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Maëva Orliac, Mickaël Mourlam, Jean-Renaud Boisserie, Loïc Costeur, Fabrice Lihoreau. Evolution of semiaquatic habits in hippos and their extinct relatives: insights from the ear region. *Zoological Journal of the Linnean Society*, 2023, 198 (4), pp.1092-1105. 10.1093/zoolinnean/zlac112. hal-04080611

HAL Id: hal-04080611

<https://hal.science/hal-04080611>

Submitted on 7 Nov 2023

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Evolution of semiaquatic habits in hippos and their extinct relatives: insights from the ear region

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ABSTRACT - Since molecular data identified hippopotamids as the closest living relatives of cetaceans, a common aquatic/semiaquatic ancestor hypothesis for these modern taxa has naturally been proposed. However, recent molecular studies concluded that most molecular adaptations in extant cetaceans occurred after their split from hippopotamids. If the question of aquatic affinities of the first cetaceans has been investigated at large, it has not been the case for the forebears of hippopotamids. Sensory organs are drastically affected by underwater perception. In this work, we question the aquatic affinities of fossil hippopotamoids through an investigation of the morphology and morphometrics of the petrosal bone and cochlea of 12 extinct hippopotamoid taxa. Petrosal and bony labyrinth morphological characters constitute a source of structured phylogenetic signal, both supporting major hippopotamoid clades and bringing original relationships. The morphometric study of functional measurements of the cochlear canal shows that anthracotheres bridge the morphological gap between terrestrial artiodactyls and modern hippopotamids. The integrated functional signal of the ear region further supports convergent acquisitions of semiaquatic behaviour in hippopotamids and cetaceans and indicates that terrestrial hearing was an ancestral trait among Hippopotamoidea. We highlight multiple convergent acquisitions of semiaquatic traits in the ear region of hippopotamoids and new robust phylogenetic characters.

KEYWORDS: amphibiosis – artiodactyls – cochlea – Hippopotamidae – petrosal.

INTRODUCTION

Since molecular data identified hippopotamids as the closest living relatives of cetaceans (e.g. Miyamoto & Goodman, 1986; Irwin *et al.*, 1991; Montgelard *et al.*, 1997), anatomists have been looking for palaeontological evidence to fill the morphological gap between these two highly divergent taxa. Indeed, extant hippopotamids and cetaceans have little in common, except for some characters related to their strong relationship, albeit different, with the aquatic environment. They share striking behavioural and morphological similarities, such as underwater suckling (Vevers, 1926), a loss or drastic diminution of sebaceous glands (Springer & Gatesy, 2018), underwater communication by bubble blasts (Barklow, 2004a, b) and a special orientation of the vocal folds (Reidenberg, 2017). Both also present morphological similarities

of the ear region related to the perception of underwater sounds (Luo & Gingerich, 1999; O’Leary, 2010; O’Leary *et al.*, 2012). A common aquatic/semiaquatic ancestor hypothesis for Cetancodonta (the group gathering Hippopotamidae and Cetacea; Árnason *et al.*, 2000) has naturally been proposed and is recurrent in the literature (e.g. Gatesy *et al.*, 1996, 2013; Gatesy & O’Leary, 2001; Thewissen *et al.*, 2007; Geisler & Theodor, 2009; Cooper *et al.*, 2016). However, recent molecular studies concluded that most molecular adaptations in extant cetaceans occurred after their split with hippopotamids (Tsagkogeorga *et al.*, 2015; Springer & Gatesy, 2018), altering the common aquatic/semiaquatic hypothesis. If the question of the aquatic affinities of the first cetaceans has been investigated at large based on palaeontological evidence (e.g. Geisler & Luo, 1998; Luo & Gingerich, 1999; Gatesy & O’Leary, 2001; Thewissen *et al.*, 2001; Spoor *et al.*, 2002; Nummela *et al.*, 2004; Mourlam & Orliac, 2017), it has not been the case for the forebears of hippopotamids. Recent phylogenetic studies including a large sample of extant and extinct hippopotamids (e.g. Boisserie *et al.*, 2010; Orliac *et al.*, 2010; Lihoreau *et al.*, 2015, 2017) demonstrated that Hippopotamidae were nested in the paraphyletic ‘Anthracotheriidae’ (forming the Hippopotamoidea *sensu* Gentry & Hooker, 1988; for alternative hypothesis see Spaulding *et al.*, 2009). The ancestral ecological behaviour of hippopotamoids has therefore to be searched for among anthracotheres, which have an evolutionary history dating back to the Middle Eocene, around 40 Mya (Lihoreau & Ducrocq, 2007). Interestingly, some anthracotheriids have been interpreted as amphibious animals, first because of their frequent presence in lignite and lacustrine deposits (Cuvier, 1822; Pickford, 1983, 1991), but also based on isotopic analyses (e.g. $\delta^{18}O$; Nelson, 2007; Clementz *et al.*, 2008), on skeletal morphology [e.g. *Arretotherium* Douglass, 1901 (Kron & Manning, 1998); *Brachyodus* Depéret, 1895 (Orliac *et al.*, 2013); *Elomeryx* Marsh, 1894 (Geais, 1934); *Libycosaurus* Bonnarelli, 1947 (Lihoreau *et al.*, 2006, 2014); *Merycopotamus* Falconer & Cautley, 1847 (Colbert, 1935, Lihoreau *et al.*, 2007)] or on long bone microanatomy (Houssaye *et al.*, 2021), suggesting that the evolutionary history of hippopotamoids is intimately linked to the aquatic environment. Specifying the lifestyle of anthracotheres is crucial to reconstruct the evolutionary history of amphibiosis in hippopotamoids and to determine if they were primitively semiaquatic, a necessary step to identify whether or not hippopotamids and cetaceans inherited their underwater skills from their common ancestor. Among morphological features, the sensory organs are drastically affected by an adaptation to underwater perception. The middle and inner ear are of particular interest to discuss the palaeobiology of taxa as they are responsible for hearing (transmission and integration of sound via the middle ear and the cochlea; Fleischer, 1978; Luo *et al.*, 2011) and involved in coordinating head posture and body movements during locomotion (vestibulo-ocular reflex via the vestibular system; e.g. Gray, 1918; David *et al.*, 2016). Here, we question the aquatic affinities of fossil hippopotamoids through an investigation of the auditory region morphology, including both the petrosal bone and the bony labyrinth, of 12 extinct hippopotamoid taxa and of the two living hippos. We investigate the phylogenetic and ecological signals carried by this morphological complex. This sets the bases for discussing the history of amphibiousness in Hippopotamoidea and, at a wider scale, at the level of Cetancodonta (Cetacea + Hippopotamoidea).

