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Brief Communication

The endocranial anatomy of protocetids and its implications for early whale evolution

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Abstract

Extant whales, dolphins, and porpoises result from a major macroevolutionary lifestyle transition that transformed land-dwelling cetaceans into fully aquatic species. This involved significant changes in sensory systems. The increase in brain size relative to body size (encephalization quotient) is an outstanding feature of modern cetaceans, especially toothed whales. Conversely, olfactory capabilities are assumed to have diminished along this transition, with airborne olfaction becoming less relevant. The extent and timing of olfactory reduction remain obscure due to challenges in accessing well-preserved fossil endocranial anatomy. This study shows that early cetaceans had already evolved an increased encephalization quotient, and that their olfactory apparatus was likely not yet under selective pressure leading to its reduction. We demonstrate this through an analysis of the extinct whale, *Protocetus atavus*, a member of the middle Eocene semiaquatic cetacean group Protocetidae. We provide the first documentation of its endocranial anatomy using high-resolution computed tomography and compare it to other early cetaceans as well as extant mammals. We conclude that cetaceans increased their brain size earlier than previously thought, while relying on a well-developed olfactory system at a time when they were still partly terrestrial.

Keywords: brain, Cetacea, encephalization, endocast, olfaction, Protocetidae

Introduction

The evolutionary history of cetaceans represents one of the most remarkable transitions from terrestrial to fully aquatic lifestyle. Their fossil record documents an exceptional array of species that shifted from small-bodied, hoofed terrestrial mammals into highly specialized, aquatic carnivores (Marx et al., 2016). This shift entailed profound changes in their sensory systems, notably in their encephalization and olfactory capabilities (Kishida et al., 2015). Encephalization, the relative increase of brain size, is a key feature in the evolution of modern cetaceans, especially within the odontocetes (toothed whales [Marino et al., 2004]). This process has been associated with the development of complex social behaviors, echolocation, and advanced cognitive functions (Racicot, 2022). In contrast, olfaction is assumed to have undergone a reduction in early cetaceans as they adapted to an aquatic environment (Thewissen & Nummela, 2008). Terrestrial mammals rely heavily on airborne olfaction (e.g., to detect food [Negus, 1958]). As cetaceans evolved to live underwater, it has been argued that airborne olfaction became less relevant, leading to a regression of the olfactory apparatus, particularly in odontocetes (Kishida et al., 2015).

Despite this general trend, our understanding of the extent and timing of olfactory reduction in early cetaceans remains incomplete (Berta et al., 2014). This has been hampered by the difficulty of accessing the endocranial anatomy of well-preserved fossils. Encephalization and olfactory capabilities in extinct species have been assessed through the study of natural brain endocasts (sediment-filled cranial cavities) and opportunistic observations of other bony structures associated with olfaction, such as the ethmoturbinals (Stromer, 1903). More recently, computed tomography (CT) has also been used to access early whale endocranial anatomy (Bajpai et al., 2011; Marino et al., 2000; Peri et al., 2020).

Here, we explore the endocranial evolution of cetaceans focusing on protocetids. This middle Eocene group (likely paraphyletic; Antar et al., 2023; Gohar et al., 2021; Lambert et al., 2019) occupies a key position in cetacean evolution, comprising its last semiaquatic representatives. Protocetid endocranial anatomy has hitherto been documented in *Indocetus* and *Aegyptocetus*, for whom a partial, natural

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endocast has been described and turbinates have been virtually reconstructed, respectively (Bajpai et al., 1996; Peri et al., 2020). We assess the early evolution of encephalization and olfactory capabilities in cetaceans, documenting for the first time the endocranial anatomy of *Protocetus atavus*, the type species of the family. Using high-resolution microtomography, we reconstructed its endocranial anatomy and extracted quantitative data. We also reanalyzed the endocranium of *Aegyptocetus*, which complements that of *Protocetus* and allows for an unprecedented understanding of the family's brain and nasal cavity anatomy. We then analyzed these data within a wide comparative framework to show the evolutionary timing of changes in encephalization and sensory specialization to clarify the adaptive pathways that shaped the cetacean brain and olfactory apparatus in the context of their lifestyle transition.

Methods

Specimens and CT data acquisition

Protocetus atavus (holotype skull: SMNS-P-11084; Staatliches Museum für Naturkunde Stuttgart), from the Lower Building Stone Member, Mokattam Formation (Gebel Mokattam, northern Egypt), is most likely middle Lutetian (middle Eocene; Gingerich, 1992). The skull was CT-scanned with a Nikon-XT-H320 system as a multiscan (fivefold; 225 kV, 220 µ A, 1.5 mm-thick copper filter, 4,476 projections, 354 ms exposure) at an isometric resolution of 53.2 µm. The stack was downgraded to 8-bit images with an isometric resolution of 0.16 mm, and its contrast was enhanced with Contrast Limited Adaptive Histogram Equalization (CLAHE; Rueden et al., 2017) to help segmentation. Endocast segmentation and volume measurement were performed using Avizo 3D 2021.2 (Thermo Fisher Scientific) and Biomedisa (Lösel et al., 2020).

