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INVESTIGATING ECOLOGICAL AND PHYLOGENETIC CONSTRAINTS IN HIPPOPOTAMIDAE SKULL SHAPE

LUCA PANDOLFI[1*], ROBERTA MARTINO[2], LORENZO ROOK[1] & PAOLO PIRAS[3]

*Corresponding author. [1]Dipartimento di Scienze della Terra, Paleo[Fab]Lab, Università degli Studi di Firenze, Via G. La Pira 4, Firenze, 50121, Italy. E-mail: luca.pandolfi@unifi.it; lorenzo.rook@unifi.it
[2]Dipartimento di Scienze della Terra, Sapienza Università di Roma, Laurea Magistrale in Scienze Geologiche, Piazzale Aldo Moro 5, Rome, I-00185, Italy. E-mail: roberta.aska@gmail.com
[3]Dipartimento di Scienze Cardiovascolari, Respiratorie, Nefrologiche, Anestesiologiche e Geriatriche, Sapienza Università di Roma, Viale del Policlinico 155, Rome, 00161, Italy. E-mail: ppiras@uniroma3.it

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*Abstract***.** Hippopotamidae are a group of large-sized mammals of interest for testing evolutionary traits in time and space. Variation in skull shape within Hippopotamidae is here investigated by means of shape analysis (Geometric Morphometrics) and modern statistical approaches. Two-dimensional shape analysis is applied to dorsal and lateral views of extant and extinct Hippopotamidae species sufficiently preserved to allow their morphology to be captured by landmark and semi-landmark digitization. The results show that *Hippopotamus gorgops* and *H. antiquus* display similar shapes, while *Hexaprotodon palaeindicus* falls within the morphospace occupied by *H. amphibius*, suggesting similar morphology. The cranial shape of the Sicilian hippopotamus (*H. pentlandi*) still resembles that of *H. amphibius* in lateral view, suggesting that adaptation to the insular domain was yet not fully attained. Madagascan hippopotamuses (*H. madagascariensis* and *H. lemerlei*) are close to the pygmy hippo, *Choeropsis liberiensis*, in PC1 values; nevertheless, the cranial shape of the Madagascan hippos seems not to be closely related to the cranial shape of *C. liberiensis*. Despite the morphological convergences within the group, while cranial shape in Hippopotamidae is phylogenetically structured, this does not hold for size. Although further investigations are needed to test the influence of ecological and palaeoecological parameters on the general shape to provide additional information for understanding Hippopotamidae evolution and adaptation, the present study provides an insight into the evolutionary framework of Hippopotamidae.

INTRODUCTION

Hippopotamidae are a group of large-sized mammals that allow observation of variation in morphological traits in response to different environmental conditions (aquatic to terrestrial). Specifically, the development of the orbits extending above the cranial roof has been interpreted as an important semiaquatic adaptation within the group, especially in the *Hippopotamus* and *Hexaprotodon* lineages (Boisserie 2005, 2007; Boisserie et al. 2011). The cranial morphology of the extant pygmy-hippo, *Choeropsis liberiensis*, with maintenance of terrestrial characters such as orbits below the cranial roof, has been considered as a model for several extinct species. The convergent appearance of terrestrial characters, related to a dietary shift and dwarfism, occurred within *Hippopotamus*-lineages during the Pleistocene, in particular after colonization of islands (e.g., Crete, Malta, Sicily and Madagascar) (Sondaar 1977; Houtemaker & Sondaar 1979; Stuenes 1989; Caloi & Palombo 1994; Spaan 1996).

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A selection of some insular hippo's species (*H. lemerlei*, *H. madagascariensis* and *Phanourios minor*) from Pleistocene and Holocene of Cyprus and Madagascar was recently the subject of a study aimed at comparing their shape change due to insularity with those occurring in Elephantidae (van der Geer et al. 2018). Here we focus on a broader taxonomic and temporal sampling of Hippopotamidae solely, from Miocene to Recent, by including both insular and non-insular species.

Despite several studies focused on the cranial morphology of Hippopotamidae, the variation in skull shape within this group has seldom been investigated by means of shape analysis (Geometric Morphometrics) and modern statistical approaches in order to quantify differences and degree of homoplasy among the species, and in order to test the influence of phylogenetic and ecological signals on the variation in skull shape. This paper aims to investigate these aspects by means of two-dimensional shape analysis applied to dorsal and lateral views of extant and extinct Hippopotamidae species sufficiently well preserved in order to allow their morphology to be captured by landmark and semi-landmark digitization in lateral and dorsal views.

Material and methods

Material

We collected images of 49 skulls of hippopotamids from both original photos and published pictures in lateral and dorsal views (Supplementary Material). Eleven hippopotamid species are represented in the sample (two extant and nine extinct species). In order to eliminate inter-observer error, the same operator (L.P.) digitized the entire landmark dataset. Hippopotamid species represented by at least one complete skull are recorded in the sample. Fossil material is poorly represented by complete skulls due to the difficulties of preservation. As sex is directly observable in extant species only, and as we are dealing with taxonomic diversity spanning from the Miocene to Recent and among different genera, we did not include sex as a variable in our analyses. In addition, we excluded morphological features strongly influenced by sexual dimorphism (tusks size and jowl area; Stevenson-Hamilton 1912; Coughlin & Fish 2009) from the shape configuration. The specimen list and the number of individuals for each species, as well as the list of institutions and references from which the images used for shape acquisition were collected are reported in Supplementary Material.

Geometric Morphometrics

We adopted Geometric Morphometrics as our shape analysis tool in order to analyze morphological variation. GM is demonstrated to be an effective method for analysis of anatomical variation and disparity in both extinct and extant taxa (Piras et al. 2009, 2010, 2014; Maiorino et al. 2013; Pandolfi & Maiorino 2016; Pandolfi et al. 2019).