MATERIAL AND METHODS

Dataset of hippopotamoid species

The morphology of the petrosal bone and bony labyrinth was investigated using μ -CT scan data for a sample of 14 hippopotamoid taxa including the extant hippopotamine *Choeropsis liberiensis* (Morton, 1844) and *Hippopotamus amphibius* Linnaeus, 1758, as well as the recently extinct *Hippopotamus madagascariensis* Guldberg, 1883, along with 11

nonhippopotamine hippopotamoids: *Arretotherium* sp., *Bothriodon velaunus* von Meyer, 1832, *Bothriogenys* cf. *gorringei* (Andrews & Beadnell, 1902), *Brachyodus onoideus* (Gervais, 1859), *Elomeryx armatus* (Marsh, 1894), *Elomeryx borbonicus* Geais, 1934, *Heptacodon curtus* (Marsh, 1894), *Libycosaurus bahri* Lihoreau *et al.*, 2014, *Merycopotamus nanus* Falconer, 1868, *Microbunodon minimum* (Cuvier, 1822) and cf. *Sivameryx palaeindicus* (Lydekker, 1877). When possible, more than one specimen was considered in this study. A specimen list and associated information as well as details regarding μ -CT scanner facilities and scanning acquisition parameters are summarized in Supporting Information, Appendix S1 (Tables S1–S2). Segmentation, linear and volumetric measurements of specimens were performed using Avizo v.9.3 (ThermoFisher Scientific Inc.) and visualization was carried out using MorphoDig v.1.5.3 (Lebrun, 2018).

Phylogenetic analyses and ancestral character states

The phylogenetic signal carried by the external morphology of the petrosal bone and by the bony labyrinth was investigated through a phylogenetic analysis based on an original data matrix including 34 morphological characters coded for 26 taxa comprising 14 Hippopotamoidea and 12 non-cetancodont taxa [Supporting Information, Appendix S1 (Tables S1–S2, character list)]. The data matrix comprises only 2.3% of missing data; it is provided in nexus format in Supporting Information, Appendix S2. The parsimony analysis was carried out with the software PAUP v.4.0a (built 162) through an exact Branch and Bound Search, using the furthest option. More details about outgroups, characters treatment and illustration of character states are available in Supporting Information, Appendix S1 (Figs S1–S3). Ancestral values for tegmen tympani relative volume have been estimated with the squared-change parsimony method (Maddison, 1991) using the R package ‘ape’ (Paradis *et al.*, 2004) and the function ‘anc.ML’ of the R package ‘phytools’ (Revell, 2012), with all branch lengths set to 1 and assuming a Brownian motion model (Schluter *et al.*, 1997; see also Martins, 1999; Paradis, 2006). Precisions on relative tegmen tympani volume calculation and ancestral states values are provided in Supporting Information, Appendix S1 (Figs S5–S6) and Supporting Information, Appendix S4 (Script 01, Dataset 01).

Functional analyses of the cochlea

In order to assess the ecological diversity in Hippopotamoidea, we analysed the functional parameters of the cochlea of hippopotamoids compared to other artiodactyl taxa. We performed a principal component analysis (PCA; Spearman type) on nine cochlear measurements related to hearing physiology following the method described in Mourlam & Orliac (2017; see also Fleischer, 1976; Ekdale & Racicot, 2015; Churchill *et al.*, 2016). For this analysis, the dataset is composed of a total of 75 taxa including 12 hippopotamoids, 52 cetaceans and 11 ‘non-cetancodont’ terrestrial artiodactyls. The dataset and more specimen details are provided in Supporting Information, Appendices S3 and S4 (Dataset 02). The Mahalanobis distance (Mahalanobis, 1936) was calculated on the five first PCs. To quantify the differences between groups, enlightened by the PCA, a Wilcoxon–Mann–Whitney test was performed. All analyses have been performed with R v.3.4.2 (R Core Development Team, 2015). Missing data were estimated using the R package ‘missMDA’ (Josse & Husson, 2016). The script for the cochlear analysis is also available in Supporting Information, Appendix S4 (Script 02).

Semicircular canal shape variation

We also quantified semicircular canal shape variation through a three-dimensional (3D) semilandmark approach based on the protocols of Gunz *et al.* (2012; see also Grohé *et al.*, 2016; Perier *et al.*, 2016; Dickson *et al.*, 2017; Schweizer *et al.*, 2017). The sample is composed of 12 hippopotamoids (including ten fossils), four tylopods (including one fossil), seven suoids (including three fossils), seven ruminants (including one fossil) and two extinct cetaceans (protocetids). We digitized 3D Bezier curves on each semicircular canal and on the common crus [Supporting Information, Appendix S1 (Fig. S7)] using MorphoDig. We then exported each curve as a set of 20 equidistant landmarks [landmark coordinates dataset provided in Supporting Information, Appendix S4 (Dataset 03)]; the first and the last landmark as type I landmarks, others in-between as semilandmarks; Bookstein, 1992, 1996; Green *et al.*, 1996; MacLeod, 1999; Adams *et al.*, 2004; Gunz *et al.*, 2005; Gunz & Mitteroecker, 2013). A General Procrustes Analysis (GPA; Gower, 1975; Rohlf & Slice, 1990) was performed using the R package ‘geomorph’ (Adams & Otárola-Castillo, 2013). During the Procrustes superimposition, the semilandmarks were allowed to slide along the curves to minimize the Procrustes distance (Perez *et al.*, 2006). This Procrustes alignment has been carried out on the whole vestibular dataset and on four different subsets: the lateral, anterior and posterior semicircular canals, and the common crus. Procrustes coordinates were used to perform a PCA (Pearson type). To quantify the morphological disparity of the different groups, the surface (on the first factorial plane; PC1 and PC2) and volume (on the first factorial space; PC1, PC2 and PC3) in the convex hull (Eddy, 1977) of each group was calculated (Euclidean geometry; using R code from Logez & Terraz, 2015 for the surface and the R package ‘geometry’ of Habel *et al.*, 2015 for the volume) and compared to the surface and volume of the morphospace of the full dataset. We also calculated the Procrustes variance (Zelditch *et al.*, 2004) and performed pairwise comparisons using the R package ‘geomorph’ (Adams & Otárola-Castillo, 2013) to estimate the differences between groups. The script for the vestibular system analysis is available in Supporting Information, Appendix S4 (Script 03). Unfortunately, the promontorium of fossil hippopotamoid taxa, and concurrently the apex of the cochlear canal, is nearly systematically broken, which prevented us performing an accurate landmark-based geometric morphometric analysis on the cochlea.

