

ARTICLES

Whales originated from aquatic artiodactyls in the Eocene epoch of India

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Although the first ten million years of whale evolution are documented by a remarkable series of fossil skeletons, the link to the ancestor of cetaceans has been missing. It was known that whales are related to even-toed ungulates (artiodactyls), but until now no artiodactyls were morphologically close to early whales. Here we show that the Eocene south Asian raoellid artiodactyls are the sister group to whales. The raoellid *Indohyus* is similar to whales, and unlike other artiodactyls, in the structure of its ears and premolars, in the density of its limb bones and in the stable-oxygen-isotope composition of its teeth. We also show that a major dietary change occurred during the transition from artiodactyls to whales and that raoellids were aquatic waders. This indicates that aquatic life in this lineage occurred before the origin of the order Cetacea.

Phylogenetic analyses of molecular data on extant animals strongly support the notion that hippopotamids are the closest relatives of cetaceans (whales, dolphins and porpoises)^{1–3}. In spite of this, it is unlikely that the two groups are closely related when extant and extinct artiodactyls are analysed, for the simple reason that cetaceans originated about 50 million years (Myr) ago in south Asia, whereas the family Hippopotamidae is only 15 Myr old, and the first hippopotamids to be recorded in Asia are only 6 Myr old⁴. However, analyses of fossil clades have not resolved the issue of cetacean relations. Proposed sister groups ranged from the entire artiodactyl order^{5,6}, to the extinct early ungulates mesonychians⁷, to an anthracotheroid clade⁸ (which included hippopotamids), to weakly supporting hippopotamids (to the exclusion of anthracotheres^{9,10}).

The middle Eocene artiodactyl family Raoellidae^{11–14} is broadly coeval with the earliest cetaceans, and both are endemic to south Asia. Raoellids, as a composite consisting of several genera, have been added to some phylogenetic analyses^{5,10}, but no close relation to whales was found because raoellid fossils were essentially limited to dental material^{11–14}. We studied new dental, cranial and postcranial material for *Indohyus*, a middle Eocene raoellid artiodactyl from Kashmir, India (Fig. 1). All fossils of *Indohyus* were collected at a middle Eocene bone bed extending for about 50 m at the locality Sindkhatudi in the Kalakot region of Kashmir on the Indian side of the Line of Control. Our analysis identifies raoellids as the sister group to cetaceans and bridges the morphological divide that separated early cetaceans from artiodactyls. This has profound implications for the character transformations near the origin of cetaceans and the cladistic definition of Cetacea, and identifies the habitat in which whales originated. Taken together, our findings lead us to propose a new hypothesis for the origin of whales.

Cetaceans and raoellids are sister groups

To investigate the importance of raoellids in cetacean phylogeny, we excluded raoellids from our initial phylogenetic analysis of artiodactyls plus cetaceans. Our data set differed from previous analyses¹⁰ by the addition of several archaic anthracotheres, and some corrected scores for pakicetid cetaceans. This analysis found stronger support for hippopotamid–cetacean sister-group relations than the previous analysis¹⁰, consistent with molecular studies^{1–3}. However, the base of

the artiodactyl cladogram is poorly resolved (see Supplementary Information for details on phylogenetic runs). In a second cladistic analysis (Fig. 2), we added the raoellids *Khirtharia* and *Indohyus* as well as several archaic ungulate groups (condylarths) and found that raoellids and cetaceans are sister groups and that they are the basal node in the Cetacea/Artiodactyla clade, consistent with some previous analyses that used different character sets^{5,6}. Our analysis is the first to show that raoellids are the sister group to cetaceans, resolving the biogeographic conundrum and closing the temporal gap between cetaceans and their sister. Relations between most artiodactyl families higher in the tree are poorly resolved, and our data lack implications for the relations between these families. Our analysis strongly argues that raoellids and cetaceans are more closely related to each other than either is to hippopotamids.

