Stable isotope evidence for an amphibious phase in early proboscidean evolution

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The order Proboscidea includes extant elephants and their extinct relatives and is closely related to the aquatic sirenians (manatees and dugongs) and terrestrial hyracoids (hyraxes). Some analyses of embryological, morphological, and paleontological data suggest that proboscideans and sirenians shared an aquatic or semiaquatic common ancestor, but independent tests of this hypothesis have proven elusive. Here we test the hypothesis of an aquatic ancestry for advanced proboscideans by measuring $\delta^{18}O$ in tooth enamel of two late Eocene proboscidean genera, *Barytherium* and *Moeritherium*, which are sister taxa of Oligocene-to-Recent proboscideans. The combination of low $\delta^{18}O$ values and low $\delta^{18}O$ standard deviations in *Barytherium* and *Moeritherium* matches the isotopic pattern seen in aquatic and semiaquatic mammals, and differs from that of terrestrial mammals. $\delta^{13}C$ values of these early proboscideans suggest that both genera are likely to have consumed freshwater plants, although a component of C_3 terrestrial vegetation cannot be ruled out. The simplest explanation for the combined evidence from isotopes, dental functional morphology, and depositional environments is that *Barytherium* and *Moeritherium* were at least semiaquatic and lived in freshwater swamp or riverine environments, where they grazed on freshwater vegetation. These results lend new support to the hypothesis that Oligocene-to-Recent proboscideans are derived from amphibious ancestors.

Barytherium | Eocene | Fayum | *Moeritherium* | Proboscidea

The elephants *Elephas* and *Loxodonta* (order Proboscidea) are the only living remnants of a major adaptive radiation whose origin can now be traced back to the earliest Eocene (≈55 million years ago) in Africa (1). Genomic data place proboscideans within the placental mammalian superorder Afrotheria (2) and the more restricted supraordinal clade Paenungulata, which also contains the aquatic manatees and dugongs (order Sirenia) and terrestrial hyraxes (order Hyracoidea). Genetic evidence has thus far failed to resolve relationships among paenungulate orders (2, 3), but a recent analysis of genomic and morphological evidence provided weak support for a Proboscidea–Sirenia clade (Tethytheria) to the exclusion of Hyracoidea (4). A monophyletic Tethytheria has long been seen as the best explanation for available morphological evidence (5) and is key to the hypothesis [also based on developmental (6) and paleontological (5, 7) evidence] that the common ancestor of elephants and sea cows might have been at least semiaquatic.

Eocene proboscideans were radically different from living elephants in their size, skeletal and dental morphology, and presumably many aspects of their ecology and behavior as well (5, 7–9). *Moeritherium* was tapir-sized and possibly had a prehensile upper lip rather than a trunk (8). *Moeritherium*'s lifestyle has been debated for over a century, with different lines of evidence supporting an aquatic, semiaquatic, or terrestrial mode of life (7, 9–13). Cranial features of *Moeritherium* that are seen in some aquatic or semiaquatic mammals, such as a long, tubular cranium and anteriorly positioned orbits, have previously been cited as evidence for an amphibious lifestyle, but they are not restricted to aquatic mammals (10). The auditory region of *Moeritherium* is more similar to that of extant elephants than to those of sirenians or older proboscideans, and it exhibits none of the characteristics that would facilitate underwater hearing (14). *Moeritherium*'s postcranial morphology is not well known, but similarities to members of the extinct aquatic or semiaquatic clade Desmostylia have been noted (15). The pelvis associated with the most complete axial skeleton of *Moeritherium* has a very small hip socket (E.L.S., unpublished observation on Yale *Moeritherium* skeleton), suggesting hindlimb reduction as in aquatic mammals. It has been argued that many of the features *Moeritherium* shares with sea cows might be convergences resulting in part from adaptation to shared aquatic lifestyle (13, 16, 17).

Much less is known about contemporaneous *Barytherium*. It is likely that the genus occupies a key position in proboscidean phylogeny, being either a sister group of Oligocene-to-Recent elephantiforms (ref. 4, Fig. 1), or perhaps a derived sister group of an older (early Eocene) genus such as *Daouitherium* or *Numidotherium* (1). *Barytherium grave* was first recovered from the Dir Abu Lifa Member of the Qasr el-Sagha Formation in the Fayum region of Egypt, which preserves both nearshore and alluvial deposits (18), and is estimated to have approached the size of modern Asian elephants (11). *Barytherium* remains from Libya derive from evaporite deposits that are probably diagenetic in origin (19). The genus was evidently more plantigrade than modern elephants (20).