RESULTS

Phylogenetic signal of the ear region

The phylogenetic signal carried by the ear region of hippopotamoids is congruent with major points of their inferred evolutionary history as supported by dental and cranial characters (e.g. Boisserie *et al.*, 2010; Orliac *et al.*, 2010; Alloing-Séguier *et al.*, 2014; Lihoreau *et al.*, 2015; Scherler *et al.*, 2018). The strict consensus [Fig. 1A; Supporting Information, Appendix S1 (Fig. S4)] of the two most-parsimonious trees [$L = 87$; consistency index (CI) = 0.42; retention index (RI) = 0.78] retrieved by the cladistic analysis indeed supports a deep nesting of Hippopotaminae in the paraphyletic anthracotheres. Despite of an extraordinary morphological diversity (Fig. 1A), the petrosals of hippopotamoids all share three unambiguous synapomorphies: an inflation of the mastoid/tegmen tympani at the external acoustic meatus (21; RI = 1.00), a large ventrolateral tuberosity [131; RI = 1.00; Supporting Information, Appendix S1 (Fig. S1E)], and a fenestra cochleae opening directed toward the external acoustic meatus [261; RI = 1.00; Supporting Information, Appendix S1 (Fig. S3D)]. In agreement with other analyses (e.g. Lihoreau & Ducrocq, 2007; Boisserie *et al.*, 2010; Orliac *et al.*, 2010; Lihoreau *et al.*, 2015), *Heptacodon curtus* (Anthracotheriinae) and *Microbunodon*

minimum (Microbunodontinae) appear as first offshoots of Hippopotamoidea and show the most primitive morphology of the petrosal. The phylogenetic signal carried by the ear region of hippopotamoids also supports the monophyly of major clades such as Hippopotaminae and Merycopotamini (e.g. Boisserie *et al.*, 2010, 2017b; Lihoreau *et al.*, 2017) and provides further support for the monophyly of the bothriodontines (see Lihoreau & Ducrocq, 2007), and to the inclusion of Hippopotamidae (*sensu* Orliac *et al.*, 2010) in this clade, in agreement with most of previous craniodental (Boisserie *et al.*, 2005, 2010, 2017a; Orliac *et al.*, 2010; Lihoreau *et al.*, 2015, 2019) or enamel microstructure analyses (Alloing-Séguier *et al.*, 2014; Lihoreau *et al.*, 2017). Merycopotamini are unambiguously supported by the presence of a blunt ventrolateral tuberosity [140; RI = 0.33; Supporting Information, Appendix S1 (Fig. S1E)]; in this clade, *Libycosaurus bahri* and *Merycopotamus nanus* are united by three non-ambiguous synapomorphies: a wide internal acoustic meatus [102; RI = 0.72; Supporting Information, Appendix S1 (Fig. S1F)] with a hypertrophied foramen acusticum superius [121; RI = 1.00; Supporting Information, Appendix S1 (Fig. S1F)], and a caudal tympanic process separated from the promontorium by a groove [251; RI = 0.5; Supporting Information, Appendix S1 (Fig. S3C)]. A clade gathering *Bothriogenys cf. gorringei* and Hippopotaminae is unambiguously supported by the presence of a supra hiatal fossa (= prefacial commissure fossa of O’Leary, 2010) (51; RI = 1.00), an intercalation of the ventrolateral tuberosity between the external meatal tube and the squamosal [151; RI = 1.00; Supporting Information, Appendix S1 (Fig. S2)], and by a weak extension of the secondary bony lamina on the basal turn (less than 20%) (321; RI = 0.25). Hippopotaminae share the presence of a groove on the tegmen tympani [41; RI = 0.66; Supporting Information, Appendix S1 (Fig. S1B)], the presence of a subarcuate fossa [81; RI = 0.88; Supporting Information, Appendix S1 (Fig. S1F)] and an enlarged common crus on the bony labyrinth [341; RI = 1.00; Supporting Information, Appendix S1 (Fig. S3H)].

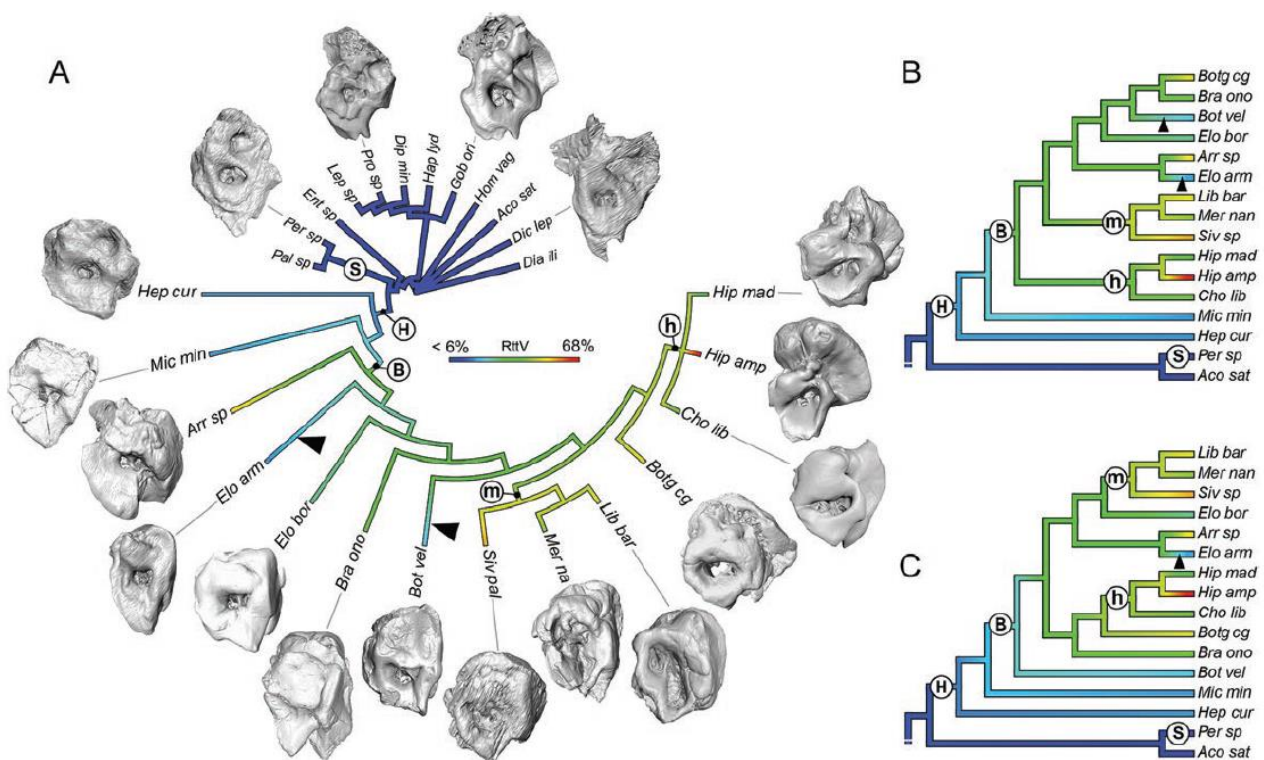


Figure 1. Results of the phylogenetic analysis based on petrosal and bony labyrinth characters (A) and ancestral character state reconstructions (B, C) mapped on the phylogenetic hypotheses of Lihoreau *et al.*'s (2015) parsimony analysis (B) and maximum likelihood analysis (C). Colours refer to the relative volume of the tegmen tympani (RttV). Abbreviations: B, Bothriodontinae;

H, Hippopotamoidea; h, Hippopotamidae; m, Merycopotamini; S, Suoidea. Black arrowheads indicate reversions to a smaller RttV. The data matrix is available in Supporting Information, Appendix S2.

