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Molecular phylogeny of cetaceans prompts revision of morphological transformations

Michel C. Milinkovitch

The echolocating toothed whales and the filter-feeding baleen whales are traditionally considered as two monophyletic lineages that originated from the extinct cetacean suborder Archaeoceti. While current interpretation of the morphological and behavioural data sets supports toothed-whale monophyly, molecular phylogenies contradict this long-accepted taxonomic subdivision. The molecular data indicate that one group of toothed whales, the sperm whales, is more closely related to the morphologically highly divergent baleen whales than to other odontocetes. Furthermore, these molecular analyses tentatively suggest a more recent origin of baleen whales than has been generally accepted. Although a thorough cladistic analysis of all relevant morphological data is still needed, reevaluation of some of the most important of these characters helps to reconcile the morphological and the molecular approaches.

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During the transition from the terrestrial to the aquatic environment, whales (Order Cetacea) experienced dramatic transformation of many biological systems[1–3] and acquired a fusiform body shape giving modern families a superficial resemblance to fish. Extant whales are well characterized by telescoping of the skull, posterior movement of the narial openings, isolation of the earbones, shortening of the neck, loss of external hind limbs, reduction of the pelvic girdle and addition of vertebrae[3].

The cetaceans are generally considered (e.g. Ref. 1) to form a clade (a truly monophyletic group), although a diphyletic origin of the two morphologically highly divergent suborders of extant whales, the Odontoceti (toothed whales) and the Mysticeti (baleen whales), has been favoured by others (but see Ref. 4). The monophyly of extant cetaceans is also strongly supported by molecular-character analyses (e.g. Refs 5,6) and this hypothesis is very widely accepted.

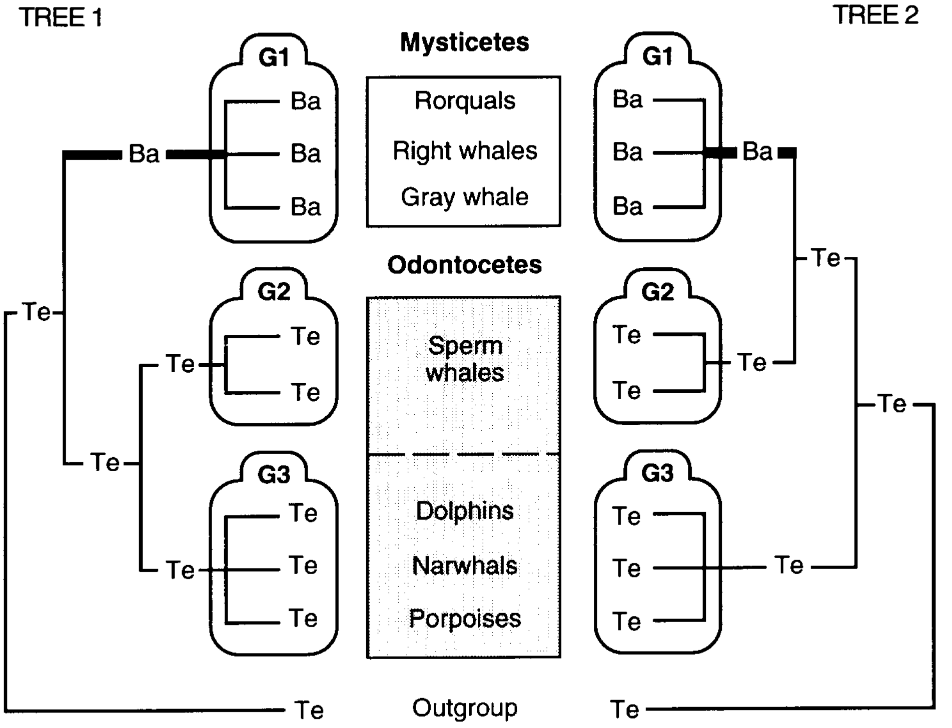
A close phylogenetic relationship between cetaceans and ungulates was first suggested more than a hundred years ago[7] and was more recently confirmed by paleontological (e.g. Refs 2,8,9, and see references in Ref. 10) and molecular (e.g. Refs 5,6,11–14) studies. More specifically, these multiple data sets suggest a sistergroup relationship between cetaceans and artiodactyls (even-toed) and, accordingly, the latter are more closely related to cetaceans than they are to perissodactyl (odd-toed) ungulates (e.g. Refs 5,6,8,13). In addition, the recent discovery and description of new fossil specimens provided valuable insights into the transitional modes of locomotion in early cetaceans[15,16]. Several analyses of molecular data suggested that cetaceans are nested within the artiodactyl phylogenetic tree. However, these studies disagreed on which of the three artiodactyl suborders (Tylopoda[13], Suiformes[17] and Ruminantia[18]) might constitute the sistergroup to cetaceans. Additional data are needed to test these interesting hypotheses.