We digitized 15 landmarks and 32 semi-landmarks from photographs in lateral view (Fig. 1A) and 9 landmarks and 13 semi-landmarks in dorsal view (Fig. 1B), on each specimen using the tpsDig2 v2.17 software (Rohlf 2013). Given that the rostral region is often damaged, or enterely lacking, in fossil specimens we chose a shape configuration allowing inclusion of the maximum number of extinct species, without losing relevant morphological signal but excluding incomplete or severely damaged skulls. Semi-landmarks were used to capture the morphology of complex outlines where anatomical homology is difficult to recognize. Semi-landmarks assume that curves or contours are homologous among specimens (Adams et al. 2004; Perez et al. 2006). Generalized Procrustes Analysis (GPA) (Bookstein 1986; Goodall 1991), implemented in the procSym( ) function from the R-package "Morpho" (Schlager 2014), was used to rotate, translate and scale landmark configurations to unit centroid size (CS = the square root of the sum of squared distances of the landmarks from their centroid) (Bookstein 1991). We used the minimization of bending energy during the sliding of semi-landmarks (cfr. Gunz & Mitteroecker 2013). After GPA, a Principal Components Analysis (PCA) was performed in order to visualize orthogonal axes of morphological variation. The Unweighted Pair Group Method with Arithmetic mean (UPGMA) algorithm was used on the per-species averaged Procrustes distances to assess similarities among taxa. The results are dendrograms of morphological similarities (skulls in lateral and dorsal views) among species included in the sample. In order to visualize shape changes in ordination plots we choose to use the method described in Márquez

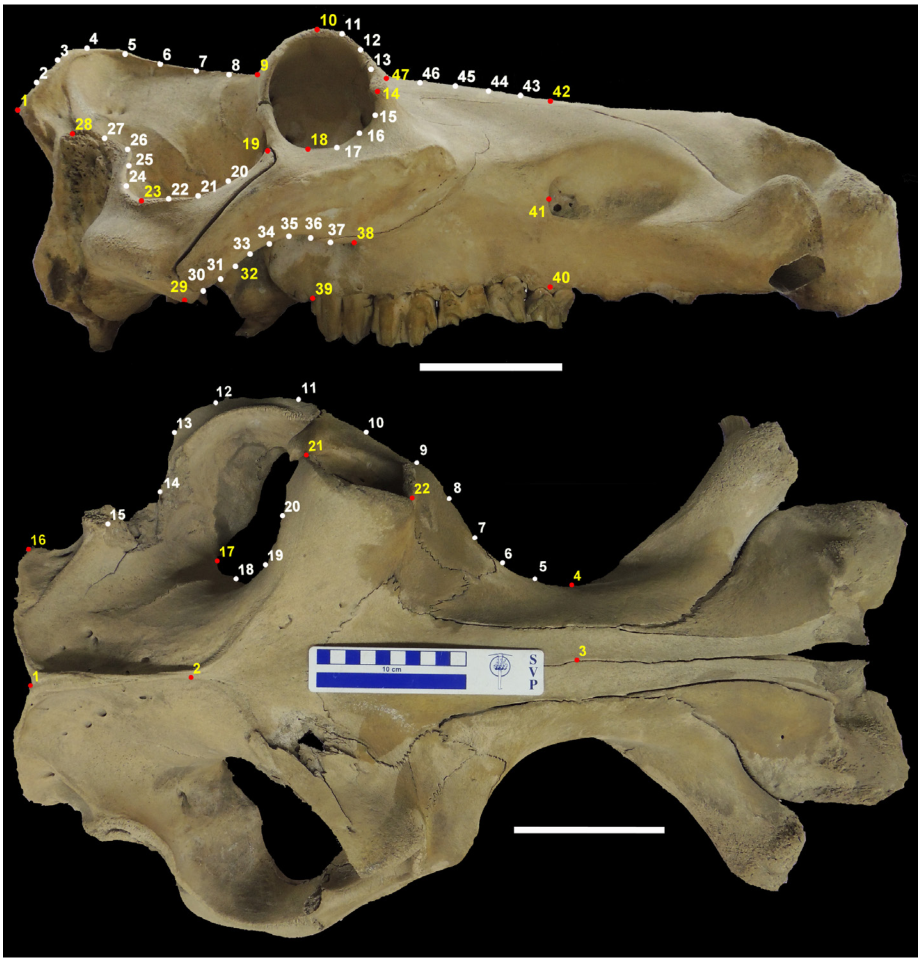


Fig. 1 - Landmark (red points) configurations of Hippopotamidae skulls in lateral (A) and dorsal (B) views. A: 1, posterior tip of the nuchal crest; 9, contact beween the orbit and the skull roof; 10, tip of the dorsal border orbit; 14, anterior border of the orbit; 18, tip of the ventral border of the orbit; 19, contact between the dorsal borde of the zygomatic arch and the posterior border of the orbit; 23, lowermost tip of the dorsal border of the zygomatic arch; 28, dorsal-posterior tip of the zygomatic arch; 29, lowermost tip of the ventral border of the zygomatic arch; 38, contact between the zygomatic arch and the maxilla; 39, posterior tip of the dental row; 40, orthogonal projection, relative to the horizontal axis identified by landmarks 39-40, of point 41 at the base of the dental row; 41, posterior border of the infraorbital foramen; 42, orthogonal projection, relative to the horizontal axis identified by landmarks 39-40, of point 41 at the cranial roof; 47, contact between the cranial roof and the anterior border of the orbit. Semilandmarks (white points): 2-8, 11-13, 15-17, 20-22, 24-27, 30-37, 43-46. B: 1, middle point of the posterior border of the nuchal crest; 2, anterior tip of the frontal-parietal crest; 3, orthogonal projection, relative to the horizontal axis identified by landmarks 1-2, of the point 4 in the middle of the cranial roof; 4, inflexion point on the dorsal view of right maxilla; 16, posterior-lateral tip of the nuchal crest; 17, posterior tip of the zygomatic cavity; 21, posterior tip of the dorsal border of the orbit; 22, anterior tip of the dorsal border of the orbit. Semilandmarks (white points): 5-15, 18-20.

et al. (2012). There it was suggested that a useful way to visualize local, infinitesimal variation within a deformation grid is to use the Jacobian (*J*) of the Thin Plate Spline interpolation function. This measures the rate of shape deformation at any point along all directions simultaneously. As *J* contains the first partial derivatives of the TPS, the affine component, which is a first-order polynomial, becomes a constant and for this reason *J* captures information as localized variation in the non-affine component of the deformation. In 2D *J* is a 2x2 matrix that can be evaluated at any point within a body. The logarithm of its determinant represents change in the area in the region about the interpolation point. Values < 0 indicate that, with respect to the source (here the sample' consensus), the target (here the PC's extremes) experiences a reduction in the local area, while values > 0 indicate an enlargement.