On the other hand, some scenarios proposed in the literature, such as the origin of *Arretotherium* from an American *Elomeryx* stock (Scott, 1940; Macdonald, 1956; Kron & Manning, 1998) or the origin of Merycopotamini from an Eurasian *Elomeryx* stock (Lihoreau & Ducrocq, 2007; Rincón *et al.*, 2013; Böhme *et al.*, 2014), are not supported by the analysis of the petrosal characters alone. The genus *Brachyodus* (here *Brachyodus onoideus*), proposed to have originated from African *Bothriogenys*, based on shared dental characteristics with the derived *Bothriogenys andrewsi* Schmidt, 1913 (Dineur, 1982; Lihoreau & Ducrocq, 2007; Holroyd *et al.*, 2010; Lihoreau *et al.*, 2015), is here retrieved in a more basal position, sister taxon to the wide clade gathering *Bothriodon velaunus*, Merycopotamini + (Hippopotamidae, *Bothriogenys*). Finally, *Bothriodon velaunus* from the Early Oligocene of western Europe, considered as a basal representative of the Laurasian Bothriodontinae (Lihoreau & Ducrocq, 2007), appears here as a sister taxon to a clade gathering (Hippopotamidae, *Bothriogenys* cf. *gorringei*) + Merycopotamini, a position that has never been proposed in previous hypotheses. These results echo the difficulty to clarify the early radiation of bothriodontines, notably due to the scarcity of the fossil record for the Late Eocene representatives of this clade (Lihoreau *et al.*, 2017).

Ancestral character state reconstruction of the relative volume of the tegmen tympani

A ‘pachyostotic and osteosclerotic’ petrosal was hypothesized to play a substantial role in the origin of directional underwater hearing in cetaceans (Luo & Gingerich, 1999). O’Leary *et al.* (2012) subsequently showed that aquatic and semiaquatic artiodactyls, including hippopotamids and extinct cetaceans indeed had pachyostotic (i.e. hyperinflated) tegmen tympani and that tegmen tympani inflation could be an indicator of adaptation to a semiaquatic lifestyle. Indeed, compared to terrestrial artiodactyls, common hippopotamuses show a hypertrophy of the tegmen tympani of the petrosal bone (O’Leary, 2010; O’Leary *et al.*, 2012; Orliac *et al.*, 2014; this work), a character considered to be a hallmark of underwater adaptation related to directional hearing in the aquatic environment (Luo & Gingerich, 1999). Ancestral character states reconstruction of the relative tegmen tympani volume shows that, regardless of the phylogenetic hypothesis chosen (Fig. 1A-C), the early diverging hippopotamoids, *Heptacodon curtus* (Anthracotheriinae) and *Microbunodon minimum* (Microbunodontinae), present a weak inflation of the tegmen tympani. Yet, these early hippopotamoid representatives show a relative proportion of the tegmen tympani superior to that of other artiodactyls of our sample (i.e. around 20% vs. < 10%). A general trend toward inflation of the tegmen tympani is observed in the rest of the hippopotamoid clade [internal node reconstruction value between 30–40%, Fig. 1; Supporting Information, Appendix S1 (Fig. S6)] with, independently of the phylogenetic assumption chosen, both convergent acquisitions of high degree of inflation in various taxa (around 50% of total volume in *Arretotherium* sp., *Bothriogenys* cf. *gorringei*, *Brachyodus onoideus*, *Hippopotamus amphibius* and cf. *Sivameryx palaeindicus*) and reversions to a smaller, less inflated tegmen tympani in others (around 20% or below, in *Elomeryx armatus* and, depending of the chosen hypothesis, in *Bothriodon velaunus*), suggesting less semiaquatic habits.

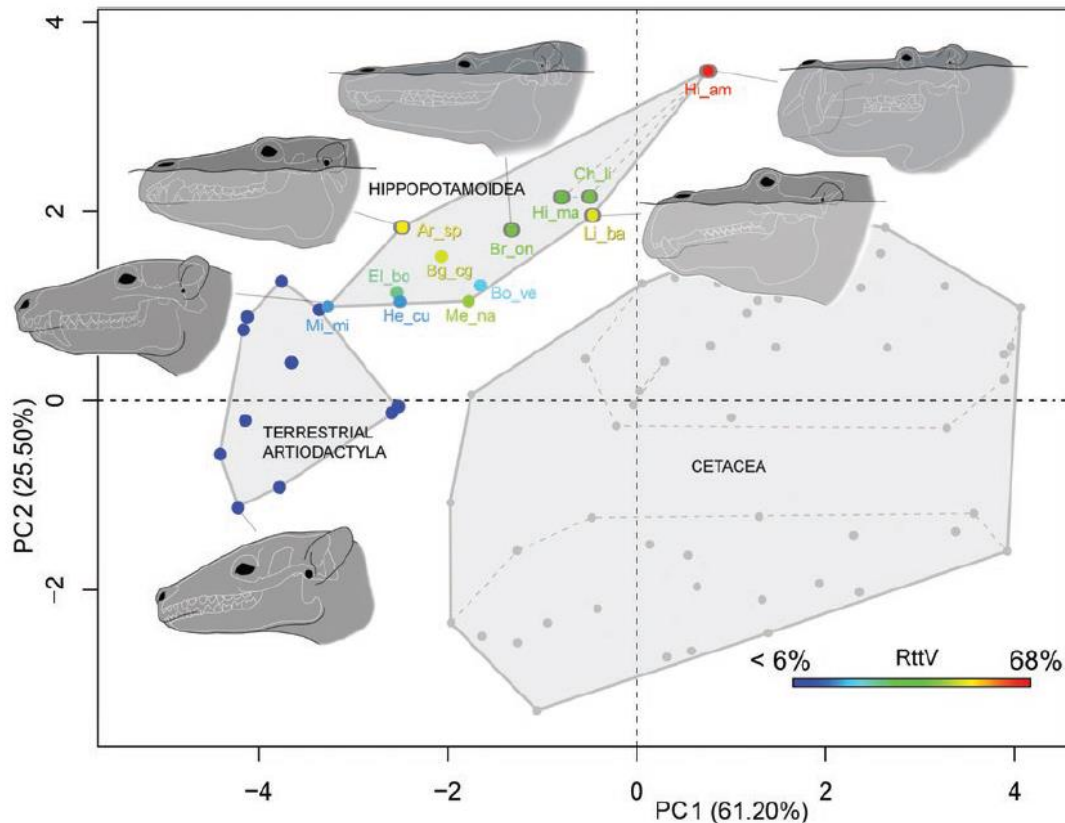


Figure 2. PCA of nine parameters of the cochlea in artiodactyls. The dataset has been compiled based on the PCA of Mourlam & Orliac (2017) and Churchill *et al.* (2016) augmented by the hippopotamoid sample of this study. Circled taxa represent hippopotamoids with an amphibious disposition of the head orifices (i.e. nostrils, eyes and ears in elevated position). Colour range for non-cetacean taxa refers to the relative volume of the tegmen tympani [Supporting Information, Appendix S1 (Table S3)]. Datasets and scripts available in Supporting Information, Appendix S4.

