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To cite this version:

Alexandra Houssaye, Florian Martin, Jean-Renaud Boisserie, Fabrice Lihoreau. Paleoecological Inferences from Long Bone Microanatomical Specializations in Hippopotamoidea (Mammalia, Artiodactyla). Journal of Mammalian Evolution, 2021, 28, pp.847-870. 10.1007/s10914-021-09536-x . hal-03170283

HAL Id: hal-03170283
https://hal.science/hal-03170283

Submitted on 16 Mar 2021

Paleoecological inferences from long bone microanatomical specializations in Hippopotamoidea (Mammalia, Artiodactyla)

Alexandra Houssaye[1,*], Florian Martin[2], Jean-Renaud Boisserie[2,3], Fabrice Lihoreau[4]

[1]UMR 7179 CNRS/Muséum National d'Histoire Naturelle, Département Adaptations du vivant, 57 rue Cuvier CP-55, 75005 Paris, France.
[2]Laboratoire Paléontologie Evolution Paléoécosystèmes Paléoprimatologie, CNRS, Université de Poitiers – UFR SFA, Bât B35 - TSA 51106, 86073 Poitiers Cedex 9, France
[3]Centre Français des Etudes Ethiopiennes, CNRS et Ministère de l'Europe et des affaires étrangères, PO BOX 5554, Addis Ababa, Ethiopia
[4]Institut des Sciences de l'Evolution de Montpellier, Université Montpellier CNRS IRD EPHE, Montpellier, France

RH: Hippopotamoid long bone microanatomical specializations

* Corresponding author
houssaye@mnhn.fr
Tel: +33140794866

Abstract

Hippopotamoids are herbivorous mammals that originated in the late middle Eocene. This taxon includes animals with a great variety of sizes and body proportions, from small and gracile forms with slender limbs to heavy massive ones. Many hippopotamoids have previously been considered semi-aquatic but recent studies have highlighted a diversity of ecologies. This study focuses on bone microanatomy, one of the various proxies that enable inferring the ecology of extinct taxa. The comparative analysis of the inner structure of the stylopod bones in various hippopotamoids, based on both transverse and longitudinal virtual sections, highlights a diversity of patterns and clarifies previously proposed hypotheses about the ecology of the sampled hippopotamoids. The filling of the medullary area by spongious deposits in the pygmy hippopotamus, *Choeropsis liberiensis*, appears associated with frequent incursions into the water by an animal that essentially forages in forests. The common hippopotamus, *Hippopotamus amphibious*, which spends most of the day submerged in water, shows a greater filling of the medullary area by spongious bone and a thicker cortex. These observations coupled with comparisons with diverse terrestrial and semi-aquatic mammals of various sizes confirm that semi-aquatic lifestyle and heavy weight-bearing are associated with similar microanatomical specializations causing an increase in bone mass. However, for a given mass, comparisons enable determining if an additional increase in bone compactness occurs, as in *Hippopotamus amphibious*, in which case a semi-aquatic lifestyle could be inferred. Accordingly, this study suggests an essentially terrestrial lifestyle for *Microbunodon minimum*, *Bothriodon velaunus*, *Elomeryx borbonicus, Merycopotamus medioximus*, *Paenanthracotherium bergeri*, and probably also *Saotherium* cf. *S. mingoz*, a slight degree of water dependence in *Brachyodus onoideus*, and a stronger one in *Libycosaurus bahri* and *Hexaprotodon garyam*, though less intense than in *Hippopotamus amphibius*. Comparisons with other large terrestrial and semi-aquatic taxa, and based on a large part of the diaphysis, are required to better decipher the microanatomical changes associated with a semi-aquatic lifestyle from those linked to loading in heavy quadrupedal mammals.

Keywords: Anthracotheriinae, bone microanatomy, Bothriodontinae, Hippopotamoidea, Microbunodontinae, semi-aquatic lifestyle, weight-bearing.

Introduction

Hippopotamoids, including hippopotamids and anthracotheres, are herbivorous mammals that emerged in the late middle Eocene (Soe et al. 2017). This clade is assumed to correspond to the sister group of Cetacea based on both molecular (Irwin and Arnason 1994; Gatesy et al. 1996; Gatesy 1997; Montgelard et al. 1997; Ursing and Arnason 1998; Nikaido et al. 1999, Arnason et al. 2002; Zhou et al. 2011) and morphological (Geisler and Uhen 2003; Boisserie et al. 2005a; Geisler et al 2007; Gatesy et al. 2013) studies. Its abundant fossil record illustrates a wide spatio-temporal distribution. Among hippopotamoids, anthracotheres lived from the late middle Eocene to the early Pleistocene in Eurasia, northern America, and Africa (Kron and Manning 1998; Dennel 2005; Lihoreau and Ducrocq 2007; Holroyd et al. 2010; Tsubamoto 2010; Rincon et al. 2013; Lihoreau et al. 2016; Grandi and Bona 2017; Soe et al. 2017; Scherler et al. 2019; Lihoreau et al. 2019; Grossman et al. 2019). Anthracotheres are assumed to correspond to stem Hippopotamidae (Boisserie et al. 2005a, 2010; Boisserie and Lihoreau 2006; Orliac et al. 2010; Lihoreau et al. 2015). Currently 27 genera are recognized and distributed in three subfamilies: the Anthracotheriinae, the Microbunodontinae, and the Bothriodontinae (Lihoreau and Ducrocq 2007), including animals with a great variety of sizes, from small (< 25 kg) to medium and large (> 1,000 kg) forms. Anthracotheriinae and Bothriodontinae were medium to very large-sized animals, whereas Microbunodontinae were exclusively small-sized anthracotheres with slender limbs. Anthracotheres have been previously considered as essentially semi-aquatic hippo-like forms (e.g., Falconer and Cautley 1836; Rütimeyer 1857). More recent studies have highlighted their great diversity of habitats and diets (Lihoreau 2003) and that anthracotheres can be used as ecological markers to reconstruct the paleoenvironmental history of the landmasses they occupied (Lihoreau and Ducrocq 2007). Hippopotamids are first known from the early Miocene in Africa, and then spread to Asia and Europe (Boisserie 2007; Orliac et al. 2010). These small-sized (~30 kg for *Morotochoerus*; Orliac et al. 2010) to very large (mean weight of 1500 kg for the extant genus *Hippopotamus*; Klingel 2013) animals are assumed to have adopted semi-aquatic habits in the latest Miocene (Boisserie 2007; Boisserie et al. 2011).

Semi-aquatic forms occurred independently in various hippopotamoid lineages, so that the question of the habitat of numerous fossil taxa, and notably their degree of water dependence, remains unresolved (Lihoreau and Ducrocq 2007; Boisserie et al. 2011; Lihoreau et al. 2014; Grandi and Bona 2017) and cannot be answered by sedimentological and morphological data only. For this purpose, various approaches can be added, such as studies on sensorial abilities (e.g., Orliac et al. 2014), on dental microwear and mesowear (e.g., Lihoreau 2003; Merceron et al. 2010; Boisserie and Merceron 2011; Lihoreau et al. 2014), and isotopic investigations (e.g., Boisserie et al. 2005a; Nelson 2007; Clementz et al. 2008; Lihoreau et al. 2014; Tütken and Absolon 2015). Morphofunctional studies on the postcranial skeleton are also required. However, to date, such data relative to hippopotamoids are extremely limited. They consist of limited studies on the skeleton of *Hippopotamus amphibius* (Gratiolet 1867) and fossil taxa (Kowalesky 1873; Scott and Jepsen 1940; Dineur 1981; Pickford 2008), and on detailed descriptions of the forelimb and hind limb myology of *Choeropsis liberiensis* and *H. amphibius*, respectively (Fisher et al. 2007; 2010). In addition, there are data on swimming and aquatic bottom-walking abilities in *H. amphibius* (Coughlin et al. 2009; Endo et al. 2019).

Beyond studies on bone shape and muscle insertions, the analysis of bone microanatomical adaptations can also be very useful for paleobiological inferences. Indeed, bone inner structure reflects the functional constraints imposed on the skeleton and thus the functional requirements of the organism (e.g., Ruff 1992; Ruimerman et al. 2005; Volpato et al. 2008; Mielke et al. 2018). The form-function relationships of bone microanatomy have been widely used in the context of adaptation to an aquatic lifestyle, focusing on both aquatic and semi-aquatic forms (e.g., Canoville and Laurin 2010; Dumont et al. 2013; Amson et al. 2014; Diaz-Berenguer et al. 2019; Klein et al. 2019). Yet, hippopotamoids also include large quadrupedal taxa. Adaptations to heavy weight bearing are also observed in the bone microanatomy of large quadrupeds (Wall, 1983; Houssaye et al. 2016a). However, they can be hard to decipher from those linked to a semi-aquatic lifestyle, as both are characterized by an increase in bone compactness (Cooper et al. 2016; Houssaye et al. 2016b). Indeed, both semi-aquatic taxa swimming or walking on the bottom at shallow depths and heavy terrestrial quadrupeds generally exhibit an increased compactness characterized by a more or less extensive thickening of the cortex and filling of the medullary cavity by cancellous bone (Nakajima and Endo 2013; Houssaye et al. 2016a,c; Houssaye and Botton-Divet 2018). The increased bone mass in semi-aquatic taxa is assumed to hydrostatically counter buoyancy to dive and stay at shallow depths but also to counter waves to improve stability in rough waters (Wall 1983; Taylor 2000). Conversely, in heavy animals, such a structure is believed to increase bone strength and improve the absorption of impact energy (Oxnard 1990; Augat and Schorlemmer 2006). Investigations on phylogenetically close taxa showing light and massive terrestrial and semi-aquatic forms thus constitute a great opportunity to better characterize these microanatomical specializations that have convergently evolved multiple times in heavy and/or semi-aquatic (and aquatic) amniotes (Houssaye et al. 2016a,c). Only limited microanatomical data are available for hippopotamoids beyond the mid-diaphyseal sections of modern hippopotamids (Cooper et al. 2016; Houssaye et al. 2016a). Cooper et al. (2016) investigated the microanatomical features of three extinct hippopotamoid species from two genera, the microbunodontine *Microbunodon silistrense* and the bothriodontines *Merycopotamus dissimilis* and *Merycopotamus medioximus*, in order to infer their paleoecology, suggesting a terrestrial lifestyle for *Mi. silistrense* and a semi-aquatic one for the two *Merycopotamus* species.

The objectives of the present study are to investigate the microanatomical adaptive features of hippopotamoid bones by analyzing the inner structure of limb long bones of diverse hippopotamoids supposed to illustrate various degrees of water dependency, taking into account their body proportions, and to discuss inferences related to the paleoecology of the sampled fossil forms.

It is predicted that 1) the bones of terrestrial forms will show a tubular organization, i.e., a compact cortex surrounding an empty medullary cavity, typical of terrestrial amniotes (Laurin et al. 2011; Houssaye et al. 2018), 2) the bones will be more compact in the largest terrestrial species, with a thicker cortex and some trabeculae in the medullary area, 3) the species most dependent on the aquatic environment will also show more compact bones, and 4) the species that are both large and highly dependent on the aquatic environment will be much more compact.

Material and Methods

Material

The material examined consists of the humeri and femora of various hippopotamoids. Stylopod bones are assumed to bear a strong morphofunctional signal in their microanatomy (e.g., Quemeneur et al. 2013). They were therefore selected in order to investigate the long bone microanatomical adaptations related to the posture and lifestyle of these extinct taxa. Moreover, most of the comparative data for ungulates are available only for these bones. Fossil material includes the bones of four hippopotamoid subfamilies (Tables1, 2).