The skull was also surface-scanned (SHINING-EinScan-ProHD, EXScanPro 3.7.0.3) for illustrative purposes. The partial endocast of *Aegyptocetus tarfa* (MSNUP I15459, holotype; Museo di Storia Naturale dell'Università di Pisa) was segmented from published CT data (Peri et al., 2020). All models were rendered in orthographic views using Blender 4.1 (Blender Online Community, 2018).

The resulting endocast was compared to a set of extant mammals (Martinez et al., 2024c). High-resolution micro-CT scans of intact skulls or heads from one representative species for each of the 29 extant orders were either newly acquired or acquired from MorphoSource (Boyer et al., 2016) (data available on figshare, doi: 10.6084/m9.figshare.28218473). Only these were included in what is referred to herein as the "strict" dataset, for which we performed all segmentations, following the same protocol.

Encephalization

Statistical analyses were done with R v4.4.2 (R Core Team, 2024) (data and scripts available on figshare, doi: 10.6084/m9.figshare.28218473). Assessing the encephalization quotient (EQ) requires measuring (or estimating) brain mass and body mass. All brain masses were estimated based on endocast volume, following the approach of Waugh and Thewissen (2021; see equation 3 therein), except for the actual masses taken from Burger et al. (2019). Waugh and Thewissen's analysis yielded negative allometry between brain mass and endocast volume. They pointed out that the bowhead whale they sampled was outlying, and cautioned against EQ estimates for fossil mysticetes in general, mostly because of their very developed rete mirabile (blood vessel network part of the brain adnexa). Indeed, removing the bowhead whale from their dataset and rerunning the same analysis (pgls function using Pagel's lambda, caper package; Orme et al., 2023) yielded a very tight relationship (pseudo $R^2 = 0.997$; piecewiseSEM; Lefcheck, 2016) that is not significantly different from isometry (slope = 0.98; $p_{\text{isometry}} = 0.13$). But even for this dataset devoid of the outlying bowhead whale, there is a rather large range of ratios of brain volume to endocast volume (71%–98%). For our "strict" analyses, which do not include taxa with large rete mirabile (i.e., basilosaurids and at least some mysticetes; see *Results*), we, therefore, used the minimum, maximum, and median of these ratios to estimate brain mass based on endocast volume. For the analysis that includes other cetaceans, we used the estimate stemming from the brain mass ∼ endocast volume regression previously published (Waugh & Thewissen, 2021).

To estimate body mass, we have also followed the latter study and used maximum width at occipital condyles (OCW). An OCW of 68 mm (our measurement) yielded a mass of 148 kg for *Protocetus*. A linear regression of log10-transformed variables was done with pgls function to use a similar approach (as above), using an existing maximum clade credibility DNA-only node-dated timetree (Upham et al., 2019) pruned to match our sample.

A broader comparison was also performed, combining datasets encompassing various extant mammals (Burger et al., 2019), extinct cetaceans (Waugh & Thewissen, 2021), and other extinct mammals (Bertrand et al., 2022).

The EQ discussed herein was finally calculated as the ratio between the brain mass (measured or estimated) and the expected mass given by the allometric regression of Burger et al. (2019).

Olfactory-relevant part of the endocast

Because of the uncertainty in the nature of the tissues filling the part of the endocast herein referred to as the olfactory tract, three regions were considered to assess the volume viewed as olfactory relevant (Figure 1; Supplementary Table S1). The smallest region only comprises the olfactory bulb (OB). The two other regions additionally include the olfactory tract, with two possible definitions of its posterior end.

To assess the relative size of the olfactory-relevant part of the endocast, the same analytical approach as for the EQ was used: One analysis was run with the "strict" dataset and the other was run with an extended dataset. In this case, the latter only includes the data of Bertrand et al. (2022), as other datasets did not document individual OB volumes.

Results

Brain endocast

The cranial CT scan of *Protocetus* revealed a crucial documentation of early cetacean endocranial anatomy, including well-preserved endocast volumes for both the overall brain and the OBs, which are both relatively large. Most of the shape of the endocast is clearly visible on the scan, with areas of suboptimal contrast being restricted to the cerebellar and