While the monophyly of cetaceans is widely accepted, the origin of and evolutionary relationships among the major groups of cetaceans is more problematic since morphological and molecular analyses reach very different conclusions. Indeed, based on the conventional interpretation of the morphological and behavioural data set, the echolocating toothed whales (about 67 species) and the filter-feeding baleen whales (10 species) are considered as two distinct monophyletic groups[3,19–21] that, supposedly, separated from the extinct suborder Archaeoceti 35–45 million years ago (e.g. Refs 19,21,22). On the other hand, phylogenetic analyses of DNA (three mitochondrial gene fragments[6,10]) and amino acid (myoglobin gene[6,12]) sequences contradict this long-accepted taxonomic subdivision. One group of toothed whales, the sperm whales, appears to be more closely related to the morphologically highly divergent baleen whales than to other odontocetes, while all other resolvable relationships are consistent with traditional groupings[6,10]. Therefore, these molecular studies suggest that the suborder Odontoceti constitutes a paraphyletic group (see Box 1) and challenge the conventional scenario of a long, independent evolutionary history of odontocetes and mysticetes. If this hypothesis is correct, and if one subscribes to the view that taxonomic classification should reflect cladogenesis (the splitting of evolutionary lineages) such that each taxonomic group is truly monophyletic, the classification of cetaceans needs to be revised[6,10].

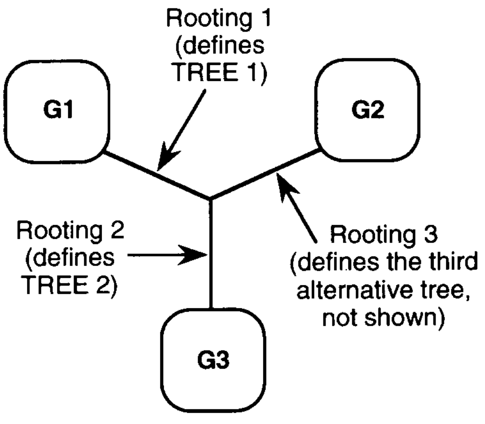
Furthermore, based on the analysis of complete cytochrome *b* DNA sequences of seven cetacean genera, Arnason and Gullberg[23] recently challenged both the classical hypothesis of toothed whale monophyly and the hypothesis[6,10] of sister relationship between sperm and baleen whales. Indeed, in a parsimony analysis with transition substitutions (Ti, that is, A↔G and T↔C) and transversion substitutions (Tv, that is, A↔T, A↔C, G↔T and G↔C) unweighted but with a weight of 4, 17 and 1 for the first, second and third codon positions, respectively, they obtained a tree where mysticetes and dolphins form a clade (supported by a low 52% bootstrap value) to the exclusion of sperm whale (this tree is defined by 'rooting 3' in Box 1).

Box 1. Character information and tree rooting

Since baleen are not present in any non-cetacean mammals, the presence of baleen (Ba) is a **synapomorphy** (a shared derived, hence cladistically informative, character state) that appeared somewhere along the branch in bold and that supports the grouping of all baleen-bearing cetaceans in a **clade** (a truly monophyletic group, that is, a group containing one ancestor not shared by any species outside that group). On the other hand, since non-cetacean mammals possess teeth, the presence of teeth (Te) in some cetacean taxa is a **symplesiomorphy** (a shared ancestral, hence cladistically uninformative, character state) retained by extant teeth-bearing cetaceans. The presence of teeth provides no information allowing us to choose between TREE 1 (where odontocetes form a clade) and TREE 2 (where odontocetes form a **paraphyletic group**, that is, a group containing an ancestral species together with some, but not all, of its descendants).