Indohyus shares with cetaceans several synapomorphies that are not present in other artiodactyls. Most significantly, *Indohyus* has a thickened medial lip of its auditory bulla, the involucrum (Figs 1 and 3), a feature previously thought to be present exclusively in cetaceans. Involucrum size varies among cetaceans, but the relative thickness of medial and lateral walls of the tympanic of *Indohyus* is clearly within the range of that of cetaceans and is well outside the range of other cetartiodactyls (Fig. 3). Other significant derived similarities between *Indohyus* and cetaceans include the anteroposterior arrangement of incisors in the jaw, and the high crowns in the posterior premolars.

Characterizing Cetacea

Until now, the involucrum was the only character occurring in all fossil and recent cetaceans but in no other mammals^{5,15,16}. Identification of the involucrum in *Indohyus* calls into question what it is to be a cetacean: it requires either that the concept of Cetacea be expanded to include *Indohyus* or that the involucrum cease to characterize cetaceans. We argue that the content of Cetacea should remain stable and include Pakicetidae, Ambulocetidae, Remingtonocetidae, Protocetidae, Basilosauridae, Mysticeti and Odontoceti^{6,7,9,10,17}. Thus, Cetacea remains a monophyletic group, whereas Artiodactyla remains a paraphyletic group (because Raoellidae are included but Cetacea are excluded). An alternative classification would render both Cetacea and Artiodactyla monophyletic by including Raoellidae in Cetacea and

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by limiting Artiodactyla to those clades one node above the raoellid/cetacean node (Fig. 2). We do not prefer this classification because it causes instability by significantly altering the traditional content of both Artiodactyla and Cetacea.

Characters identified as synapomorphies for Cetacea in some of our most parsimonious trees include: long external auditory meatus, double-rooted P3/, lack of P4/ protocone, M1-2/ metacones present but small, and lack of M1-2/ hypocone. None of these features characterize all modern and extinct cetaceans; the dental characters, for instance, cannot be scored in toothless mysticetes. In addition, all of these characters are found in some mammals unrelated to cetaceans.

We attach particular importance to two character complexes that characterize basal cetaceans, constitute synapomorphic suites for the order, and are of great functional importance. All fossil and recent cetaceans differ from most other mammals in the reduction of crushing basins on their teeth: there are no trigonid and talonid basins in the lower molars, and the trigon basin of the upper molars is very small (for example in pakicetids and ambulocetids) or absent.

