



ELSEVIER

Contents lists available at ScienceDirect

Comptes Rendus Palevol

www.sciencedirect.com



General Palaeontology, Systematics and Evolution (Vertebrate Palaeontology)

Was the Early Eocene proboscidean *Numidotherium koholense* semi-aquatic or terrestrial? Evidence from stable isotopes and bone histology



Numidotherium koholense, un proboscideen primitif de l'Eocène inférieur, était-il terrestre ou semi-aquatique ? Contribution de l'analyse des isotopes stables et de l'histologie osseuse

Salamet Mahboubi^{a,*}, Hervé Bocherens^b, Michael Scheffler^b,
Mouloud Benammi^a, Jean-Jacques Jaeger^a

^a Institut de Paléoprimatologie, Paléontologie Humaine : Évolution et Paléoenvironnements (IPHEP), UMR-CNRS 7262, Bâtiment Sciences Naturelles, 6, rue M.-Brunet, 86022 Poitiers Cedex, France

^b Fachbereich Geowissenschaften Forschungsbereich Paläobiologie, Biogeologie Universität Tübingen Hölderlinstr. 12, 72074 Tübingen, Germany

ARTICLE INFO

Article history:

Received 22 July 2013

Accepted after revision 13 January 2014

Available online 24 February 2014

Handled by Lars W. van den Hoek Ostende

Keywords:

Numidotherium koholense

Eocene

Algeria

Terrestrial life

Stable isotopes

Histology

ABSTRACT

The Early Eocene deposits of El Kohol, Algeria, have yielded numerous remains of *Numidotherium koholense*, one of the most primitive and oldest known proboscideans in Africa. The Upper Eocene proboscideans of the Fayum locality (Egypt), *Barytherium* sp. and *Moeritherium* sp., were recently interpreted as aquatic or semi-aquatic, according to the stable isotopic compositions ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) of their tooth enamel. These data led us to reinvestigate the adaptations of *N. koholense*. Stable isotopic analysis and observations of histological sections of its long bones reveal that it was essentially terrestrial. According to its position within the phylogenetic tree of Eocene proboscideans, the adaptation to semi-aquatic life appears to have evolved independently in different lineages of Middle and Upper Eocene proboscideans during their adaptive radiation in Africa. Moreover, these new results reopen the debate about the hypothesis that Eocene to Recent proboscideans are derived from semi-aquatic ancestors.

© 2014 Académie des sciences. Published by Elsevier Masson SAS. All rights reserved.

R É S U M É

Le gisement de l'Eocène inférieur d'El Kohol (Algérie) a livré de nombreux restes de *Numidotherium koholense*, l'un des proboscidiens les plus primitifs et les plus anciens de la radiation de cet ordre en Afrique. Les proboscidiens de l'Eocène supérieur du Fayum en Égypte, comme *Barytherium* sp. et *Moeritherium* sp. étaient semi-aquatiques d'après des données des isotopes stables ($\delta^{13}\text{C}$ et $\delta^{18}\text{O}$) de leur émail dentaire. Ces données nous ont conduits à réexaminer le mode de vie de *N. koholense*. Les analyses des isotopes stables et l'examen des coupes histologiques des os longs révèlent qu'il était plutôt terrestre. Compte-tenu de la position qu'il occupe dans l'arbre phylogénétique des proboscidiens

Mots clés :

Numidotherium koholense

Algérie

Éocène

Vie terrestre

Isotopes stables

Histologie

* Corresponding author.

E-mail address: salamet.mahboubi@univ-poitiers.fr (S. Mahboubi).

éocènes, l'adaptation à la vie semi-aquatique serait donc développée indépendamment dans différentes lignées de proboscidiens primitifs au cours de leur radiation adaptative éocène. Par ailleurs, ces résultats rouvrent le débat concernant l'hypothèse de l'adaptation semi-aquatique de l'ancêtre des proboscidiens primitifs.

© 2014 Académie des sciences. Publié par Elsevier Masson SAS. Tous droits réservés.

1. Introduction

The evolutionary history of proboscideans is documented over 60 million years (Gheerbrant, 2009). The first radiation is that of the Eocene lophodont taxa (Gheerbrant and Tassy, 2009), whose phylogenetic relationships were established by Delmer (2009) (Fig. 1). His phylogeny supports the hypothesis of an ancestral morphotype with lophodont molars that gave rise to semi-aquatic forms such as *Moeritherium* that displayed bunolophodont molars, and later to bunodont forms, including the elephantiforms (Gheerbrant et al., 1998, 2005). Previous stable isotopic work showed that *Moeritherium* and *Barytherium*, proboscideans from the Upper Eocene of Fayum, were semi-aquatic mammals (Liu et al., 2008). *Numidotherium koholense*, one of the oldest and basalmost proboscideans, comes from the Early Eocene of El Kohol (Algeria). It was described on the basis of rich and well-preserved material that documents almost its entire skeleton (Mahboubi et al., 1986). An Ypresian age, between 52 Ma and 51 Ma, was recently estimated for this deposit, based on a magnetostratigraphic study (Coster et al., 2012).

According to the detailed description of Court (1994a), the postcranial morphology of *N. koholense* differs significantly from that of the later elephantiform proboscideans. The fusion of the distal radius and ulna in a semi-supinated position, a character also observed in aquatic mammals such as Pinnipedia and Sirenia, could indicate an aquatic mode of life (Court, 1994a; Savage, 1957). However, Court (1994a) concluded that there is "little else in the limb skeleton of *N. koholense* to suggest an aquatic mode of life". Furthermore, the abducted members and ambulatory gait of this animal reflect a compromise between aquatic and terrestrial lifestyles (Court, 1994a).

Here, we reconstruct the ecology of *N. koholense*, through stable isotope analyses of tooth enamel. Using carbon isotopes, we assessed feeding preference. Then, we used oxygen isotopes of tooth enamel to know its lifestyle, as well as histological studies of its long bones. Our study aims to complete and re-evaluate the interpretations of mode of life proposed by Court (1994a). In addition, to obtain more relevant data, we also analyzed the tooth enamel of several proboscideans from Dur At Talah (Central Libya, late Middle Eocene) such as *Arcanotherium savagei*, *Moeritherium* sp., and *Barytherium grave* (Jaeger et al., 2010).

1.1. Stable isotopes of carbon and diet

Stable carbon isotope analysis of tooth enamel offers a good proxy for reconstructing terrestrial paleoenvironments, including climate and vegetation, notably providing valuable information regarding the proportions of C₃ and

C₄ vegetation in the diet of the herbivores and thus indirectly in their habitat. Although one of the earliest record of C₄ plants is dated around 14 Ma–12.5 Ma (Cerling et al., 1997a; Nambudiri et al., 1978; Tidwell and Nambudiri, 1989), these plants do not constitute a significant part of ecosystem ground before 8 Ma–7 Ma, and became common worldwide only from 6 Ma–5 Ma, (Cerling et al., 1997b).