Ideally, diverse lines of evidence, such as sedimentology, taphonomy, functional morphology, and analysis of coexisting fauna and flora could be used to determine the paleoecology and habitat of these early proboscideans, but in this case the situation is complicated by the large number of possible environments involved and a dearth of well-preserved postcranial skeletal material. This study employs stable isotopic evidence from tooth enamel to test the hypothesis that early proboscideans were amphibious, and it focuses on two late Eocene genera, *Barytherium* and *Moeritherium*, based on samples recently collected from early late Eocene (≈37 million-year-old) deposits of the Birket Qarun Formation in northern Egypt. The fossil-bearing sediments at the primary fossil locality in the area, Birket Qarun Locality 2 or BQ-2, were deposited during periodic flooding events of a landscape that otherwise might have regularly become ponded and stagnant; vertebrate fossils are predominantly of terrestrial animals, but the presence of shark and marine fish remains, and marine sediments above and below BQ-2, indicate that deposition occurred very close to the coast (21). Therefore the Birket Qarun proboscideans could have inhabited a wide range of habitats, including fluvial, coastal, lagoonal, or estuarine environments.

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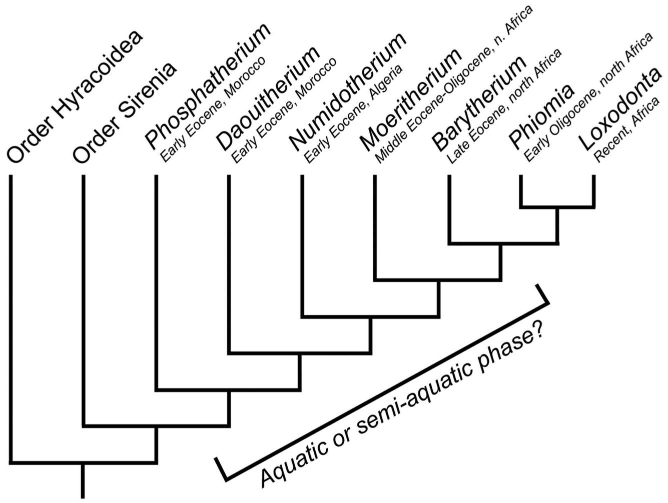

Fig. 1. Phylogenetic relationships of Paleogene proboscideans, from Seiffert (4).

Inferring the Habitat and Dietary Preferences of Early Proboscideans Through Stable Isotope Analysis

The stable isotopes analyzed in this study are those of carbon (in the form of $\delta^{13}C$) and oxygen (as $\delta^{18}O$). Because the degree of isotope fractionation differs in different organic tissues, comparative analyses of fossil taxa should be based on one tissue type (22). Tooth enamel bioapatite is favored for isotopic studies of extinct taxa because of its durability and abundance in the fossil record. Enamel is also not easily altered during diagenesis, giving it an advantage over dentin and bone (23).

Tooth enamel grows in layers from the crown to the base at a rate of ≈30–60 mm/yr in large herbivores (24). Mixing of information from multiple layers during sample preparation provides a mean value of the isotopes incorporated into the enamel structure during amelogenesis; therefore the isotopic ratio of a finite volume of tooth enamel represents a time-averaged value of the individual's body water and diet over the period of enamel formation (24). Eocene mammalian enamel has previously been studied using these methods (25), which are suitable provided that diagenetic alteration has not changed the original *in vivo* isotopic signatures of the teeth.

Studies incorporating carbon and oxygen isotope analyses of tooth enamel hydroxyapatite have demonstrated that they are legitimate proxies for the habitat and diet of aquatic and terrestrial mammals in modern environments (22). Carbon isotope ratios recorded in mammalian tooth enamel can be used as a proxy for diet (26) because different types of vegetation have distinct $\delta^{13}C$ values (27). Such differences result from isotopic fractionation during photosynthesis, productivity changes, and dissolved CO_2 and bicarbonate levels in the surrounding environment (22). Carbon is integrated into mammalian body tissue in a ratio that is proportional to that in which it is taken up by the animal in the form of food (22). Therefore the carbon isotopes incorporated into body tissues record the isotopic signature of the food the mammal eats, and, by correcting for several fractionation steps, allow the $\delta^{13}C$ values of the plants in an environment to be correlated with the $\delta^{13}C$ of tooth enamel in their consumers all of the way up the food chain (22). There is considerable overlap between the $\delta^{13}C$ ranges of certain vegetation types (Fig. 2*B*), but the use of other isotopic proxies (e.g., strontium) can potentially resolve such problems (28).