Microbunodon minimum (Cuvier 1822) is a microbunodontine from the late Oligocene of Europe. The sampled specimens are from the collections from La Milloque, France (Lihoreau et al. 2004). This small-sized species (around 20 kg) is lightly built with long and slender limbs, the hind limb being longer and more robust than the forelimb (Cabard 1976; Lihoreau 2003; Lihoreau et al. 2004; Lihoreau and Ducrocq 2007). *Microbunodon* is assumed to be terrestrial and to have lived in closed habitats (dense forest) based on its morphology (e.g., low orbits, no specialization for subaquatic hearing, autopod showing a digitigrade stance, weakly elongated metapodials; Cabard 1976; Boisserie 2002; Lihoreau 2003; Lihoreau and Ducrocq 2007), which is corroborated at the generic level by micro- and mesowear data, and isotopic studies (Lihoreau 2003; Lihoreau and Ducrocq 2007; Nelson 2007).

The sampled anthracotheriine is *Paenanthracotherium bergeri*, from the Oligocene of La Bénissons-Dieu, France. It is large (< 950 kg; estimation based on talus measurement following Martinez and Sudre [1995]) and heavily-built with short and robust limb bones (Roman and Boucher 1936) and may have lived in swamps as was historically supposed for the closely related *Anthracotherium* (Cuvier 1822). However, the low position of its orbits on the skull (see plates in Roman and Boucher 1936; Scherler et al. 2019), though not incompatible with a semi-aquatic lifestyle, does not indicate a hippo-like lifestyle at the interface between air and water.

Within Bothriodontinae, *Bothriodon velaunus*, from the early Oligocene of Ronzon, France (Lihoreau and Ducrocq 2007) is a medium sized animal (< 150 kg; estimation based on talus measurement following Martinez and Sudre [1995]) displaying a lightly-built skeleton with gracile legs (Filhol 1881; Lihoreau and Ducrocq 2007). Though little is known about its ecology, its diet is supposed to have been predominantly folivorous, based on cusp morphology (Lihoreau and Ducrocq 2007). It is similar to *Elomeryx borbonicus* (<130 kg; estimated from Martinez and Sudre [1995]), from the late Oligocene of Saint-Henri/Saint-André, Marseille, France (Lihoreau and Ducrocq 2007). The hind limbs of *Elomeryx borbonicus* are longer and more robust than its forelimbs (Geais 1934). If the proposed occurrence of webbed feet (Geais 1934) suggests a semi-aquatic lifestyle, supposedly in marshy habitats, the position of the superior border of the orbit and the morphology of the tympanic bulla and of the auditory tube are typical of terrestrial animals (Lihoreau 2003). *Libycosaurus bahri*, from the late Miocene of Toros-Ménalla, Chad (Vignaud et al. 2002), displays a heavy (< 1,600 kg) hippo-like morphology with short and stocky limbs, although being more elongated and slender than those of the common hippopotamus (Lihoreau et al.

2014). This sexually body-mass-dimorphic taxon appears to be semi-aquatic, based among others on its highly elevated orbits and external nares, the morphology of the tympanic bulla, and on isotopic studies (Lihoreau et al. 2014, 2006). Finally, *Brachyodus onoideus*, from the early Miocene of Nancray and Neuville, near Orléans, France (Lihoreau and Ducrocq 2007), is much larger (< 2,350 kg; estimate based on talus measurement following Martinez and Sudre [1995]). This large-sized species displays strong limb bones albeit being more elongated and slender than those of the common hippo (Dineur 1981). A terrestrial mode of life has been suggested for this species based on the conformation of its limbs (Dineur 1981) but the amphibious position of the sensory organs on the head along with the morphology of the tympanic bulla potentially enabling underwater directional hearing rather suggest hydrophilic and water immersive habits (Orliac et al. 2013). Trackways from the lower Miocene of Spain possibly attributable to this species seem to indicate a gregarious behavior close to that of hippos with trails in palustrine environments (Diaz Martinez et al. 2020).

Within hippopotamines, *Saotherium* cf. *S. mingoz* (Boisserie et al. 2003), from the early Pliocene of Kossom Bougoudi, Chad (Boisserie 2007; Weston and Boisserie 2010), is of intermediate size (<1,000 kg; estimated from Martinez and Sudre [1995]) between the extant *C. liberiensis* (< 275 kg) and *H. amphibius* (< 2,000 kg). It does not show adaptations to a semi-aquatic lifestyle (Boisserie et al. 2003; Boisserie 2005, 2007; Weston and Boisserie 2010) but isotopic data suggest water dependence (Zazzo et al. 2000). As for *Hexaprotodon garyam*, from the late Miocene of Toros-Ménalla, Chad (Boisserie 2007; Vignaud et al. 2002; Weston and Boisserie 2010), weighting up to 2,200 kg (Lihoreau et al. 2014; estimated from Martinez and Sudre [1995]), it displays a set of morphological (Boisserie 2002) and isotopic features (Jacques 2007) that might indicate a semi-aquatic lifestyle (Boisserie 2002; Boisserie et al. 2005b; Lihoreau et al. 2014). The extant hippopotamine, *C. liberiensis*, about six times lighter than *H. amphibius*, is more gracile with slender elongated limbs (Eltringham 1999; Nowak and Paradiso 1983; Robinson, 2013), whereas *H. amphibius* displays a massive barrel-shaped body supported by a heavily-built skeleton with short and stocky limbs (Eltringham 1999; Klingel 2013; Nowak and Paradiso 1983). Common hippopotamuses inhabit wetlands with permanent water bodies deep enough to enable immersion, such as lakes, rivers, pools, swamps, marshes, and ponds, in the vicinity of reed beds and grasslands (Eltringham 1999; Klingel 2013; Nowak and Paradiso 1983). *Choeropsis* inhabits wetter, closer, and denser environments than the common hippopotamus, which is consistent with its less webbed toes and its nocturnal habits. It lives close to streams, rivers, or swamps, and can dive, thus showing a lifestyle close to that of extant forest tapirs (Eltringham 1999; Nowak and Paradiso

1983; Robinson 2013), despite additional adaptations for a semi-aquatic lifestyle, such as the ability to occlude ears and nostrils when diving.

The comparative material includes terrestrial and semi-aquatic ungulates covering and extending the possible ecologies as well as the size range of the hippopotamoid sample (see Table 1; Fig. 1). It includes the basal cetancodont *Indohyus indirae* and archaeocetes belonging to the Pakicetidae, Remingtonocetidae, and Protocetidae, in order to illustrate various ecological steps in the progressive adaptation to an aquatic lifestyle in the cetacean lineage. It also includes small to medium-sized even-toed ungulates with long and slender legs (e.g., *Hyemoschus aquaticus*, *Potamochoerus porcus*), but also medium- to large-sized even-toed and odd-toed ungulates, from gracile (e.g., *Giraffa Camelopardalis*, *Alces alces*) to heavily-built (e.g., *Syncerus caffer*, *Rhinoceros unicornis*) animals (Table 1).

Methods

Data acquisition

Bones were scanned using high-resolution computed tomography at: 1) the IC2MP, University of Poitiers (UMR 7285; EasyTom XL duo, RX Solutions), 2) the ISEM, University of Montpellier (UMR 5554; EasyTom 150, RX Solutions) 3) the Steinmann-Institut, University of Bonn (Germany; GE phoenix|X-ray v|tome|xs 240), 4) the AST-RX platform of the Muséum National d'Histoire Naturelle, Paris (UMS 2700; GE phoenix|X-ray v|tome|xs 240), and 5) the Equine Diagnostic Imaging Centre at the Royal Veterinary College, London (GE Lightspeed), with reconstructions performed using X-Act (RX Solutions), DATOX/RES, phoenix datos|x or MEDVIEW software (MedImage). Voxel size naturally varies pending on specimen size (Table 1).

Transverse virtual thin-sections were made for each bone at the plane assumed to cross the growth center (where the cortex is the thickest in the case of a non-uniform thickness, see Houssaye et al. 2018, and at the intersection of the nutrient arteries the central axis of the medullary cavity, see Houssaye and Prévoteau 2020). Sagittal and coronal virtual sections were made at the planes crossing the core of the transverse section. Image visualization and virtual sections were performed using VGSTUDIOMAX, versions 2.2 (Volume Graphics Inc.).

Data analysis

Quantitative parameters were used to describe the transverse sections, except those of extinct taxa showing an insufficient contrast between bone and the infilling sediment (see Table 1). The choice of parameters follows Houssaye et al. (2018), using the BoneJ plugin (Doube et al. 2010) of ImageJ (Wayne Rasband National Institutes of Health, USA) and the software Bone Profiler (Girondot and Laurin, 2003): A) CSAb (Cross-Sectional Area of bone), representing the surface occupied by bony tissue; B) CSAt (total Cross-Sectional Area), as the surface of the whole transverse section; C) C (Compactness), as the ratio of CSAb over CSAt; D) R, as the radius of the section approximated as a circle calculated based on the perimeter/2π, and used as a proxy of size; E) CSS (Cross-Sectional Shape), as the ratio between the maximal and minimal second moments of area (Imax/Imin); F) Zpol (Polar Section Modulus) representing the resistance of a section to torsion and bending (see Ruff 2002; Ksepka et al. 2015); G) RMeanT, as the relative mean thickness of cortical bone (after separation by segmentation of the cortical and medullary areas), calculated as the absolute mean thickness of cortical bone divided by R; H) RSDT, as the relative standard deviation of cortical bone thickness; I) P: the extent of the medullary cavity as measured by the relative distance from the center of the section to the point where the most abrupt change in compactness occurs; and J) S: the width of the transitional zone between the compact cortex and the medullary cavity as measured by the reciprocal of the slope of the compactness profile at the inflection point.

We tested the influence of size on all parameters by performing linear regressions of each parameter to R (lm function). We comparatively analyzed the values of each parameter for the hippopotamoids separately, all rhinos, the (dominantly) terrestrial and the (dominantly) aquatic ungulates (Table 1; Figs S2; S4).

We tested the phylogenetic signal for each parameter on the raw data. For that we averaged the values obtained for each species when several specimens from the same species were available. Then we calculated the K-statistic following Blomberg et al. (2003) for each parameter and performed randomization tests. The K-statistic compares the observed phylogenetic signal in a trait with the signal under a Brownian motion model of trait evolution. A K-value > 1 implies more similarity between relatives than expected under Brownian motion; K < 1 highlights convergences.

For this testing, we used two different consensus trees (Fig. 1) based on phylogenies from Lihoreau et al. (2015, 2019), Boisserie et al. (2011, 2017), and Gomes Rodrigues (2019) for the relationships within Hippopotamoidea, the proposition of Thewissen et al. (2007) and

Vautrin et al. (2020) for the position of *Indohyus* and Cetacea, and recent phylogenetic hypotheses based on molecular data for extant representatives of modern clades of laurasiatherian mammals (Gilbert et al. 2006; Bibi 2013; Foley et al. 2016; Springer et al. 2019). These phylogenies differ by the relationships observed within Hippopotamoidea. The first hypothesis links Hippopotamidae to archaic bothriodontines, i.e., the African Paleogene clade including *Bothriogenys* and *Brachyodus*. The second hypothesis proposes the Hippopotamidae as sister-group of the whole Bothriodontinae.

In order to analyze quantitatively the distribution of the different specimens in the microanatomical morphospace, but also for the purpose of evaluating how the different microanatomical and cross-sectional parameters explain the variations observable in our sample, we conducted normalized PCAs (David and Jacobs 2014).

All statistical analyses were performed using the statistical software R (R Core Team 2014).

