Los cambios alométricos de los huesos largos del esqueleto apendicular de los carnívoros se asocian a dos mecanismos que disminuyen el estrés generado por una masa corporal elevada: (*i*) aumentar la robustez de los huesos de forma paralela al incremento en tamaño, lo que aumenta también su capacidad de resistir los esfuerzos; y (*ii*) adquirir una postura más vertical, lo que aumenta la ventaja mecánica para los músculos y reduce los esfuerzos de torsión.
- La correlación entre la actividad o el modo de locomoción, por un lado, y la morfología de los elementos del esqueleto apendicular, por otro, no resultó tan evidente como se esperaba, debido a la influencia de otros aspectos, como el legado filogenético o las limitaciones biomecánicas, que pueden enmascarar dicha asociación.
- La mayor parte de la variabilidad morfológica de los huesos largos se concentra en un eje que mide el grado de robustez. Este gradiente se podría asociar a una contraposición entre la resistencia a los esfuerzos (maximizada con la posesión de huesos robustos) y la eficiencia energética durante la locomoción (potenciada por los huesos gráciles). Una consecuencia de este grado limitado de variación es que las especies con diferentes adaptaciones ecológicas pueden tener morfologías similares, debido a que tanto la resistencia al estrés como la eficiencia energética, cuyos óptimos funcionales son opuestos, resultan adaptativas en diferentes escenarios ecológicos. Por lo tanto, los huesos largos del esqueleto apendicular de los carnívoros constituyen un ejemplo paradigmático sobre el hecho de que una misma solución morfológica permite resolver

diferentes problemas ecológicos. Además, esta puede ser la causa principal de la débil correlación observada entre morfología y ecología.

- Las cinturas pectoral y pélvica se caracterizan por un patrón morfológico diferente al de los otros huesos del esqueleto apendicular, pues ambas muestran mayor variabilidad. De hecho, se ven más influenciadas por la herencia filogenética que por otros aspectos. El caso de la cintura pélvica es el más llamativo, por ser su variación morfológica más compleja, lo que probablemente se debe a su estructura peculiar, al incluir tres huesos fusionados y conservar una estrecha conexión con el esqueleto axial.
- El esqueleto apendicular de los carnívoros está muy integrado en su conjunto, tanto dentro de cada extremidad como entre las extremidades anteriores y las posteriores. El grado de integración morfológica entre los huesos largos es muy alto y se asocia con el grado de robustez. Este cambio morfológico tan concentrado probablemente pueda venir determinado por la limitación biomecánica mencionada anteriormente, el compromiso funcional entre resistencia a los esfuerzos y eficiencia energética durante la locomoción. La contraposición de ambos factores afecta a ambas extremidades por igual al funcionar coordinadamente durante la locomoción cuadrúpeda. Dentro de este escenario, dicho tipo de locomoción impondría demandas biomecánicas similares a ambas extremidades, lo que se traduciría en la consecución de un patrón integrado bajo la acción de la selección natural.
- El grado de integración dentro y entre las extremidades aumenta desde los huesos proximales a los distales. Este hecho sugiere la existencia de razones funcionales para dicho patrón, dado que los huesos distales están, por lo general, más sujetos a modificaciones funcionales que los proximales.
- En el caso de las comparaciones entre extremidades, las hipótesis de equivalencia funcional entre elementos mostraron un nivel similar de integración al de los elementos homólogos seriales. Por lo tanto, la presencia de procesos del desarrollo comunes a ambas extremidades no parece ser la única causa que subyace a dicho patrón.

- Nuevamente, se observó que las cinturas pectoral y pélvica no siguen el patrón de los huesos largos, pues están poco integradas entre sí y con los demás elementos, lo que se puede deber a su distinto origen embrionario o a que poseen diferentes condicionantes biomecánicos y/o funcionales.
- La especialización funcional en las extremidades anterior y posterior aumenta el grado de integración morfológica entre ambas, según sugiere la comparación del grado de covariación de dos categorías funcionales: los corredores especializados (especies que utilizan su extremidad anterior principalmente para la locomoción, de manera similar a la posterior) tienen ambas extremidades más integradas que los no especializados (especies que utilizan su extremidad anterior para otras actividades aparte de la locomoción, como manipular objetos o capturar presas). Este hallazgo representa un ejemplo destacado de coincidencia entre una estrecha interacción funcional y una mayor integración morfológica, evidenciando el papel de la selección natural.
- La morfología de los huesos de las extremidades anteriores de los carnívoros se encuentra muy influenciada por la adaptación a las diferentes estrategias de caza. Por el contrario, otras clasificaciones ecológicas posibles, tales como el modo de locomoción o la preferencia de hábitat, parecen tener menos influencia.
- Las características morfológicas más importantes de la extremidad anterior asociadas a las diferentes estrategias de caza reflejan adaptaciones hacia distintos aspectos biomecánicos, como la resistencia a los esfuerzos, la eficiencia energética durante la locomoción, el grado de movilidad del antebrazo, la ventaja mecánica para algunos músculos y la postura. Cada estrategia de caza se caracteriza, pues, por una combinación particular de tales adaptaciones.
- Los cánidos borofaginos muestran una combinación única de características morfológicas, no representada en los carnívoros actuales, de lo que se deduce que su estrategia de caza no era completamente equiparable a la de ninguno de ellos. Sin embargo, sus características tienen una serie de

implicaciones biomecánicas y funcionales interpretables en términos de adaptaciones, por ejemplo para ejercer fuerza con la extremidad anterior en *Aelurodon ferox,* lo que indica una cierta capacidad de manipular a sus presas, o hacia una postura erguida en el caso de *Epicyon haydeni,* lo que sugiere que se trataba de un buen corredor.

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