We tested for significant phylogenetic signal in size (CS) using the function phylosig( ) in the 'phytools' R package (Revell 2012). The degree of phylogenetic signal in shape data for a given phylogenetic tree was quantified using the geomorph

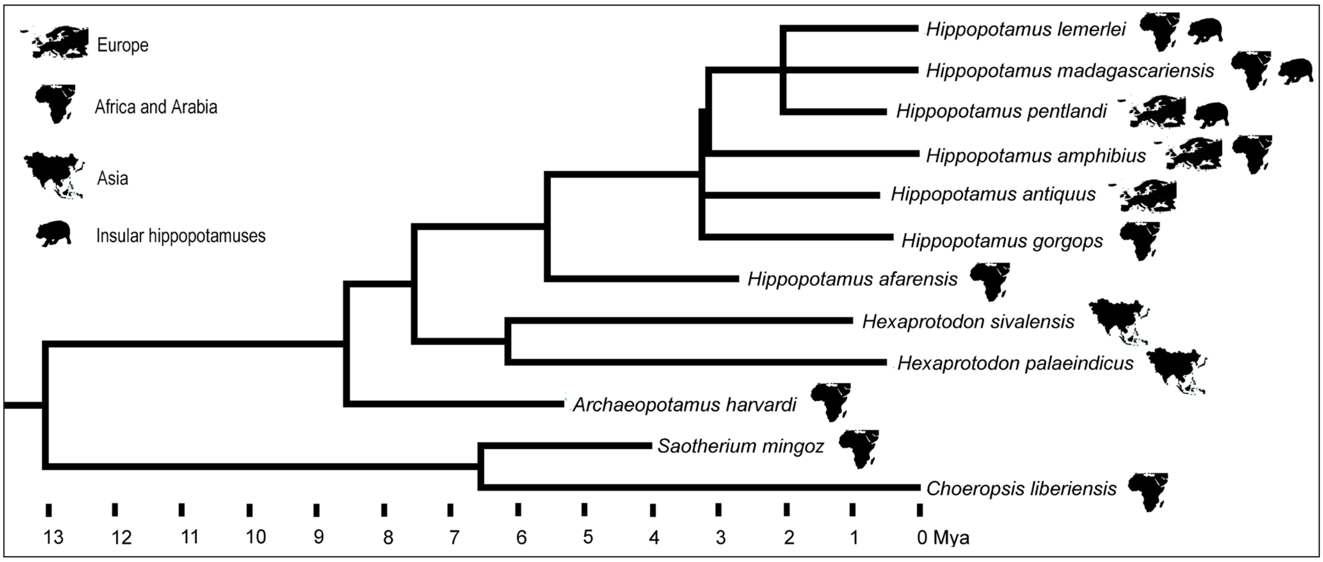


Fig. 2 - Time-calibrated phylogenetic tree of the considered species of Hippopotamidae used in this study (details are reported in methods).

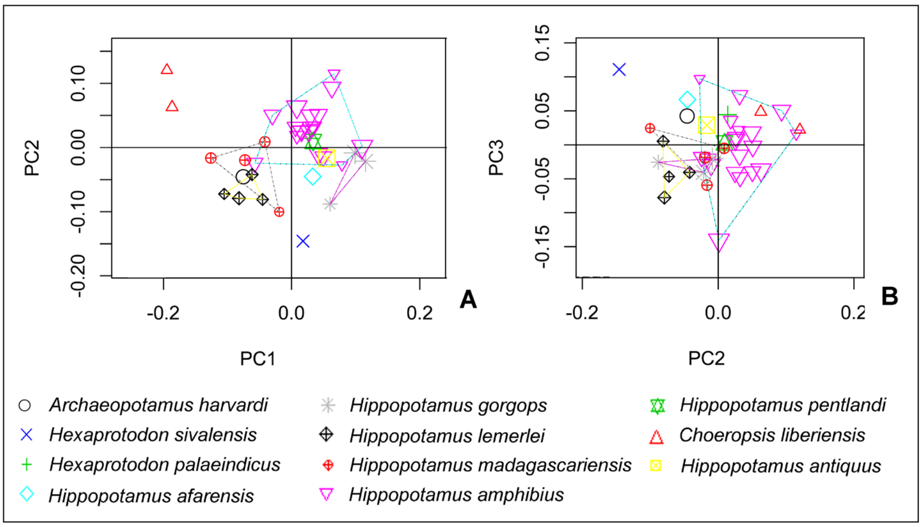
function ‘physignal’ (Adams et al. 2019). The function ‘phylo.heatmap’ has been used to create a multivariate phylogenetic heatmap (Revell 2012). We used phylogenetic generalized least squares (PGLS) regressions from package ‘geomorph’ to evaluate the relationship between size and shape of the skull averaged by species (Grafen 1989; Rohlf 2001, 2006; Martins et al. 2002; Mundry 2014); inference is based on null-hypothesis significance testing (P-value). In order to present results of regressions even in the absence of comparative methods we included the results of standard regression. Phylomorphospace was created by projecting the hippopotamid phylogeny presented in Figure 2 onto the morphospace delimited by the first three PC axes.

Phylogeny

We built a synthetic phylogenetic tree (Fig. 2) using the software Mesquite 2.75 (Maddison & Maddison 2011), based on the most recently proposed phylogenetic relationships (Petronio 1986; Boisserie 2006; Mazza & Bertini 2013) and including all hippopotamine species considered valid. We calibrated branch lengths in millions of years (Ma) based on stratigraphic range in the fossil record.

The phylogeny of the family Hippopotamidae has been recently revised by Boisserie (2005). The earliest representatives of the group include the genera *Archaeopotamus* and *Saotherium*. *Archaeopotamus* occurred during the Late Miocene and it has been considered as sister taxon to the *Hippopotamus*-*Hexaprotodon* clade (Boisserie 2005). *Hippopotamus* occurred for the first time at least during the Early Pliocene (Faure 1994), and it has been documented from several Pleistocene localities in Africa (*H. kaisensis*, *H. gorgops*, *H. amphibius*), Europe (*H. antiquus*, *H. amphibius*) and the Near East (*H. behemoth*) (Dietrich 1928; Hopwood 1926; Caloi et al. 1980; Capasso Barbato et al. 1982; Stuenes 1989; Faure & Guérin 1990; Mazza 1991, 1995; Caloi & Palombo 1994; Petronio 1995; Boisserie 2006; Pandolfi & Petronio 2016). During the Pleistocene, *Hippopotamus* also reached several Mediterranean islands and Madagascar, evolving into different dwarf species: *H. pentlandi*, *H. melitensis*, *H. creutzburgi*, *H. madagascariensis*, *H. lemerlei*, *H. laloumena*. All the above-mentioned taxa seem to be derived from *H. amphibius*. The phylogenetic relationships among Pleistocene European hippopotamuses were not investigated by Boisserie (2005) but have been discussed in other papers (Petronio 1986, 1995; Mazza & Bertini 2013). *Hexaprotodon* mainly occurred in the Indian subcontinent and south-east Asia from the Miocene-Pliocene transition to the late Pleistocene (Boisserie 2005 and references therein). The earliest representative of this group is *Hex. sivalensis*, while *Hex. palaeindicus* displays some apomorphic features such as increased elevation of the orbits, high molar crowns and wide I/3 diameter (Boisserie 2005 and references therein). The genus *Saotherium* is latest Miocene in age and it is supposed to be the sister taxon of the extant pigmy-hippo *Choeropsis liberiensis* (Boisserie 2005).