Results

Humerus

Qualitative description

Humeral sections of *Choeropsis* do not show a particular thickening of the cortex (Figs. 2A-B, 3A), as compared to most quadrupedal mammals (see Houssaye et al. 2018; Canoville and Laurin 2010; Fig. S1). However, whereas these taxa show a generally tubular diaphysis with a compact cortex surrounding an empty medullary cavity, the humerus medullary space of *Choeropsis* is filled with a spongious tissue. The thickness of the cortex increases distal to the deltoid tuberosity but is then rather homogeneous in the rest of the diaphysis. The organization appears rather similar in *Saotherium* (Figs. 2C, 3B), *Libycosaurus* (Figs. 2J, 3H) and in one specimen of *Hexaprotodon* (TM 55-XX-05; Figs. 2F, 3D), but also in *Brachyodus* (Figs. 2H, 3F), though its spongiosa is poorly preserved. The spongiosa is also poorly preserved in *Bothriodon* whose cortex is thinner than in the other taxa (Figs. 2I, 3G). In *Hippopotamus* the thickness of the cortex varies along the diaphysis distal to the deltoid tuberosity, with a pronounced thickening around the growth center (Figs. 2D-E, 3C). As in *Choeropsis* a spongiosa fills the medullary area. Such a thickening of the cortex is also observed in one specimen of *Hexaprotodon garyam* (TM 258-01-27; Figs. 2G, 3E) and, to a lesser extent, in *Paenanthracotherium* (Figs. 2M, 3K). The spongiosa in the medullary cavity of the later is not preserved or was absent. As for *Elomeryx* and *Microbunodon*, they show a tubular organization (Figs. 2K-L, 3I-J). As for *Merycopotamus*, sections of *Me. medioximus* (Lihoreau et al. 2004) from Cooper et al. (2016) show an organization close to that of *Elomeryx* and *Microbunodon* (Fig. S1A) but that of *Me. dissimilis* (Falconer and Cautley 1836) is filled with some trabeculae (Fig. S1B).

Quantitative comparisons

Compactness (C) is the highest in *Hippopotamus*. It is higher than in rhinos and in *Hexaprotodon* (TM 258-01-27), which show higher values than the other taxa (even than most aquatic ones; Fig. S2A). *Choeropsis* globally displays a higher compactness than the other terrestrial ungulates, but it is much lower than in rhinos. The other hippopotamoids show compactness values slightly (especially *Brachyodus*) lower than those of *Choeropsis*. The cross-sectional shape (CSS) is strongly higher in aquatic taxa (Fig. S2B); as for the others, it is maximal in the comparative terrestrial taxa and globally much higher in rhinos, *Hippopotamus*, *Microbunodon*, and *Bothriodon* than in *Choeropsis*, *Elomeryx*, *Brachyodus*, and *Hexaprotodon*, which thus present more rounded sections (Fig. S2C). The relative mean cortical thickness (RMeanT) is the highest in *Hippopotamus* and *Hexaprotodon* (TM 258-01-27). It varies strongly within rhinos where it is generally higher than in other terrestrial ungulates. RMeanT is much higher in *Brachyodus* than in *Choeropsis*, *Elomeryx*, *Bothriodon*, *Microbunodon*, and the comparative terrestrial and aquatic taxa (Fig. S2D). The cortical thickness is rather homogeneous in most terrestrial and in aquatic taxa, which show the lowest RSDT values (Fig. S2E). This is also the case for *Microbunodon*, *Elomeryx*, and *Bothriodon*. However it is slightly higher in *Choeropsis* and much higher in *Brachyodus* and even more in *Hippopotamus* and *Hexaprotodon garyam* (TM 258-01-27). RSDT varies strongly among rhinos, from values close to those of *Brachyodus* to values higher to those of *Hippopotamus*. The extent of the medullary cavity (P) is the highest in *Bothriodon*, which shows values similar to most terrestrial forms (Fig. S2F). P is rather similar in *Microbunodon*, *Elomeryx*, *Choeropsis*, and *Brachyodus*, being slightly lower than in most terrestrial taxa. It is lower in rhinos and *Hexaprotodon*, and clearly low in *Hippopotamus*. Values vary strongly in aquatic taxa. S, the transition zone (between the compact cortex and the medullary cavity), is rather similar in *Choeropsis*, *Microbunodon*, *Elomeryx*, and *Hexaprotodon* compared to most other terrestrial ungulates (Fig. S2G). It is higher in rhinos and much higher in *Hippopotamus*, and intermediate between *Choeropsis* and rhinos for *Bothriodon* and *Brachyodus*. It varies a lot among aquatic taxa. As for the polar section modulus (Zpol), it varies a lot within rhinos where it is the highest but is extremely constant in *Hippopotamus* and *Choeropsis*. It is high in *Hippopotamus*, *Brachyodus*, and *Hexaprotodon* and very high in rhinos (Fig. S2H).

Quantitative analyses

All parameters, except Zpol (in mm^3), are dimensionless ratios. All parameters except CSS are correlated with size (Table 3). This shows, beyond a size effect, a strong allometry in the data, except for the shape of the diaphyseal sections (CSS).

All parameters show a significant phylogenetic signal (at 5%; Table 3), whatever the chosen phylogeny (Fig. 1), with K values however always below 1. This signal, as significant in R, could be partly associated with the allometry, but not only, as CSS is not significantly correlated with size.

A first PCA was performed on the humerus data but as the specimen of *Maiacetus* was driving too much the variation along PC2, another one was conducted without this specimen. The two first axes of the PCA on humerus data represent 78.30% of the variance with 64.55 for the first axis and 13.75% for the second one. They are both correlated with size (Table 3) and commented hereafter. The first axis rather clearly discriminates most terrestrial ungulates (negative values) from semi-aquatic and heavy ones (Fig. 4). Specimens of *Hippopotamus* group together on the positive part. Specimens of *Choeropsis* are rather intermediary between most terrestrial ungulates and the heavy and semi-aquatic ones. Both specimens of *Microbunodon* and the specimen of *Bothriodon* clearly group with most terrestrial ungulates; it is the same for both specimens of *Elomeryx*, which are close to *Choeropsis'*. Conversely, the two specimens of *Brachyodus* are between *Choeropsis* and rhinos. The specimen of *Hexaprotodon* TM 258-01-27 is very close to *Hippopotamus*. The first axis is essentially driven by RSDT, P, C, and Zpol (RMeanT and S to a slightly lower extent), which all show allometry. Zpol and S covary, and P varies antagonistically with C. This axis shows that larger humeri show a higher compactness (C), a reduced medullary area (P, RMeanT), resulting in a higher resistance to bending and torsion (Zpol), but also a wider transition zone between the compact cortex and the medullary cavity (S), associated with the filling of the medullary cavity by a spongiosa, and also a higher variation of the cortical thickness in the transverse section (RSDT), which means a more heterogeneous growth in width of the bone. The second axis poorly discriminates groups. This axis is essentially driven by the cross-sectional geometry (CSS), and to a lesser extent by the transition zone (S) and Zpol.

Femur

Qualitative analyses

Choeropsis shows a femoral inner organization similar to that of the humerus, except that the trabecular network appears much looser in most of the diaphysis (Fig. 5A-B). In *Hippopotamus*, the cortex is thicker than in *Choeropsis* but its thickness is rather homogeneous along the diaphysis (Fig. 5C-D). Both taxa show a medullary space partially filled by a loose spongiosa. Conversely, in all the other hippopotamoids sampled, the medullary space appears empty (Figs. 5E-J, 6C-H) so that their organization is tubular. Only the thickness of the cortical layer varies, being the lowest in *Elomeryx*, whose section is similar to those of *Me. medioximus* and *Mi. silistrense* (Cooper et al. 2006).

Quantitative comparisons

Femoral compactness in transverse sections of *Elomeryx* is rather similar to that of most terrestrial ungulates (Fig. S3). However, it is progressively higher in *Choeropsis*, *Hexaprotodon*, rhinos, *Brachyodus*, *Libycosaurus*, and *Hippopotamus* that show the highest compactness values, with aquatic ungulates (Fig. S4). CSS values are much higher in rhinos than in all other taxa; hippopotamoids show values similar to (e.g., *Elomeryx*) or lower (especially *Choeropsis* and *Hippopotamus*) than those of the terrestrial ungulates. *Elomeryx*, like rhinos and most terrestrial ungulates, has rather low values of relative cortical thickness (RMeanT), which are minimal in the aquatic ungulates. Values are higher in *Choeropsis* and *Libycosaurus*, and much higher in *Hippopotamus*, *Brachyodus*, and *Hexaprotodon*. RSDT is rather low in *Elomeryx*, most terrestrial and aquatic ungulates. It is slightly higher in *Choeropsis* and progressively much higher in *Hexaprotodon*, *Libycosaurus*, and rhinos, *Hippopotamus*, and *Brachyodus*. The size of the medullary cavity in *Elomeryx* is like in most terrestrial ungulates, whereas it is smaller in rhinos and *Choeropsis*, and much smaller in *Brachyodus*, *Hexaprotodon*, *Libycosaurus*, and *Hippopotamus*. It is minimal in aquatic taxa. The transition zone (S) is much wider in rhinos than in all other taxa and minimal for most terrestrial ungulates. As for the polar section modulus (Zpol), it is similar as in most terrestrial ungulates in *Choeropsis, Elomeryx*, and aquatic ungulates, but higher in *Hippopotamus* and *Libycosaurus*, much higher in rhinos and *Hexaprotodon*, and very high in *Brachyodus*.

Quantitatives analyses

In the femora, all variables, except CSS, are significantly correlated with size (Table 4).

All parameters show a significant phylogenetic signal, for both phylogenies (Table 4), with high K values for C and, to a lesser extent, P. Again, the signal could be partly associated with the allometry.

The first two axes represent 72.93% (54.07, and 18.86%, respectively) of the variance for the femur data. Only PC1 is correlated with size (Table 4). Again, C and RMeanT vary antagonistically to P. Zpol covaries with RSDT. As for the humerus, PC1 distinguishes most terrestrial ungulates from rhinos and massive hippopotamoids (Fig. 7). *Choeropsis* specimens, like those of *Tapirus terrestris* and some large terrestrial ungulates (e.g., *Alces americanus*, *Bubalus bubalis*, *Syncerus caffer*), are intermediate between most terrestrial ungulates and rhinos and large hippopotamoids. *Elomeryx* groups with most terrestrial ungulates, whereas *Libycosaurus* and *Hexaprotodon* are between *Choeropsis* and *Hippopotamus*, and *Brachyodus* gathers with *Hippopotamus*. The first axis essentially discriminates based on RSDT, C, P, Zpol, and RMeanT. Bones on the positive part of the axis show a higher compactness, a reduced medullary cavity, and a wider transition zone, with a cortical thickness being more heterogeneous along the transverse section. As it is correlated with size (but not only), this trend can be generalized to femora when they become larger in our sample. Along the second axis, rhinos are clearly distinct from hippopotamoids and from most terrestrial ungulates. They group with tapirs and the aquatic *Remingtonecetus*. The second axis is essentially driven by CSS and S and, to a lesser extent, by RMeanT.

Discussion

Hippopotamoid microanatomical features

Humerus

This study reveals a high variability in humerus microanatomy within hippopotamoids. It clearly shows for the first time, thanks to longitudinal sections, that the medullary area of *Choeropsis liberiensis* is completely filled by a spongious tissue. This is also the case for *Hippopotamus amphibius*, as previously shown in Wall (1983) and in other hippopotamoids. It is clear in *Saotherium* cf. *S. mingoz*, *Hexaprotodon garyam*, and *Libycosaurus bahri*, likely in *Brachyodus onoideus*, but it is unclear for *Bothriodon velaunus* and *Paenanthracotherium bergeri*. A spongious medullary area occurs in only a few terrestrial mammals with most of them showing a tubular organization (Canoville and Laurin 2010; Laurin et al. 2011; Houssaye et al. 2018; Fig. S1). The spongiosa is tighter and more extended along the whole diaphysis in these hippopotamoids than in some sloths, anteaters and armadillos (Montanez-Rivera et al. 2018) as well as some otters (Houssaye and Botton-Divet 2018), but rather close to what is observable in tapirs (AH, pers. obs.), the giant anteater, large ground sloths (Amson and Nyakatura 2018), and large otters (Houssaye and Botton-Divet 2018), whereas it is tighter in rhinos (AH, pers. obs.). The thickening of the cortex near the growth center in the humerus of *Hippopotamus* and one specimen of *Hexaprotodon garyam* is similar to what is observed in the sea otter (Houssaye and Botton-Divet 2018), some rhinos (Fig. S1X; AH, pers. obs.), and maybe also in the ground sloth *Hapalops* (Amson and Nyakatura, 2018). It evokes what is observed in the astrapothere *Parastrapotherium* sp., though the cortex of the latter is less thick – approximating the condition in *Libycosaurus bahri* and *Brachyodus onoideus* – and in the notungulate *Nesodon imbricatus*, which nevertheless shows an empty medullary cavity and is maybe closer to *Paenanthracotherium bergeri* (Houssaye et al. 2016b). The organization in *Choeropsis* appears close to that of *Tapirus terrestris* (Fig. S1U). *Elomeryx borbonicus* and *Mi. minimum*, like most specimens of *Merycopotamus* (except that of *Me. dissimilis*; Cooper et al. 2016; Fig. S1A-B), display a tubular inner structure rather close to that of large terrestrial mammals, like *Alces* and *Bubalus bubalus*, but also of the small semi-aquatic *Hyemoschus aquaticus* and *Indohyus indirae*.