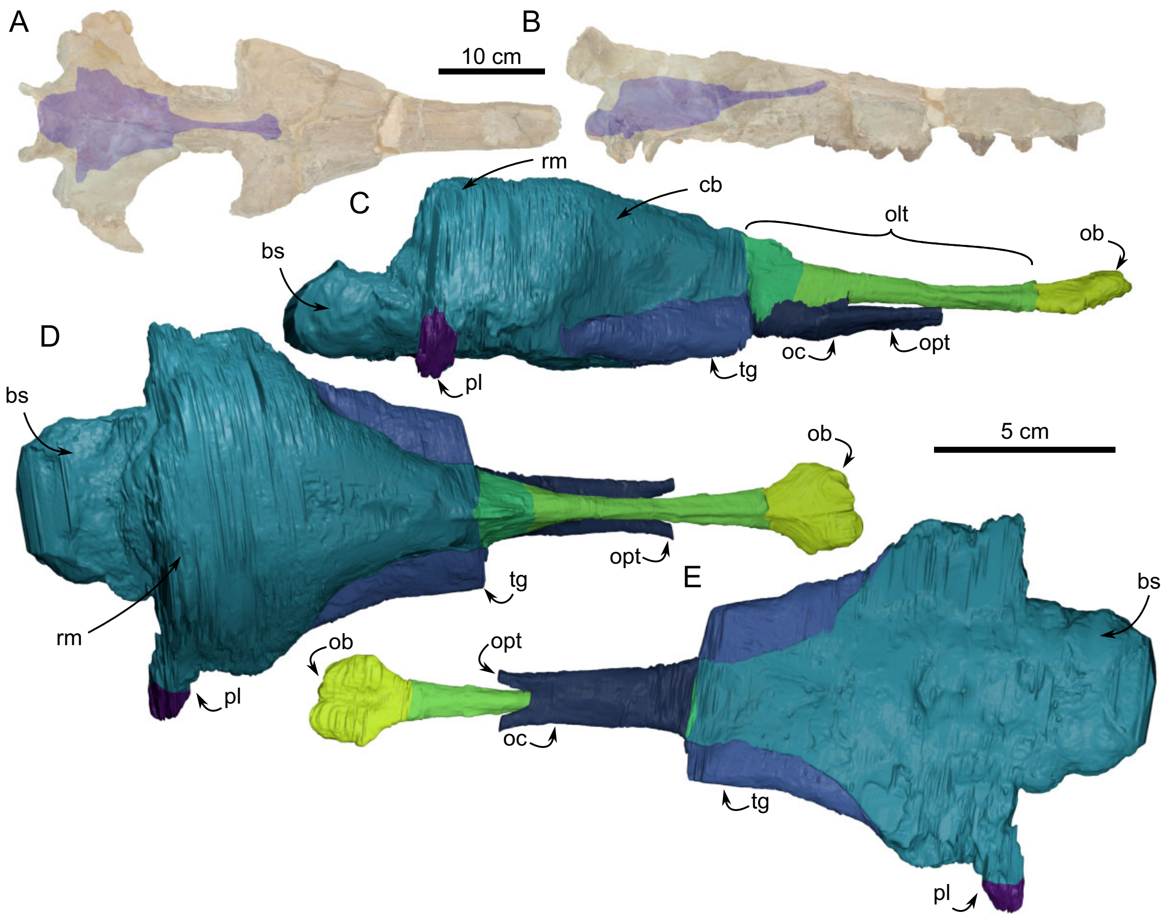

Figure 1. Brain endocast of *Protocetus atavus* (SMNS-P-11084, holotype). (A–B) Overall views of the skull (semitransparent) in dorsal and right lateral views, respectively. (C–E) Isolated endocast in right lateral, dorsal, and ventral views, respectively. Two potential positions of the posterior end of the olfactory tract are displayed (shades of green). The trigeminal ganglion and issuing nerve were cut at the level of the split with the olfactory tract (posterior-most position). bs = brainstem; cb = cerebrum; ob = olfactory bulb; oc = optic chiasm; olt = olfactory tract; opt = optic tract; pl = petrosal lobule; rm = rete mirabile; tg = trigeminal ganglion. Scale bars apply to A–B and C–E, respectively.

petrosal regions (Figure 1; Supplementary Table S1). The overall shape of the posterior region of the endocast and the fact that the cerebellar hemispheres are not visible suggest that a posterior rete mirabile was present. Its extent seems to be relatively modest, as in other non-Pelagiceti cetaceans (Orliac & Thewissen, 2021), though it reaches roughly the same level as the cerebrum dorsally, as in *Indocetus* (Bajpai et al., 1996) (and probably *Georgiacetus*; Smith et al., 2019). This differs from basilosaurids, where the rete is more extensive, especially dorsally (Dart, 1923; Uhen, 2004).

The posterior half of the cerebrum is triangular in dorsal view, as it strongly tapers anteriorly to produce the olfactory tract. This tapering is progressive, it is hence difficult to locate the exact position of the posterior end of this tract. The latter is relatively long and narrow, quite more so than in *Remingtonocetus* (Bajpai et al., 2011) or *Aegyptocetus* (Supplementary Figure S1). The olfactory tract widens anteriorly to produce well-formed OBs, of which the ventral side (limited by the cribriform plate) faces anteroventrally. The bulbs occupy 2.9 cm^3, which represents 1.2% of the whole endocast (and up to 3.8% together with the olfactory tract, see the *Methods* section and Figure 1). A substantial volume of the endocast is occupied by a lateroventral bulge running along part of the posterior half of the cerebrum. We tentatively ascribe it to the trigeminal ganglion (= gasserian ganglion) and surrounding adnexa. The unpaired cavity for the optic chiasm starts ventral to the base of the olfactory tract. It quickly separates into the optic canals, which end anteriorly in the large canal for the trigeminal nerve. The latter follows the olfactory tract along its lateral side to exit the skull through the sphenorbital fissure. A very similar configuration is seen in *Aegyptocetus*. The bulges ascribed to the trigeminal ganglia are better preserved in the latter, which shows that the left and right bulges are distinct from each other and convex ventrally (Supplementary Figure S1). Such a bulge is not seen in the segmentation made for *Remingtonocetus* (Bajpai et al., 2011) but has been described in basilosaurids (Dart, 1923; Gingerich, 2015; Marino et al., 2000).