Fig. 3 - Scatterplots between PC1 and PC2 (A) and between PC2 and PC3 (B) of the Hippopotamidae skulls in lateral view.



Results

Lateral view

The first 5 principal components of the PCA, performed on the skulls in lateral view, summarize 78.77% of total shape variance. Figure 3A shows the relationship between PC1 (29.84% of total shape variance) and PC2 (18.61% of total shape variance). Figure 3B shows the relationship between PC2 and PC3 (13.37% of total shape variance). Figure 1S shows the 3D plot with the relationship between PC1, PC2 and PC3.

Positive PC1 values are associated with a massive skull having a thick and posteriorly high zygomatic arch, elevated orbit with respect to the cranial roof, a concave profile of the cranial roof posterior to the orbit, and a foramen infraorbitalis placed distant from the anterior border of the orbit. This cranial shape corresponds to an *Hippopotamus amphibius*-like morphology. Negative PC1 values are associated with a shorter and less massive skull, with slender zygomatic arch, a rather flat cranial roof posterior to the orbit, and a lower orbit relative to the cranial roof. This shape can be observed in *Choeropsis liberiensis*.

At positive PC2 values the skull is massive, with a thick zygomatic arch, a convex and downward-directed dorsal profile of the neurocranial portion, and an elevated orbit, whereas at negative PC2 values the skull is somewhat slender, with a longer and upward-directed neurocranial portion, a less elevated orbit and slender zygomatic arch.

A skull that is less high, with a moderate elevation of the orbit, longer neurocranial portion, slender zygomatic arch, and anteriorly projected orbit is associated with positive PC3 values. The neurocranial portion of the skull is shorter and the zygomatic arch is antero-posteriorly compressed at negative PC3 values.

Variations along the three axes are shown in Figure 4.

Specimens with *Hippopotamus amphibius*-like shape are placed within the second and the third quarters of the PC1-PC2 morphospace (Fig. 3); *Archaeopotamus* and the Madagascan hippopotamids are placed within the fourth quarter and *C. liberiensis* within the first quarter.

In the UPGMA dendrogram of cranial shape similarities in lateral view (Fig. 5A), *H. amphibius* clusters with *Hex. palaeindicus* and *H. antiquus* with *H. gorgops*. The Madagascan hippopotamuses cluster together. The Miocene *A. harvardi* lies close to the Madagascan hippopotamuses. All these taxa are well separated from the extant *C. liberiensis*.

A PGLS regression between shape and size of skulls in lateral view is not significant (P = 0.073), nor is a standard linear regression (P = 0.07). Shape is phylogenetically structured in the skulls in lateral view (Fig. 3S) as revealed by the results of the physignal( ) function ($P < 0.005$). Using the phylosig( ) function, we found that size (CS) was not phylogenetically structured ($P = 1$).

A phylogenetic heat map, plotting the input tree and the first five PCs, for skulls in lateral view is shown in Figure 6. Similarity in PC values among species is expressed by similar color tones; the plot summarizes the variation of PC values for each species taking into account the phylogenetic tree.

Dorsal view

The first 5 principal components of the PCA,

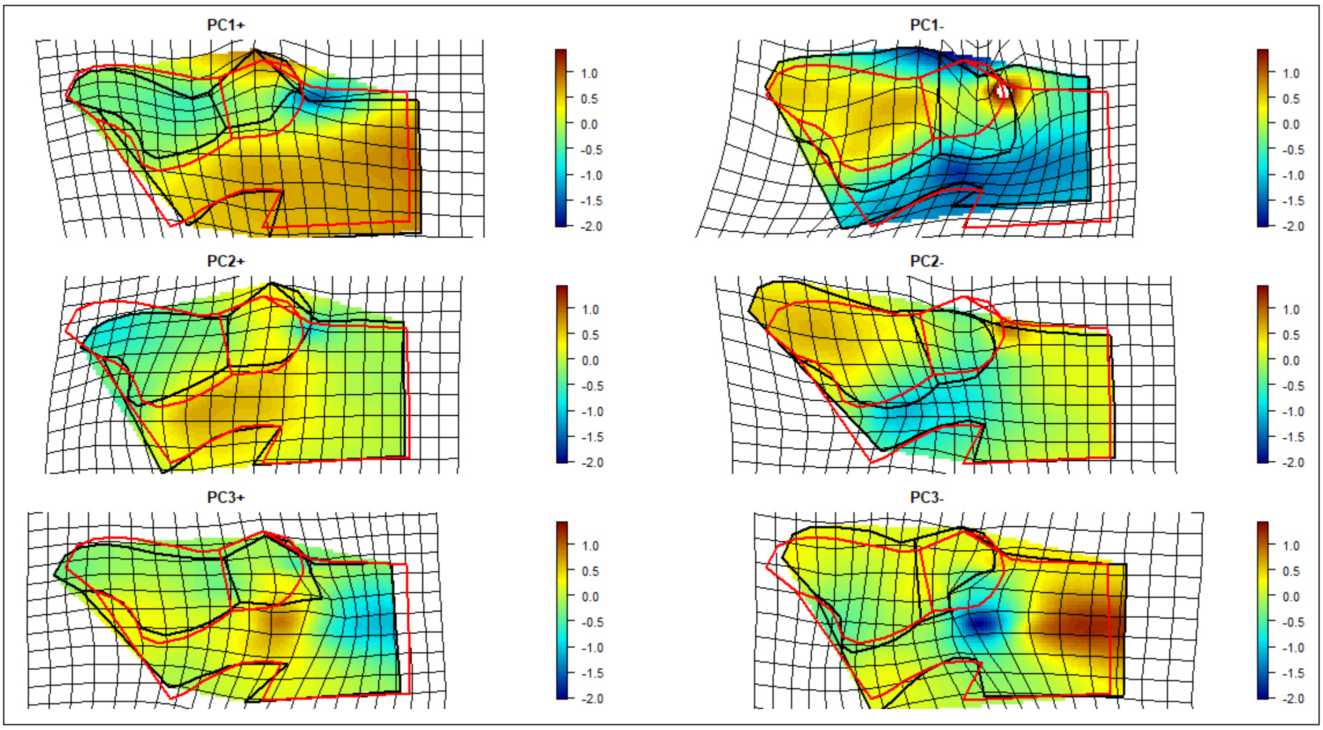


Fig. 4 - Deformation grids refer to the first three PC axis extremes (positive and negative) for Hippopotamidae skulls in lateral view.