A high compactness, as compared to most terrestrial ungulates, is observed in all hippopotamoids except *Elomeryx*, *Microbunodon*, and *Me. medioximus*, and it is maximal in *Hippopotamus* and *Hexaprotodon*, which show a much thicker cortex around the growth center. The more massive taxa (*Hippopotamus*, *Hexaprotodon*, *Brachyodus*, and rhinos) show the most heterogeneous distribution of cortical bone along the transverse section, and thus a heterogeneous bone growth in diameter. Their bone structure suggests a high resistance to torsion and bending (though less than for rhinos) as indicated by their high polar section modulus values.

Femur

A spongious organization is also observed in the femur of *Choeropsis* and *Hippopotamus*. In *Choeropsis*, it evokes what is observed in some large otters (*Lontra*) and *Tapirus terrestris* with an empty core of the medullary area (Houssaye and Botton-Divet 2018; Fig. S3T). The spongiosa is also less tight than in *Hippopotamus*, where it is less tight than in rhinos (Fig. S3V-Y). *Hippopotamus* does not show a thickening of the cortex around the growth center as in the humerus though its cortex is thicker than in *Choeropsis*. All other hippopotamoids sampled display a medullary area with as much spongious deposits as in *Choeropsis*, or less. There is less variability in microanatomical features among hippopotamoid femora. Most, except *Elomeryx borbonicus*, show a cortex thicker than in the biggest terrestrial ungulates, like *Syncerus caffer*, or than the putative semi-aquatic *Indohyus indirae* (Fig. S3B,J), and rather evoking *Pteronura brasiliensis* (Houssaye and Botton-Divet, 2018). *Elomeryx borbonicus* sections are similar to those of *Hyemoschus*, *Bubalus*, and *Alces*. As for *Tapirus terrestris*, *Parastrapotherium* sp., and *Nesodon imbricatus*, they display a relatively thinner cortex with a spongious transition zone between the compact cortex and the medullary cavity and an open medullary cavity (Fig. S3T; Houssaye et al. 2016b), which thus differs from the patterns observed in the sampled hippopotamoids, though only slightly to *Choeropsis*'. The organizations observed in most hippopotamoids sampled (i.e., except *Choeropsis*, *Hippopotamus*, and *Elomeryx*) appear thus only similar to what is observed in various otter femora (Houssaye and Botton-Divet 2018) and *Syncerus caffer*, based on the comparative material available to date.

We generally observe the same interspecific variations in compactness in the femur than in the humerus. All hippopotamoids except *Elomeryx* show a high compactness as compared to terrestrial ungulates, especially *Hippopotamus*, though to a much lesser extent than in the humerus (like for one specimen of *Hexaprotodon*). The cortex is proportionally thicker in *Hippopotamus*, *Brachyodus*, and *Hexaprotodon*. As for the humerus, the more massive taxa show the most heterogeneous organization along the section. Contrary to what is observed for the humerus, the cross-sectional shape (as indicated by CSS) differs significantly between hippos (whose femoral diaphysis is more cylindrical) and rhinos, which also show a larger transition zone linked to their tighter spongiosa. The bone structure of *Brachyodus* and *Hexaprotodon* (and of rhinos) suggests a higher resistance to torsion and bending than in the other taxa.

Microanatomical features linked to size

There is a phylogenetic signal in all parameters analyzed. However, as size also follows the phylogeny (whatever the phylogeny chosen), and as all parameters (except CSS, for both bones) are correlated with size, it is difficult to distinguish the phylogenetic signal from the allometry. In the quantitative analyses, the impact of size appears to be the main driver of variation. The analyses have shown that in both humeri and femora an increase in bone absolute width is associated with a relative increase in compactness, a more heterogeneous growth in width of the bone, a wider transition zone between the compact cortex and the open medullary cavity, a more reduced medullary area, and the filling of the medullary cavity by a spongiosa. This confers to the bone a higher resistance to bending and torsion. This is consistent with previous studies including heavy taxa (Houssaye et al. 2016a,b). The intensity of the observed microanatomical specializations - increase in cortical thickness and spongiosa in the medullary area - are more intense in humeri than in femora in many hippopotamoids (see below).

If the size parameter (radius of the section approximated as a circle), which reflects animal weight because long bone diameter is known to be correlated with body mass (Anderson et al. 1985), appears as the main driver distinguishing hippopotamoids and rhinos from other ungulates in the quantitative analyses, the qualitative observations allow to better distinguish and characterize the microanatomical specializations of the various taxa analyzed. Indeed, qualitative observations are also based on longitudinal sections and not only transverse ones and thus cover a much larger proportion of the bone. Moreover, the quantitative parameters do not include enough information about the trabecular bone that would allow to better decipher between taxa, notably between rhinos and hippos in our case.

Ecological inferences

In *Choeropsis*, humeri and femora display rather similar microanatomical organizations, characteristically differing from most terrestrial ungulates by showing a filling of the medullary area by spongious bone. Both bones show microanatomical features very similar to those of *Tapirus terrestris* (despite a slightly thinner cortex in the latter). Both animals are of approximately the same weight and forage in similar habitats, i.e., lowland forests close to streams, rivers, or swamps (Medici et al. 2001). The filling of the medullary area is also observed in other semi-aquatic mammals (e.g., large otters [Houssaye and Botton-Divet 2018]) and is probably associated with their frequent forays into the water.

Hippopotamus displays a thickening of the cortex - stronger in the humerus than in the femur, especially near the growth center - and the filling of the medullary area by spongious bone. This is consistent with *Hippopotamus* being more specialized than *Choeropsis* for an aquatic lifestyle as it spends most of the day submerged in water. Its nevertheless still active locomotion on land, impeding more drastic functional aquatic adaptations (Fisher et al. 2007; 2010), is consistent with the absence of osteosclerosis (strong increase in whole bone compactness; see Houssaye 2009).

Both extant hippopotamids show muscular adaptations associated with body weight support and increased resistance against water, especially in the forelimb to propel the trunk forward and in the autopod loading for stability on wet ground (Fisher et al. 2007). Modifications of the pectoral muscles might explain the stronger compactness observed in hippo humeri than femora.

The humeral sections of *Hippopotamus* evoke what is observed in some rhinos. However, *Hippopotamus* femora differ from those of rhinos, whose diaphyses show a heterogeneous (proximo-distal) organization along the diaphysis (Wall, 1983; AH pers. obs.) whereas it is homogeneous in *Hippopotamus*. Longitudinal sections of the long bones of diverse extant rhino species would be required in order to better analyze the variation observed among rhino transverse sections (Fig. S1W-A') considering the strong changes occurring along the diaphysis (Wall, 1983; AH, pers. obs.). This would also allow to determine more precisely to which rhinos *Hippopotamus* is the closest, some rhinos being exclusively terrestrial whereas others spend most of their time in shallow waters (Dinerstein 2011), and to link more precisely the microanatomical features observed with the habitat and mass.

Saotherium cf. *S. mingoz* has a humerus inner structure very close to that of *Choeropsis*, despite being much larger, so that this filling could be only related to mass and not necessarily to a semi-aquatic lifestyle. Femur data were unfortunately not available to verify this hypothesis.

Humeri in *Mi. minimum* and humeri and femora in *Elomeryx borbonicus*, as for *Me. medioximus* (Cooper et al. 2016; Fig.S1A), have a tubular inner structure close to that of large terrestrial ungulates, like *Alces* and *Bubalus* but also to the small *Hyemoschus*, and *Indohyus* for the humerus. The cortical layer is slightly thicker than in ungulates of similar sizes. This suggests a lifestyle possibly similar to that of *Hyemoschus*, which is essentially terrestrial, living in closed habitats, but foraging along the banks of rivers at night, which is in agreement with previous ecological reconstructions (Lihoreau 2003; Lihoreau and Ducrocq 2007; Table 2). No femur is available for *Microbunodon*. The higher morphological robustness of the hind limb in *Elomeryx borbonicus* is not reflected in the microanatomy of its stylopod bone.

The humerus of *Bothriodon velaunus* is close to those of *Microbudondon* and *Elomeryx*, though it shows a thinner cortex and a spongious transition zone between a compact cortex and a probably empty core (at least) of the medullary area, with a transverse section close to that of *Tapirus terrestris*. This taxon, very close in size (< 150 kg) and morphology to *Elomeryx borbonicus*, probably also shared a similar lifestyle (Table 2).

The humerus of *Paenanthracotherium bergeri* shows a rather thick cortex, slightly thicker than in *Bubalus bubalis* for a similar diameter, and the medullary area appears essentially void of trabeculae (if it is not a preservation artefact), except a few at the transition zone. This organization is rather similar to that of the notungulate *Nesodon imbricatus* (< 500 kg; Cassini et al. 2012; Forasiepi et al. 2015; Houssaye et al. 2016b), which suggests an essentially terrestrial lifestyle for this rather massive (< 950 kg) and heavily built animal, in accordance with Scherler et al. (2019; Table 2). This could be compatible with walking, but not underwater swimming or bottom walking in swamps. Unfortunately, no femur was available to further explore this comparison.

The humerus of *Brachyodus onoideus* resembles that of *Choeropsis*. The bad preservation of the core of the bone prevents us from knowing to which degree the medullary cavity is filled with a spongiosa and thus to state if it was more similar to *Choeropsis*, to some rhinos, or to the astrapothere *Parastrapotherium* (Houssaye et al. 2016b). Its femur seems clearly void of trabeculae in the medullary area and thus differs from *Choeropsis*, *Hippopotamus*, and rhinos. It also differs from *Parastrapotherium* whose cortex is thinner and the transition zone large (Houssaye et al. 2016b). In *Brachyodus onoideus*, humerus and femur, like in *Choeropsis*, present similar microanatomical organizations, which might suggest comparable relative use of the forelimbs and hind limbs in these two taxa. More specimens would naturally be required to confirm this hypothesis. The relatively thick cortex is consistent with its heavy weight (< 2,350 kg), the spongious tissue in the medullary area of the humerus with a semi-aquatic lifestyle, but animals with aquatic affinities of similar size, i.e., *Hippopotamus* and the Asiatic rhinos combine a thick cortex and spongious tissue in the medullary cavity. *Brachyodus onoideus*, if semi-aquatic (Orliac et al. 2013), would probably have had reduced immersive habits as compared to these species.

The humerus of *Libycosaurus bahri* is close to those of *Choeropsis* and *Brachyodus onoideus*. As opposed to in *Brachyodus onoideus*, the spongiosa is clearly well developed and extended in the medullary area. As for the femur, it is also void of trabeculae but with a spongious transition zone. *Libycosaurus bahri* is a heavy (< 1,600 kg) animal with a hippo-like morphology. Its microanatomical features are consistent with a semi-aquatic lifestyle as suggested by previous works (Lihoreau et al. 2006, 2014; Table 2).