Nasal cavity

Combined observations of the bony olfactory apparatus in the nasal cavity of *Protocetus* and *Aegyptocetus* suggest that none of this apparatus' elements were substantially reduced in such early whales. In *Protocetus*, the dorsal nasal meatus (dnm) forms a deep notch along the entire length of the nasal (Figure 2). This meatus is found in *Remingtonocetus*

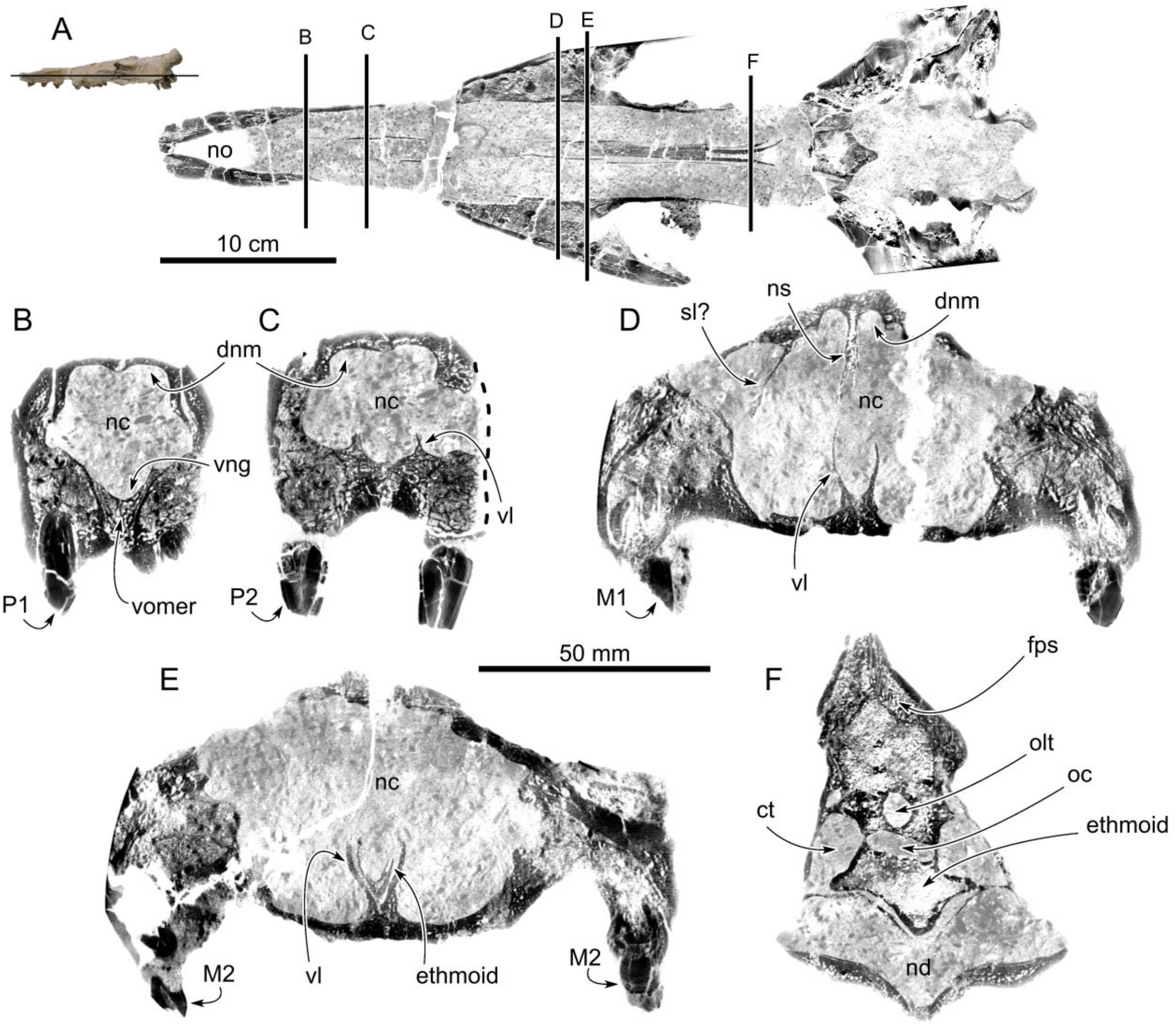

Figure 2. CT sections through the skull of *Protocetus atavus* (SMNS-P-11084, holotype). (A) Horizontal section (located in the inset), where the positions of the following coronal sections are located. (B–F) Selected coronal sections. Grayscale was inverted, and the sediment filling the nasal cavity and nasopharyngeal duct was lightened for clarity. ct = canal for trigeminal nerve; dnm = dorsal nasal meatus; fps = frontal–parietal suture; nc = nasal cavity; nd = nasopharyngeal duct; no = nasal opening; ns = nasal septum; oc = optic chiasm; olt = olfactory tract; sl? = potential semicircular lamina; vl = vomer lamina; vng = vomeronasal groove. Scale bars apply to (A) and (B–F), respectively.