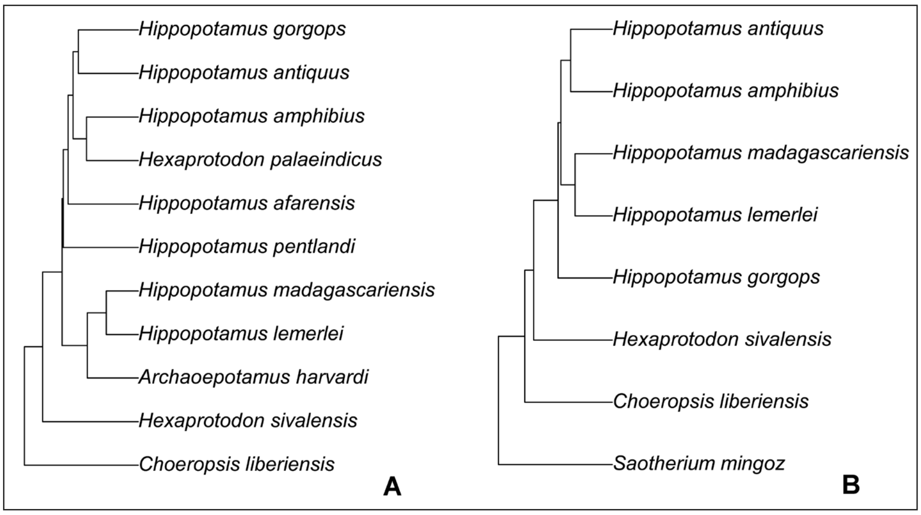


Fig. 5 - UPGMA dendograms for Hippopotamidae skulls in lateral (A) and dorsal (B) views.

performed on the skulls in dorsal view, summarize 82.31% of total shape variance. Figure 7A shows the relationship between PC1 (33.63% of total shape variance) and PC2 (20.50% of total shape variance). Figure 7B shows the relationship between PC2 and PC3 (12.83% of total shape variance). Figure 2S shows the 3D plot with the relationship between PC1, PC2 and PC3.

Positive PC1 values are associated with a rather narrow skull having a roughly tapered shape, with a zygomatic arch that is not particularly projected laterally relatively to the orbit; the latter is slightly oblique with respect to the antero-posterior direction. Negative PC1 values are associated with a wider and enlarged skull, with a zygomatic arch that is much more laterally projected with respect to the orbit. This shape corresponds to large-sized *H. amphibius*.

At positive PC2 values the zygomatic arch is slightly laterally projected, the orbit is anteriorly turned a few degrees, and the neurocranium is short, whereas at negative PC2 values the skull is longer, and the orbit is more anteriorly placed. At positive PC3 values, the skull is narrow, and the zygomatic

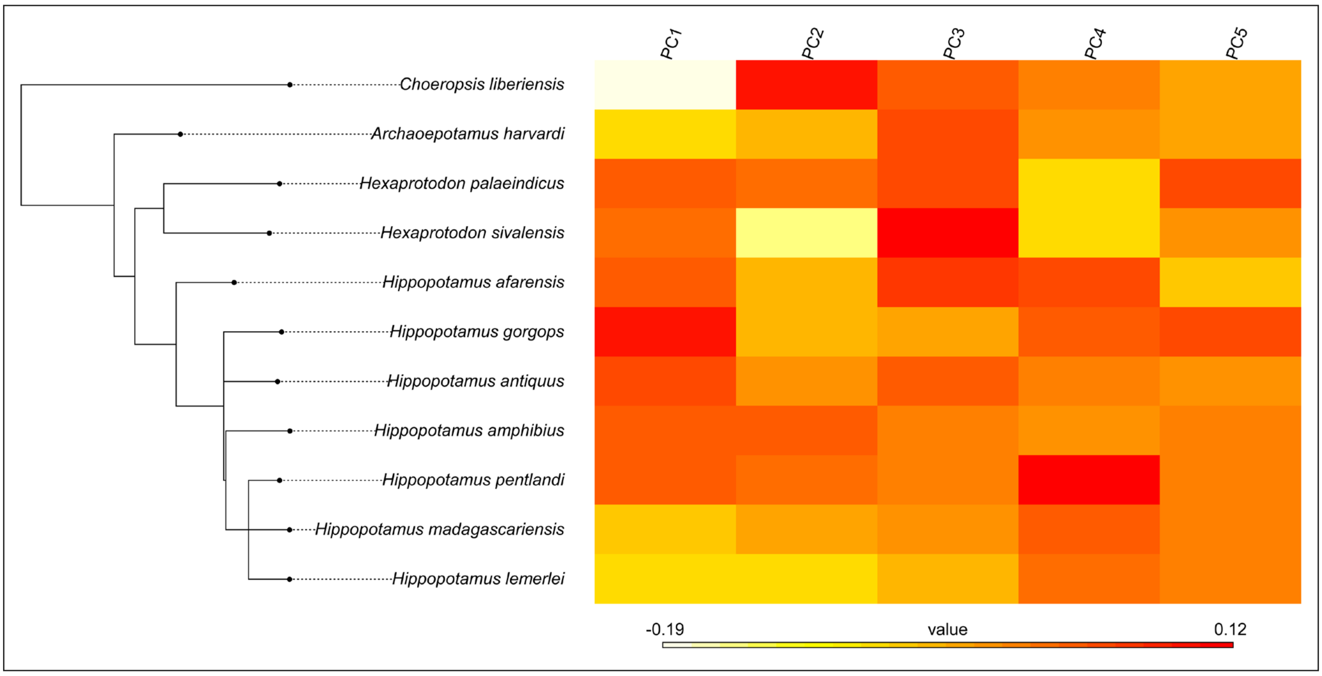
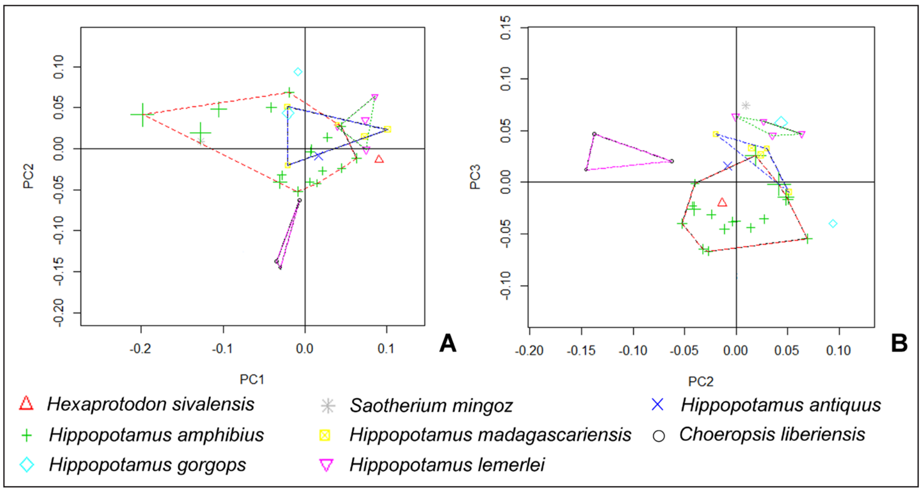


Fig. 6 - A phylogenetic heat map, plotting the input tree and the first five PCs, for Hippopotamidae cranial shape in lateral view.