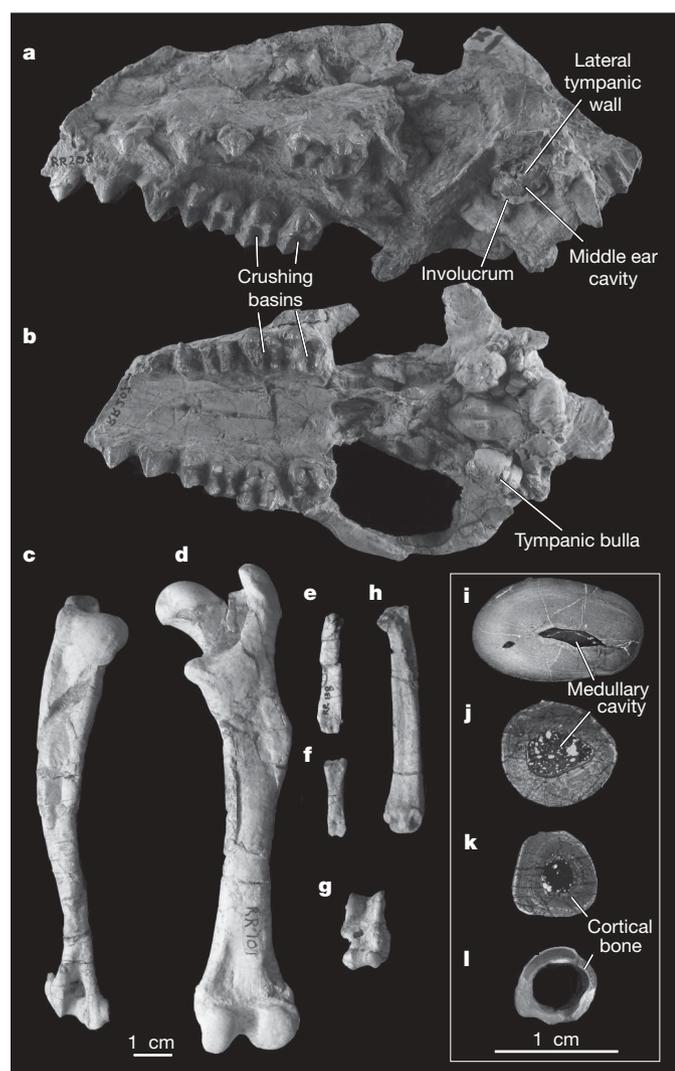


Figure 1 | Osteology of *Indohyus* and cross-sections of long bones of Eocene cetartiodactyls. **a, b**, Oblique lateral view of skull RR 208 (**a**) and ventral view of skull RR 207 (**b**). **c–h**, Posterior views of humerus (RR 149, **c**) and femur (RR 101, **d**), plantar views of metacarpal (RR 138, **e**) and proximal manual phalanx (RR 19, **f**), dorsal view of astragalus (RR 224, **g**), and posterior view of metatarsal (RR 139, **h**). **i–l**, Histological mid-shaft sections for humerus of the pakicetid *Ichthyolestes* (H-GSP 96227, **i**), humerus of *Indohyus* (RR 157, **j**), femur of *Indohyus* (RR 42, **k**) and femur of the artiodactyl *Cainotherium* (IVAU unnum, **l**). Both scale bars are 1 cm; the scale bar near **d** goes with **a–h**, and that near **l** goes with **i–l**.

Crushing basins are large in raoellids (Fig. 1a, b) and other basal ungulates. This implies that a major change in dental function occurred at the origin of cetaceans, probably related to dietary change at the origin¹⁸. Reduced crushing basins also occur in mesonychids, archaic ungulates long thought to be closely related to cetaceans. However, mesonychian molars have wear facets very unlike those of cetaceans^{7,18}, whereas wear facets in raoellids are more similar to wear facets in early cetaceans¹⁴.

The second character complex that identifies cetaceans is the shape of the postorbital and temporal region of the skull. In early cetaceans, this region is long and narrow¹⁹. This affects the sense organs: the olfactory peduncle is long and narrow and the orbits are set close together near the roof of the skull. It also affects oral function, the nasopharyngeal duct is narrow, and the out-lever of the masticatory muscles is long, increasing the closing speed of the jaws. We speculate that the importance of different sense organs was related to these changes, or that changing diet led to a change in food-processing organs.