Most phylogenetic methods produce **unrooted trees**. The tree is rooted by using an **outgroup** taxon, that is, a taxon that diverged from G1, G2 and G3 before these three groups diverged from each other. The phylogenetic analysis will position the branch leading to the outgroup on one of the three branches connecting G1, G2 and G3. These three alternative rootings will define three alternative cladogenetic relationships between the groups G1 (mysticetes), G2 (sperm whales) and G3 (non-sperm-whale odontocetes). Using G1 as an outgroup would force the grouping of G2 and G3 in a clade, hence, the monophyly of toothed whales (G2 + G3) would be constrained and not assessed.



However, it is well known that, in the mitochondrial genome, Ti accumulate much more rapidly than Tv (e.g. Refs 13,24,25). Consequently, it is crucial to assess whether or not multiple hits are likely to conceal the phylogenetic signal in the variability range of the relevant comparisons. Inspection of the number of Tv and Ti across all positions in Arnason and Gullberg's data set demonstrated[26] that Ti are saturated (while Tv are not) in all comparisons between any of the dolphins, sperm whale, baleen whales and the cow (*Bos taurus*) (the only outgroup included in their DNA sequence analysis). Consequently, Ti need to be down-weighted (e.g. Ref. 13) to improve substantially the performance of the parsimony analysis[27]. Re-analyses of Arnason and Gullberg's data set using only Tv substitutions resulted in high bootstrap support[26] for the hypothesis of sister relationship between sperm whales and baleen whales[6,10]. Parsimony analyses excluding Ti only in third position of all codons and in first position of leucine codons (but using all substitutions in other positions) and maximum likelihood (ML) analyses also yielded[26] unambiguous support for the baleen + sperm whale[6,10] topology rather than for the one reported by Arnason and Gullberg[23].

As it is the case for many phylogenetic controversies, the problem of rooting of the tree is central to the question of cetacean phylogeny. Indeed, alternative rootings define alternative branching relationships (cf. Box 1) between the three main groups of cetaceans: sperm whales, baleen whales and the non-sperm-whale odontocetes. It is well known[28,29] that the rooting of a phylogenetic tree can be particularly problematic. More specifically, molecular character states shared by one taxon and a divergent outgroup can be based on random similarity rather than on history[28]. Adachi and Hasegawa[30] suggested that the grouping of the dolphins and baleen whales in Arnason and Gullberg's analysis is not only due to saturated transitions, but also to such a 'random rooting' artifact. Indeed, using ML inference of protein phylogeny, Adachi and Hasegawa[30] demonstrated that Arnason and Gullberg's data[23] support Milinkovitch *et al.*'s hypothesis[6,10,26] when several alternative artiodactyl outgroups are used.

Figure 1 illustrates the fundamental phylogenetic concept of character polarization and demonstrates that 'random rooting' can be avoided by including additional outgroup taxa (e.g. Ref. 29). Indeed, since Arnason and Gullberg[23] gave a much higher weight to second than to first and third codon positions (see above), second positions greatly influenced the phylogenetic analysis. However, inspection of this cytochrome *b* data set reveals that only two second positions (788 and 1046) support the grouping of baleen whales and dolphins in a clade. Figure 1 shows that, when the cow V00654 is used as the only outgroup, the position 1046 is erroneously interpreted (through 'random rooting') as supporting the grouping of dolphins and baleen whales in a monophyletic lineage, while inclusion of additional available artiodactyl taxa (Fig. 1) unambiguously demonstrates that it is a phylogenetically uninformative character for the species included in the data set.

Although all mitochondrial DNA (mtDNA) data analysed to date[6,10,26,30] (in addition to analyses of the myoglobin gene[6,12]) support the hypothesis that sperm whales and baleen whales form a monophyletic group to the exclusion of other toothed whales, these conclusions remain strikingly inconsistent with the common interpretation of the morphological data set. However, the morphological (including paleontological) data can be re-interpreted[31], and some morphological and behavioural characters can be convincingly reevaluated[31] in the light of the molecular hypothesis[6,10,26] of toothed-whale paraphyly.