The $\delta^{13}\text{C}$ values for C₃ plants range between -38% and -22% , with an average of -27% (Cerling and Harris, 1999; Farquhar et al., 1989; Tieszen, 1991). The $\delta^{13}\text{C}$ values of C₄ plants range between -17% and -9% , with an average of -13% (Smith and Epstein, 1971). In general, aquatic plants have lower concentrations of $\delta^{13}\text{C}$, and some algae and other aquatic plants can have values of $\delta^{13}\text{C}$ in the range of C₄ plants (Cerling et al., 1997b).

Herbivorous mammals usually exhibit a $+14\%$ enrichment for the $\delta^{13}\text{C}$ values of carbonate in bioapatite in bones and enamel relative to the type of plant consumed (Cerling and Harris, 1999; Cerling et al., 1997b; Lee-Thorp and Van der Merwe, 1989; Passey et al., 2005). This enrichment for tooth enamel in large mammals compared to their diet is greater than observed in laboratory experiments on very small mammals (Cerling et al., 1997b; DeNiro and Epstein, 1978). In carnivores, the enrichment is $+9\%$ for bones and enamel apatite (Bocherens and Drucker, 2013; Cerling et al., 1997b; Koch, 1998; Kohn and Cerling, 2002; Lee-Thorp and Van der Merwe, 1989). We take into consideration this enrichment for the interpretation of our results.

1.2. Oxygen isotopes environment/habitat use

Two isotopic approaches have been proposed to distinguish terrestrial mammals from aquatic or semi-aquatic ones. First, the hippopotamids of Plio-Pleistocene age in eastern Africa show lower $\delta^{18}\text{O}$ values than other associated terrestrial herbivores, which have been related to their semi-aquatic lifestyle (Bocherens et al., 1996). Another approach, proposed by Clementz and Koch (2001) and by Clementz et al. (2008), showed that $\delta^{18}\text{O}$ values within a same population of terrestrial animals display considerable variation, significantly higher than in semi-aquatic mammals. The standard deviation of $\delta^{18}\text{O}$ measured on a minimum of five specimens of a terrestrial population is higher than 1% . Aquatic mammals living in isotopically homogeneous water show a much lower variation in their $\delta^{18}\text{O}$ values, their standard deviation of $\delta^{18}\text{O}$ being generally less than 0.5% (Clementz and Koch, 2001; Yoshida and Miyazaki, 1991). It shows that $\delta^{18}\text{O}$ of tooth enamel reflects water values during the mineralization of teeth (Andersen and Nielsen, 1983; Hui, 1981). This model was previously applied to distinguish terrestrial and semi-aquatic fossil mammals. According to the results obtained on *Moeritherium* sp. and *Barytherium* sp. from Upper Eocene

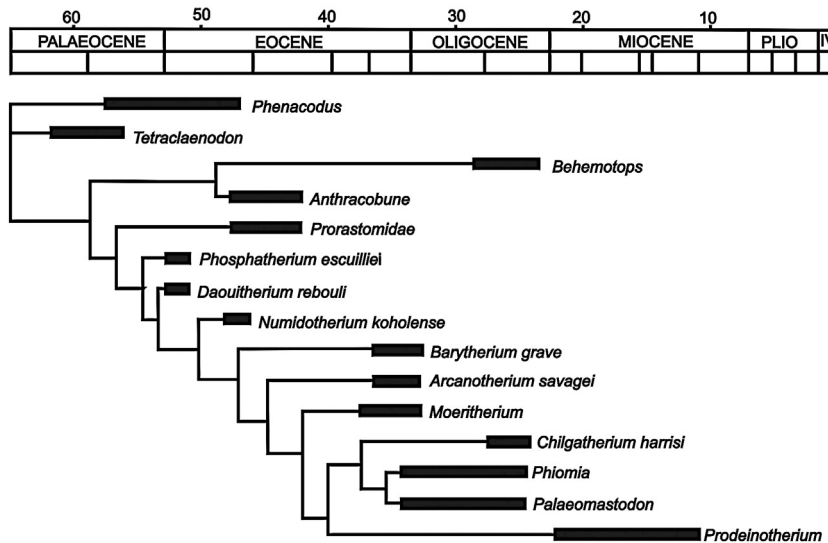


Fig. 1. The phylogeny of Proboscidea.

Fig. 1. Phylogénie des Proboscidea.

From Delmer (2009).

of Fayum, their standard deviation of $\delta^{18}\text{O}$ was lower (respectively 0.45‰ and 0.44‰) (Liu et al., 2008). Also, the $\delta^{18}\text{O}$ values were very low compared to those of terrestrial animals from the same sites, suggesting that *Moeritherium* sp. and *Barytherium* sp. were aquatic or semi-aquatic, living near to the river or the seacoast.

Within that framework, we analyzed the carbon and oxygen stable isotopes in the tooth enamel and dentin of *N. koholense*. We also analyzed some samples of three other primitive proboscideans and other mammals from Dur At Talah locality in central Libya, dated of Bartonian age (Jaeger et al., 2010). Although the site of El Kohol delivered some very rare remains of terrestrial animals, they could not be integrated into this study due to their scarcity.

2. Materials and methods

2.1. Stable isotope analyses

For stable isotope analyses, the enamel and dentin were obtained from mammals of El Kohol in Algeria, and Dur At Talah in central Libya. All samples belong to adult individuals. *N. koholense* samples are from specimens on deposit at the Paleontological Collection of the University of Oran in Algeria. The samples of Dur At Talah mammals are housed in the Paleontological Collections of the University of El Fateh in Tripoli, Libya.

2.2. Sampling protocol and analyses

Stable isotope analyses were conducted on the carbonate within tooth enamel and dentin bioapatite. We took nine samples of premolar and molar enamel from the cap of tooth and dentin of different adults specimens of *N. koholense* (El Kohol, Algeria). Similarly, we analyzed

nine samples obtained from molars of three proboscideans *A. savagei*, *B. grave*, *Moeritherium* sp., one molar fragment of creodont *Apterodon langebadreae* (Grohé et al., 2012), and one hyracoid indet. From Dur At Talah (Central Libya) (Jaeger et al., 2010; Tab. 1). The samples were cleaned, and then the tooth surfaces were air-abraded to remove surface contaminants on the outer layers of enamel. A diamond drill bit was used to extract samples from the teeth, although for some larger samples, around 20 mg of fragments of enamel could be snapped off and ground using an agate mortar.

Before being analyzed, the hydroxylapatite carbonate needs to be purified to remove any contamination. We followed the procedure described by Bocherens et al. (1996). First, powder is poured in a solution of sodium hypochlorite at 2.5% NaOCl for 24 h at 20 °C to oxidize organic residues, and then rinsed three times with distilled water. After that, the powder is treated with acetic acid (pH = 4.66) for 24 h at 20 °C to remove exogenous carbonate (Kohn et al., 1998), and finally rinsed with distilled water three times and dried at 29 °C for 24 h. Then, hydroxylapatite carbonate is reacted with phosphoric acid H_3PO_4 at 70 °C in a Thermo Finnigan Gasbench II on a Finnigan Delta Plus XL CFIRMS.