Fig. 7 - Scatterplots between PC1 and PC2 (A) and between PC2 and PC3 (B) of Hippopotamidae skulls in dorsal view.



arch has a rather flat external profile. The skull is wider, with an enlarged zygomatic arch at negative PC3 values.

Variation along the three axes is shown in Figure 8.

Hippopotamus madagascariensis and *H. lemerlei* are plotted towards positive values of PC1 (Fig. 7); *C. liberiensis* is plotted at negative values of PC1 and PC2; *Saotherium mingoz* is plotted at negative PC1 and positive PC2 whereas *H. amphibius* occupies several quarters but with larger specimens placed in the first quarter. *Choeropsis liberiensis*, *H. lemerlei* and *H. madagascariensis*, and *S. mingoz* are plotted at positive values of PC3 but the first occupies very negative values of PC2 (Fig. 7).

In the UPGMA dendrogram of cranial shape similarities in dorsal view (Fig. 5B), *H. amphibius* clusters with *H. antiquus*. The Madagascan hippopotamuses cluster together. *H. gorgops* lies close to the previously mentioned two clusters. All these taxa are well separated from the extant *C. liberiensis* and from *S. mingoz*.

A PGLS regression between shape and size of skulls in dorsal view is not significant (P = 0.414), nor is a standard linear regression (P = 0.569). Shape is phylogenetically structured for skull

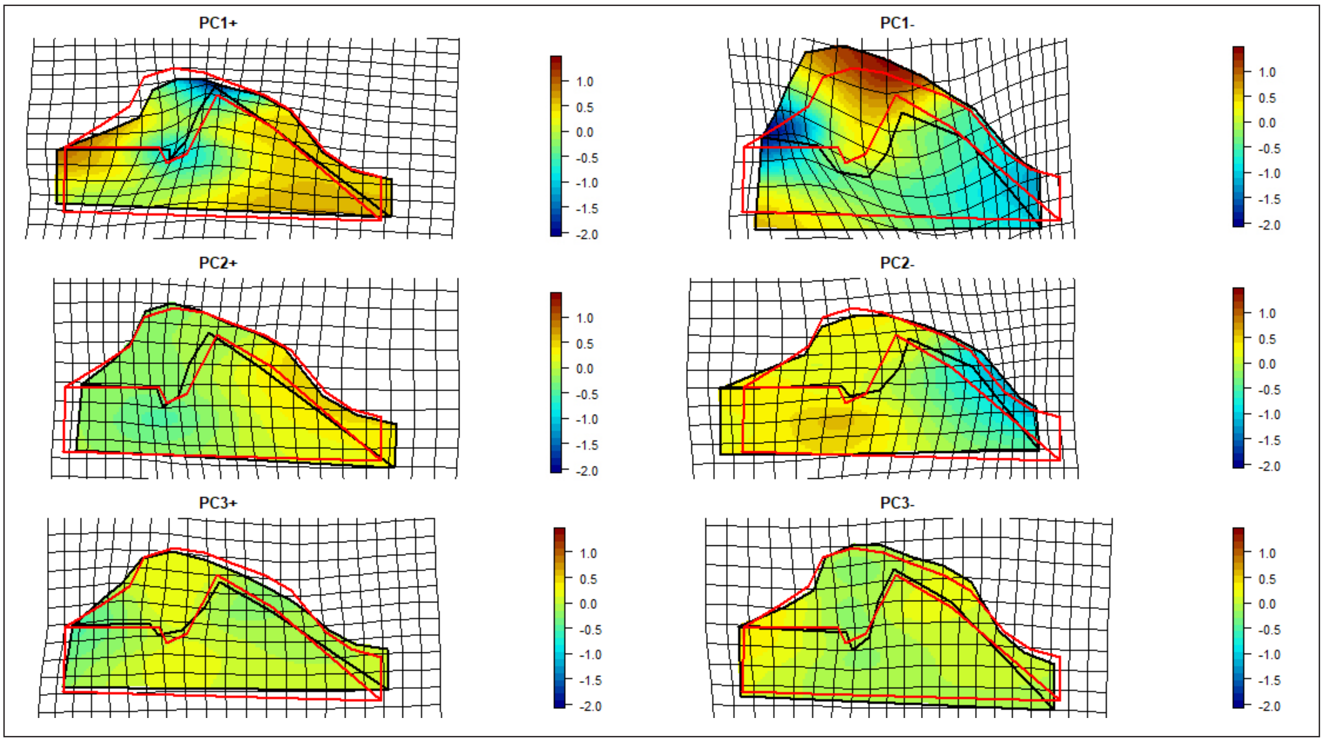


Fig. 8 - Deformation grids refer to the first three PC axis extremes (positive and negative) for Hippopotamidae skulls in dorsal view.