The specimens of *Hexaprotodon garyam* differ in their microanatomical specialization, despite a similar size. One humerus (TM 115-06-01) is close to that of *Saotherium* cf. *S. mingoz*, whereas the other (TM 258-01-31) has a much thicker cortex, especially near the growth center. This difference in cortical thickness, and thus of compactness, is also observed, though to a lesser extent, between the two femora. Both humeri show spongious bone extending in the medullary area. Like for *Brachyodus onoideus* and *Libycosaurus bahri*, the femora show a relatively thick cortex but an open medullary cavity. Both microanatomical patterns are compatible with a semi-aquatic lifestyle for this heavy taxon (<2,200 kg), as suggested by previous studies (Boisserie 2002; Boisserie et al. 2005b; Lihoreau et al. 2014; Table 2). However, if one specimen (TM 115-06-01) is more compatible with the lifestyle of *Choeropsis*, the other (TM 258-01-31) is more similar to *Hippopotamus*.

Hexaprotodon garyam and *Libycosaurus bahri*, like *Hippopotamus amphibius* and contrary to *Choeropsis* and *Brachyodus onoideus*, display a stronger compactness in the humerus than in the femur. *Libycosaurus bahri* and the most compact *Hexaprotodon garyam* specimens evoke what is observed in *Parastrapotherium*. This taxon, supposedly with some species as heavy as *Hippopotamus*, was assumed to be a megaherbiore frequently foraging in aquatic environments (Avilla and Vizcaino, 2005; Flynn et al. 2012). *Parastrapotherium* nevertheless shows a lower increase in compactness and extension of the spongiosa than *Hippopotamus*. *Hippopotamus* is so far the hippopotamoid displaying the strongest increase in bone compactness. *Hippopotamus* is among the heaviest hippopotamoids but not strongly heavier than some others (e.g., *Hexaprotodon* and *Brachyodu*s). This taxon might thus illustrate the strongest degree of adaptation to a semi-aquatic lifestyle among hippopotamoids. Only *Pyrotherium romeroi* (of about 3,500 kg; Shockey and Daza, 2004), considered showing extreme microanatomical adaptation to heavy-weight support, exhibits more compact stylopod bones than *Hippopotamus*, at least near the growth center (no longitudinal section is available for *Pyrotherium romeroi*). This extreme increase in bone mass, if indeed not associated with a semi-aquatic lifestyle, remains poorly misunderstood and requires further investigation. Indeed, heavier terrestrial taxa do not show such a high inner compactness (Houssaye et al. 2016a). Only the sea otter shows a similarly high compactness near the growth center (Houssaye and Botton-Divet, 2018) but this specialization is linked to its almost exclusively aquatic lifestyle and not to loading. Comparisons with other large terrestrial and semi-aquatic taxa are required to better decipher the microanatomical changes associated with a semi-aquatic lifestyle from those linked to loading in heavy quadrupedal mammals.

Conclusion

This microanatomical study of the stylopod bones of various hippopotamoids and comparisons with diverse terrestrial and semi-aquatic quadrupedal ungulates highlight converging specializations for a semi-aquatic lifestyle and loading. This consists of an increase in bone compactness by filling the medullary area with cancellous bone and a thickening of the cortex. However, comparisons between animals of similar weight suggest, in the case of excessive compactness, a semi-aquatic lifestyle. In this context, the microstructure of *Choeropsis liberiensis* appears to be consistent with an animal that is mainly foraging in forests but frequently invades the water, whereas that of *Hippopotamus amphibius* shows a stronger compactness consistent with an animal that spends a large part of its time standing in the water or walking/swimming on the bottom. Accordingly, on the ground of microanatomical features, the extinct taxa *Microbunodon minimum*, *Bothriodon velaunus*, *Elomeryx borbonicus, Merycopotamus medioximus*, *Paenanthracotherium bergeri*, and probably also *Saotherium* cf. *S. mingoz* are inferred as essentially terrestrial animals, *Brachyodus onoideus* as slightly water-dependent, and *Libycosaurus bahri* and *Hexaprotodon garyam* as clearly semi-aquatic, although less specialized for this ecology than *Hippopotamus amphibius*. In order to better decipher the microanatomical changes associated with a semi-aquatic lifestyle in relation to those associated with loading in heavy quadrupedal mammals, microanatomical investigations, preferentially including a large part of the diaphysis, and functional analyses on limb bones of other large terrestrial and semi-aquatic taxa are strongly needed.

Acknowledgments

We warmly thank J. Lesur, A. Verguin, C. Argot and G. Billet (MNHN, Paris, France); F. Saragoza, A. Bonnet and E. Magne (Musée Crozatier, Le Puy-en-Velay, France); D. Berthet and F. Vigouroux (Musée des Confluences, Lyon, France); E. Robert (Université Claude Bernard Lyon I, Lyon, France); L. Costeur and F. Dammeyer (Naturhistorisches Museum Basel, Basel, Switzerland); G. Garcia and M. Brunet (PALEVOPRIM, Université de Poitiers, Poitiers, France); C. Nekoulnang Djetounako and M. Adoum, (Centre National de Recherche pour le Dévelopement, N'Djamena, Chad) with support of L. Andossa and M. Hassane Taïsso (Université de N'djaména, Chad) for the loan of the hippopotamoid specimens. We thank A. Mazurier (IC2MP, UMR 7285 CNRS-UP, Poitiers, France), R. Lebrun (ISEM, plateforme MRI, Montpellier, France), M. Garcia Sanz (AST-RX platform, UMS 2700, MNHN), and the Steinmann-Institut (University of Bonn, Germany), for providing beamtime and support, and for performing scans and reconstructions. We are very grateful to L.N. Cooper (Northeast Ohio Medical University, USA) for sending us virtual sections of *Merycopotamus* and *Microbunodon*. We also thank F. Guy, X. Valentin, J. Surault (PALEVOPRIM) and A.-L. Charruault (ISEM) for their precious help for the preparation of the specimens before scanning, A. Gekme and L. Martin for borrowing and transporting some of the studied specimens, as well as the Mission Paléoanthropologique Franco-Tchadienne (MPFT, PI: M. Brunet) for providing access to the Chadian material. We also thank two anonymous reviewers for constructive comments that enabled to improve the manuscript. We acknowledge financial support from the ANR SPLASH (ANR-15-CE32-0010). AH also acknowledges financial support from the ERC-2016-STG-715300.

Declarations

Funding We acknowledge financial support from the ANR SPLASH (ANR-15-CE32-0010). AH also acknowledges financial support from the ERC-2016-STG-715300.

Conflicts of interest/Competing interests None

Ethics approval Not applicable

Consent to participate Not applicable

Consent for publication Not applicable

Availability of data and material Microtomographic scans will be available under request to the authors.

3D image data (.tif format) obtained by micro-tomography of MNHN specimens will be permanently saved by the MNHN DSI and identified by the inventory number of the specimen. They will be visible as work carried out on the interface https://3dtheque.mnhn.fr/ and made available via the interface http://colhelper.mnhn.fr/. All 3D image data obtained by micro-tomography at the IC2MP (University of Poitiers) are permanently saved by PALEVOPRIM under the responsibility of the director of the research unit (currently: JRB). Inventory will be communicated upon request, and data will be made available depending on the policy of the original specimen repository institution.

Code availability Not applicable

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Tables

Table 1. List of the material analyzed in this study. H: humerus; F: femur. Abb: abbreviations, as in Figs 4,7). Mass estimates from Bongianni et al. (1988), Cooper et al. (2011), and Dinerstein (2011), in kilograms. Resol: resolution of the microtomographic scans, in micrometers. B: Bonn; L: London; M: Montpellier; P: Poitiers; Pa: Paris; °: taxa considered as aquatic ungulates in the comparative analyses (Figs. S2, S4). Institutional abbreviations: H-GSP: Howard University-Geological Survey of Pakistan, Quetta, Pakistan; GSP-UM: Geological Survey of Pakistan-University of Michigan, specimens archived in Quetta, Pakistan; JRHRVC: uncatalogued research collection of John R. Hutchinson at The Royal Veterinary College, Hatfield, United Kingdom; KB: Kossom Bougoudi, Centre National de Recherche pour le Développement, N'Djamena, Chad; MHNL: Musée des Confluences, Lyon, France; MNHN: Muséum National d'Histoire Naturelle, Paris, France; NHMUK, Natural History Museum, London, UK; NMB: Naturhistorisches Museum Basel, Switzerland; RON: Ronzon, Musée Crozatier, Le Puy-en-Velay, France; RR: A. Ranga Rao Collection of India currently housed at the Department of Anatomy and Neurobiology at the Northeastern Ohio Universities College of Medicine, Ohio; STIPB: Steinmann-Institut, Universität Bonn, Germany; TM: Toros-Ménalla, Centre National de Recherche pour le Développement, N'Djamena, Chad; UCBL-FSL: Faculté des Sciences de la Terre, Université Claude Bernard Lyon 1, France; UMZC: University Museum of Zoology, Cambridge, United Kingdom; UP: Université de Poitiers, France; ZFMK: Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany. *: sections not used in quantitative analyses

(sub)Family	**Species**	**Abb.**	**Mass**	**Inventory number**	**Bone**	**Resol**
Equidae	*Equus caballus*	Ec	380-600	MNHN ZM AC 1880-29	H,F	78,90(Pa)
Tapiridae	*Tapirus pinchaque*	Tp	150-200	MNHN ZM AC 1982-34	H,F	80,80(M)
	Tapirus terrestris	Tt	180-300	MNHN ZM MO 1939-225	H,F	55,55(P)
				ZFMK 462	H,F	48,82(B)
Rhinocerotidae	*Ceratotherium simum*	Cs	1,350-3,500	MNHN ZM MO 2005-297	H,F	78,68(Pa)
				JRHRVC uncat.	H,F	684,771(L)
	Dicerorhinus sumatrensis	Ds	600-775 kg	MNHN ZM AC 1903-300	H,F	58,65(Pa)
	Diceros bicornis	Db	800-1,300	UMZC H.6481	H	518(L)
				NHMUK M92402	H	561(L)
	Rhinoceros sondaicus	Rs	1,200-1,500	MNHN ZM AC A7970	H,F	76,76(Pa)
	Rhinoceros unicornis	Ru	< 2,000 kg	MNHN ZM AC 1960-59	H,F	76,76(Pa)
Tayassuidae	*Pecari tajacu*	Pt	15-42	MNHN ZM MO 1917-263	H,F	38,38(P)
				MNHN ZM MO 2000-352	H,F	40,40(P)
Camelidae	*Lama guanicoe*	Lg	90-140	STIPB M7388	H,F	55,55(B)
Suidae	*Potamochoerus porcus*	Pp	50-115	MNHN ZM MO 1944-234	H,F	53,53(P)
				MNHN ZM MO C.G. 1971-34	H,F	61,61(P)
	Sus scrofa	Ss	44-320	STIPB M56	H,F	173,206(B)

Tragulidae	*Hyemoschus aquaticus*	Haq	7-16	MHNL 50.002142	H,F	54,54(P)
				MNHN ZM MO 1914-97	H,F	41,41(P)
Giraffidae	*Giraffa camelopardalis*	Gc	450-1,930	JRHRVC uncat.	H,F	703,703(L)
	Okapia johnstoni	Oj	180-320	UMZC H.20302	H,F	352,389(L)
Cervidae	*Hydropotes inermis*	Hi	11-15,5	MNHN ZM MO 1874-274	H,F	74,74(P)
				MNHN ZM MO 1971-36	H,F	74,74(P)
Cervidae	*Alces alces*	Aa	280-600	MNHN ZM MO 2013-15	H,F	60,51(P)
Cervidae	*Alces americanus*	Aam	280-600	UMZC H.17691	H,F	352,416(B)
Cervidae	*Capreolus capreolus*	Cc	17-30	MNHN ZM AC 1993-221	H,F	Laurin *et al.*, 2011
Bovidae	*Bubalus bubalis*	Bb	400-1000	MNHN ZM MO 1866-56	H,F	79,79(P)
Bovidae	*Cephalophus sylvicultor*	Cs	45-80	NHMUK ZD 1961.8.9.80-1	H,F	242,316(B)
Bovidae	*Rupicapra rupicapra*	Rr	25-60	STIPB M1639	H,F	189,201(B)