2.3. Cross-section of long bones

Bone is a dynamic and multifunctional tissue able to adapt constantly through a variety of processes through changes in biology and environment occurring during the life of the animal (Skerry, 2000). Previous work has shown that distinct histological specializations suggest different modes of life (Buffrénil and Mazin, 1989; Buffrénil and Schoevaert, 1988; Hua and Buffrénil, 1996). As well as its physiological and biological functions, the primary function of bone is biomechanical.

Table 1

Isotopic values of carbon and oxygen samples of Eocene mammals of El Kohol and Dur At Talah. $\delta^{13}\text{C}$ of the diet were obtained by subtracting an enamel-diet fractionation value of 14‰ from the enamel values for herbivore mammals and 9.5‰ for *Apterodon langebadreae* (Cerling et al., 1997b; Lee-Thorp and Van der Merwe, 1989).

Tableau 1

Valeurs isotopiques du carbone et de l'oxygène des échantillons analysés des mammifères éocènes d'El Kohol et Dur At Talah. Les valeurs de $\delta^{13}\text{C}$ des aliments consommés sont estimées en soustrayant 14‰ aux valeurs brutes de $\delta^{13}\text{C}$ obtenues pour les herbivores et 9,5‰ pour *Apterodon langebadreae* (fraction enamel/aliments) (Cerling et al., 1997b; Lee-Thorp et Van der Merwe, 1989).

Samples	Tax	$\delta^{13}\text{C}$ P DB (‰)			$\delta^{18}\text{O}$ SMOW (‰)		$\delta^{18}\text{O}$ (σ ‰)
		$\delta^{13}\text{C}$		$\delta^{13}\text{C}$ Diet	$\delta^{18}\text{O}$		
		Dentin	Enamel		Dentin	Enamel	
El Kohol							
PENA- 1 (P)	<i>N. koholense</i>	−10.5	−9.3	−23.3	25.9	28.7	1.94
PENA- 2 (P)	<i>N. koholense</i>	−9.8	−10.6	−24.6	26.4	30.6	
PENA- 3 (M1)	<i>N. koholense</i>	−9.8	−8.5	−22.5	25.8	32.8	
PENA- 4 (M1)	<i>N. koholense</i>	−10.5	−9.8	−23.8	26	31.5	
PENA- 5 (M1)	<i>N. koholense</i>	−9.1	−11	−25	25.7	29.6	
PENA- 6 (M1)	<i>N. koholense</i>	−10.4	−9.1	−23.1	26.1	29.3	
PENA- 7 (M3)	<i>N. koholense</i>	−9.2	−8.9	−22.9	26.1	26.4	
PENA- 8 (M3)	<i>N. koholense</i>	−9.9	−8.9	−22.9	26.5	31	
PENA- 9 (M3)	<i>N. koholense</i>	−9.7	−8.2	−22.2	26.5	32	
Dur At Talah							
PNEA- 10	<i>B. grave</i>		−10.5	−24.5		27.5	
PENA- 11	<i>M. sp.</i>		−13.3	−27.3		23.4	
PENA- 12	<i>M. sp.</i>		−10.9	−24.9		30.9	
PENA- 13	<i>A. savagei</i>		−11.1	−25.1		27.9	
PENA- 14	<i>A. savagei</i>		−10.6	−24.6		27.8	
PENA- 15	<i>A. savagei</i>		−11.2	−25.2		22.9	
PENA- 16	Hyracoid		−11.0	−25.0		27.6	
PENA- 17	Hyracoid		−9.6	−23.6		33.4	
PENA- 18	<i>A. langebadreae</i>		−13.7	−22.2		24.9	

N. koholense: *Numidotherium koholense*; *A. savagei*: *Apterodon langebadreae*; *A. savagei*: *Arcanotherium savagei*; *B. grave*: *Barytherium grave*; *M. sp.*: *Moeritherium sp.*; SMOW: standard mean ocean water.

The paleontological sample in this study included two fragments of humerus and femur of *N. koholense*. These bones are catalogued in the University of Oran, Algeria. These fossils are catalogued under the numbers (KA 68, KA 170) at the El Kohol locality. Long bones of *N. koholense* were encased in resin; in this method, it is possible to sever them with a diamond saw (George and Vashishth, 2005; Jepsen et al., 1999). From these histological sections, we analyzed the thickness of the medullar cavity and the cortical bone, which is supposed to indicate the lifestyle of mammals. The medullar cavities of semi-aquatic and aquatic mammals are relatively reduced compared to terrestrial mammals (e.g., Hayashi et al., 2013; Thewissen et al., 2007).

3. Results

3.1. Carbon Oxygen isotope, results and interpretation

Many studies have shown that some Eocene mammals enamel is suitable for isotope analyses provided that diagenetic alteration has not changed the original *in vivo* isotopic signatures of the teeth (Clementz et al., 2006). The isotopic abundances of carbon and oxygen in *N. koholense* teeth show differences between the enamel and dentin values: within the same tooth these reach 1 ‰ for $\delta^{13}\text{C}$ and 2.8 to 6.1 ‰ for $\delta^{18}\text{O}$ values. These results are evidence for different behaviors of two tissues subjected to diagenesis (Jacques et al., 2008; Zazzo et al., 2004).

Moreover, the oxygen isotopic signal is less stable than the carbon signal (Jacques et al., 2008). This phenomenon is probably related to the complex behavior of oxygen and sources of oxygen contamination during diagenesis. The dense tooth enamel structure better resists diagenesis than bone or dentin (Bocherens et al., 1996; Koch et al., 1992; Quade et al., 1992). The following interpretations are therefore based only on results obtained from tooth enamel, the results are summarized in Table 1.

The $\delta^{13}\text{C}$ values of the dentin and enamel of *N. koholense* reflect a diet composed exclusively of C_3 plants. However, $\delta^{18}\text{O}$ values exhibit significant differences between dentin and enamel values, which can reach 6.1‰ within a single tooth (Fig. 2). This probably results from post-mortem alteration of the dentin: the high crystallinity and low porosity of the tooth enamel resists to the recrystallization and isotopic exchanges much better than dentin (Lee-Thorp and Van der Merwe, 1989; LeGeros, 1981). The $\delta^{13}\text{C}$ values of Dur At Talah indicate that they are exclusive consumers of C_3 plants, which corresponds to the absence of C_4 plants at that period (Cerling et al., 1997a; Nambudiri et al., 1978) (Fig. 3).