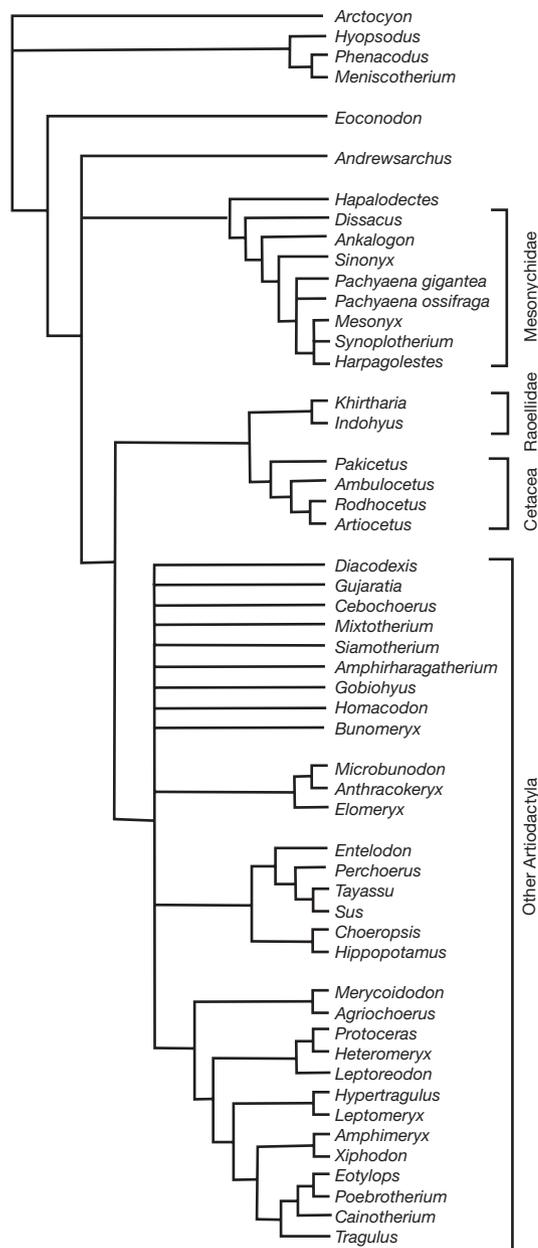


Figure 2 | Phylogeny of artiodactyls, cetaceans and archaic ungulates. The figure shows a consensus cladogram produced by heuristic searches with PAUP (random addition sequence, 1,000 repetitions), using a published data set¹⁰. See Supplementary Information for further details.

Indohyus was aquatic

Behaviourally, the earliest whales (pakicetids) were aquatic waders^{5,20–23}. This led us to investigate whether *Indohyus* was aquatic too. Cortical bone thickness in secondarily aquatic tetrapods is commonly increased at the expense of the medullary cavity, a pattern called osteosclerosis²⁴. Osteosclerosis occurs in early whales^{20,21,25}, manatees^{26,27}, sea otters²⁸, *Hippopotamus*²⁹, beavers²⁹, pinnipeds²⁹ and Mesozoic marine reptiles^{26,30}. Osteosclerosis provides ballast that allows some aquatic taxa to be bottom walkers (hippopotamids) and others to maintain neutral buoyancy in water (manatees)³⁰. Histological sections indicate that the limb bones of *Indohyus* are also osteosclerotic (Fig. 1i–l), in a similar manner to those of pakicetid cetaceans. Our survey of cortical bone thickness in the limb bones of terrestrial artiodactyls shows that this pattern is unusual for that order: in mid-bone cross-sections of the femur, the medullary cavity makes up between 0.60 and 0.75 of the width of the bone, whereas in aquatic mammals the values are lower (*Hippopotamus*, 0.55; pakicetids and ambulocetids, 0.25–0.57). In *Indohyus* this ratio is 0.42, suggesting that *Indohyus* was osteosclerotic and thus aquatic. We interpret the limb osteosclerosis of *Indohyus* to be related to bottom walking and not to slow swimming, because the limbs are gracile and not modified into paddles.

To investigate further the hypothesis that *Indohyus* was aquatic, we studied the stable isotopes of its enamel, a tissue relatively resistant to preburial and postburial alteration of isotopic composition³¹. Enamel $\delta^{18}\text{O}$ values are influenced by the oxygen isotope composition of the food and water ingested by an animal as well as by certain physiological processes (such as sweating, panting and respiration)³². For aquatic species, the flux of environmental water by means of direct ingestion and transcutaneous exchange overwhelms all other oxygen sources³³ and can cause the enamel $\delta^{18}\text{O}$ values of freshwater taxa (for example *Hippopotamus*) to be 2–3‰ lower than those for terrestrial mammals^{33,34}. Mean $\delta^{18}\text{O}$ values for four individuals of *Indohyus* are at least 2‰ lower than those for our comparative sample of Eocene terrestrial and semi-aquatic mammals from formations of India and Pakistan of similar or slightly older age (Fig. 4)^{22,23}. Although not representative of the specific deposits from which *Indohyus* was collected, oxygen isotope values for each ecological type from these sites (namely terrestrial, 24–28‰; semi-aquatic, 23‰ or less) are surprisingly consistent regardless of age or location (Fig. 4). This suggests that temporal and spatial variation in environmental isotope values was relatively minor and was most probably insufficient to account for the extremely low $\delta^{18}\text{O}$ we have reported for *Indohyus*. We did not recover tooth material of other mammals at the