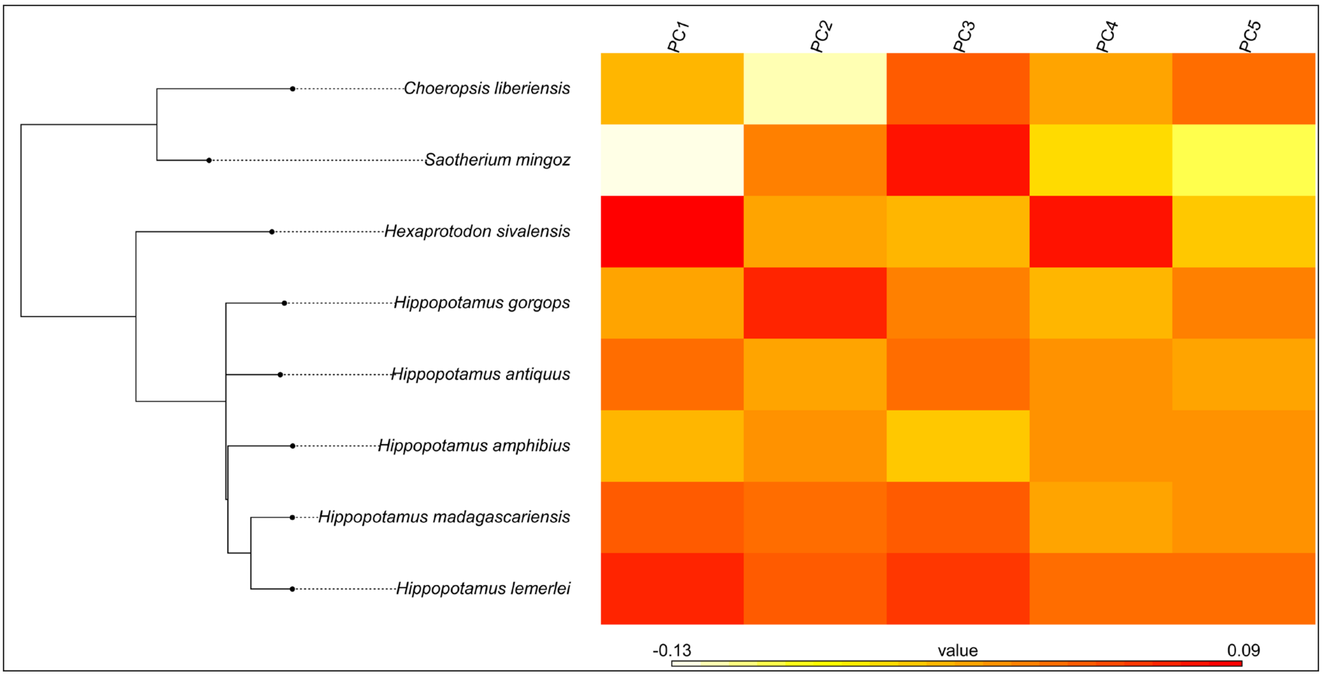


Fig. 9 - A phylogenetic heat map, plotting the input tree and the first five PCs, for the Hippopotamidae cranial shape in dorsal view.

shape in dorsal view (Fig. 4S) as revealed by the results of the physignal( ) function ($P = 0.0074$). Using the phylosig( ) function, we found that size (CS) was not phylogenetically structured ($P = 0.71$).

A phylogenetic heat map, plotting the input tree and the first five PCs, for skulls in dorsal view is shown in Figure 9. The similarity in PC values among species is expressed by similar color tones; the plot summarizes the variation of PC values for each species taking into account the phylogenetic tree.

DISCUSSION

The Miocene *Archaeopotamus*

One of the oldest known hippopotamid is the Miocene *A. harvardi (= Hexaprotodon harvardi* in Coryndon 1977*)*. This species was gracile with unelevated orbits (Coryndon 1977; Boisserie 2005). The main distinguishing features of the genus *Archaeopotamus* are canine processes poorly extended and a highly elongate mandibular symphysis (Boisserie 2005). In our study the species *A. harvardi* is represented by one specimen that displays negative values of PC1 and PC2 and positive values of PC3 in lateral view. These values are associated with a shorter and less massive skull, low orbits and a slender zygomatic arch. The latter features are also present in the Madagascan hippos.

Continental *Hippopotamus* and *Hexaprotodon*

Elevated orbits, a long facial region and a short postorbital part of the skull in Hippopotamidae are related to specialization for a semiaquatic lifestyle (Coryndon 1977). *Hippopotamus gorgops* and *H. antiquus* share these features, and probably were more aquatic than *H. amphibius* (Stuenes 1989). According to Mazza (1991) *H. antiquus* from Upper Valdarno is morphologically similar to *H. gorgops* of Olduvai Bed II. In our analysis, *H. gorgops* and *H. antiquus* display similar shapes, with negative values of PC2 and positive values of PC1. The similarity in shape of these two species is reinforced by the UPGMA analysis of skull shape acquired in lateral view.

The adaptation to a semiaquatic life style evolved independently in *Hippopotamus* and *Hexaprotodon* suggesting a convergence between the two lineages, as previously discussed in several studies (Boisserie 2005; Boisserie et al. 2007, 2011 and references therein).

In the present analysis, *Hex. palaeindicus* falls within the morphospace occupied by *H. amphibius*, suggesting similar shape of the skull. Six incisors have been used in the past as a distinguishing character in *Hexaprotodon* (Coryndon 1977) but this genus also differs from *Hippopotamus* by having a very high robust mandibular symphysis and canine processes that are not laterally extended (Boisserie 2005). In addition, *Hexaprotodon* is identified by low-crowned molars, while *Hippopotamus* has high-crowned ones (Weston 2000). *Hexaprotodon* is also characterized by slender and less massive postcranial remains, suggesting that it was less well adapted for walking on mud (Weston 2000). Boisserie (2005) recognized an evolutionary trend within the genus *Hexaprotodon* with increase in orbit elevation and increase in molar crown height.

Insular hippopotamuses

Insular hippopotamids are characterized by reduction in size with respect to their continental ancestors, by a general decrease of the height of the orbits (Caloi & Palombo 1994) and by brain size reduction (Weston & Lister 2009). According to van der Geer et al. (2018) the largest amount of morphological variation in dwarfed hippos is in muzzle shape, which becomes anteriorly low. This new arrangement of the anterior skull requires a restructuring of the dental battery that in some cases even includes the loss of a premolar (van der Geer et al. 2018). In addition, some works suggest that insular hippopotamids show an increasing trend toward terrestrialization (Boisserie et al. 2011). This hypothesis is based on three main morphological comparisons: limb, cranial and tooth morphology. Limbs become shorter, more erect and with restricted lateral movement (Houtemaker & Sondaar 1979). This new structure of the limbs is linked to a 'low gear locomotion' adaptation to the different rocky grounds of the Mediterranean islands conquered by the insular species (Sondaar 1977).

Hippopotamus pentlandi from Sicily and Malta shows a slight reduction in size with respect to its continental ancestor *H. amphibius* and more robust limb bones (Boisserie 2005). It is unclear if limb robustness can provide information on water dependence (Boisserie et al. 2011). *Hippopotamus pentlandi* displays a shorter muzzle, less developed nasal region and a wider diastema C/P2 relative to *H. antiquus* (Caloi & Palombo 1986). The shape of the Sicilian hippopotamus still resembles that of *H. amphibius* in lateral view and falls well within the morphospace occupied by the extant species, suggesting that its adaptation to the insular domain was not yet fully attained. Unfortunately, the shape analysis of *H. pentlandi* is not exhaustive; the remains attributed to this species are not well preserved and only one skull was well enough preserved to be included in our study.

The dwarfed hippos of Madagascar exhibit different degrees of muzzle shortening. *Hippopota-*

mus madagascariensis is characterized by a moderately short muzzle, whereas *H. lemerlei* does not exhibit shortening but has an anterior narrowing instead. The results obtained by van der Geer et al. (2018) that mainly focus on the differences in the anterior portion of the skull reinforce the idea that dwarfed hippos are not 'downscaled mainland hippos'.