Bovidae	*Syncerus caffer*	Sc	350-900	MNHN ZM MO 1936-72	H,F	56,56(P)
				NHMUK ZD 1874.11.2.4	H,F	352,545(B)
Raoellidae	*Indohyus indirae* °	Ii	~2	RR 157	H	Thewissen et al. 2007
				RR 42	F	Thewissen et al. 2007
Pakicetidae	*Ichthyolestes pinfoldi* °	Ip	~7	H-GSP 96227	H	Thewissen et al. 2007
Remingtonocetidae	*Remingtonocetus domandaensis* °	Rd	-	GSP-UM 3054	F	49(B)
Protocetidae	*Qaisracetus arifi* °	Qa	-	GSP-UM 3318	H	58(B)
	Maiacetus inuus °	-	-	GSP-UM 3551	H	46(B)
Microbunodontinae	*Microbunodon minimum*	Mm	~20	UP-L.M. 1967 MA 350	H	46(P)
				UP-L.M. 1968 MA 377	H	46(P)
Anthracotheriinae	*Paenanthracotherium bergeri*	Pb	< 950	UCBL-FSL 213779	H*	89(P)
Bothriodontinae	*Bothriodon velaunus*	Bv	< 150	2004-6-1792-RON	H	44(P)
Bothriodontinae	*Brachyodus onoideus*	Bo	< 2,350	NMB S.O. 5897	F	93(P)
				MNHN Neu 75	H	57(P)
				MNHN Neu 76	H	78(P)
Bothriodontinae	*Elomeryx borbonicus*	Eb	<130	UCBL-FSL 8540	F	69(P)
				UCBL-FSL 9285	F	46(P)
				UCBL-FSL 8565	H	59(P)
				UCBL-FSL 8572	H	59(P)
Bothriodontinae	*Libycosaurus bahri*	Lb	< 1,600	TM 104-00-003	F	93(P)
				TM 254-02-010	F	93(P)
				TM 098-99-001	H*	69(P)

Hippopotaminae	*Choeropsis liberiensis*	Cl	160-270	MNHN ZM MO 1921-309	H,F	62,73(P)
				MNHN ZM MO 1944-146	H,F	73,77(P)
				MNHN ZM MO 1978-104	H,F	69,76(P)
				ZFMK 65 570	H,F	59,66(B)
Hippopotaminae	*Hexaprotodon garyam*	Hg	< 2,200	TM 115-06-001	F	93(P)
				TM 258-01-031	F	93(P)
				TM 055-XX-05	H*	93(P)
				TM 258-01-027	H	93(P)
Hippopotaminae	*Hippopotamus amphibius*	Ha	1,000-4,500	MHNL 50.002123	H,F	93,93(P)
				MNHN ZM MO 1917-249	H,F	93,93(P)
				MNHN ZM MO 1971-308	H,F	93,93(P)
Hippopotaminae	*Saotherium* cf. *S. mingoz*	Sm	<1,000	KB 03-97-170	H*	93(P)

Table 2. Supposed lifestyle of the hippopotamoids sampled based on literature and on this study. H: humerus; F: femur.

Taxon	Data from the literature	Supposed lifestyle	Microanatomical data	Mass (kg)	Inferred lifestyle
Microbunodontinae					
Microbunodon minimum	- Skull morphology: no elevated sense organs (Cabard 1976) - Diet: frugivorous-folivorous (micro and	Terrestrial and solitary in forested habitats	H: tubular	~20	Essentially terrestrial

	mesowear; Lihoreau 2003), - Postcranial morphology: autopod showing a digitigrade stance with weekly elongated metapodials (Lihoreau 2003) - Gregarious habits: proposed to be solitary (Lihoreau 2003) - no data for subaquatic hearing - oxygene isotopic ratio: proposed to be terrestrial (Sherler 2011) and a closely related species is considered as a terrestrial forest dweller (Nelson 2007)				
Anthracotheriinae					
Paenanthracotherium bergeri	- Skull morphology: no elevated sense organs (Sherler et al. 2018) - Diet: folivorous (Sieber 1936), browser on C3 plants in a mesophytic forest (Tütken and Absolon 2015 for a close relative). - Postcranial morphology: heavily-built with short and	Terrestrial forest-dweller	H: thick cortex; open medullary cavity No F	< 950	Essentially terrestrial

	robust limb bones - Gregarious habits: no data - no data for subaquatic hearing - Oxygene isotopic ratio: a close relative is proposed to be terrestrial (Tütken and Absolon 2015) -Taphonomy: relatives have historically been found in lignit deposits (Cuvier 1822)				
Bothriodontinae					
Bothriodon velaunus	- Skull morphology: no data - Diet: supposed to have been predominantly folivorous (Lihoreau and Ducrocq 2007) - Postcranial morphology: lightly-built skeleton with gracile legs (Filhol 1881) - Gregarious habits: no data - no data for subaquatic hearing - Oxygene isotopic ratio: no data -Taphonomy: swamp deposit (Filhol 1881)	Regularly considered to live in swamp habitat	H: tubular with spongious transition zone	< 150	Essentially terrestrial
Brachyodus onoideus	- Skull morphology: amphibious	Terrestrial -	H: thick cortex; spongious filling	< 2,350	Possibly

	position of the sensory organs on the head (Orliac et al. 2013) - Diet: no data - Morphology of the tympanic bulla potentially enabling underwater directional hearing (Orliac et al. 2013) - Postcranial morphology: terrestrial (Dineur 1981) - Gregarious habits: trackways suggest multi-aged group and important gregarism (Diaz Martinez et al. 2020) - Oxygene isotopic ratio: no data -Taphonomy: shoreline and palustrine environment	hydrophilic and water immersive habits	of the medullary area F: thick cortex; open medullary cavity		slightly semi-aquatic (with reduced immersive habits)
Elomeryx borbonicus	- Skull morphology: low position of the superior border of the orbit and lack of specialization of the tympanic bulla and of the auditory tube (Lihoreau 2003) - Diet: no data - no data for subaquatic hearing	Semi-aquatic lifestyle, supposedly in marshy habitats	H&F: tubular	<130	Essentially terrestrial

	- Postcranial morphology: hind limb more robust than forelimb and proposed occurrence of webbed feet (Geais 1934) - Gregarious habits: sexual dimorphism (Geais 1934) - Oxygene isotopic ratio: no data -Taphonomy: swamp deposit (Chateauneuf and Nury 1995)				
Libycosaurus bahri	- Skull morphology: highly elevated orbits and external nares, compressed and dense tympanic bulla (Lihoreau et al. 2014) - Diet: fresh mixed feeder (microwear and isotopic ratio of Carbon; Lihoreau et al. 2014) - no data for subaquatic hearing - Postcranial morphology: no data - Gregarious habits: large sexual size dimorphism suggests gregarism and polygenous mating system	Semi-aquatic	H: thick cortex; extensive spongious filling of the medullary area F: thick cortex; open medullary cavity with spongious transition zone	< 1,600	Semi-aquatic

	(Lihoreau et al. 2014) - Oxygene isotopic ratio: aquatic signal compared to the terrestrial fauna (Lihoreau et al. 2014) -Taphonomy: peri-lacustrine deposit (Vignaud et al. 2002)				
Hippopotaminae					
Saotherium cf. *S. mingoz*	- Skull morphology: no elevation of the orbit (Boisserie et al. 2003) - Diet: mixed C_3-C_4 to pure C_4 (Zazzo et al. 2000). - No data for subaquatic hearing - Postcranial morphology: not published yet - Gregarious habits: no data - Oxygene isotopic ratio: negative values compared to terrestrial fauna (notably bovids, suids – from Jacques 2007). -Taphonomy: peri-lacustrine environment	Terrestrial - Water dependence	H: spongious filling of the medullary area No F	<1,000	Terrestrial
Hexaprotodon garyam	- Skull morphology: moderate elevation of the sensory organs on the head	Semi-aquatic	H: (very) thick cortex; thickening around the growth center; extensive spongious filling	< 2.200	Semi-aquatic

	- Diet: opportunistic mixed feeder (Boisserie et al. 2005b; Jacques 2007) - No data for subaquatic hearing - Postcranial morphology: not published yet - Gregarious habits: high frequency in assemblages; moderate sexual dimorphism and agonistic weaponry (Boisserie 2002; Boisserie et al. 2005b) - Oxygene isotopic ratio: aquatic signal compared to the terrestrial fauna (Lihoreau et al. 2014) -Taphonomy: peri-lacustrine deposit (Vignaud et al. 2002)		F: rather thick cortex; open medullary cavity		

Table 3 Values obtained for the tests of a size effect and phylogenetic signal in the various parameters used in the humerus analyses. In bold when significant at 5%

	C	CSS	Zpol	RMeanT	RSDT	S	P	PC1	PC2	R
Size	**r=0.50 p<0.01**	r =-0.16 p=0.28	**r =0.92 p<0.01**	r =-**0.77 p<0.01**	**r =0.92 p<0.01**	**r =0.42 p<0.01**	r =-**0.44 p=0.03**	**r =0.85 p<0.01**	**r =0.29 p=0.05**	**-**

Phylogeny (A)	**K =0.79** **p< 0.01**	**K=0.47** **p=0.03**	**K=0.67** **p<0.01**	**K=0.56** **p<0.01**	**K=0.84** **p<0.01**	**K=0.74** **p<0.01**	**K=0.56** **p=0.01**	**-**	**-**	**K=0.71** **p<0.01**
Phylogeny (B)	**K =0.79** **p< 0.01**	**K=0.47** **p=0.03**	**K=0.67** **p<0.01**	**K=0.56** **p<0.01**	**K=0.84** **p<0.01**	**K=0.75** **p<0.01**	**K=0.56** **p=0.02**	**-**	**-**	**K=0.71** **p<0.01**

Table 4 Values obtained for the tests of a size effect and phylogenetic signal in the various parameters used in the femur analyses. In bold when significant at 5%

	C	CSS	Zpol	RMeanT	RSDT	S	P	PC1	PC2	R
Size	**r=0.58** **p<0.01**	r =0.26 p=0.08	**r =0.93** **p<0.01**	**r =0.66** **p<0.01**	**r =0.83** **p<0.01**	**r =0.53** **p<0.01**	**r =-0.49** **p<0.01**	**r =0.85** **p<0.01**	r =0.18 p=0.23	
Phylogeny (A)	**K=0.97** **p<0.01**	**K=0.51** **p<0.01**	**K=0.47** **p=0.01**	**K=0.56** **p<0.01**	**K=0.58** **p<0.01**	**K=0.59** **p<0.01**	**K=0.84** **p<0.01**	**-**	**-**	**K=0.57** **p<0.01**
Phylogeny (B)	**K=1.02** **p<0.01**	**K=0.52** **p<0.01**	**K=0.48** **p=0.01**	**K=0.51** **p<0.01**	**K=0.60** **p<0.01**	**K=0.63** **p<0.01**	**K=0.88** **p<0.01**	**-**	**-**	**K=0.58** **p<0.01**