Morphometric parameters of the cochlea

The projection of the individuals on the first two PCs [86.70% of the variance; see also Supporting Information, Appendix S1 (Tables S4–S5)] results in three non-overlapping groups corresponding to Hippopotamoidea, Cetacea and terrestrial Artiodactyla (Fig. 2). Within the hippopotamoid morphospace, Hippopotamidae and anthracotheres occupy distinct morphospaces, with anthracotheres bridging the gap between hippopotamids and non-cetancodont terrestrial artiodactyls. On PC1 (61.20% of the variance), the two main positive contributions come from the shorter diameter of the basal turn, perpendicular to the cochlear width [W2; contribution (ctr) = 16.84%] and the cochlear width (Cw; ctr = 15.62%), whereas the number of turns (#T; ctr = 2.60%) has a negative contribution on that axis. Compared with non-cetancodont terrestrial artiodactyls, aquatic and semiaquatic extant taxa possess more positive scores on PC1. PC2 (25.50% of the variance) mostly isolates high and low frequency specialists. The principal positive drivers of variation along this axis are #T (ctr = 31.70%), cochlear canal length (Cl; ctr = 11.46%), cochlear height (Ch; ctr = 9.86%) and the area of the fenestra cochlearis (FC; ctr = 9.12%), whereas the variables interturn distance (ITD; ctr = 13.56%), maximal diameter of the spiral ganglion canal (GAN; ctr = 12.74%) and length of the secondary bony lamina (SBL; ctr = 8.78%) have a negative contribution. Interestingly, the centroid of the anthracotheres is closer to that of the noncetancodont terrestrial artiodactyls [Mahalanobis distance (Md) = 3.25; see Supporting Information, Appendix S1 (Table S6)] than to that of Cetacea (Md = 4.88), but it is even further apart from the centroid of the hippopotamid

(Md = 6.5), dragged upward by the extreme values of *Hippopotamus amphibius*. However, when considered separately, the locations of *Bothriogenys* cf. *gorringei*, *Brachyodus onoideus* and *Libycosaurus bahri* in the general morphospace are closer to the hippopotamid centroid (Md = 3.01; Md = 2.38; Md = 19.75, respectively) than to that of terrestrial artiodactyls (Md = 3.53; Md = 4.42; Md = 22.12, respectively).

Hippopotamoidea significantly differs from the noncetancodont terrestrial artiodactyls [see Supporting Information, Appendix S1 (Table S7)] by having a greater CI ($W = 131$; $P < 0.001$), Cw ($W = 119$; $P < 0.001$), Ch ($W = 132$; $P < 0.001^*$; * estimated P -value due to *ex aequo*), W2 ($W = 123$; $P < 0.001$) and FC ($W = 101$; $P = 0.016$). Within hippopotamoids, Hippopotamidae have a greater CI ($W = 2$; $P = 0.018$), W2 ($W = 2$; $P = 0.018$), ITD ($W = 1$; $P = 0.013$) and FC ($W = 0$; $P = 0.005$). Hippopotamoid taxa with an amphibious disposition of sensory organs [i.e. *Arretotherium* sp., *Brachyodus onoideus* (see Orliac *et al.*, 2013), *Libycosaurus bahri* (see Lihoreau *et al.*, 2014), *Hi. madagascariensis* (see Rakotovoao *et al.*, 2014) and the two extant species] have greater values on PC2. Their morphospaces do not overlap with that of other anthracotheres and differ significantly from the latter [Supporting Information, Appendix S1 (Table S7)] in having greater CI ($W = 34$; $P = 0.004$), Cw ($W = 32$; $P = 0.013$), ITD ($W = 32$; $P = 0.015$) and Ch ($W = 31$; $P = 0.021$). Taxa with bigger tegmen tympani also tend to have higher values on PC2.