The standard deviation of $\delta^{18}O$ values in tooth enamel has been found to differ between terrestrial and aquatic mammals, with most terrestrial species showing markedly higher standard deviations than aquatic ones (22). The oxygen isotope ratio recorded in body tissues depends on three factors: the temperature at which the tissue/mineral is deposited, fractionation during metabolic and biological activity within the organism, and the fluxes and isotopic composition of the oxygen-containing matter entering the animal (29). The physiological fractionation of isotopes varies by species (30, 31), being determined by the animal's metabolism and the oxygen exchanges into and out of its body (32), which are, in turn, dependent on its size and mode of life. Because the fractionations for fossil taxa cannot be accurately established, this study does not consider physiological fractionation of oxygen isotopes in any calculations. Assuming that the mammals in this study are homeothermic [and therefore had a constant temperature-dependent fractionation between the enamel oxygen isotopes and body water ratios (33)] removes the temperature-dependent fractionation of $\delta^{18}O$. This assumption leaves the $\delta^{18}O$ values of the oxygen sources and fluxes

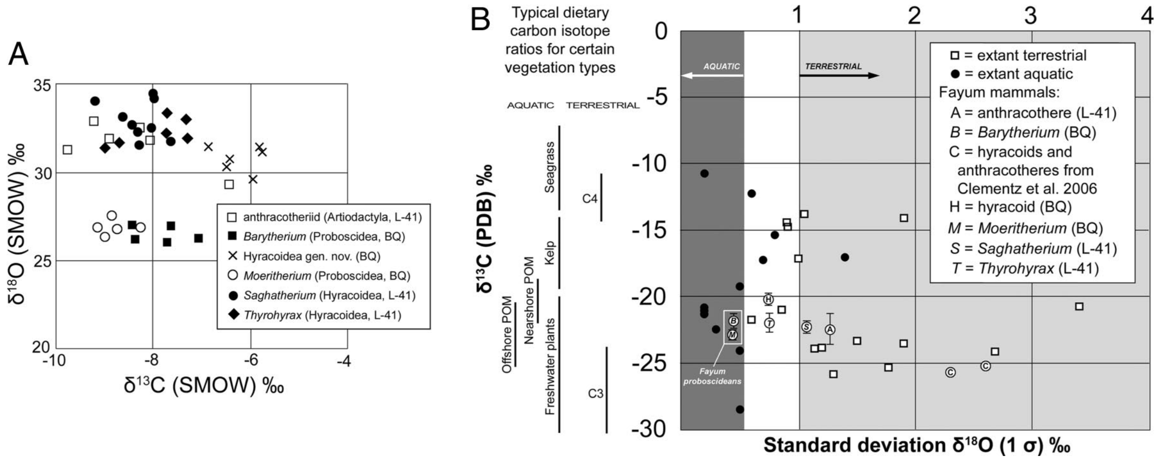

Fig. 2. Carbon and oxygen isotype ratios in fossil mammalian teeth from the Fayum Depression, Egypt, compared with similar data from living and extinct mammals. (*A*) $\delta^{18}O$ and $\delta^{13}C$ data from late Eocene mammals from Egypt. (*B*) $\delta^{13}C$ versus standard deviation of $\delta^{18}O$ for a range of mammalian taxa. Each data point represents average values for one taxon. Taxa falling in the dark gray band (SD $\delta^{18}O < 0.5‰$), are considered aquatic, whereas taxa in the larger light-gray field (SD $\delta^{18}O > 1.0‰$) are likely to be terrestrial. Additional data are from Clementz *et al.* (25, 26) and Kingston and Harrison (39). All $\delta^{18}O$ standard deviations are calculated from values normalized relative to standard mean ocean water (SMOW) (modern value). $\delta^{13}C$ error bars are shown at 1σ for Fayum taxa only. PDB, Pee Dee Formation belemnite standard; POM, particulate organic matter.