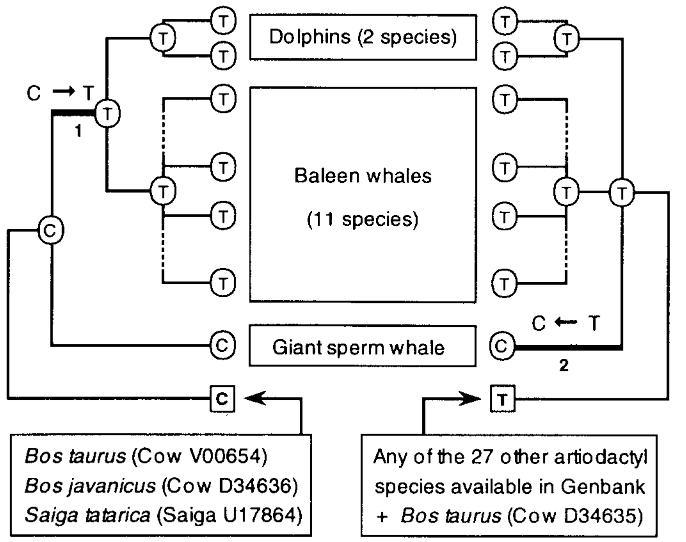


Fig. 1. States of character 1046 for each species of cetaceans in the Arnason and Gullberg's cytochrome *b* data set[24]. Thirty species of Artiodactyl for which cytochrome *b* DNA has been sequenced are available in GenBank and EMBL databanks (January 1995): pigs, peccaries and hippopotamuses; camels, dromedaries, llamas and alpacas; and chevrotains, deers, pronghorns, cows and sheep. *Bos taurus* (accession number V00654), *B. javanicus* and *Saiga tatarica* have a state C in position 1046. All other 27 species have a T. Furthermore, a second individual of *B. taurus* has been sequenced (GenBank D34635), and its state at character 1046 is a T. Left tree: because Arnason and Gullberg[24] used only the individual cow V00654 as an outgroup, they polarized C→T the character 1046 in branch 1 (in bold) and, consequently, they considered the T as a shared derived character (a synapomorphy) grouping baleen whales and dolphins in a clade. Right tree: when all other available artiodactyl cytochrome *b* DNA sequences are considered, the character 1046 is polarized T→C in the branch leading to the giant sperm whale (branch 2, in bold). Consequently, the character state T in position 1046 is now indisputably interpreted as an ancestral character state (a symplesiomorphy) for all non-sperm whale cetaceans included in the data set. Character 1046 is therefore phylogenetically non-informative for the cetacean species included in the data set, and this demonstrates that including several outgroups (as long as they are closely related to the ingroup taxa) is crucial in order to reduce errors in polarization of the characters.

Cladogenetic relationships among cetaceans

The problem of the Archaeoceti

Although the fossil record of cetaceans has not provided unequivocal evidence on the relationships among the three recognized suborders of cetaceans (the Odontoceti, Mysticeti and the extinct Archaeoceti)[1,3,19,22], the general consensus among morphologists is that odontocetes and mysticetes originated from archaeocetes (e.g. Refs 1,3,21). However, archaeocetes (which retain the eutherian dental formula and do not have telescoping of the cranial bones) constitute a 'wastebasket' group, defined as those Cetacea lacking the derived characters of Mysticeti or Odontoceti[22,32,33]. Consequently, the Archaeoceti are most likely a paraphyletic (cf. Box 1) assemblage, and many of the fossils identified as archaeocete specimens (e.g. in Ref. 34) probably represent extinct parallel lineages that are not direct ancestors to any modern cetacean family. Obviously, questions requiring the inclusion of comparative analysis of archaeocetes are beyond the scope of molecular methods. Only phylogenetic character-based analyses (such as a cladistic approach) on well-preserved fossils can define what, if any, group(s) of Archaeocetes is (are) likely to be the sistergroup(s) to the major extant clades of cetaceans. Unfortunately, the relevant described fossil record is fragmentary (see Box 2) and lacks many transitional forms. In addition, the highly modified and specialized morphology of all cetaceans (1) makes the homology statements and polarization of the morphological characters difficult even in well-preserved fossils (this might explain why some fossil genera have been variously interpreted as belonging to Archaeoceti, Mysticeti or Odontoceti) and (2) makes the choice of a non-cetacean outgroup (crucial for testing subordinal classifications since it defines