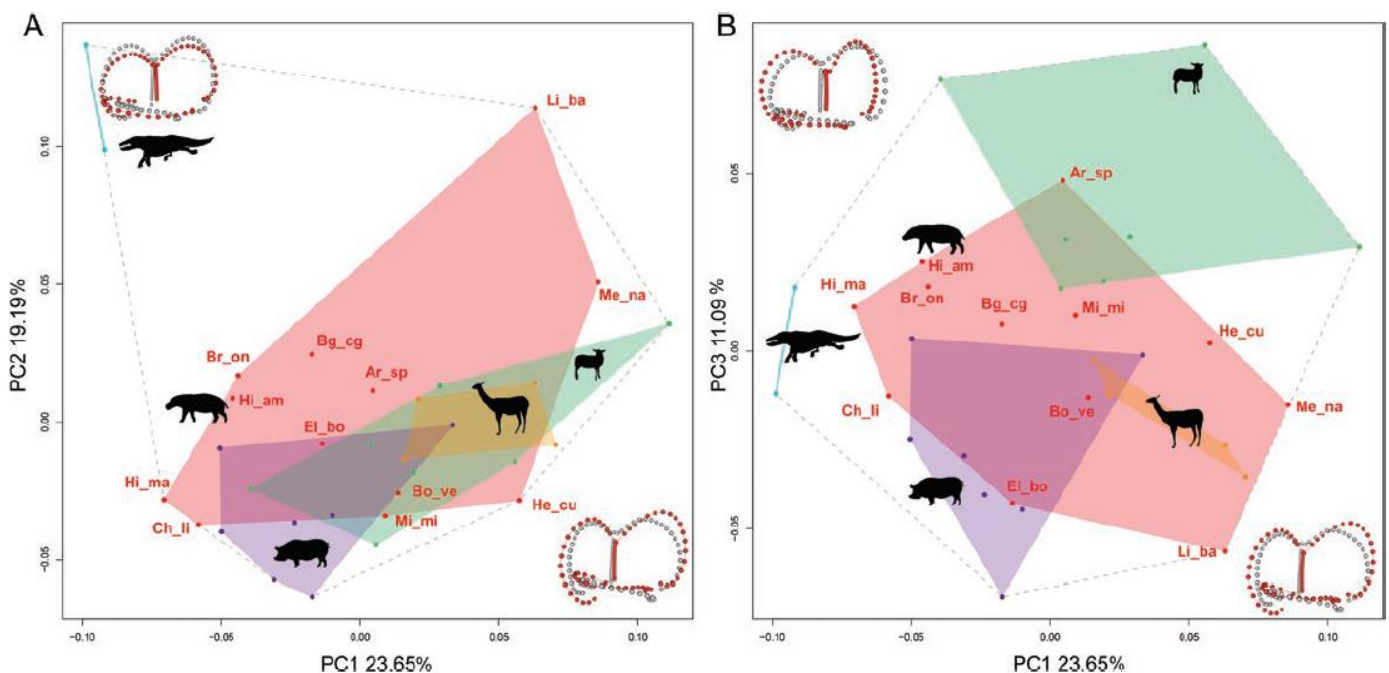


Figure 3. PCA of the shape of the three semicircular canals. Projection of the individuals on the first factorial plane (PC1-PC2) (A); and on the second factorial plane (PC1-PC3) (B). The maximum shape of every axis is shown in red, superimposed on the minimum shape in grey. Cyan, Cetacea; green, Ruminantia; orange, Tylopoda; pink, Hippopotamoidea; purple, Suoidea. Datasets and script available in Supporting Information, Appendix S4.

Shape of the semicircular canals

In order to compare the shape variation of the semicircular canals of the early hippopotamoids with those of extant hippopotamids, exclusively terrestrial artiodactyls and early cetaceans, we performed a GPA on a dataset of 3D semilandmarks located on the three semicircular canals (SCC) and the common crus for a sample of 32 taxa. We carried out the analysis on the whole vestibular dataset and on the four different anatomical structures separately [cf. Material and

methods; Supporting Information, Appendix S1 (Tables S8–S11)]. For the whole semicircular dataset, the first factorial plane (Fig. 3) explains 42.84% of the variance, while the first factorial space (PC1, PC2 and PC3) explains up to 53.93% of it. The projection of the individuals on the first (Fig. 3A) and the second (Fig. 3B) factorial planes presents four overlapping groups corresponding to Hippopotamoidea, Ruminantia, Suoidea and Tylopoda; the two protocetids are clearly apart from these artiodactyls, with more negative values on PC1 and more positive values on PC2. Despite an apparently wide overlap on the first factorial plane, the hippopotamoid morphospace presents a low similarity to that of ruminants, suoids and tylopods, with Jaccard indexes of 0.25, 0.15 and 0.08, respectively [Supporting Information, Appendix S1 (Table S12)]. Furthermore, in three dimensions [Supporting Information, Appendix S1 (Fig. S8)], hippopotamoids only slightly overlap with ruminants. Besides, ruminants, suoids and tylopods occupy distinct morphospaces with only a slight intersection between suoids and tylopods. The hippopotamoid morphospace is considerably expanded by the taxa likely to present an ‘aquatic audition’ and is stretched toward high values on PC2 by *Arretotherium* sp., *Bothriogenys* cf. *gorringei*, *Brachyodus onoideus*, *Hippopotamus amphibius*, *Merycopotamus nanus* and *Libycosaurus bahri*. The two latter taxa, belonging to the Merycopotamini clade (*sensu* Lihoreau *et al.*, 2017), lie far apart from other hippopotamoids, in strong opposition with Hippopotaminae (*Hi. amphibius*, *Hippopotamus madagascariensis*, *C. liberiensis*). Representatives of Hippopotaminae have negative values on PC1, and in this respect are closer to protocetids than any other non-cetacean taxa of our sample. *Brachyodus onoideus* lies close to *Hippopotamus amphibius* in both the first and second factorial plane. Hippopotamoids occupy the largest morphospace with 22.08% of the volume of the whole sample [see Supporting Information, Appendix S1 (Table S10); in comparison, Ruminantia represent only 5.88% of the morphospace of the whole sample]. In terms of shape variation, the Procrustes variance (Zelditch *et al.*, 2004) of Hippopotamoidea is also the highest with a variance of 0.00936 (while Ruminantia = 0.00800). That said, there is no significant difference between groups for this whole dataset [Supporting Information, Appendix S1 (Table S11)]. On PC1 (23.65% of the variance), compared to the positive values, the negative values stand for a subtle angular deviation of the anterior SCC, a posterior SCC smaller than the anterior SCC, a smaller angle between the posterior and anterior SCCs, a more circular and nearly straight (no angular deviation) lateral SCC, a posterior end of the lateral SCC distant from the basis of the common crus, a longer common crus and a short secondary common crus (absent on the more positive values). On PC2 (19.19% of the variance), the negative values describe a longer and more circular anterior SCC, a more ovoid posterior SCC, a shorter lateral SCC and a straighter common crus. On PC3 (11.09% of the variance), the negative values present a longer anterior SCC, a longer and more ovoid lateral SCC and a longer common crus. Results of the four different subsets (lateral, anterior and posterior SCCs and common crus) are similar to those of the whole semicircular dataset [Supporting Information, Appendix S1 (Table S9)].

DISCUSSION

Palaeoecological signal of the ear region and amphibiosis in Hippopotamoidea

The petrosal bones of hippopotamoids show a great morphological diversity (Fig. 1a) highlighting the complex evolutionary history of this highly diversified group, found in Eurasia, northern and central America and Africa (Lihoreau & Ducrocq, 2007; Rincón *et al.*, 2013). This history is intimately linked to the aquatic environment and illustrates a nice case of multiple convergent acquisitions of semiaquatic habits—likely of different degrees—resulting from different combinations of morphological traits. Semiaquatic lifestyle or amphibiosis varies in intensity and covers a wide range of adaptations linked with a range of behaviours from

spending part of the day with the body partly immersed to spending most of the daytime with most of the body underwater. These different degrees of amphibiosis are likely to correspond to different degrees of morphological modifications from the exclusively terrestrial Bauplan. The extant common hippopotamus, *Hi. amphibius*, exemplifies a high degree of specialization to a semiaquatic lifestyle including complex underwater communication (Barklow, 2004a, b; Maust-Mohl *et al.*, 2015, 2018). The latter skill is related to a derived morphology of the skull, which displays an amphibious disposition of the sensory organs, nostrils, eyes and ears: with their ‘periscopic’ eyes, their ears and nostrils above water, and the mouth and throat below water, common hippopotamuses produce and hear sounds in air and underwater simultaneously allowing ‘amphibious communication’ (Barklow, 2004a). Concerning the petrosal morphology, compared to terrestrial artiodactyls, common hippopotamuses show a hypertrophy of the tegmen tympani (O’Leary, 2010; O’Leary *et al.*, 2012; Orliac *et al.*, 2014; this work), a character considered to be a hallmark of underwater adaptation related to directional hearing in the aquatic environment (Luo & Gingerich, 1999; O’Leary *et al.*, 2012). Our results indicate that there is congruence between petrosal morphology (tegmen tympani inflation), cochlear morphology and the disposition of the sense organs of the head in hippopotamoids (Fig. 2). In addition, the phylogenetic signal carried by the petrosal is not overwhelmed by the ecological signal, and convergent acquisitions of tegmen tympani inflation are identified (Fig. 1). Put altogether, these parameters allow us to discuss the different character combinations related to aquatic adaptations in fossil taxa.