Table 1. Mean values of carbon and oxygen isotope ratios for each taxon

Taxon	n	Mean δ^{13}C, ‰ PDB		Mean ($\pm 1\sigma$) δ^{18}O, ‰ PDB	Mean ($\pm 1\sigma$) δ^{18}O, ‰ SMOW
		Enamel ($\pm 1\sigma$)	Ecosystem		
Fossils					
Moeritherium sp.	5	−8.882 ± 0.36	−22.882	−3.887 ± 0.42	26.901 ± 0.44
Barytherium sp.	5	−7.847 ± 0.56	−21.847	−4.255 ± 0.44	26.523 ± 0.45
Anthracotheriid	6	−8.499 ± 1.16	−22.499	0.747 ± 1.23	31.680 ± 1.27
Thyrohyrax meyeri	6	−7.961 ± 0.70	−21.961	1.336 ± 0.73	32.287 ± 0.75
Saghatherium bowni	9	−8.276 ± 0.45	−22.276	2.019 ± 1.04	32.991 ± 1.07
Hyracoid (BQ-2)	6	−6.216 ± 0.44	−20.216	−0.079 ± 0.73	30.828 ± 0.75
Rocks					
Rock from L-41	4	−0.842 ± 0.69		−1.734 ± 2.22	29.122 ± 2.29
Sample L-41a	1	−9.475		1.770	32.735
Rock from BQ-2	9	−0.538 ± 0.31		6.975 ± 1.10	38.100 ± 1.13

"Rock from L-41" does not include sample L-41a, which is displayed separately to show the distinct difference between it and other L-41 results. n = number of specimens successfully sampled for each mammal group. Ecosystem δ^{13}C values were obtained by subtracting an enamel-diet fractionation value of 14‰ from the enamel values.

entering the body, and therefore the environmental surroundings, as the main factor influencing the isotopic values preserved in enamel. The sources of δ^{18}O in the mammal's surroundings are in turn dominated by the oxygen isotope ratio of local meteoric water (33), which is predominantly dependent on latitude and amount of rainfall. Global marine δ^{18}O values change because of global temperature or ice volume variations and are relatively unimportant in this context (34, 35).

Because the body water δ^{18}O of aquatic mammals is dominated by diffusion of water through the skin [up to 98% of total flux (36)], and aquatic mammals are often limited to just a few water sources during their lives, aquatic mammal populations tend to have low standard deviations for enamel δ^{18}O measurements (22). When compared with aquatic mammals, terrestrial mammals have a greater variety of sources from which to obtain their oxygen (including the atmosphere, more varied water sources, and food material), and a terrestrial population will therefore have a relatively high δ^{18}O standard deviation compared with strictly aquatic populations (22). By combining the δ^{13}C ranges of vegetation types with the δ^{18}O standard deviation fields for different habitats, an ecology diagram can be produced, from which the habitat and diet of fossil mammals can be determined (25) (Fig. 2*B*).

Results

The proboscidean samples from the Birket Qarun localities, and the control group (hyracoid and anthracothere) samples from BQ-2 and the terminal Eocene Quarry L-41, exhibit a well defined spread in their isotopic values [Fig. 2*A*, supporting information (SI) Table S1] and do not show any patterns that would suggest diagenetic or chemical alteration of isotopic ratios. Furthermore, the isotopic signature of the BQ-2 rock matrix plots well away from that of the teeth from BQ-2 (Table 1), suggesting that the values obtained from the BQ-2 mammals are reliable proxies for diet and habitat. *Barytherium* and *Moeritherium* are significantly different from all other taxa sampled in both δ^{18}O mean and variance (Kruskal–Wallis and ANOVA, $P < 0.01$, Tukey post hoc test $P < 0.01$; Tables S2–S4). However, the proboscidean δ^{13}C data are different only when compared with the hyracoids (Kruskal–Wallis and ANOVA, $P < 0.01$). When compared with each other, *Barytherium* and *Moeritherium* show a significant difference in carbon isotope values (ANOVA, Kruskal–Wallis, Mann–Whitney, $P < 0.05$, Table S5), but not oxygen isotope values (Kruskal–Wallis, $P = 0.347$, Tukey test $P = 0.9765$). The BQ-2 hyracoid's δ^{13}C values are significantly different in mean and variance from those of all taxa sampled from both quarries (ANOVA, Kruskal–Wallis, Mann–Whitney all $P < 0.02$); its δ^{18}O values are significantly different from those of all other taxa except for the L-41 anthracothere (ANOVA, $P < 0.01$; Mann–Whitney and Kruskal–Wallis, $P < 0.05$).