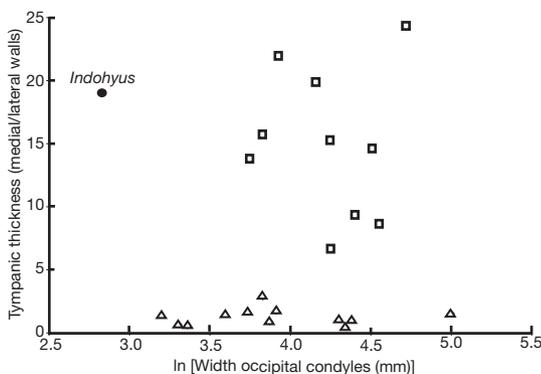


Figure 3 | Plot of the ratio of the thickness of the medial tympanic wall to that of the lateral tympanic wall against the natural logarithm of the width across occipital condyles, showing that the ratio in *Indohyus* is similar to that in cetaceans. In cetaceans (open squares), the medial tympanic wall is inflated and called the involucrum, and the lateral tympanic wall is thinned and called the tympanic plate. In artiodactyls (open triangles), the medial and lateral tympanic walls are more similar in thickness, causing values on the y axis to be closer to 1. See Supplementary Information for further details.

Indohyus site; until such material can be analysed, the most consistent interpretation is that these low values are a result of the aquatic habits of this species.

Supporting evidence for a semi-aquatic life of *Indohyus* comes from examination of its inter-individual variation in $\delta^{18}\text{O}$ values. The overwhelming influx and mitigating influence of isotopically homogeneous environmental water causes the variation in individual $\delta^{18}\text{O}$ values for semi-aquatic and aquatic species (s.d. < 0.5‰) to be much lower than that of terrestrial species (s.d. > 1.0‰)³³. This line of evidence is especially relevant in our study because it does not require an approximation of the mean environmental isotope values for a site through analysis of the associated fauna. Variation in $\delta^{18}\text{O}$ values for *Indohyus* (s.d. = 0.4‰) is extremely low and when compared with species of sufficient sample size ($n > 3$) it is similar to that of the semi-aquatic archaeocete *Pakicetus*. Given that the influence of physiological and environmental factors on body water $\delta^{18}\text{O}$ values is more strongly felt at smaller body sizes³⁵, this low level of variation is particularly compelling for *Indohyus*, with a body mass of less than 50 kg.

To explore the diet of *Indohyus* we studied carbon isotopes. Enamel $\delta^{13}\text{C}$ values are defined by the carbon isotope composition of an animal's diet and can be used to identify the food webs and resources used by an animal³⁶. The $\delta^{13}\text{C}$ values of primary producers at the base of aquatic and terrestrial food webs overlap, but values for freshwater phytoplankton are typically depleted in ^{13}C relative to freshwater macrophytes³⁷, and both types of aquatic producer are depleted in ^{13}C relative to terrestrial C_3 plants^{37–39}. Consumers foraging within food webs fuelled by freshwater phytoplankton (for example freshwater and brackish-water foraging Eocene whales) typically have lower $\delta^{13}\text{C}$ values than species foraging on aquatic macrophytes³⁷ or on terrestrial resources³³ (Fig. 4). Enamel $\delta^{13}\text{C}$ values for *Indohyus* are higher than those for most early cetaceans and are most similar to the $\delta^{13}\text{C}$ values in enamel for terrestrial mammals from early and middle Eocene deposits in India and Pakistan. *Indohyus* could have been feeding on land or in water, but it was clearly eating something different from archaeocetes such as *Pakicetus* and *Ambulocetus*. If the large crushing basins in the molars of *Indohyus* were used for processing vegetation, these $\delta^{13}\text{C}$ values in enamel could come from the ingestion of terrestrial plants or aquatic macrophytes. Alternatively, a more omnivorous diet would suggest that *Indohyus* might have foraged on benthic, aquatic invertebrates in