3.2. $\delta^{13}\text{C}$ and paleodiets

Stable carbon isotopic analyses conducted on samples of tooth enamel of *N. koholense* show an average $\delta^{13}\text{C}$ of -23.3 ± 1.6 ‰, reflecting a diet of C_3 plants. The $\delta^{13}\text{C}$ values of Dur At Talah proboscideans are slightly lower

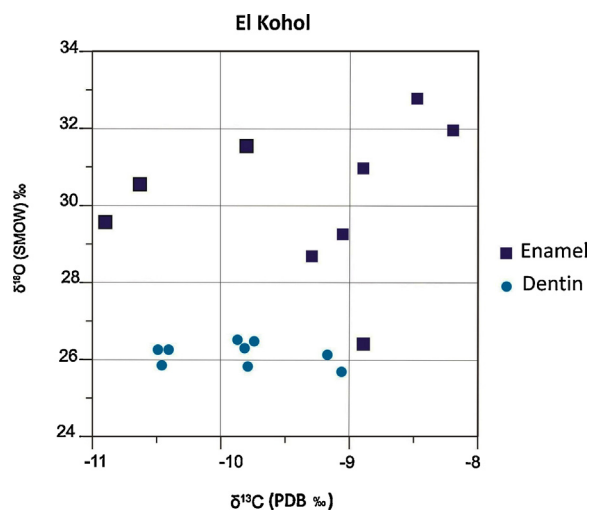


Fig. 2. Carbon and oxygen isotope on enamel teeth and dentin of *Numidotherium koholense* from El Kohol, Algeria.

Fig. 2. Distribution des valeurs isotopiques du carbone et de l'oxygène de l'émail et de la dentine de *Numidotherium koholense* d'El Kohol, Algérie.

(respectively -24.5% , -26.0% and -24.9% for *B. grave*, *Moeritherium sp.* and *A. savagei*), indicating a slightly different diet from that of *N. koholense*.

By contrast, the dietary $\delta^{13}\text{C}$ values of *Barytherium sp.* and *Moeritherium sp.* of the Fayum locality are respectively $-21.8 \pm 0.56\%$ and $-22.8 \pm 3.6\%$, indicating that they fed on freshwater plants or offshore particulate organic matter, thus supporting evidence for a semi-aquatic life, and preference of aquatic habitat (Clementz and Koch, 2001; Liu et al., 2008) (Table 2).

3.3. Oxygen isotopes and aquatic/terrestrial habitat use

The remains of *N. koholense* were deposited during the Early Eocene, which experienced the warmest conditions of the Cenozoic (Early Eocene Climatic Optimum), while *Moeritherium*, *Barytherium*, and the other comparative taxa were deposited during the much cooler and drier conditions of the Late Eocene. In fact, taking into account climate change during this period, we cannot compare our results with those obtained from other proboscideans of

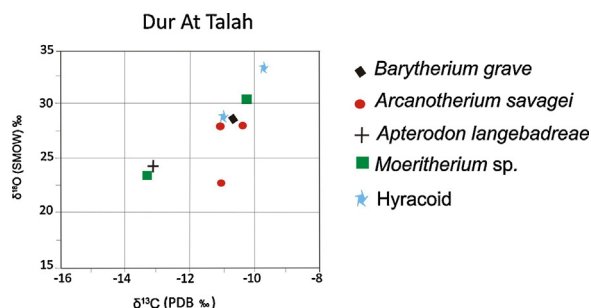


Fig. 3. Carbon and oxygen isotopes of enamel teeth of Eocene mammals from Dur At Talah.

Fig. 3. Distribution des valeurs isotopiques du carbone et de l'oxygène des mammifères éocènes du site Dur At Talah.

Table 2

Mean values of carbon and oxygen isotope ratios of tooth enamel for each taxa of Eocene mammals of northern Africa. Isotope ratios of the Fayum locality are from Liu et al. (2008).

Tableau 2

Valeurs isotopiques moyennes du carbone et de l'oxygène de l'émail dentaire des mammifères éocènes d'Afrique septentrionale. Les valeurs isotopiques du Fayum sont celles de Liu et al. (2008).

Taxon	n	$\delta^{13}\text{C} \text{ ‰ PDB}$	$\delta^{18}\text{O} \text{ SMOW (‰)}$
Fayum			
<i>Moeritherium sp.</i>	5	-22.8	26.9 ± 0.44
<i>Barytherium sp.</i>	5	-21.8	26.5 ± 0.45
Anthracotheriid	6	-22.4	31.6 ± 1.27
<i>Thyrohyrax meyeri</i>	6	-21.6	32.2 ± 0.75
<i>Saghatherium bowmi</i>	9	-22.2	32.9 ± 1.07
Hyracoid	6	-20.2	30.8 ± 0.75
El Kohol			
<i>Numidotherium koholense</i>	9	-23.3	30.1 ± 1.94
Dur At Talah			
<i>Moeritherium sp.</i>	2	-26.0	27.17
<i>Barytherium grave</i>	1	-24.5	27.46
<i>Arcanotherium savagei</i>	3	-24.9	26.21
Hyracoid	2	-23.8	30.51
<i>Apterodon langebadrae</i>	1	-22.7	24.94

SMOW: standard mean ocean water.

previous studies. The standard deviations of $\delta^{18}\text{O}$ values were calculated after conversion of all values (from the current study) to the SMOW standard, using the formula: $\delta^{18}\text{O} \text{ SMOW} = 30.91 + (1.03091 \times \delta^{18}\text{O} \text{ OPDB})$. The standard deviation obtained for $\delta^{18}\text{O}$ in tooth enamel of *N. koholense* is considerable, around 1.94‰, suggesting a terrestrial lifestyle (Clementz and Koch, 2001; Clementz et al., 2008) (Fig. 4). Hence, the standard deviation of $\delta^{18}\text{O}$ obtained from *N. koholense* is higher than those obtained for semi-aquatic proboscideans of Fayum (Liu et al., 2008), which confirms a terrestrial life for *N. koholense*. Dur At Talah proboscideans show $\delta^{18}\text{O}$ values more impoverished than the $\delta^{18}\text{O}$ values obtained from hyracoids, which are exclusively terrestrial mammals. However, too few samples of proboscideans were analyzed in this work to estimate the standard deviation and to reconstruct the presumed lifestyle. In order to confirm the lifestyle of Dur At Talah proboscideans, the number of samples should be increased to estimate their standard deviation and to better know their lifestyle.

Notably, the creodont *Apterodon langebadrae* displays an impoverished value of $\delta^{18}\text{O}$, suggesting a surprising semi-aquatic mode of life. This is congruent with the recent study by Grohé et al. (2012), who recently demonstrated that the postcranial anatomy of this creodont displays adaptations to a semi-aquatic life. This study, along with our results, implies that *A. langebadrae* found its food in the river or in the sea.

3.4. Histology and lifestyle adaptations

Bone displays a particularly dense inner organization with an extremely compact and thick cortex and a compacted medullary region (Hayashi et al., 2013). Wall (1983) considered the thickness of the cortical bone to be correlated with a specific mode of life. He suggested that if the cortical thickness exceeds 30 percent of the average

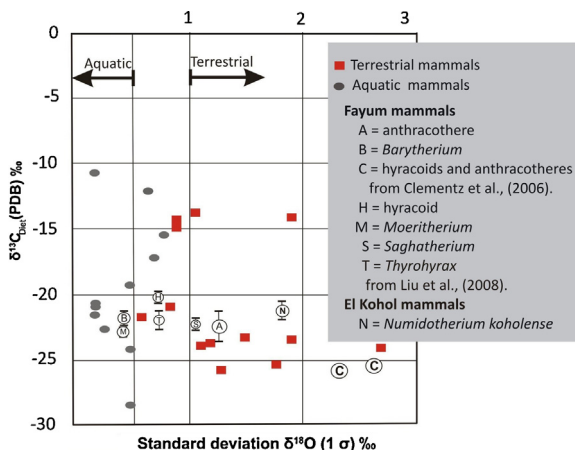


Fig. 4. $\delta^{13}\text{C}$ versus standard deviation of $\delta^{18}\text{O}$ (σ) for a range of aquatic and terrestrial modern and Eocene mammalian taxa. Each data point represents average values for one taxon. Taxa falling in ($\text{SD } \delta^{18}\text{O} < 0.5\text{‰}$), are considered aquatic, while taxa in falling in ($\text{SD } \delta^{18}\text{O} > 1.0\text{‰}$) are terrestrial. Additional data are from Clementz et al., 2006 and Liu et al., 2008. All $\delta^{18}\text{O}$ standard deviations are calculated from values normalized relative to standard mean ocean water (SMOW).