(Bajpai et al., 2011), *Saghacetus*, and at least some extant mysticetes, but not in extant odontocetes (Godfrey et al., 2013). Most turbinals were eroded during fossilization. The roots of some turbinals are visible, starting anteriorly at the level of P3 (and up to the suture with the frontal, up to three to four roots of ethmoturbinals are visible on a single coronal cross-section). The nasal septum is partly preserved, anteriorly from the level between P4 and M1 to the distal side of M3. It sends ventrally two thin laminae that join the dorsal ends of the ethmoid (described below). The vomer starts anteriorly from the level of the canine. Anteriorly, it forms a median gutter on the floor of the nasal cavity (vomeronasal groove; Patel et al., 2024). From the level of P2, it forms dorsoventrally directed laminae (also described in *Dorudon*; Uhen, 2004). They extend posteriorly up to M3. The ethmoid starts anteriorly at the level between M1 and M2, as a narrow bone with a V-shaped cross-section, lodged medially within the furrow formed by the vomer laminae. Proceeding posteriorly, it widens mediolaterally and dorsoventrally and becomes very spongy. A posterior break in the fossil makes the identification of its suture with the presphenoid difficult. The endocranial anatomy of *Aegyptocetus* seems to closely match that of *Protocetus*. The holotype of *Aegyptocetus* further displays a full set of exquisitely preserved, highly convoluted turbinals. It is hence quite likely that this phenotype was found in *Protocetus*, too.

The nasopharyngeal duct is large. The palatine's horizontal lamina starts cranially at the level of P4, and then forms the anterior end of the nasopharyngeal duct. The palatine–pterygoid ventral suture is difficult to locate (because of preservation); posteriorly, the lateral recess of the nasopharyngeal duct (see Bajpai et al., 2011) is well developed, and exits the skull rather posteriorly.

The newly acquired CT data allowed us to identify a few additional features that were not observed in previous descriptions solely based on the external morphology of the skull (Fraas, 1904; Kellogg, 1936). The exact posterior extension of the ascending process of the premaxilla could not be identified as it ends in a region not preserved in the holotype (Supplementary Figure S3; an old restoration has been

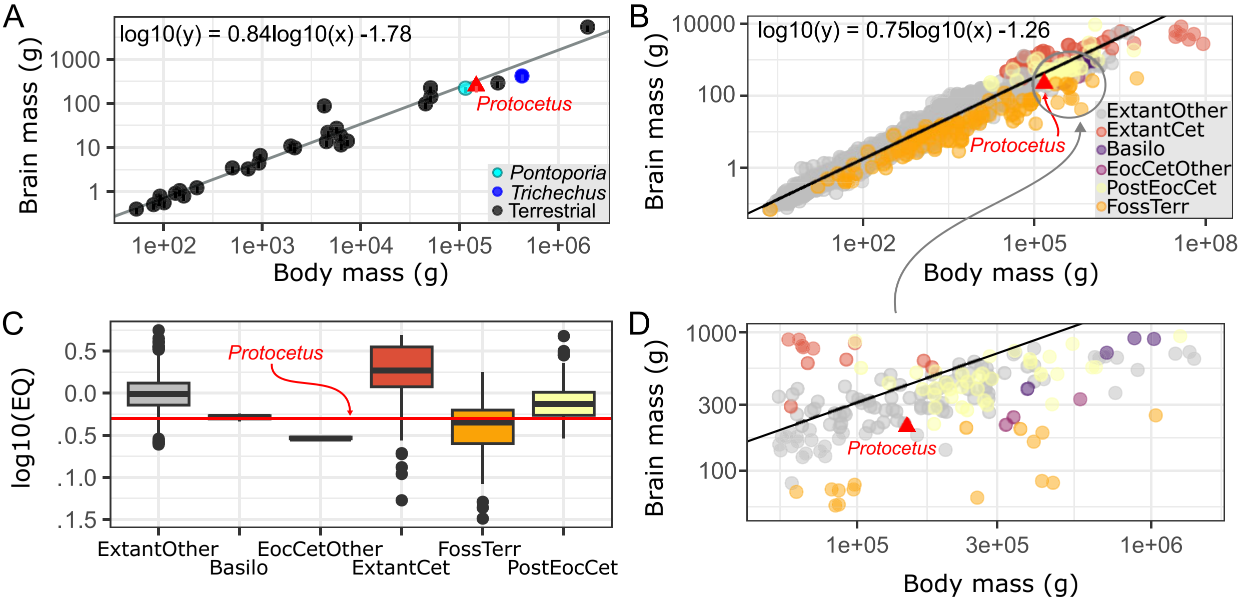

Figure 3. Scaling of the whole brain mass across mammals. The data stem from our acquisition ("strict" dataset; A) or a combination with previous studies (B–D). For the plots that do not include taxa with large rete mirabile (A), the points are the median brain mass estimates, and error bars correspond to the minimum and maximum estimates. For (B–D), the estimate based on equation (3) of Waugh and Thewissen (2021) was used. For (C), the encephalization quotient (EQ) was calculated as the ratio between the measured brain mass (or estimates based on the regression of Waugh & Thewissen 2021) and the expected mass given by the regression of Burger et al. (2019). (D) is a detailed view from (B). Regression lines correspond to pgls regressions made with our strict dataset (A) or from other studies (B, D) (Burger et al., 2019). Basilo = basilosaurids; EocCetOther = non-basilosaurid Eocene cetaceans; ExtantCet = extant cetaceans; ExtantOther = non-cetacean extant mammals; FossTerr = extinct terrestrial taxa; Post-EocCet = post-Eocene extinct cetaceans.