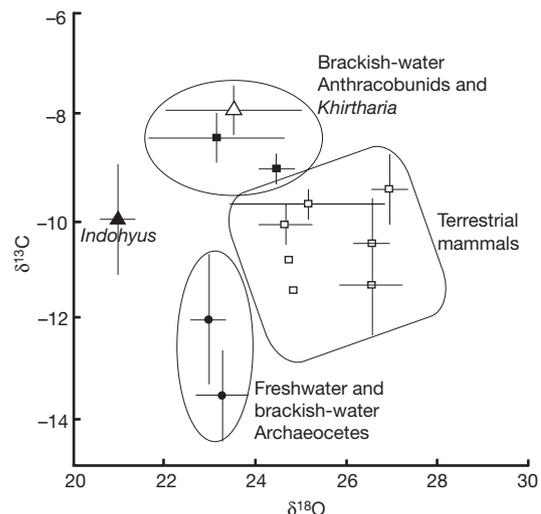


Figure 4 | Bivariate plot of $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values for enamel samples of early and middle Eocene mammals from India and Pakistan. Results are shown as means \pm s.d. for the sample population. Filled triangle, *Indohyus*; open triangle, *Khirtharia*; open squares, terrestrial mammals; filled squares, brackish-water anthracobunids; filled circles, freshwater/brackish-water archaeocetes. See Supplementary Information for details.

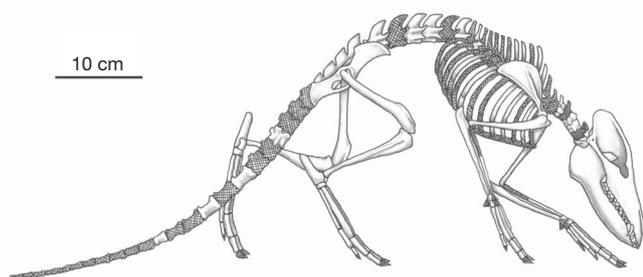


Figure 5 | Skeletal reconstruction of *Indohyus*. Hatched elements are reconstructed on the basis of related taxa.

freshwater systems. Although we cannot exclude the possibility of aquatic foraging by *Indohyus*, $\delta^{13}\text{C}$ values in enamel do suggest that the diet of *Indohyus* differed significantly from that of Eocene whales. A more refined interpretation of the dietary preferences of *Indohyus* will require a study of tooth wear and tooth morphology.

Evolutionary hypothesis for whale origins

Indohyus was a small, stocky artiodactyl, roughly the size of the raccoon *Procyon lotor* (Fig. 5). It was not an adept swimmer; instead it waded in shallow water, with its heavy bones providing ballast to keep its feet anchored. *Indohyus* may have fed on land, although a specialized aquatic diet is also possible.

The modern artiodactyl morphologically most similar to *Indohyus* is probably the African mosedeer *Hyemoschus aquaticus*. *Hyemoschus* lives near streams and feeds on land, but flees into the water when danger occurs⁴⁰. *Indohyus* had more pronounced aquatic specializations than *Hyemoschus* does, and it probably spent a considerably greater amount of time in the water either for protection or when feeding. As indicated by the evidence from stable isotopes, *Indohyus* spent most of its time in the water and either came onshore to feed on vegetation (as the modern *Hippopotamus* does) or foraged on invertebrates or aquatic vegetation in the same way that the modern muskrat *Ondatra* does.