The data from quarry L-41 (hyracoids and anthracotheres) show considerable overlap, and with one exception there are no significant differences between L-41 species in either mean or variance at the $\alpha = 0.05$ level for either δ^{18}O or δ^{13}C (Kruskal–Wallis, ANOVA, Mann–Whitney, all $P > 0.05$). Only the δ^{18}O values of *Saghatherium* and the L-41 anthracothere are significantly different ($P < 0.1$). The similarity of isotopic values from all L-41 taxa could be an indication that their isotopic compositions were homogenized by diagenetic processes, for instance by fluids that might have passed through the unit after burial. This hypothesis is weakly supported by the fact that one rock sample plots among the teeth (37). However, the molar teeth of hyracoids and anthracotheres are quite similar in their functional morphology, which may indicate that they were consuming similar foods; furthermore, anthracotheres and hyracoids previously sampled by Clementz *et al.* (25) from a slightly younger Fayum quarry also show very similar δ^{18}O and δ^{13}C values. As such, the isotopic overlap of L-41 hyracoids and anthracotheres need not be due to diagenesis, although the possibility should be explored through future sampling of additional, distantly related, mammalian species from the same quarry.

The mean variability between isotope values within individual proboscidean teeth was ±0.11‰ for carbon, and ±0.17‰ for oxygen, far lower than the population variability (approximately ±0.4‰). Where sufficient data were available, intratooth variability in the control taxa was approximately ±0.2‰ for carbon and approximately ±0.3‰ for oxygen. These values are slightly higher than the variability of the standard, which never exceeded ± 0.17‰ for either isotope.

The standard deviations of δ^{18}O values were calculated after conversion of all values (from the current study and the literature) to the SMOW standard. Available data from extant mammals show a clear trend, with terrestrial mammals having δ^{18}O standard deviations of >0.8‰ and aquatic mammals having deviations of <0.8‰. *Barytherium* and *Moeritherium*'s δ^{18}O standard deviations (0.45‰ and 0.44‰, respectively) are lower than the 0.5‰ boundary below which only obligatorily aquatic taxa fall (Fig. 3). The anthracotheres and *Saghatherium bowni* plot within the range of definitively terrestrial taxa. Interestingly, *Thyrohyrax meyeri* and the new hyracoid genus from BQ-2 both plot in the intermediate zone between definitively aquatic and definitively terrestrial taxa (along with taxa as diverse as extant sirenians and elephants; Pliocene pigs, rhinos, and horses, and Oligocene *Desmostylus*). These data unexpect-

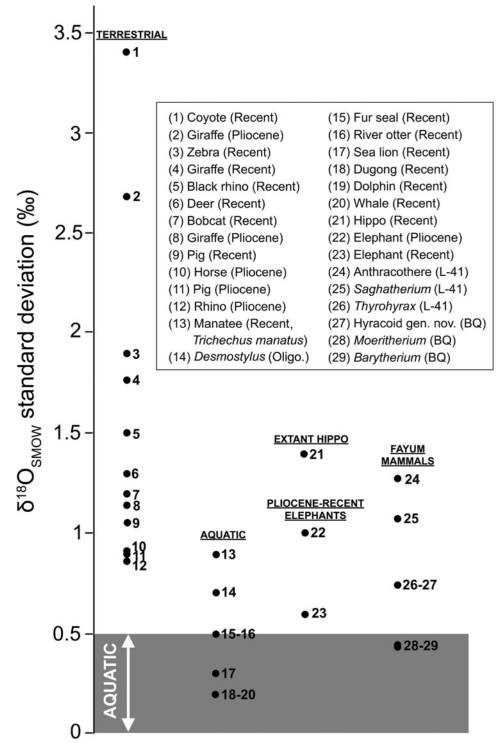

Fig. 3. $\delta^{18}O$ standard deviations for a variety of aquatic and terrestrial taxa and late Eocene mammals from Egypt. Note the sharp break at ≈0.5‰, below which are definitively aquatic extant mammals. Additional data are from Clementz *et al.* (25, 26), Kingston and Harrison (39), and MacFadden *et al.* (41).

edly raise the possibility that, like their Paleogene paenungulate relatives, some early hyracoids might have also been, to some extent, semiaquatic.