Differences in cranio-dental morphology of the Madagascan species, such as the different orbit heights (Stuenes 1989), suggest that they occupied different ecological niches (Rakotovao et al. 2014), with *H. madagascariensis* being more terrestrial than *H. lemerlei*. *Hippopotamus madagascariensis* presents a different orbit orientation, with orbits more elevated than *H. lemerlei*, and it has smaller dimensions relative to *H. amphibius* (Rakotovao et al. 2014). The skull of *H. madagascariensis* is robust and characterized by a facial prolongation with a thin supraorbital margin, a short postorbital part, and orbits as high as they are wide (Stuenes 1989). Despite the adaptation to terrestriality, cranial shape in the Madagascan hippos seems not to be closely related to the cranial shape of *C. liberiensis*. These species can be found at negative values of PC1, but the morphospace occupied is different, supporting the distance between the hippos of Madagascar and *C. liberiensis*. The UPGMA analysis supports the closeness of *H. madascariensis* and *H. lemerlei*.

The skull morphology of the Malagasy hippos displays features typical of the genus *Hexaprotodon* (Stuenes 1989). These features are a double rooted first premolar and the tip-to-tip occlusion in *H. madascariensis,* while, regarding both species, the greatest breadth of the nasals falls within the variation typical of *Hexaprotodon* more than that of *Hippopotamus*. In our study the Madagascan species fall in the same morphospace as *Hippopotamus* at positive values of PC1 and negative values of PC2 in lateral view, and at negative values of PC2 and PC3 for dorsal view. The analyses here performed do not highlight similarities between the hippos from Madagascar and the genus *Hexaprotodon* (albeit the latter group is here represented by very few specimens).

The extant pygmy hippopotamus

It has been suggested that *C. liberiensis* is a dwarfed version of the common hippo (Gould 1975). It is tempting to link this hypothesis to the forest refugia hypothesis (Mayr & O'Hara 1986), stipulating major contractions of forest habitats into small areas during past episodes of aridity. *Choeropsis liberiensis* could thus have evolved an insular morphology in restricted forest patches. It displays some features common to island hippopotamid morphology, including reduced transverse movement of the front limb (Houtemaker & Sondaar 1979) and a somewhat lophodont cheek dentition, but these are not sufficient to support a dwarfing event. It has been clearly demonstrated that *C. liberiensis* is not a dwarfed *Hippopotamus* (Weston 2003a), but rather evolved as a separate lineage, distinct from all other hippopotamids, since the latest Miocene (Boisserie 2005), and its size could be a plesiomorphic trait, in contrast to Mediterranean dwarf species that evolved from larger relatives.

The genus names *Choeropsis* and *Hexaprotodon* appear interchangeably in the scientific literature, although Boisserie (2005) concluded that the combination of primitive and derived characteristics of the extant pygmy hippo place it in a distinct lineage, validating the genus *Choeropsis*, and restricting the genus *Hexaprotodon* to the fossil lineage mostly found in Asia. According to Weston (2000) the pygmy hippo was labelled as a 'living fossil' because it shares more traits with extinct ancestral clades than with *H. amphibius*. Most of its cranial traits are plesiomorphic, while the mandible and the dentition display some very derived features. The main plesiomorphic traits in *Choeropsis* are a weak extension of the canine processes, a slender zygomatic arch in ventral view, a lachrymal separated from the nasal by a long maxillary process of the frontal, and elongated and transversally rounded braincase (Boisserie 2005). In addition, the pigmy hippo shows a downturned sagittal crest, a feature that is generally regarded as a plesiomorphy, reinforcing the 'primitive aspect' of the Liberian hippos (Boisserie 2005). The theory of the 'different lineage' of Weston (2003) is supported also by the work of van der Geer et al. (2018): the mainland dwarf hippo *Choeropsis* is not a downscaled version of the species *Hippopotamus*. The cranial shape of the considered taxa revealed that *C. liberiensis* does not fit well within insular hippos derived from *H. amphibius* and it occurs in different areas of the morphospace.

The genus *Saotherium*

Another genus that shows a mosaic of characters is *Saotherium*. According to Boisserie (2003) the species *S. mingoz*, from the Mio-Pliocene boundary, combines features generally considered derived (e.g., a short premolar row) and primitive characters (e.g., low orbits and a weak development of canine processes). Another important feature of this species is the relative height of the skull above the molars. These characteristics are unique in hippopotamids and the introduction of the new genus *Saotherium*, replacing *Hexaprotodon,* is therefore justified (Boisserie 2003). Parsimony analysis of Hippopotamidae relates *Saotherium* with the genus *Choeropsis* (Boisserie 2005). The Liberian hippos lack the cranial structure typical of *Saotherium* and therefore these two species share features that are mainly plesiomorphic or convergent (Boisserie 2005).

Boisserie (2007) gave a new interpretation of the mosaic features of *Choeropsis*, this species presents physiological adaptations to semiaquatic environments and some of the archaic features of its skull could therefore be interpreted as a secondary adaptation to the shaded rainforest. This adaptation would explain the low position of the orbits and the sagittal crest on the cranium of *Choeropsis*, which could be useful for a better penetration of the dense rainforest vegetation. This feature is also present in *Saotherium* (Boisserie 2005) and it could be interpreted as a link between these two species. This hypothesis may be reinforced by the UPGMA analysis on dorsal view data, where these two species are closely related. However, the relationships between *Choeropsis* and *Saotherium* is still difficult to understand.

Conclusions

The present paper represents a contribution towards the understanding of the variation in skull shape in Hippopotamidae by means of shape analysis (Geometric Morphometrics) and modern statistical approaches. Two-dimensional modern shape analysis applied to dorsal and lateral views of extant and extinct Hippopotamidae species highlighted several points that will be further investigated in future works.

The skull shape of the pigmy hippo doesn't match those of insular hippopotamuses; the different specimens are plotted in different areas of the morphospace. *Choeropsis liberiensis* cannot be considered as a model for insular fossil species and the appearance of terrestrial characters within *Hippopotamus* evolved independently following a different morphological pattern that needs to be further investigated (Fig. 10). Similarities between *Hexaprotodon* and *Hippopotamus* have been detected but need further investigation, as well as the similarities between *H. gorgops* and *H. antiquus*. Despite the morphological convergences within the group, cranial shape (for the chosen configurations) in Hippopotamidae is phylogenetically structured while this does not hold for size. Small-sized hippos occur within different lineages but are related to different cranial shapes.

Fig. 10 - Head life appearance comparing *Choeropsis liberiensis* and *Hippopotamus amphibius* as reconstructed by Edoardo Previato (Msc student in Natural Sciences, Sapienza University of Rome).

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