Raoellids are the sister group to cetaceans, and this implies that aquatic habitats originated before the Order Cetacea. The great evolutionary change that occurred at the origin of cetaceans is thus not the adoption of an aquatic lifestyle. Here we propose that dietary change was the event that defined cetacean origins; this is consistent with the cranial and dental synapomorphies identified. Molars of *Indohyus* are markedly different from those of pakicetids, and it is widely assumed that pakicetids ate aquatic prey^{18,23}.

Our working hypothesis for the origin of whales is that raoellid ancestors, although herbivores or omnivores on land, took to fresh water in times of danger. Aquatic habits were increased in *Indohyus* (as suggested by osteosclerosis and oxygen isotopes), although it did not necessarily have an aquatic diet (as suggested by carbon isotopes). Cetaceans originated from an *Indohyus*-like ancestor and switched to a diet of aquatic prey. Significant changes in the morphology of the teeth, the oral skeleton and the sense organs made cetaceans different from their ancestors and unique among mammals.

METHODS SUMMARY

We chose an existing character matrix¹⁰ as the basis for our phylogenetic analysis. We corrected scores of some of the taxa, and made some changes in the taxa included. Details on these taxa, the rationale for using them, and their scores are given in Methods and in Supplementary Table 1.

Tympanic wall thickness was investigated to address the presence of the involucrum quantitatively. We calculated the ratio of medial tympanic wall thickness divided by lateral tympanic wall thickness (see Methods and Supplementary Table 2). Bone histology was studied to investigate the presence of osteosclerosis. It was quantified as the ratio of medullary cavity width divided by bone width in the mediolateral plane (see Methods and Supplementary Table 3).

For analysis of stable isotopes, we prepared powders by following published methods (see Methods and Supplementary Tables 4 and 5). Multiple samples were collected for each species to provide an estimate of population means for carbon and oxygen isotope values³³. About 5 mg of enamel powder was collected from each specimen (tooth) for study of carbon and oxygen isotope values.

Most fossils of *Indohyus* were collected by the late Indian geologist A. Ranga Rao, who discovered the locality about 25 years ago (acronym RR); additional fossils were collected by S.B. and B.N.T. at the same locality (acronym IITR-SB-Kal-S).

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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METHODS

Systematic study. We chose an existing, published character matrix¹⁰ as the basis for our phylogenetic analysis because this matrix is rich in characters and contains most relevant taxa. From this matrix we deleted those artiodactyls and cetaceans that are geologically young or modern and are well represented by fossil relatives (*Odocoileus*, *Bos*, *Ovis*, *Remingtonocetus*, *Protocetus*, *Georgiacetus*, *Basilosaurus*, *Balaenoptera*, *Physeter*, *Tursiops*, *Delphinapterus*, *Camelus* and *Lama*), as well as perissodactyls and non-ungulate taxa.

To this matrix, we added the anthracotheres *Siamotherium*, *Anthracokeryx* and *Microbunodon*, because they are near the base of the anthracotheroid clade (Anthracotheriidae plus Hippopotamidae) and are sometimes thought to be close to early whales⁴. Scores for these taxa, and the sources on which we based the scores, are listed in Supplementary Table 1 (refs 4, 6, 41–46).

We chose *Gujaratia pakistanensis* (formerly *Diacodexis pakistanensis*)⁴⁷ as outgroup for the analyses of cetaceans plus artiodactyls, and we chose *Arctocyon* and *Hyopsodus* as outgroups for the (second) analysis that included all taxa (Fig. 2).

We corrected some of the scores for Pakicetidae¹⁰, because new fossils have been published for this family, in particular cranial material¹⁹ and postcranial material²¹. Corrected scores for pakicetids are also listed in Supplementary Table 1 and were based on original material in the Howard-Geological Survey of Pakistan (H-GSP) collections, currently curated by J.G.M.T.