Discussion

The new species of *Barytherium* from the Birket Qarun localities has a low oxygen standard deviation (0.45‰) and primary-producer carbon isotope values of −21.847 ± 0.56‰. These data suggest that *Barytherium* was an aquatic or semiaquatic mammal that consumed freshwater plants, near-shore phytoplankton, or offshore particulate organic matter. The molars of *Barytherium* are strongly bilophodont (double-crested) (9), which, by analogy with living bilophodont mammals, are likely to have been used to shear through tough, resistant plant matter. Therefore it seems highly unlikely, based on functional morphology, that *Barytherium* would have been a filter feeder, or consumed large amounts of phytoplankton (if any). Based on the combined isotopic and dental evidence, we suggest that *Barytherium* consumed large amounts of freshwater vegetation.

In interpreting *Barytherium*'s oxygen isotope data, one possible caveat is that extant mammals that weigh over 1,000 kg obtain a larger proportion of their oxygen intake from drinking water than do smaller mammals (38), and if large mammals drink from a water source that is isotopically homogenous, their $\delta^{18}O$ values will be less variable and may falsely indicate an aquatic habitat (26). This may explain the low $\delta^{18}O$ standard deviations of modern elephants, despite their largely terrestrial existence (26). The *Barytherium* species analyzed here was certainly a large mammal, although it was smaller than *B. grave* and extant elephants; body mass estimates for the new species are, unfortunately, currently unavailable. However, unlike extant elephants, *Barytherium* not only shows a very low $\delta^{18}O$ standard deviation but also is relatively depleted in $\delta^{18}O$ (by ≈4‰) compared with the contemporaneous and younger (and presumably largely terrestrial) hyracoids from BQ-2 and L-41. This is another important source of information, because many extant aquatic herbivores are similarly depleted in ^{18}O compared with contemporaneous terrestrial taxa (25, 39). *Barytherium*'s depleted ^{18}O values increase the likelihood that its restricted $\delta^{18}O$ values are due to at least semiaquatic lifestyle and not body mass alone.

Another concern is that mammals inhabiting humid climates may show lower $\delta^{18}O$ variability because they lose little water through evaporation (26). Judging from the diverse arboreal primate fauna preserved at BQ-2 (40), it is quite likely that the Fayum region was covered by lush tropical rain forest during the earliest part of the late Eocene, and so high humidity could conceivably have restricted mammalian $\delta^{18}O$ variability. However in the same region, the hyracoid *Saghatherium* and the anthracotheres from L-41 and younger localities show $\delta^{18}O$ variability typical of terrestrial mammals, suggesting that humidity did not restrict $\delta^{18}O$ variability of Fayum mammals in the late Eocene unless it had a nonuniform effect across different taxa. We do not see high humidity as being a likely cause for *Barytherium* or *Moeritherium*'s low $\delta^{18}O$ standard deviations.

Moeritherium samples show low $\delta^{18}O$ standard deviations (0.44‰) and dietary $\delta^{13}C$ values of −22.882 ± 0.36‰. Again, as with *Barytherium*, this indicates that *Moeritherium* was probably a largely aquatic animal that fed on freshwater plants or offshore particulate organic matter, although a semiaquatic existence with reliance on both freshwater and terrestrial C_3 vegetation cannot be ruled out by the data for either proboscidean genus. As with *Barytherium*, we consider it unlikely that nearshore or offshore particulate organic matter was a major component of *Moeritherium*'s diet because of dental functional morphology, as well as the fluvial nature of the sediment in which the remains were found. The body mass-related caveat detailed above for *Barytherium* could not explain *Moeritherium*'s low $\delta^{18}O$ variability, because members of the latter genus probably fell within the size range of living tapirs. Given the combined evidence from morphology and isotopes, *Moeritherium* probably spent a considerable amount of time in the water, and fed largely on freshwater vegetation. When compared with *Barytherium*, *Moeritherium*'s lower, and significantly different, $\delta^{13}C$ values suggest that the two proboscideans were eating different freshwater plants that were available in the Fayum ecosystem in the earliest late Eocene.

Did these early proboscideans ever move into marine waters? Extant manatee populations have higher $\delta^{18}O$ standard deviations than other aquatic mammals (Fig. 3), presumably because they migrate between freshwater and marine settings that have different isotopic compositions (41). The relatively low $\delta^{18}O$ variability of *Barytherium* and *Moeritherium* suggests that these proboscideans were relatively restricted in their habitat preferences. MacFadden *et al.* (41) suggested that, among living and extinct fully aquatic sirenians, $\delta^{18}O$ SMOW values of ≈25 are indicative of freshwater lifestyle, whereas values of ≈30 are indicative of marine habitat. *Moeritherium*'s $\delta^{18}O$ SMOW mean of 26.9, and *Barytherium*'s mean of 26.5, provide additional evidence that these early proboscideans are more likely to have inhabited freshwater, and not marine, habitats. Given that remains of the two proboscidean genera are now documented at Locality BQ-2 in a purely fluvial setting, we consider it likely that they did not venture far from purely freshwater, riverine, or swampy habitats.