made filling the missing area with synthetic material). The posterior end of the nasals and anterior end of the frontals are not well preserved either, and the exact position and shape of the dorsal suture between these bones could not be determined. The lacrimals are poorly preserved, and their suture with the other bones—the jugal in particular—is hard to trace. As in *Dorudon* (Uhen, 2004), the jugal extends far anteriorly. Internally, we could trace it up to the level of the posterior edge of P3. The infraorbital canal can be traced along the maxilla and the jugal; the position of the infraorbital foramen (where the canal exits the skull anteriorly) is probably slightly anterior to the level of P4.

Discussion

Encephalization

The brain of cetaceans has long been recognized to be exceptionally large (e.g., Lacépède, 1804). It is now understood that all extant whales have large brains in absolute terms, but relative to their body, only toothed whales stand out at the scale of mammals (Burger et al., 2019). At least in some cetaceans, the substantial volume of nonbrain tissues in the cranial endocast prevents it from being a straightforward proxy for brain size (Waugh & Thewissen, 2021). A large rete was most likely present in the first Pelagiceti (basilosaurids), whereas earlier whales differ in this regard (Bajpai et al., 1996; Orliac & Thewissen, 2021). The endocranial anatomy of *Protocetus* agrees with this observation. Compared to extant mammals that have been segmented in a strictly consistent fashion (one arbitrarily selected species for each extant mammalian order; Figure 3A), the whole brain of *Protocetus* appears to be as large as expected for its body size (falling close to the regression line). Within a broader dataset, including both extant and extinct species (Figure 3B–D), the encephalization of *Protocetus* falls within the lower distribution of extant non-cetacean mammals and basilosaurids. Other Eocene cetaceans have relatively smaller brains, and most post-Eocene (including extant) cetaceans have relatively much larger brains. A significant increase in encephalization in late Eocene cetaceans (basilosaurids) has previously been detected (Waugh & Thewissen, 2021). Adding *Protocetus* to the dataset nuances this view, as we found its EQ to be quite higher than the three other middle Eocene cetaceans included herein, i.e., *Remingtonocetus*, *Dalanistes*, and *Rodhocetus*. Protocetidae is widely viewed as a paraphyletic family, and *Protocetus* has been recovered as more closely related to basilosaurids than any of the three other aforementioned cetaceans (Antar et al., 2023; Gohar et al., 2021; Lambert et al., 2019). It is hence possible that an increase of encephalization took place in a clade including *Protocetus* and more recent cetaceans but excluding these three other early-diverging taxa. At a broader scale, the Eocene has been argued to be a time when several mammalian lineages—including artiodactyls—independently acquired increased encephalization (Bertrand et al., 2022). Relatively high EQ was most likely present before the divergence of cetaceans from other artiodactyls. The further increase of EQ, so far associated with basilosaurids (hence the clade Pelagiceti; Waugh & Thewissen, 2021), might hence have taken place as early as the middle Eocene, within a more inclusive clade (that includes *Protocetus*).

This conclusion has to be tempered by the fact that the body masses of *Remingtonocetus*, *Dalanistes*, and

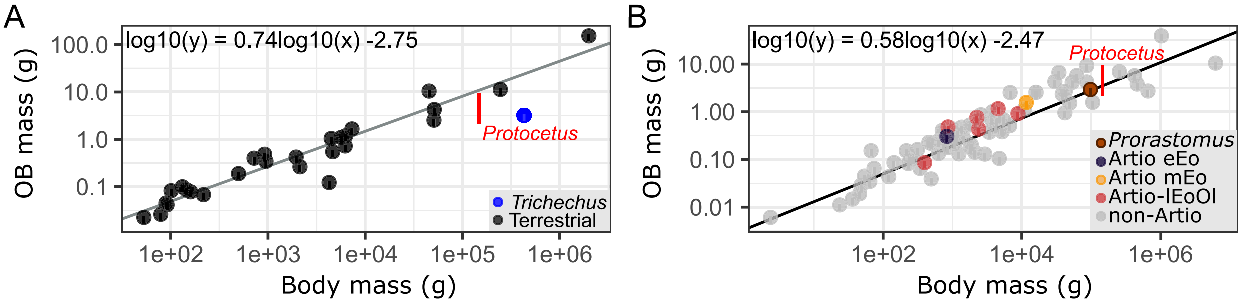

Figure 4. Scaling of the olfactory bulb mass across mammals. The data stem from our acquisition ("strict" dataset; A) or a combination with a previous study (B). The points are the median olfactory bulb mass estimates; error bars correspond to the minimum and maximum estimates. Regression lines correspond to pgls regressions made with our strict dataset (A), or from other studies (B; Bertrand et al., 2022). The error bar of *Protocetus* also includes the uncertainty associated with the olfactory-relevant part of the endocast (see the *Methods* section). Artio eEo/mEo/lEooOl = artiodactyls from the early Eocene, middle Eocene, late Eocene, or Oligocene, respectively.