The (*Bothriogenys* cf. *gorringei*, Hippopotaminae) clade has an ancestral estimated tegmen tympani volume close to 50% [Supporting Information, Appendix S1 (Fig. S6a)] vs. non-hippopotamoid taxa < 11%. It gathers taxa with marked inflation of the tegmen tympani that also show a homogeneous morphology of the petrosal bone (Fig. 1) and auditory area. They all present a tight connection of the petrosal bone with the area surrounding the glenoid surface via a tegmen tympani/bulla/tympanic neck complex (Orliac *et al.*, 2014) and the ventrolateral tuberosity [Supporting Information, Appendix S1 (Fig. S2)]. This complex is likely to participate to underwater directional hearing in extant hippopotamids by providing a pathway for the sound to the middle ear (mandibular transmission of the sound hypothesis; Barklow, 2004a) or by creating a barrier for sound penetration and helping in isolating the ear acoustically (Orliac *et al.*, 2014). They also are the only hippopotamoids to present a suprahiatal fossa (= the prefacial commissure fossa of O’Leary (2010)). According to the morphology of the brain in extant hippopotamids (Pilleri, 1962; Dell *et al.*, 2016), this fossa could accommodate a lobule of the paraflocculus or a posterior part of the cerebrum, and is closely associated with modification of the shape of the braincase in members of the (*Bothriogenys* cf. *gorringei*, Hippopotaminae) clade.

Among Hippopotaminae, *Hi. amphibius* represents an extreme in terms of petrosal morphology, tegmen tympani inflation and functional cochlear parameters (Figs 1, 2). Compared to *Hi. amphibius*, the two other hippopotamines of our sample, *Hi. Madagascariensis* and *C. liberiensis*, present much lower degrees of tegmen tympani inflation (Fig. 1) and lower values on PC1 and PC2 of the cochlear parameter analysis in principal component (Fig. 2; Supporting Information, Appendix S3). *Choeropsis liberiensis* is much less aquatic than *Hi. amphibius*, with little webbing between the digits, proportionately longer limbs and neck, lower orbits and parturition on land, not in water. In its general ecology and social behaviour *C. liberiensis* is closer to tapirs than to the common hippopotamus (Robinson, 1970; Robinson *et al.*, 2017). The recently extinct subfossil *Hi. madagascariensis*, retrieved from the Malagasy central plateau, is close to *C. liberiensis* in ear parameters and was also probably less aquatic than *Hi. amphibius* (Rakotovao *et al.*, 2014). For both *Hi. madagascariensis* and *C. liberiensis*, this

situation could either reflect a primitive condition or result from secondary terrestrial adaptations linked to particular habitats (subhumid highlands of Madagascar and equatorial rain forest of western Africa, respectively). The lack of fossil records documenting the evolution of these taxa precludes testing those competing hypotheses for now.

Merycopotamini have been reconstructed as semiaquatic hippopotamoids based on cranial morphology and isotopic analyses (Lihoreau *et al.*, 2007, 2014, 2019; Nelson, 2007). The functional signal of the auditory region supports these conclusions. *Libycosaurus bahri*, considered as the most aquatic merycopotamine, has higher values of the tegmen tympani volume and cochlear parameters close to extant hippopotamids, while *Merycopotamus nanus*, hypothesized as the less aquatic species of the genus (Lihoreau *et al.*, 2007, 2019), has a weaker tegmen tympani inflation and lower values on PC1 and PC2 (Fig. 2). Compared to Hippopotaminae and *Bothriogenys*, Merycopotamini have a different configuration of the ear region, with no tight tegmen tympani/bulla/tympanic neck complex and no suprahiatal fossa. The general profile of the skull of *Libycosaurus bahri* is similar to that of the modern common hippopotamus with an amphibious disposition of the sensory organs, which is congruent with high aquatic specialization. Even if it did not achieve the same degree of tegmen tympani pachyostosis, it does show an extreme enlargement of the foramen acusticum superius [Fig. 1; Supporting Information, Appendix S1 (Fig. S1F)] and a concurrently enlarged hiatus fallopii. This enlarged pathway for the greater petrosal nerve most probably implies an enlarged nerve size and concurrently, a significant number of fibres. In extant mammals, these efferent fibres are part of a bundle that innervates the lacrimal gland and the nasal mucosa. Modifications of the lacrimal apparatus occur in aquatic (e.g. Matthes, 1912; De Burlet, 1913, 1914) and semiaquatic mammals (e.g. Starck, 1982; Sánchez-Villagra & Asher, 2002; lack of lacrimal foramen in both hippopotamines and merycopotamines, Lihoreau *et al.*, 2007), and probably relates to the physiology of the eye and nose underwater. The great enlargement of the facial nerve pathway in Merycopotamini most probably relates to strong aquatic habits. Enlargement of the greater petrosal nerve pathway is also present in Hippopotamidae, but is much more moderate. Based on the character polarity and on the position of cf. *Sivameryx palaeindicus* at the base of the Merycopotamini clade, the merycopotamine petrosal morphology seems to have derived from an inflated tegmen tympani strategy toward an extremely enlarged pathway for the main branch of the facial nerve. Common hippopotamuses and derived merycopotamines share a gregarious behavior (Lihoreau *et al.*, 2007, 2014); tegmen tympani inflation is most probably involved in directional underwater hearing, and particularly meaningful for *Hi. amphibius* in which social interactions imply underwater communication. Merycopotamini might have used a different sound transmission pathway or developed other sensory abilities to communicate underwater. Finally, in merycopotamines the shape of the semicircular canals, implied in equilibrioception, differs greatly from that of hippopotamines, with much lower and wider anterior and posterior canals, and it is worth noting that *Libycosaurus bahri* has high values on PC2, comparable to those of protocetid whales (Fig. 3A). This result further supports the hypothesis that merycopotamines adapted to a semiaquatic lifestyle in a different way compared to modern hippopotamines.