Fig. 4. Variation de l'écart-type de $\delta^{18}\text{O}$ (σ) par rapport aux valeurs de $\delta^{13}\text{C}$ pour des mammifères aquatiques et terrestres actuels et mammifères éocènes. Chaque point représente les valeurs moyennes pour un taxon. Les taxons ayant un écart-type ($\sigma \delta^{18}\text{O} < 0,5\text{‰}$) sont des mammifères aquatiques, tandis que les taxons ayant un écart-type ($\sigma \delta^{18}\text{O} > 1\text{‰}$) sont des mammifères terrestres (d'après Clementz et al., 2006; Liu et al., 2008). L'écart-type de $\delta^{18}\text{O}$ est calculé par rapport aux valeurs normalisées sur le standard de l'eau de mer (SMOW).

bone diameter in the limb bones, the animal was at least semi-aquatic like manatees, sea otters, hippopotamus, beavers, pinnipeds and Mesozoic marine reptiles (Domning and De Buffrénil, 1991; Taylor, 2000; Wall, 1983). Therefore, aquatic or semi-aquatic mammals are characterized by high cortical thickness, which suggests high bone density, which helps in overcoming buoyancy. High bone densities have also been recorded in the sirenians, cetaceans and certain aquatic birds (Buffrénil et al., 1990; Hua and Buffrénil, 1996).

To investigate further the hypothesis that *N. koholense* had a terrestrial mode of life, we studied the cross-sections of long bones, where we can see tubular bones, with a rather thick cortex and a large open medullar cavity (Fig. 5) similar to those observed in terrestrial mammals with a rather thick cortex and a large open medullar cavity (e.g. in *Nyctereutes procyonoides*) (Hayashi et al., 2013; Thewissen et al., 2007). In *N. koholense*, the femur and humerus show large medullar cavities. The studied sample of long bones shows that *N. koholense* has a terrestrial lifestyle. This result confirms our previous hypothesis of a terrestrial life for this primitive proboscidean based on stable isotope analyses.

4. Discussion

It is important to compare the two methods in the present paper to obtain more probable results. Prior works demonstrated that *Moeritherium* sp. from the Fayum locality had a semi-aquatic or aquatic lifestyle using isotope analyses. Another approach can be added to confirm its

lifestyle: the anterior situation of the orbits, which might not be an aquatic adaptation, because many mammals and reptiles profoundly adapted to an aquatic life have very posteriorly situated orbits. On the other hand, the external auditory openings are situated on a high level. As pointed out by Osborn (1909), this character might be an aquatic adaptation in a certain degree.

Matsumoto (1923) considered *Moeritherium* at least semi-aquatic; the possibility therefore exists that the auditory region of *Moeritherium* may have functioned in a similar manner to that of living seacows. *Moeritherium* from the Dur At Talah locality shows that the cochlea differs very little from that of living elephants (Court, 1994b; Tassy, 1981), exhibiting none of the specializations expected in aquatic audition via resonant reactions. In contrast, *N. koholense*, which lacks occipital extensions of the middle ear cavity (Court, 1992), has been interpreted as being moderately 'high frequency mammals' based on cochlear anatomy. The cochlea morphology of *Moeritherium* is larger in all dimensions than that of *N. koholense*, with a more apically extensive mode of coiling. Moreover, the small number of coils and mode of coiling indicate that *N. koholense* would have a rather limited frequency range, with the major portion of the basilar membrane length concentrated in the high frequency basal turn. The presence of the spiral canal in only the basal part of the cochlea is further evidence that in *N. koholense* auditory acuity was restricted to higher frequencies. The greater height of the cochlea in *Moeritherium* suggests that it had an increased frequency range over *N. koholense*, which can be interpreted by the difference of lifestyle of both mammals.

With regard to the morphology of *N. koholense*, its fore feet were evidently more plantigrade: each of the major limb joints provides evidence compatible with the thesis that *N. koholense* employed an ambulatory or semi-sprawling mode of locomotion. Generally speaking, this mode of locomotion is certainly primitive for therian mammals and stands in stark contrast to the parasagittal graviportalism of elephantiform Proboscidea. As discussed by Savage (1957), the distal radius and ulna are often fused in a semi-supinated position in aquatic mammals, such as pinnipeds and sirenians. Apart from this feature, there is little else in the limb skeleton of *N. koholense* to suggest an aquatic mode of life. Nevertheless, the abducted limbs and ambulatory gait reflect a mode of locomotion between aquatic and terrestrial life. Another morphological character which can be useful to determine its mode of life is the position of the orbits, which gives a key to distinguish aquatic mammals, although the low position of the orbits of *N. koholense*, rather support a terrestrial lifestyle.

In order to know the lifestyle of *N. koholense*, we should use alternative methods that are independent of morphology. The oxygen isotope composition of fossil materials has been used to distinguish between terrestrial and aquatic species (Bocherens et al., 1996; Clementz and Koch, 2001; Clementz et al., 2008; MacFadden, 1998). Prior studies have shown that *Barytherium* sp. and *Moeritherium* sp. of the Fayum locality and the Eocene *Coryphodon* of Bighorn Basin, Wyoming, had aquatic adaptations based on isotopic data of $\delta^{18}\text{O}$ (Koch et al., 1995; Liu et al., 2008); their $\delta^{18}\text{O}$ values were also significantly lower than the