Rodhocetus are estimated to be much larger than that of *Protocetus*, which might artificially lower their EQ. This emphasizes a more general issue that applies to traits that are considered in relative terms, but that can also be relevant in absolute terms: If body size is also under selective pressure—which is understood to be the case with its overall increase in early cetaceans, for instance (Montgomery et al., 2013)—it is not straightforward to disentangle a change of organ size from the overall size change, nor it is simple to draw conclusion about the pressures acting on the relative trait (here EQ). A seemingly stable relative trait could very well result from an increase (or decrease) of the absolute traits from which the relative trait is drawn. This is indeed what has been argued for the joint increase of body and brain sizes in early cetaceans (Montgomery et al., 2013). From a neurological perspective, assuming that neuronal density remains relatively stable within a given clade, the absolute volume of the brain or its components could serve as a relevant proxy for brain function. This is because the absolute brain volume is likely correlated with the absolute number of neurons, which is a crucial metric for certain cognitive or sensory functions, such as olfaction (Kverková et al., 2022).

Olfactory apparatus

Contrary to previous assumptions that olfactory structures diminished early during cetacean evolution, our results show that *Protocetus* retained substantial olfactory structures comparable to terrestrial mammals. Many components of the olfactory apparatus are soft tissues. Setting exceptional preservation cases aside, the paleobiology of olfaction relies on skeletal proxies. These notably comprise the OB endocast, olfactory turbinals, and cribriform plate (Bertrand et al., 2022; Bird et al., 2018; Martinez et al., 2024b). Most of the turbinals in *Protocetus* are not preserved. However, given the overall similarities in endocranial anatomy between *Protocetus* and *Aegyptocetus*, it is highly probable that their turbinal anatomy was also comparable. The nasoturbinals, maxilloturbinals, and ethmoturbinals (olfactory turbinals) were identified in *Aegyptocetus* and appear potentially reduced compared to extant terrestrial artiodactyls (Peri et al., 2020). However, the olfactory turbinals in *Aegyptocetus* are highly convoluted and densely packed, contrasting sharply with the condition observed in extant cetaceans and sirenians (Martinez et al., 2024c). Regarding the brain endocast, the OB of *Aegyptocetus* and *Protocetus* share the same general conformation, i.e., sitting at the end of an elongated tract. It is not straightforward to infer the actual nature of the tissues filling this tract. The similarly elongated tract of a dissected bowhead whale (*Balaena mysticetus*) appears to be made of different tissues from the OB (Thewissen et al., 2011), which would suggest that the volume of the tract should not be seen as olfactory relevant (as that of the OB). On the other hand, in the closest relatives of cetaceans, the raoellids (Orliac & Thewissen, 2021; Waqas et al., 2024), the OB, and olfactory tract (also referred to as olfactory peduncle therein) are anteroposteriorly elongate but not as narrow. Somewhat intermediate conditions are seen in *Remingtonocetus* and *Aegyptocetus*, which could indicate that the volume of the tract should also be considered olfactory relevant. Consequently, we measured the volume of the OB, both independently and in combination with the olfactory tract in *Protocetus* (Figure 1). Assuming the largest volume, *Protocetus* falls in the distribution of extant terrestrial mammals measured in a strictly similar way (and considering body mass scaling), while the smallest volume (OB only) yields a position between the latter and the manatee (Figure 4A). When compared to previously analyzed pre-Miocene mammaliaforms (Bertrand et al., 2022), all but one (*Prorastomus*) presumed terrestrial, *Protocetus* does not exhibit a reduction in olfactory-relevant volume, regardless of how that volume is defined (Figure 4B). In fact, the median estimate for *Protocetus* lies above the regression line derived from these taxa. This suggests that the OB did not undergo the reduction previously associated with early cetacean evolution. Limited data are available on the OB of extant mysticetes; it is presumed to be entirely absent in odontocetes. In the bowhead whale, the OB accounts for ~0.13% of the total brain mass (Thewissen et al., 2011). Assuming a comparable proportion of adnexa surrounding the OB and the rest of the brain, and considering only the OB (excluding the olfactory tract), this ratio in *Protocetus* is nearly 10 times greater. Nevertheless, the absolute volumes of the OB are similar in both species. As previously mentioned in the context of overall brain size and neuron number, studying sensory systems such as olfaction raises the challenge of choosing the best anatomical proxy for olfactory capabilities. From a neurobiological perspective, the absolute volume of the OB may be considered the most informative proxy, as it is thought to reflect the total number of olfactory neurons. However, when comparing distantly related lineages that span a broad range of body sizes, absolute volume tends to scale with body mass and may primarily reflect overall species size rather than sensory function. In such cases, accounting for body size is essential to disentangle potential ecological or functional patterns from general allometric trends.