Convergent acquisitions of substantial semiaquatic habits are also retrieved in the early bothriodontine *Arretotherium* sp. and *Brachyodus onoideus*. They both combine important tegmen tympani inflation and amphibious disposition of nostrils, eyes and ears. The petrosal bone of *Arretotherium* sp. retains a primitive morphology in Hippopotamoidea, close to that of *Elomeryx* (small auditory meatus with foramina acustica of same size) and, except for the pachyostotic tegmen tympani, exhibits no adaptation to underwater hearing. This is congruent with its position in the PCA of functional parameters of their cochlea (Fig. 2).

Overview of semiaquatic adaptation in hippopotamoid history

Setting our results on the auditory region in the big picture of hippopotamoid evolution highlights that adaptation to semiaquatic lifestyle occurred convergently on three different continents, during different geological periods. The earliest signs of semiaquatic traits in Hippopotamoidea are observed in the Early Oligocene African representative of the genus *Bothriogenys*. According to dispersion hypotheses, *Bothriogenys* would have dispersed toward Africa from south-eastern Asia during the Eocene-Oligocene transition (Ducrocq, 1997; Lihoreau *et al.*, 2015), a period of low sea level resulting from glacioeustatism (cooling event Oi1; Vandenberghe *et al.*, 2012). The semiaquatic behaviour of this taxon supported by isotopic analysis (Clementz *et al.*, 2008) and petrosal morphology (O’Leary *et al.*, 2012; this study), would have allowed *Bothriogenys* to cross the large bodies of water separating both continents at this time. Another phase of increasing amphibiosis in Hippopotamoidea is here documented during the Early Miocene in phylogenetically distant taxa: in Europe by *Brachyodus onoideus*, phylogenetically related to African *Brachyodus* and to *Bothriogenys* (Lihoreau & Ducrocq, 2007), in northern America by *Arretotherium* sp., and in Asia by cf. *Sivameryx palaeindicus*. In Asia, increasing specialization of the ear region of merycopotamines is observed between the Early Miocene cf. *Sivameryx palaeindicus* and the Late Miocene *Merycopotamus nanus*. This result is congruent with an increase of semiaquatic habits in the lineage of *Merycopotamus* (Lihoreau *et al.*, 2007, 2014), concurrent with the seasonality intensification of the Late Miocene (Badgley *et al.*, 2008). In Africa, the petrosal and inner ear characteristics of the Late Miocene *Libycosaurus bahri* point toward increased semiaquatic adaptations in this other merycopotamine genus. *Libycosaurus* has been demonstrated to show a parallel evolution with *Merycopotamus*, exhibiting an even stronger specialization to aquatic habits in relation to increased aridity of the Saharan Province (Lihoreau *et al.*, 2014, 2019). The morphology of the ear region provides additional support for this hypothesis. Bothriodontine evolutionary history shows that they experienced specialization to a semiaquatic lifestyle in context of increasing aridity (Lihoreau & Ducrocq, 2007; Lihoreau *et al.*, 2014) and submersion of the body in water could be regarded as an answer to protect the skin against sun damage. The skin of extant hippopotamid species is indeed highly specialized; hairless and deprived of sebaceous glands, it produces a viscous secretion, both preventing sunburn and infections (Hashimoto *et al.*, 2006, 2007). The loss of sebaceous glands and hairs presumably evolved in conjunction with a semiaquatic lifestyle (Gatesy *et al.*, 2013), and several clades of hippopotamoids might have evolved a special physiology of the skin convergently. The molecular results of Springer & Gatesy (2018) point to a recent loss of sebaceous glands in Hippopotamidae, estimated at between 13.2 and 11.8 Myr, but probably underestimated due to the weak number of extant species. The convergent acquisition of semi-aquatic habits highlighted in this work suggests that loss of sebaceous glands might have occurred several times in hippopotamoid history, and potentially much earlier in the history of Hippopotamidae, as indicated by the high specialization of the ear region of the Early Oligocene taxon *Bothriogenys*. Finally, it is worth noting that advanced specialization to amphibiosis often occurred in the last representatives of lineages on each continent. Successive dispersions and adaptations to water dwelling may have been ‘emergency responses’ to unfavourable environmental conditions faced by hippopotamoids in particular and to large semiaquatic herbivores in general, from the Oligocene onwards. These responses made it possible for this superfamily to persist until now.

CONCLUSION

Persuasive cases of convergent acquisitions of semiaquatic traits have been mentioned in the evolutionary history of hippopotamoids (Boisserie, 2005; Lihoreau & Ducrocq, 2007; Orliac *et al.*, 2013; Lihoreau *et al.*, 2017), underlying the lability of semiaquatic behaviour in this clade and questioning the unique, ancestral acquisition of semiaquatic habits for hippopotamids and cetaceans (see Boisserie *et al.*, 2011). Molecular studies dealing with different processes such as lipid metabolism, hypoxia (Tsagkogeorga *et al.*, 2015) or specialized features of the skin (Springer & Gatesy, 2018) conclude that several molecular adaptations related to life underwater have arisen independently in cetaceans and hippopotamids. The integrated signal of the ear region further supports convergent acquisitions of semiaquatic behaviour in hippopotamids and cetaceans. Indeed, the early diverging taxa present a terrestrial functional signal of the auditory region, implying that terrestrial hearing was present as an ancestral trait among Hippopotamoidea. We support here that significant amphibiosis is derived in Hippopotamoidea and occurred convergently in different hippopotamoid clades. Our results highlight the potential of petrosal characters to clarify the relationships in Hippopotamoidea. The inclusion of a more complete taxonomic sample in a comprehensive morphological framework would surely help to disentangle the early history of bothriodontines.

ACKNOWLEDGEMENTS

We are especially grateful to Meng Jin (American Museum of Natural History, New York), M. O’Leary (Stony Brook University, New York), E. Gilissen (Musée Royal d’Afrique Centrale, Tervuren, Belgium), P. Brewer and A. Garbout (Natural History Museum, London), B. Mallah and C. Nekoulngang (Centre National de Recherche pour le Développement, N’Djamena), F. Saragoza and A. Bonnet (Museum Crozatier, Le Puy-en-Velay), J. Barry (Harvard Peabody Museum, Cambridge) and F. Guy (PALEVOPRIM, Poitiers, France) for access to the specimens from the collections they are in charge of, or for providing us with μ -CT scan data. We acknowledge the MRI platform member of the national infrastructure France-BioImaging supported by the French National Research Agency (ANR-10-INBS-04, ‘Investments for the future’), labex CeMEB (ANR-10-LABX-0004) and NUMEV (ANR- 10-LABX-0020). This work was funded by the French National Research Agency (ANR-15-CE32-0010-01, *SPLASH*) and by labex CeMEB (ANR-10-LABX-0004), recherche exploratoire (project PygmHippoCom). This is ISEM publication no. 2022-288.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article on the publisher's website:

- Appendix S1. Supplementary figures, tables and character lists.
- Appendix S2. Data matrix in Nexus format.
- Appendix S3. Dataset on PCA values.
- Appendix S4. Datasets and scripts.