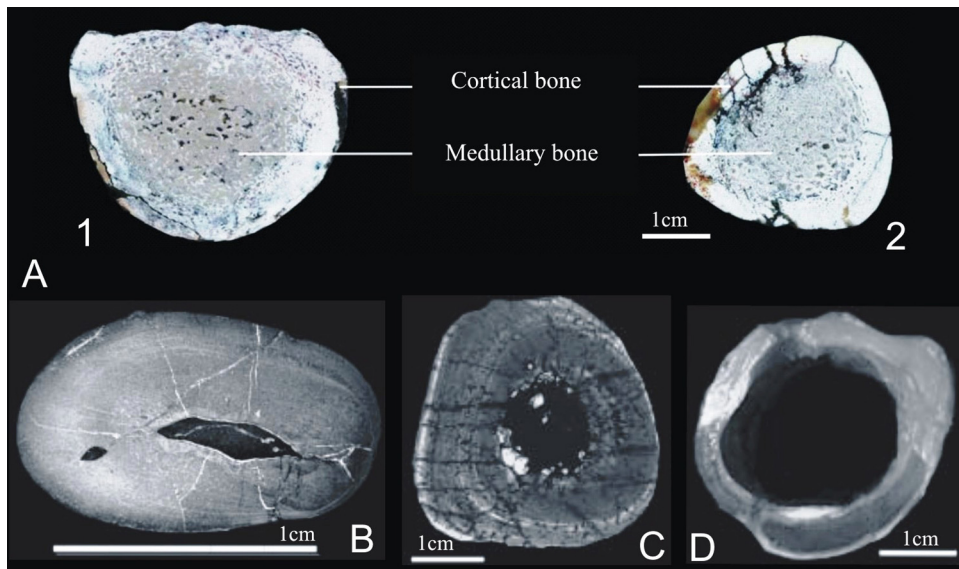


Fig. 5. Cross-sections of long bones. **A.** *Numidotherium koholense* (1: femur, 2: humerus). **B.** Aquatic mammal (humerus of the pakicetid *Ichthyolestes*). **C.** Semi-aquatic mammal (femur of *Indohyus*). **D.** Terrestrial mammal (femur of the cetartiodactyl *Cainotherium*). Both scale bars are 1 cm. Cross-sections **B**, **C** and **D** were obtained by [Thewissen et al. \(2007\)](#).

Fig. 5. Sections transverses des os longs. **A.** *Numidotherium koholense* (1 : fémur, 2 : humérus). **B.** Mammifère aquatique (humérus du pakicetidé *Ichthyolestes*). **C.** Mammifère semi-aquatique (fémur de *Indohyus*). **D.** Mammifère terrestre (fémur du cétartiodactyle *Cainotherium*). Les barres d'échelle sont égales à 1 cm. Les coupes histologiques **B**, **C** et **D** ont été obtenues par [Thewissen et al. \(2007\)](#).

associated terrestrial fauna. Preliminary results obtained on the Dur At Talah proboscideans should be interpreted with caution due to the limited number of samples, but our preliminary results suggest that they were semi-aquatic. *Numidotherium koholense* is more primitive than the other proboscideans described from Fayum and Dur At Talah, and occupies a more basal position on the phylogenetic tree (Fig. 1). *N. koholense* has a high oxygen standard deviation (1.94‰), suggesting a terrestrial lifestyle. Moreover, the cross-sections of the long bone show a great medullary cavity, which is a character present in terrestrial mammals, confirming its terrestrial lifestyle.

On the other hand, our study suggests that, contrary to the hypothesis proposed by some authors (e.g. [Clementz et al., 2006](#); [Liu et al., 2008](#)), not all primitive forms of Eocene proboscideans were semi-aquatic or aquatic. Thus, the adaptation to semi-aquatic life observed in more recent proboscideans occurs in distinct lineages. This strongly suggests that they evolved a semi-aquatic lifestyle separately, as a case of parallel evolution homoplasy in early proboscideans. This period is characterized by a very dense fluvial network, ending in impressive deltas, tropical forests and vegetation dominated by C_3 plants. This reconstruction is also supported by the sedimentological data of that period in northern Africa ([Holroyd et al., 1996](#)), because these mammals occur almost exclusively in the alluvial deposits.

5. Conclusion

This work attempts to identify the lifestyle of *N. koholense* through two independent methods, stable isotope analysis and histological cross-sections of long bones.

The isotopic results obtained from tooth enamel of Eocene proboscideans of El Kohol and Dur At Talah show that C_3 plants (leaves, fruits and/or aquatic plants for aquatic mammals) constituted the major element of their diet, which is not unexpected because C_4 plants started to dominate tropical ecosystems later than 8 Ma ([Nambudiri et al., 1978](#); [Quade et al., 1992](#)). Moreover, $\delta^{13}C$ values in enamel suggest that the diet of *N. koholense* differed significantly from that of the other proboscideans. The stable oxygen data (notably a high $\delta^{18}O$ standard deviation) also indicate that this primitive Eocene proboscidean of Algeria was terrestrial.

Our conclusion on the lifestyle is not based only on differences of absolute $\delta^{18}O$ values, but on the standard deviation of $\delta^{18}O$ of the population of *N. koholense*. This last point is valid to distinguish between aquatic and terrestrial animals which have been proven on different taxa and different environments. Study of its long bone sections and the low position of its orbits also confirm this terrestrial lifestyle, contrary to aquatic or semi-aquatic *Moeritherium* and *Barytherium*. Hence this multiple and independent evolution of adaptation to semi-aquatic life can be related to the climate and environments that prevailed in northern Africa during Eocene times. The aquatic adaptation of *Moeritherium* was relied on morphological and geological setting ([Andrew, 1906](#); [Matsumoto, 1923](#); [Osborn, 1936](#)), and recently by isotopic analyses ([Liu et al., 2008](#)). It is one thing to infer that some early proboscideans were adapted to semi or aquatic habitat, and another to suppose that living proboscideans had an aquatic ancestry ([Shoshani and Tassy, 2005](#)).

Our study suggests that *N. koholense* had a terrestrial adaptation. If this result is confirmed, it also may change

our reconstructions of the adaptation of the ancestor of early proboscideans. This ancestor may therefore also have been terrestrial, rather than aquatic as suggested by several previous authors (Seiffert, 2007).

Our results highlight the necessity of using multiple proxies to answer questions about the terrestrial adaptation of *N. koholense*. We cannot restrict our interpretations to the stable isotopic analyses because of the absence of associated terrestrial mammals. However, using the cross-sections of long bones gives us an idea of the lifestyle of this basal proboscidean.

This work opens new perspectives for further studies, on the one hand, by extending this approach to other basal proboscideans (*Phosphatherium* and *Eritherium*). On the other hand, it is preferable to incorporate microwear analysis into studies of other basal proboscideans species to identify differences in foraging preferences among co-occurring taxa, and to develop a method for testing our interpretations of these extinct animals.

Acknowledgments

This work was supported by the ANR-09-BLAN-0238-02 Program, and by the universities of Poitiers, Oran and Tlemcen. The authors would like to thank R. Tabuce (ISEM, Univ. Montpellier 2, France) for the loan of fossil material and X. Valentin (University of Poitiers) for its technical help in preparing histological sections of *Numidotherium* long bones. We are also thankful to the working group of Geochemistry at the University of Tübingen (Germany), and two anonymous reviewers for their useful comments that improved the manuscript.