Analysis of the nasal cavity in *Protocetus* further supports the retention of a specific olfactory airflow pathway, reinforcing the interpretation that airborne olfaction remained functionally important during the early stages of cetacean aquatic adaptation. The dnm, a space free of turbinals that lines the dorsal side of the cavity, has been argued to be a bypass for odorants (Craven et al., 2007). The dnm is well developed in *Protocetus* and *Aegyptocetus*, from the anteriormost nasal to the cribriform plate. This further likely implies that the rest of the nasal cavity was not free but occupied by the laminae of the vomer and turbinals (not preserved in *Protocetus*), including nonossified (cartilaginous) ones, as in most terrestrial mammals (Martinez et al., 2024b). Another relevant feature of the nasal cavity is the olfactory recess, which is formed by the lamina transversa, and separates the main respiratory airflow from the olfactory turbinals (Craven et al., 2010). This structure is also seen in *Aegyptocetus* (Supplementary Figure S2), and it was most likely present in *Protocetus*, too.

CMM-V-4536, a partial skull recovered from the Pamunkey River in Virginia, United States, and tentatively attributed to a middle Eocene protocetid (Godfrey et al., 2013), is an important fossil for understanding early cetacean endocranial anatomy. Compared to *Aegyptocetus* (and probably *Protocetus* as well), it notably features clearly more reduced, less convoluted, and thickened ethmoturbinals, along with a thick nasal septum (not seen in *Aegyptocetus*, where the nasal septum is as thin as the surrounding turbinals; Supplementary Figure S2). As previously noted (Godfrey et al., 2013), this is reminiscent of mysticetes (exemplified therein by *Balaenoptera acutorostrata*). These major differences probably imply that CMM-V-4536 should be dissociated from our concept of the protocetid condition.

The olfactory apparatus of *Protocetus* was not drastically reduced. The morphological reduction of the olfactory apparatus has repeatedly been associated with diminished olfactory capabilities, particularly in aquatic and semiaquatic mammals (Martinez et al., 2020). However, functional evidence remains scarce, and robust statistical support linking the absolute or relative size of the olfactory organs in tetrapods to their olfactory capabilities (sensitivity and discrimination) is still lacking. Nevertheless, recent studies suggest an interconnection between olfactory capabilities and other olfactory proxies, such as the number of functional olfactory receptor genes (Martinez et al., 2024a).

Conclusion

An assessment of the endocranial anatomy of the middle Eocene protocetid *P. atavus*, in combination with the closely related and coeval *A. tarfa*, enabled us to further understand the reorganization of the sensory capabilities of early cetaceans during the transition from land to sea. While different sources of bias make it difficult to assess EQ in extinct whales, it appears that some protocetids (at least *Protocetus*) already featured relatively large brains. However, our comparative analysis included only one other protocetid (*Rodhocetus kasranii*; Gingerich, 1998; Waugh & Thewissen, 2021), so the notion that increased encephalization might predate basilosaurids has to be further substantiated.

None of the olfactory proxies preserved in *Protocetus* suggest that this early whale had a reduced sense of smell, indicating that airborne olfaction was most likely not selected against (i.e., reduced as a result of a set of selective pressures) in semiaquatic whales. It has long been assumed that the olfactory apparatus regressed during early cetacean evolution, resulting in all extant species having reduced olfactory capabilities. However, a growing body of evidence shows that extant baleen whales retained a potentially well-developed sense of smell (Farnkopf et al., 2022). Their olfactory apparatus is clearly distinct from that of their closest terrestrial relatives, but so are most of their other cranial features. As a corollary, and together with the conclusion stemming from the present analysis, this challenges the notion that the olfactory apparatus went through a significant phase of reduction at any stage of cetacean evolution preceding the diversification of extant groups.

Supplementary material

Supplementary material is available online at *Evolution*.

Data availability

All data and code are provided on figshare: https://doi.org/10.6084/m9.figshare.28218473. Surface models of the skull and segmented endocast of *Protocetus atavus* (SMNS-P-11084, holotype) are freely available on MorphoMuseuM (Berger et al., 2025).

Author contributions

Conceptualization: A.S.G., E.A., H.M.S., and Q.M.; data curation: E.A., E.B., E.P., G.S.F., Q.M., and R.R.C.; formal analysis: E.A., E.B., E.P., and Q.M.; funding acquisition; E.A. and Q.M.; investigation: E.A., E.B., E.P., G.S.F., Q.M., and R.R.C.; methodology: A.S.G., E.A., H.M.S., and Q.M.; project administration: E.A. and Q.M.; resources: E.A., E.B., E.P., G.S.F., Q.M., and R.R.C.; software: E.A., E.B., E.P., G.S.F., Q.M., and R.R.C.; supervision: E.A. and Q.M.; validation: E.A. and Q.M.; visualization: E.A., E.B., E.P., Q.M., and R.R.C.; writing—original draft: E.A.; and writing—review & editing: all.

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Conflict of interest

The authors declare no conflict of interest.

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