Fig. 2*B* shows a summary of all of the data from Fayum mammals, plotted alongside modern and fossil mammals from the literature on an ecology diagram (25). The control taxa (hyracoids and anthracotheres) plot as being terrestrial, or in the intermediate zone between terrestrial and aquatic ecosystems, with diets more enriched in ^{13}C than would be expected from a purely C_3 diet. Possible reasons include (*i*) the diet–enamel isotope fractionations have been calculated by using figures that are inaccurate; (*ii*) there is another source of food supplementing the diets of these mammals—either freshwater plants or some very early C_4 vegetation [which is unlikely but not impossible (42)]; or (*iii*) the higher than expected $\delta^{13}C$ values from late Eocene mammals represent a background global $\delta^{13}C$ record that was ^{13}C enriched compared with the present. A global signal makes sense, given that data from anthracotheres and *Saghatherium* sampled from slightly younger beds in the Fayum succession by Clementz *et al.* (25) [and considered to be early Oligocene in age by Seiffert (43)] show markedly depleted ^{13}C values compared with L-41 taxa, suggesting that both creatures were terrestrial with diets composed of pure C_3 vegetation (25). However, the $\delta^{13}C$ differences between latest Eocene (L-41) and early Oligocene (Clementz *et al.* samples) lineages might also be explained by the major negative $\delta^{13}C$ excursion that occurred after the Eocene–Oligocene boundary (44).

Finally, the anthracothere results are of interest given that recent phylogenetic studies have placed these artiodactyls as stem hippopotamids (45). All but one of the data points for the L-41 anthracothere are clustered close together, with one seemingly anomalous data point falling far away from the other samples (indeed, far away from all of the other L-41 samples) (Fig. 2*A*). If this outlier is excluded, the $\delta^{18}O$ standard deviation for the L-41 anthracothere falls to ±0.6‰, and thus close to habitually aquatic mammals. This is unlike extant *Hippopotamus amphibius*, however, which has a $\delta^{18}O$ variability of ±1.4‰ despite being semiaquatic (26) (Fig. 3), perhaps because of a varied diet of terrestrial C_4 grasses, freshwater plants, leaves, and fruits (which all contain water with different isotopic ratios), or perhaps because female and young male hippos move between water bodies. *H. amphibius* is similar to aquatic mammals, and unlike terrestrial mammals (and the L-41 anthracothere), however, in having depleted ^{18}O values (26). These data suggest that the L-41 anthracothere is unlikely to have been particularly similar in its ecology to extant hippos.

Conclusions and Prospects

The combined evidence from *Barytherium* and *Moeritherium*'s low $\delta^{18}O$ standard deviations, depleted ^{18}O values, dental functional morphology, dietary $\delta^{13}C$ "ecosystem" values, and occurrence in fluvial deposits suggests that these early proboscideans were largely aquatic mammals that fed on freshwater vegetation in riverine or swampy settings. Because *Moeritherium* and *Barytherium* are placed as consecutive sister taxa of Oligocene-to-Recent proboscideans in the most comprehensive available analysis of early paenungulate relationships (4), these data provide new evidence for a semiaquatic phase in early proboscidean evolution. This hypothesis can be further tested through similar isotopic analyses of demonstrably more primitive proboscideans, such as early Eocene *Phosphatherium*, *Daouitherium*, and *Numidotherium* (5). Unfortunately, at present nothing is known of the postcranial morphology of *Phosphatherium* aside from a single phalanx (1), but Court's analysis of a more extensive collection of *Numidotherium* postcrania (46) led him to speculate that this genus might have been semiaquatic. All of these early proboscideans had bilophodont molars that were similar in morphology to those of *Barytherium* (1), suggesting exploitation of food resources with similar material properties. An additional test of the hypothesis presented here could be provided by similar stable isotopic analyses of other *Barytherium* and *Moeritherium* populations of different ages and geographical locations, for instance those from Dor el-Talha in Libya (47).