References

- Andersen, S.H., Nielsen, E., 1983. Exchange of water between the harbor porpoise, *Phocoena phocoena*, and the environment. *Experientia* 39, 52–53.
- Andrew, C.W., British Museum (Natural History). Dept. of Geology, 1906. A descriptive catalogue of the Tertiary Vertebrata of the Fayu^m, Egypt. Based on the collection of the Egyptian government in the Geological museum, Cairo, and on the collection in the British museum (Natural history), London. Printed by order of the Trustees of the British museum, London.
- Bocherens, H., Drucker, D.G., 2013. Carbonate stable isotopes: Terrestrial Teeth and Bones. In: Elias, S.A., Mock, C.J. (Eds.), *Encyclopedia of Quaternary Science*, Second Edition. Elsevier, Amsterdam, pp. 304–314.
- Bocherens, H., Koch, P.L., Mariotti, A., Geraads, D., Jaeger, J.J., 1996. Isotopic biogeochemistry (^{13}C , ^{18}O) of mammalian enamel from African Pleistocene hominid sites. *Palaaios* 11, 306–318.
- Buffrénil, V., De Ricqlès, A., Ray, C.E., Domning, D.P., 1990. Bone histology of the ribs of the archaeocetes (Mammalia: Cetacea). *J. Vert. Paleontol.* 10, 455–466.
- Buffrénil, V., Mazin, J., 1989. Bone histology of *Claudiosaurus germani* (reptilia, claudiosauridae) and the problem of pachyostosis in aquatic tetrapods. *Hist. Biol.* 2, 311–322.
- Buffrénil, V., Schoevaert, D., 1988. On how the periosteal bone of the delphini humerus becomes cancellous: Ontogeny of a histological specialization. *J. Morphol.* 198, 149–164.
- Cerling, T.E., Harris, J.M., 1999. Carbon isotope fractionation between diet and bioapatite in ungulate mammals and implications for ecological and paleoecological studies. *Oecologia* 120, 347–363.
- Cerling, T.E., Harris, J.M., Ambrose, S.H., Leakey, M.G., Solounias, N., 1997a. Dietary and environmental reconstruction with stable isotopes analyses of herbivore tooth enamel from the Miocene locality of Fort Ternan, Kenya. *J. Hum. Evol.* 33, 635–650.
- Cerling, T.E., Harris, J.M., MacFadden, B.J., Leakey, M.G., Quade, J., Ehleringer, J.R., 1997b. Global vegetation change through the Mio-Pliocene boundary. *Nature* 389, 153–158.
- Clementz, M.T., Koch, P.L., 2001. Differentiating aquatic mammal habitat and foraging ecology with stable isotopes in tooth enamel. *Oecologia* 129, 461–472.
- Clementz, M.T., Goswami, A., Gingerich, P.D., Koch, P.L., 2006. Isotopic records from early whales and sea cows. Contrasting patterns of ecological transition. *J. Vert. Paleontol.* 26, 355–370.
- Clementz, M.T., Holroyd, P.A., Koch, P.L., 2008. Identifying aquatic habits of herbivorous mammals through stable isotope analysis. *Palaaios* 23, 574–585.
- Coster, P., Benammi, M., Mahboubi, M., Tabuce, R., Adaci, M., Marivaux, L., Bensalah, M., Mahboubi, S., Mahboubi, A., Mebrouk, F., Maameri, C., Jaeger, J.J., 2012. Chronology of the Eocene continental deposits of Africa: Magnetostratigraphy and biostratigraphy of the El Kohol and Glib Zegdou Formations, Algeria. *GSA Bull.* 124, 1590–1606.
- Court, N., 1992. Cochlea anatomy of *Numidotherium koholense*: auditory acuity in the oldest known proboscidean. *Lethaia* 25, 211–215.
- Court, N., 1994a. Limb posture and gait in *Numidotherium koholense*, a primitive proboscidean from the Eocene of Algeria. *Biol. J. Linn. Soc.* 111, 297–338.
- Court, N., 1994b. The periotic of *Moeritherium* (Mammalia, Proboscidea): homology or homoplasy in the ear region of Tethytheria McKenna, 1975? *J. Zool. Linn. Soc.* 112, 13–28.
- Delmer, C., 2009. Reassessment of the generic attribution of *Numidotherium savagei* and the homologies of lower incisors in proboscideans. *Acta Palaeontol. Pol.* 54, 561–580.
- DeNiro, M.J., Epstein, S., 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochim. Cosmochim. Acta.* 42, 495–506.
- Domning, D.P., De Buffrénil, V., 1991. Hydrostasis in the Sirenia: quantitative data and functional interpretations. *Mar. Mamm. Sci.* 7, 331–368.
- Farquhar, G.D., Ehleringer, J.R., Hubick, K.T., 1989. Carbon isotope discrimination and photosynthesis. *Ann. Rev. Plant Physiol. Mol. Biol.* 40, 503–537.
- George, W.T., Vashishth, D., 2005. Influence of phase angle between axial and torsional loadings on fatigue fractures of bone. *J. Biomech.* 38, 819–825.
- Gheerbrant, E., 2009. Paleocene emergence of elephant relatives and the rapid radiation of African ungulates. *Proc. Natl. Acad. Sci.* 106, 10717–10721.
- Gheerbrant, E., Tassy, P., 2009. L'origine et l'évolution des éléphants. *C. R. Palevol* 8, 281–294.
- Gheerbrant, E., Sudre, J., Cappetta, H., Bignot, G., 1998. *Phosphatherium escuilliei*, from the Thanetian of the Ouled Abdoun basin (Morocco), oldest known Proboscidean (Mammalia) from Africa. *Geobios* 30, 247–269.
- Gheerbrant, E., Sudre, J., Tassy, P., Amaghazaz, M., Bouya, B., Iarochene, M., 2005. Nouvelles données sur *Phosphatherium escuilliei* de l'Eocène inférieur du Maroc, apports à la phylogénie des Proboscidea et des ongulés lophodontes. *Geodiversitas* 27, 229–333.
- Grohé, C., Morlo, M., Chaimanee, Y., Blondel, C., Coster, P., Valentin, X., Salem, M., Bilal, A.A., Jaeger, J.J., Brunet, M., 2012. New Apterodontinae (Hyaenodontida) from the Eocene locality of Dur At Talah (Libya): systematic, paleoecological and phylogenetical implications. *Plos One* 7, 1–19.
- Hayashi, S., Houssaye, A., Nakajima, Y., Chiba, K., Ando, T., Sawamura, H., Inuzuka, N., Kaneko, N., Osaki, T., 2013. Bone inner structure suggests increasing aquatic adaptations in *Desmostylia* (Mammalia, Afrotheria). *Plos One* 8, e59146.
- Holroyd, P.A., Simons, E.L., Bown, T.M., Polly, P.D., Kraus, M.J., 1996. New records of terrestrial mammals from the Upper Eocene Qasr el Sagha Formation, Fayum Depression, Egypt. *Palaeovertebrata* 25, 175–192.
- Hua, S., Buffrénil, V., 1996. Bone histology as a clue in the interpretation of functional adaptations in the *Thalattosuchia* (Reptilia, Crocodylia). *J. Vert. Paleontol.* 16, 703–717.
- Hui, C.A., 1981. Seawater consumption and water flux in the common dolphin *Delphinus delphis*. *Physiol. Zool.* 54, 430–440.
- Jacques, L., Ogle, N., Moussa, I., Kalin, R., Vignaud, P., Brunet, M., Bocherens, H., 2008. Implications of diagenesis for the isotopic analysis of Upper Miocene large mammalian herbivore tooth enamel from Chad. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 266, 200–210.
- Jaeger, J.J., Marivaux, L., Salem, M., Bilal, A.A., Benammi, M., Chaimanee, Y., Druinger, P., Marandat, B., Metais, E., Schuster, M., Valentin, X., Brunet, M., 2010. New rodent assemblages from the Eocene Dur At Talah escarpment (Sahara of Central Libya): systematic, biochronological and paleobiogeographical implications. *Zool. J. Linn. Soc.* 160, 195–213.