Raoellidae have been included in several previous phylogenetic analyses relating to early whales^{5,6}. These authors based raoellid scores on *Khirtharia* and *Indohyus*. In the present analyses we have split scores for these animals, with *Khirtharia* scores based mostly on published H-GSP material and one unpublished skull (H-GSP 1979; dentition published⁴⁸, specimen now lost). Scores for *Indohyus* are based on the material in the RR and IITR-SB collections; all raoellid scores are listed, with the specimen number of the fossil on which the score was based, in Supplementary Table 1.

Study of tympanic walls. Tympanic wall thickness was investigated to address the presence of the involucrum quantitatively. The involucrum is the thickened medial wall of the tympanic bone (the ossified wall of the middle ear cavity). The lateral tympanic wall of cetaceans is reduced in thickness (the tympanic plate). To quantify these differences in tympanic walls, we calculated the ratio of medial tympanic wall thickness to the lateral tympanic wall thickness. Lateral tympanic wall thickness was measured with a micrometer (Dyer gauge) just inferior to the tympanic ring, and medial tympanic wall thickness was measured directly across from this site on the other (medial) side of the middle ear cavity (see Supplementary Table 2).

Bone histology. Bone histology was studied to investigate the presence of osteosclerosis. Osteosclerosis is the thickening of the cortical bone. It was quantified as the ratio of medullary cavity width divided by bone width in the mediolateral plane, because left and right cortical thickness plus medullary cavity thickness equals bone width (see Supplementary Table 3). Measurements were taken on the femur with callipers.

Fossil limb shaft fragments were embedded in Buehler low-viscosity epoxy resin and sectioned with a diamond saw. Sections were mounted on frosted glass slides by using epoxy resin. Mounted sections were then ground down and

polished to a thickness of about 75 µm by using a precision grinder with 600, 800 and 1,200 grit paper²⁰ (Fig. 1i–l).

Study of stable isotopes. For the analysis of stable isotopes, three or more specimens of each species were analysed (when available; see Supplementary Tables 4 and 5) to provide a robust estimate of the population mean and s.d. for carbon and oxygen isotope values³³. About 5 mg of enamel powder was collected from each specimen, either by drilling directly from the tooth or by grinding enamel chips in an agate mortar and pestle. Before collection, contaminants were removed by abrading the outer surface of the specimen.

Preparation of powders for analysis of stable isotopes followed published methods³¹. Powders were first transferred to 1-ml microcentrifuge vials and then soaked sequentially overnight in about 0.20 ml of a sodium hypochlorite solution (1–2 g dl⁻¹) and then in about 0.20 ml of calcium acetate buffered acetic acid (pH about 5.1). On addition of each reagent, samples were agitated for 1 min on a Vortex Genie vortex mixer. After each soak, the supernatant was removed by aspiration and the residual powder was rinsed five times with deionized water. Samples were then freeze-dried overnight and about 1.5 mg of powder from each was weighed into individual test tubes for analysis on a Thermo-Finnigan gas bench autosampler attached to a Thermo Finnigan Delta^{plus} XP continuous-flow isotope-ratio mass spectrometer at the University of Wyoming Stable Isotope Facility.

All values for stable isotopes are reported in delta (δ) notation, using the equation $\delta(\text{‰}) = 1,000 \times (R_{\text{sample}}/R_{\text{standard}} - 1)$, where R_{sample} is the observed isotope ratio of the sample (¹³C/¹²C or ¹⁸O/¹⁶O) and R_{standard} is the accepted ratio for an appropriate international standard (Vienna Pee Dee belemnite for δ¹³C; Vienna Standard Mean Ocean Water for δ¹⁸O). Analytical precision is typically better than 0.1‰ for δ¹³C values and 0.2‰ for δ¹⁸O values (±1σ).

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