Figure legends

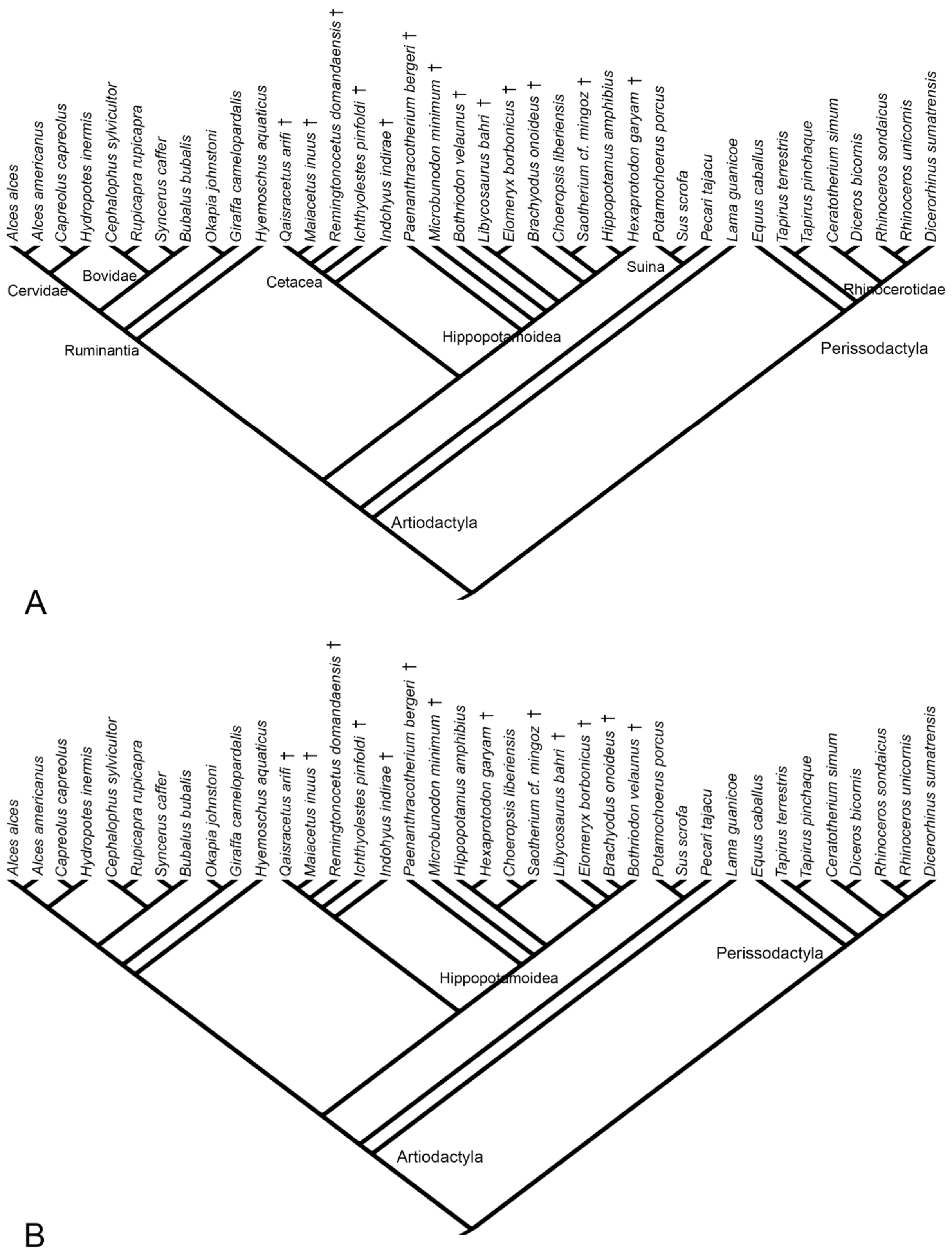

Fig. 1 Two consensus phylogenetic trees including the extant and extinct taxa sampled. **A** from Boisserie et al. (2011) for the hippopotamines, Lihoreau et al. (2015; SI fig. 17) and Boisserie et al. (2017) for the other hippopotamoids; **B** from Lihoreau et al. (2015, 2019) and Gomes Rodrigues et al. (2019) for the hippopotamoids, and **A,B** from Thewissen et al. (2007)

and Vautrin et al. (2020) for cetaceans and Gilbert et al. (2006), Bibi (2013), Foley et al. (2016), and Springer et al. (2019) for the remaining mammals. †: extinct taxa.

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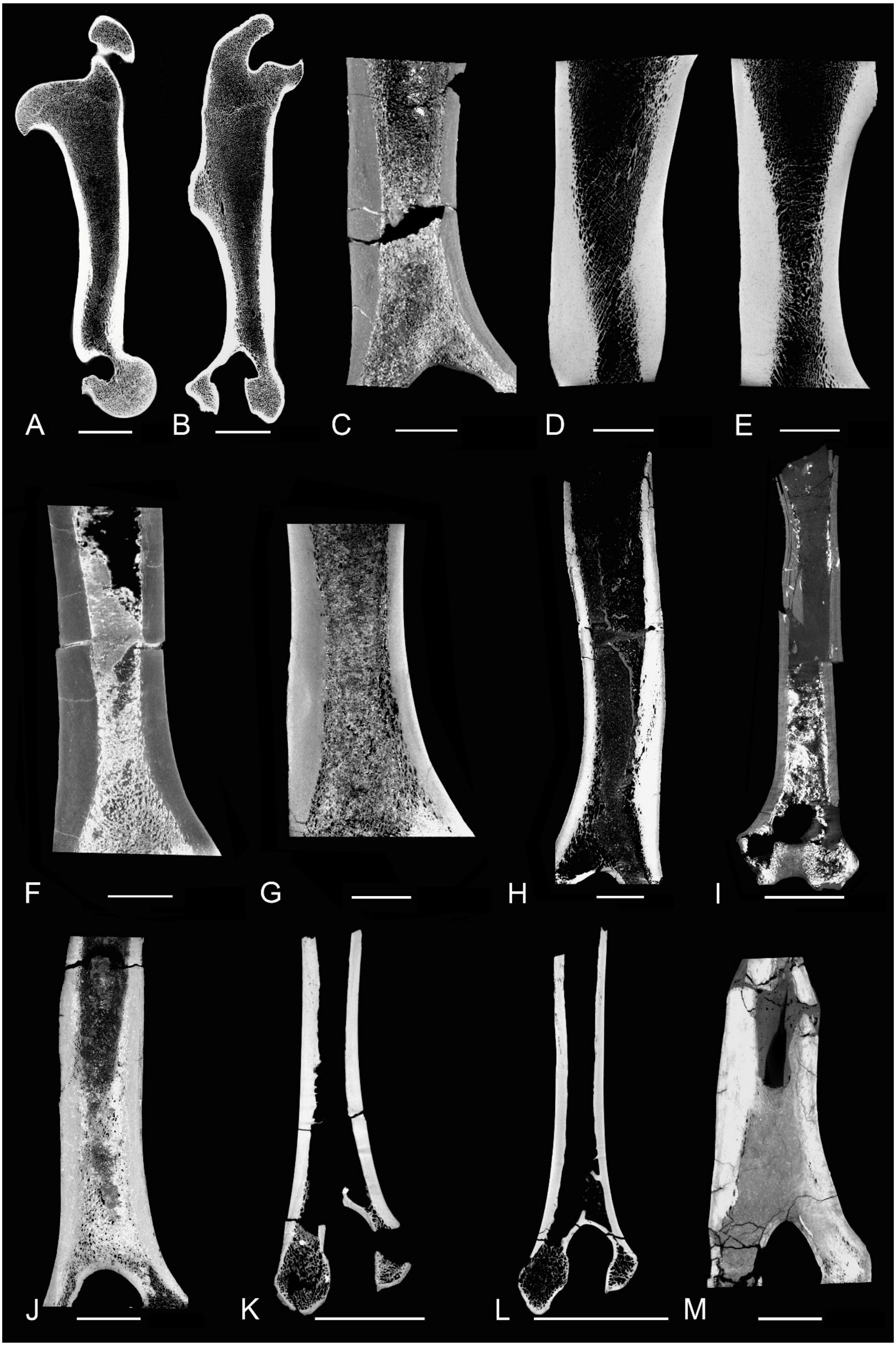

Fig. 2 Humerus longitudinal sections. **A,B** *Choeropsis liberiensis* MNHN 1944-146 sagittal (A) and coronal (B) sections; **C** *Saotherium* cf. *S. mingoz* KB 03-97-170 coronal section (CS); **D,E** *Hippopotamus amphibius* MHNL 50.002123 sagittal (C) and coronal (D) sections; **F** *Hexaprotodon garyam* TM 55-XX-05 CS; **G** *Hexaprotodon garyam* TM 258-01-27 CS; **H** *Brachyodus onoideus* MNHN Neu 75 CS; **I** *Bothriodon velaunus* 2004-6-1792-RON CS; **J** *Libycosaurus bahri* TM 98-99-01 CS; **K** *Elomeryx borbonicus* UCBL-FSL 8572 CS; **L** *Microbunodon minimum* UP-L.M. 1968 MA 377 CS; **M** *Paenanthracotherium bergeri* UCBL-FSL 213779 CS. Scale bars equal 3 cm. Partial sedimentary filling of the bone in C, F, G, H, I, J, and M.

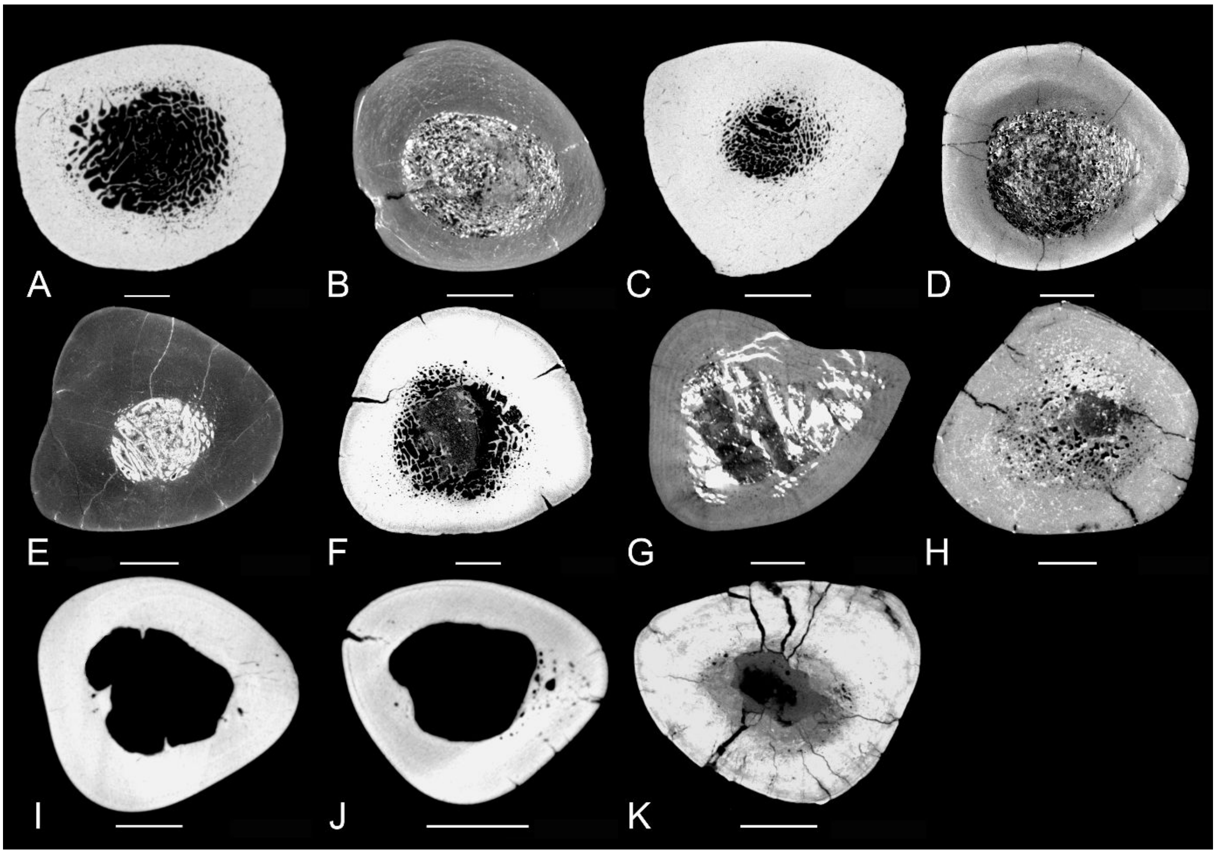

Fig. 3 Humerus transverse sections near the growth center. **A** *Choeropsis liberiensis* MNHN 1978-104; **B** *Saotherium* cf. S. *mingoz* KB 03-97-170; **C** *Hippopotamus amphibius* MNHN 1971-308; **D** *Hexaprotodon garyam* TM 55-XX-05; **E** *Hexaprotodon garyam* TM 258-01-27; **F** *Brachyodus onoideus* MNHN Neu 75; **G** *Bothriodon velaunus* 2004-6-1792-RON; **H** *Libycosaurus bahri* TM 98-99-01; **I** *Elomeryx borbonicus* UCBL-FSL 8572; **J** *Microbunodon minimum* UP-L.M. 1968 MA 377; **K** *Paenanthracotherium bergeri* UCBL-FSL 213779. Scale bars equal: A,G,I,J- 5 mm; F,H- 10 mm; B,C,D,E,K- 15 mm. Partial sedimentary filling of the bone in B, D,E,(F), G, H, and K.