Box 2. Fossils and early mysticetes

As other enigmatic isolated teeth[22], the following specimens are usually interpreted (e.g. Refs 22,44) as supporting the presence of early Oligocene (c.35 million years ago) mysticetes: *Llanocetus denticrenatus*, early Oligocene, portion of right mandibular ramus + two teeth + partial endocast; *Protosqualodont*, early Oligocene, two teeth; *ZMT62* (Ref. 33), early Oligocene, piece of mandible with three teeth. In addition, Fordyce[44] described (a) a middle or posterior cheek tooth (REF S1-67) from an undescribed late-Eocene species; (b) the left periotic (OU21939) of an undescribed early-Oligocene species; (c) a middle or posterior cheek tooth (GS10897) of the early Oligocene. Fordyce[22,44] questionably interprets these very fragmentary specimens as 'toothed mysticetes' (a) and (c) and 'baleen-bearing mysticetes' (b). All other described fossils seem compatible with a c.25 million year old origin (late Oligocene) of baleen whales. For instance, *Aetiocetus cotylalveus*[46] (Aetiocetidae, Oregon, USA) is a very important fossil specimen possessing the typical eutherian dental formula (I3, C1, PM4, M3)[21]. Considerations of some features of the well-preserved skull (e.g. Ref. 4 and E. Fordyce, pers. commun.) suggest that this c.25 million year old species belongs to the early mysticete lineage, although Emlong[46] initially classified this fossil as an archaeocete and perceived slight similarity with an early sperm whale, *Idiophyseter*. Mchedlidze[34] has referred two other late-Oligocene genera, *Mirocetus* and *Ferecetotherium*, to the aetiocetid mysticetes. Interestingly, Barnes[3] and Fordyce[22] reinterpreted the latter as a sperm whale. In addition, early sperm whales and late-Oligocene Aetiocetidae had the same dental formula (11-11/11-11; L. Barnes, pers. commun.). Consequently, *Aetiocetus* and *Ferecetotherium* could be reasonably interpreted as early representatives of the baleen- and sperm-whale lineages, respectively, just after the occurrence of the cladogenetic event that hypothetically[6,10,26] individualized the two groups. The oldest possible member of Balaenidae (an extant mysticete family), *Morenocetus parvus*, and the oldest unambiguous giant sperm whale (Physeteridae), *Diaphorocetus poucheti*, are from the same early-Miocene (22–23 million years ago) Patagonian deposits (references in Ref. 10), while the oldest members of Kogiidae (pygmy sperm whales) are from the late Miocene[3,21].

the rooting of the tree, cf. Box 1) almost impossible in morphological analyses because most characters used for cetacean classification are not comparable or even absent in non-cetacean mammals. On the other hand, some archaeocetes (such as primitive protocetids) might constitute more-appropriate outgroups.

Reevaluation of some characters classically recruited as supporting toothed-whale monophyly

Among the primary morphological characters that have been used for the separation of cetaceans into odontocetes or mysticetes is the presence of teeth in the former and baleen in the latter. While the presence of baleen (a neomorph) is a synapomorphy for Mysticeti, the presence of teeth in odontocetes is uninformative for testing toothed-whale monophyly (cf. Box 1). Clearly, the presence of teeth is a shared ancestral character state for all whales and is phylogenetically uninformative for subordinal classification; for instance, it is well established that baleen-whale embryos express teeth (e.g. Ref. 20). Interestingly, since foetal mysticetes exhibit homodont dentition, this also suggests that homodonty is not a synapomorphy for toothed whales[20] but rather a convergence among major groups of cetaceans or a synapomorphy for the order Cetacea.

The number of blowhole(s) [i.e. external nare(s)] is a more interesting character. All baleen whales have two blowholes (Fig. 2a) while all toothed whales are believed to have a single blowhole (Fig. 2b, 2c). Considering that terrestrial mammals (including the whales' closest relatives: the artiodactyls) have two external nares, the presence of a unique blowhole in toothed whales seems to be an unambiguous derived character supporting the monophyly of odontocetes (including sperm whales). Indeed, while in mysticetes the nasal passages are separate tubes all the way to the external nares (Fig. 2d), the typical condition in toothed whales is the presence of two nasal passages that become confluent just distal to the bony nares[20] (Fig. 2e); a single tube leads from that point to the unique blowhole. However, the sperm whale has a sigmoidally shaped blowhole (Fig. 2c, 2f), which is formed by two nasal tubes that remain distinct, from the bony nares to the top of the head[20] (Fig. 2f). Consequently, the blowhole of the sperm whale does not constitute a truly singular opening: the anterior and posterior curves of the sigmoid represent the apertures of the right and left nares, respectively[20] (rare examples of adult sperm whales with two distinct blowholes have even been reported). In the pygmy sperm whales (Kogiidae), the situation is only slightly different since the nasal passages remain discrete tubes until just proximal to the single blowhole. Hence, the number of blowhole(s) is a very ambiguous character and the character state 'one blowhole' should not be used as a synapomorphy for toothed whales (contra Refs 20,35). On the other hand, I proposed[31] to define a new character 'number of nasal passages distal to the bony nares' with two states: 'two nasal passages' or 'single nasal passage' (cf. Fig. 3). The former would be the ancestral state, present in baleen whales (Fig. 2d) and in all sperm whales (Fig. 2f), and the latter would be a derived character that joins the river dolphins, the delphinoids and the beaked whales (i.e. all toothed whales but sperm whales) in a clade (cf. Fig. 3).