- Jepsen, K.J., Davy, D.T., Krzypow, D.J., 1999. The role of the lamellar interface during torsional yielding of the human cortical bone. *J. Biomech.* 32, 303–310.
- Koch, P.L., 1998. Isotopic reconstruction of past continental environments. *Annu. Rev. Earth Planet. Sci.* 26, 573–613.
- Koch, P.L., Zachos, J.C., Gingerich, P.D., 1992. Correlation between isotope records in marine and continental carbon reservoirs near the Palaeocene/Eocene boundary. *Nature* 358, 319–322.
- Koch, P.L., Zachos, J.C., Dettman, D.L., 1995. Stable isotope stratigraphy and paleoclimatology of the Paleogene Bighorn Basin (Wyoming, USA). *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 115, 61–89.
- Kohn, M.J., Cerling, T.E., 2002. Stable isotope compositions of biological apatite. *Rev. Miner. Geochem.* 48, 455–488.
- Kohn, M.J., Schoeninger, M.J., Valley, J.W., 1998. Variability in oxygen isotope compositions of herbivore teeth: reflections of seasonality or developmental physiology? *Chem. Geol.* 152, 97–112.
- Lee-Thorp, J.A., Van der Merwe, N.J., 1989. Carbon isotope analysis of fossil bone apatite. *S. Afr. J. Sci.* 83, 712–715.
- LeGeros, F., 1981. Apatites in biological systems. In: Pamplin, B. (Ed.), *Inorganic Biological Crystal Growth*. Progr. Crystal. Growth. Charact. 4, 1–45.
- Liu, A.G., Seiffert, E.R., Simons, E.L., 2008. Stable isotope evidence for an amphibious phase in early proboscidean evolution. *Proc. Natl. Acad. Sci. U S A* 15, 5786–5791.
- MacFadden, B.J., 1998. Tale of two rhinos: isotopic ecology, paleodiet, and niche differentiation of *Aphelops* and *Teleoceras* from the Florida Neogene. *Paleobiology* 24, 274–286.
- Mahboubi, M., Ameur, R., Crochet, J.Y., Jaeger, J.J., 1986. El Kohol (Saharan Atlas, Algeria): a new Eocene mammal locality in North-West Africa. *Paleontographica Abteilung A* 192, 15–49.
- Matsumoto, H., 1923. A contribution to the knowledge of *Moeritherium*. *Bull. Am. Mus. Nat. Hist.* 48, 97–139.
- Nambudiri, E.M.V., Tidwell, W.D., Smith, B.N., Hebbert, N.P., 1978. A *C₄* plant from the Pliocene. *Nature* 276, 816–817.
- Osborn, H.F., 1909. On the feeding habits of *Moeritherium* and *Palaeomastodon*. *Nature* 81, 139–140.
- Osborn, H.F., 1936. *Moeritherioidea, Deinotherioidea, Mastodontoidea*. In: *Proboscidea: A Monograph of the Discovery, Evolution, Migration and Extinction of the Mastodonts and Elephants of the World*, Vol. 1. American Museum of Natural History Press, New York.
- Passey, B.H., Robinson, T.F., Ayliffe, L.K., Cerling, T.E., Sponheimer, M., Dearing, M.D., Roeder, B.L., Ehleringer, J.R., 2005. Carbon isotope fractionation between diet, breath CO₂, and bioapatite in different mammals. *J. Arch. Sci.* 32, 1459–1470.
- Quade, J., Cerling, T.E., Morgan, M.M., Pilbeam, D.R., Barry, J., Chivas, A.R., Lee-Thorp, J.A., Van der Merwe, N.J., 1992. A 16 million year record of paleodiet using carbon and oxygen isotopes in fossil teeth from Pakistan. *Chem. Geol.* 94, 183–192.
- Savage, R.J., 1957. The anatomy of *Potamotherium* an Oligocene lutrine. *Proc. Zool. Soc. Lond.* 129, 151–244.
- Seiffert, E.R., 2007. Evolution and extinction of Afro-Arabian primates near the Eocene-Oligocene boundary. *Folia Primatol.* 78, 314–327.
- Shoshani, J., Tassy, P., 2005. Advances in proboscidean taxonomy and classification, anatomy & physiology, and ecology & behavior. *Quat. Int.* 126–128, 5–20.
- Skerry, T., 2000. Biomechanical influences on skeletal growth and development. In: O'Higgins, P., Cohn, M.J. (Eds.), *Development, Growth and Evolution: Implications for the Study of the Hominid Skeleton*. Academic Press, London, pp. 29–39.
- Smith, B.N., Epstein, S., 1971. Two categories of ¹³C/¹²C ratios of higher plants. *Plant. Physiol.* 47, 380–384.
- Tassy, P., 1981. Le crâne de *Moeritherium* (Proboscidea, Mammalia) de l'Eocène de Dor El Talha (Libye) et le problème de la classification phylogénétique du genre dans les Tethytheria McKenna, 1975. *Bull. Mus. Nat. Hist., Paris* 4C3, 87–147.
- Taylor, M.A., 2000. Functional significance of bone ballastin in the evolution of buoyancy control strategies by aquatic tetrapods. *Hist. Biol.* 14, 15–31.
- Thewissen, J.G.M., Cooper, L.N., Clementz, M.T., Sunil, B., Tiwar, B.N., 2007. Whales originated from aquatic artiodactyls in the Eocene epoch of India. *Nature* 450, 1190–1194.
- Tidwell, W.D., Nambudiri, E.M.V., 1989. *Tomlinsonia thomassonii*, gen. et sp. nov., a permineralized grass from the Upper Miocene Ricardo Formation, California. *Rev. Palaeobot. Palynol.* 60, 165–177.
- Tieszen, L.L., 1991. Natural variations in the carbon isotope values of plants: implications for archaeology, ecology, and paleoecology. *J. Archaeol. Sci.* 18, 227–248.
- Wall, W.P., 1983. The correlation between high limb bone density and aquatic habits in recent mammals. *J. Palaeontol.* 57, 197–207.
- Yoshida, N., Miyazaki, N., 1991. Oxygen isotope correlation of cetacean bone phosphate with environmental water. *J. Geophys. Res.* 96, 815–820.
- Zazzo, A., Lécuyer, C., Sheppard, S.M.F., Grandjean, P., Mariotti, A., 2004. Diagenesis and the reconstruction of paleoenvironments: a method to restore original δ¹⁸O values of carbonate and phosphate from fossil tooth enamel. *Geochim. Cosmochim. Acta* 68, 2245–2258.