Methods

Sample preparation followed that outlined in Clementz and Koch (22) with the sample pretreatment methods of Koch *et al.* (23). Methods are described in greater detail in *SI Materials and Methods*. Control taxa include an unnamed new hyracoid genus from Locality BQ-2, the younger hyracoids *Saghatherium bowni* and *Thyrohyrax meyeri* from the ≈34-million-year-old Quarry L-41, and an unnamed anthracotheriid from L-41 (43). The L-41 fossils were extracted from a well-indurated green mudstone that could represent an ancient swamp or lake (48).

For carbon, a fractionation of approximately +14.0–14.3‰ in tooth enamel compared with diet is cited for herbivores, with larger mammals having the greater offset, although a value of +9.5‰ is given for carnivores (25, 27, 39). However a +12‰ diet–apatite fractionation is calculated for herbivores from South Africa (49). The values used here are +14‰ for herbivores (all of our taxa), and +9.5‰ for carnivores, in line with Clementz *et al.* (25). This was followed by a 0.8‰ fractionation for each successive trophic level an organism inhabits (22, 25, 50). Herbivores were assigned a trophic level of zero, terrestrial carnivores one, and aquatic carnivores two (consistent with ref. 25). Also, ambient $\delta^{13}C$ during the Eocene should ideally be corrected for, but as the exact ages of quarries L-41 and BQ-2 are not known, and the global isotopic curve is very variable near the time periods considered here, it is not possible to make this correction without risking large errors of ±0.5‰ (44). The suggestion that $\delta^{13}C$ values need to be shifted in fossils with respect to modern values to take into account the effect of anthropogenic CO_2 emissions cannot be investigated for similar reasons (27).

SO_2 in the CO_2 released from the teeth during analysis can offset $\delta^{13}C$ values by as much as 4‰, although this study has no way of quantifying this effect (27). Cerling *et al.* (27) found that water-stress and high light levels can both lead to enrichments in ^{13}C in C_3 plants. These fractionations are assumed to have affected all of the Fayum taxa equally because of their close spatial and temporal distribution, and they can therefore be corrected for by comparison with results from the control taxa.

In several mammal species, enamel formation begins in the womb, so isotopic ratios will include influences and fractionations related to growth and weaning, for example the isotopic signature of the mother's milk (22), offsetting isotope ratios substantially (25). It has also been noted that teeth forming later in life contain more ^{13}C and less ^{18}O than those formed earlier (51). Therefore it is important to consistently use teeth known to have formed later in life for isotopic studies wherever possible, e.g., M3 and P4 (25), and this has been done here, although the position (i.e., M1, M2, M3) of *Barytherium* molars could not be determined with confidence. Differences in isotope ratios have been found both within and between jaws and teeth of mammals (32, 52, 53).

In enamel bioapatite, up to 10% of the oxygen is in the form of carbonate, while the rest is present as phosphate and hydroxyl ions (53). Of this carbonate, 90% is in phosphorus sites of apatite (resistant to alteration), while 10% is in hydroxyl sites (54). If the hydroxyl-site oxygen has been replaced, or the isotope ratios have been affected by alteration, $\delta^{18}O$ ratios could have an error of ±1‰ (54). It has not been possible to determine whether this has affected these samples. The isotopic ratios of the carbonate (CO_3) fraction of apatite are considered by many to be liable to diagenetic alteration, whereas the hydroxyl component can be extremely modified by chemical alteration (54). The amount of diagenetic alteration is lower in the enamel phosphate component than in the carbonate component, so oxygen values should be taken from the phosphate sites where possible (37), a recommendation supported by enamel carbonate $\delta^{18}O$ values having a larger range than their respective phosphate $\delta^{18}O$ values (32). However, the carbonate fraction has retained a usable oxygen signal in some fossil specimens (29), and this study considers these carbonate values to give reliable and useful information. $\delta^{13}C$ values are more reliable than $\delta^{18}O$ from apatite structural carbonate, because oxygen is more prone to diagenetic alteration than carbon (55). Diagenesis has also been hypothesized to cause $\delta^{18}O$ values to equilibrate with those of the diagenetic fluid (37), but teeth may retain original oxygen isotope values if the diagenetic system is of both low temperature and water/biomineral ratio (55). If diagenesis has altered the samples significantly, it would be expected to have homogenized all $\delta^{18}O$ values of the vertebrate remains in the rock unit (56). Therefore more than one mammal type has been analyzed from the two main localities, and samples of bulk rock from the localities were tested to determine the extent of any diagenetic overprint.

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