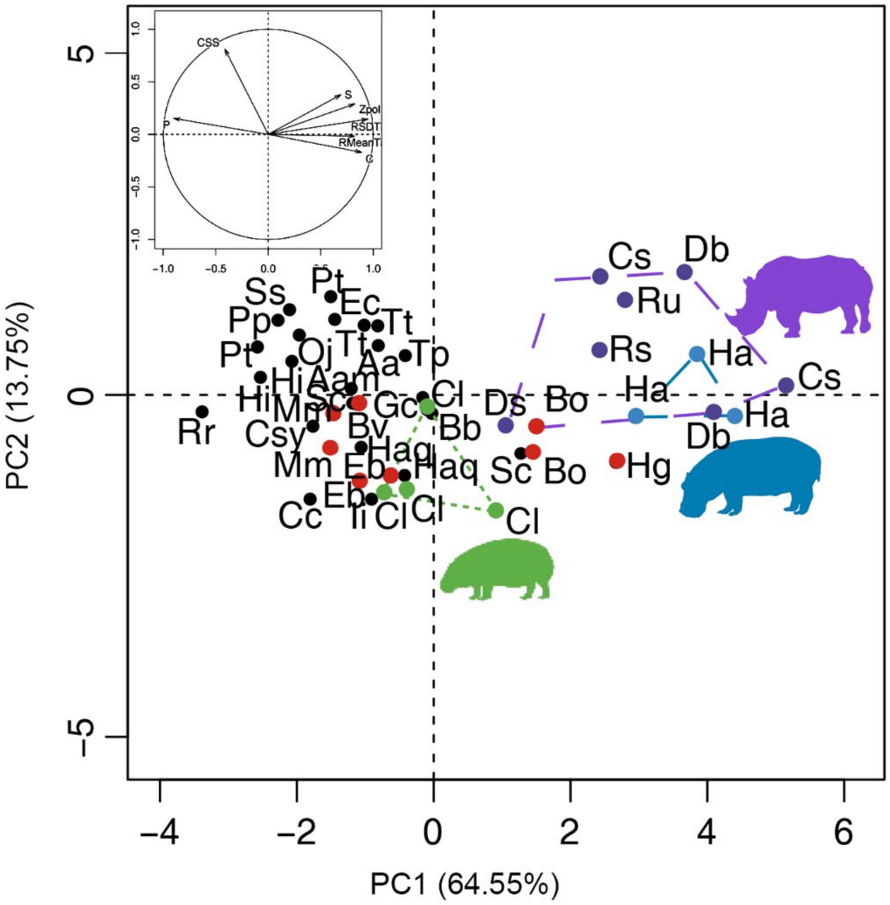

Fig. 4 Distribution of the specimens in the morphospace along the two first axes of the humerus PCA with the contribution of the different parameters according to the principal component (PC)1 and PC2 axes. Abbreviations as listed in Table 1

Fig. 5 Femur longitudinal sections. A,B *Choeropsis liberiensis* MNHN 1978-104 SS(A) and CS(B); **C,D** *Hippopotamus amphibius* MNHN 1971-308 SS(C) and CS(D); **E** *Hexaprotodon garyam* TM 115-06-01 SS; **F** *Hexaprotodon garyam* TM 258-01-31 SS; **G** *Brachyodus onoideus* NMB S.O. 5897 CS; **H** *Libycosaurus bahri* TM 104-00-03 SS; **I** *Libycosaurus bahri* TM 254-02-10 SS; **J** *Elomeryx borbonicus* UCBL-FSL 8540 SS. Scale bars equal 30 mm. Partial sedimentary filling of the bone in E, F, G, H, I, and J.

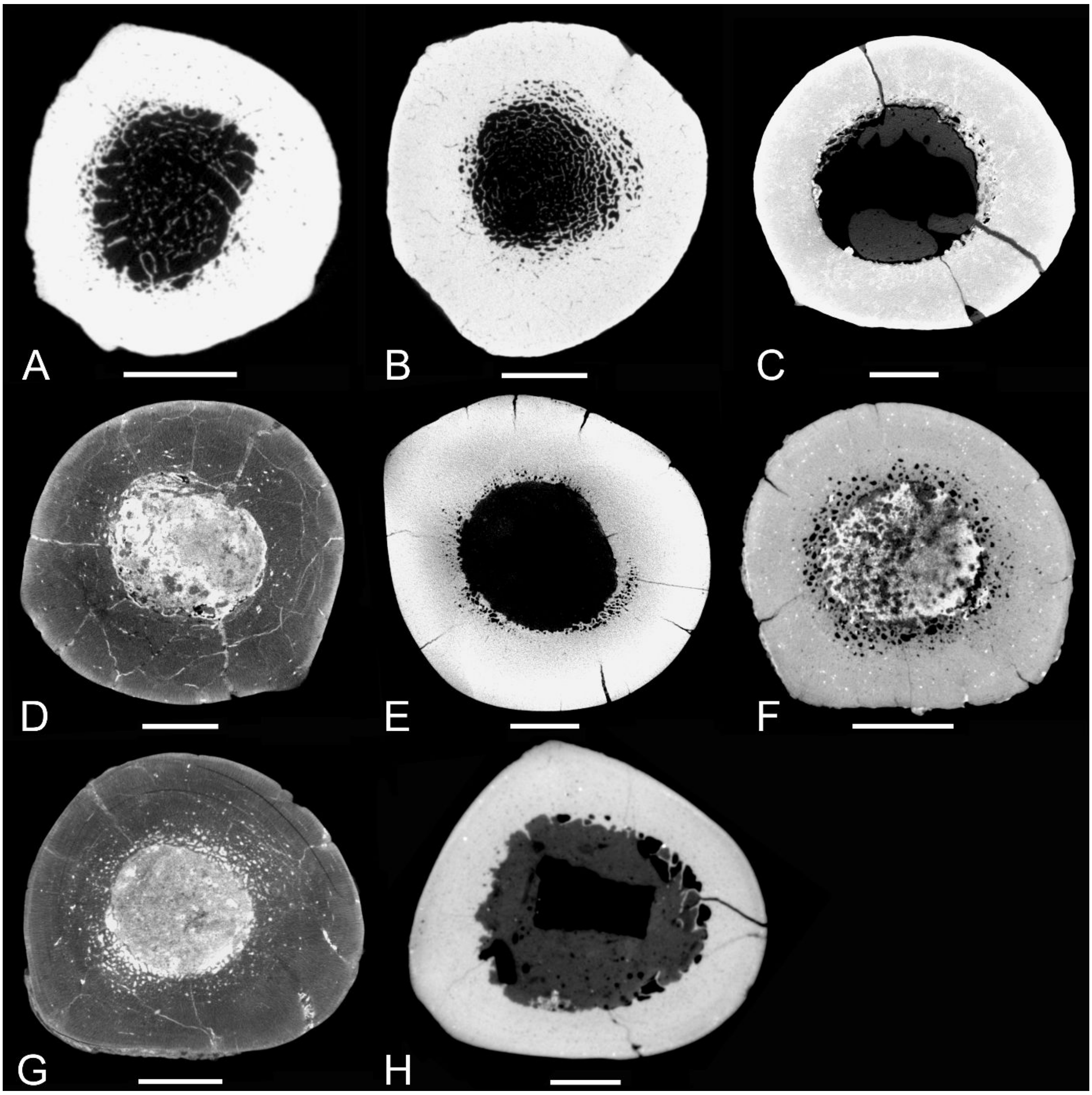

Fig. 6 Femur transverse sections near the growth center. **A** *Choeropsis liberiensis* MNHN 1944-146; **B** *Hippopotamus amphibius* MNHN 1971-308; **C** *Hexaprotodon garyam* TM 115-06-01; **D** *Hexaprotodon garyam* TM 258-01-31; **E** *Brachyodus onoideus* NMB S.O. 5897; **F** *Libycosaurus bahri* TM 104-00-03; **G** *Libycosaurus bahri* TM 254-02-10; **H** *Elomeryx borbonicus* UCBL-FSL 8540. Scale bars equal: A- 10 mm; B-G- 15 mm; H- 5mm. Partial sedimentary filling of the bone in (C), D, F, G, and H.

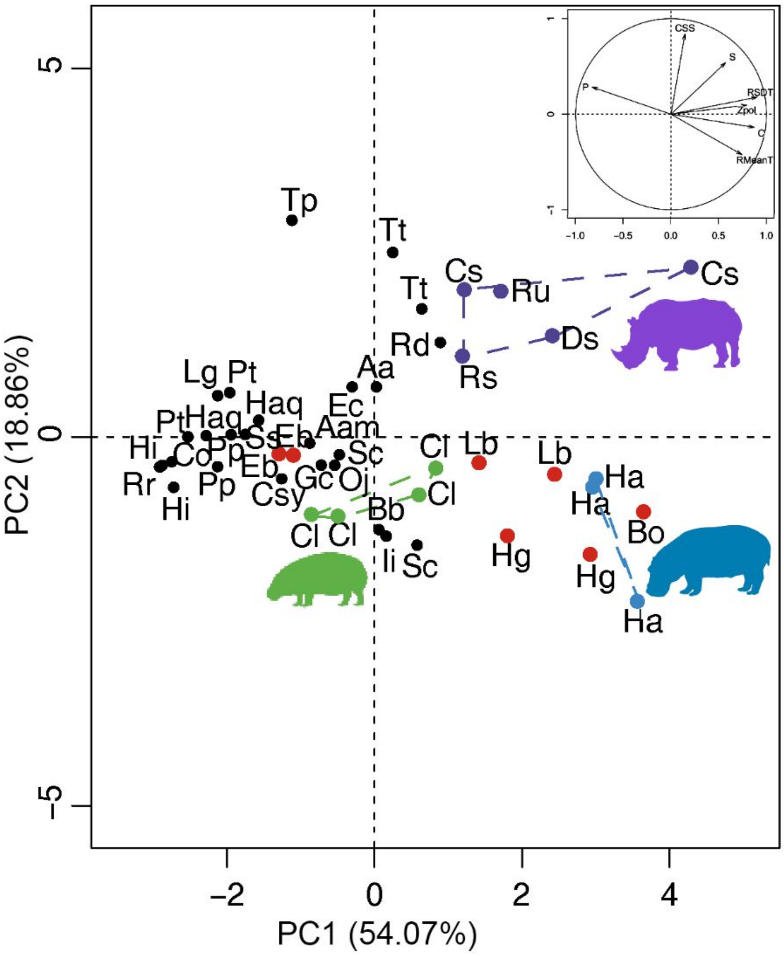

Fig. 7 Distribution of the specimens in the morphospace along the two first axes of the femur PCA with the contribution of the different parameters according to the principal component (PC)1 and PC2 axes. Abbreviations as listed in Table 1. Color code as in Fig. 4