The presence or absence of echolocation abilities is also a character recruited as unambiguously supporting the monophyly of toothed whales. Indeed, active echolocation is believed to occur in all toothed whales but, supposedly, has never been developed in baleen whales[3,21]. Contrary to this classic hypothesis, we suggested[6,10] that echolocation capabilities are likely to have been present in the ancestor of all extant whales (making the presence of echolocation in odontocetes an ancestral character state). Indeed, if the molecular-phylogenetic hypothesis of (sperm + baleen whale) sister relationship is correct, and if baleen whales never developed echolocation capabilities, one must assume that the biological sonar evolved twice, independently, in cetaceans: once in sperm whales and once in other toothed whales. However, because it is easier to lose a complex biological function than to develop it several times *de novo*, it is more likely[6,10] that echolocation is ancestral for all whales and that this function (together with other related adaptations) was lost or greatly reduced in baleen whales.

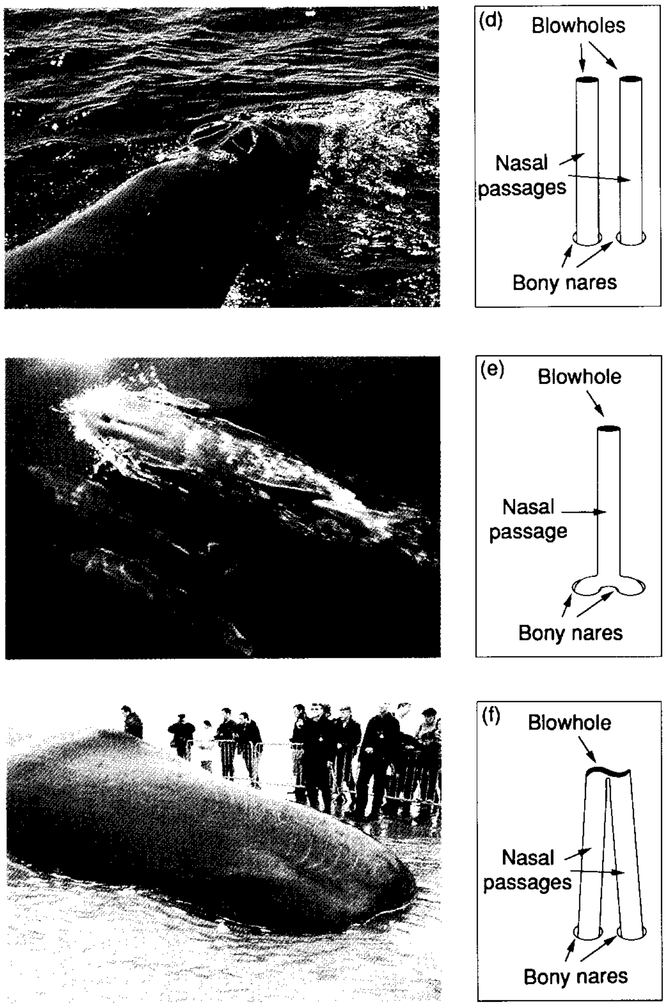


Fig. 2. Appearance of the blowhole(s) in (a) a right whale, (b) a dolphin and (c) a giant sperm whale, and a schematic description of the situation of nasal passages in (d) a mysticete, for example, a right whale, (e) a non-sperm whale odontocete, for example, a dolphin and (f) a giant sperm whale.

There are behavioural and morphological data that support this line of reasoning. For instance, a vestigial melon has been described in baleen whales[35] (the melon – a fatty acoustic lens located in the forehead of toothed whales – is an important component of the echolocation system). This suggests that the ancestor of all extant whales may have possessed a well-developed melon (cf. Fig. 3) and correspondingly well-developed echolocation capabilities. Furthermore, it has been suggested that the morphology of the auditory region of some archaeocetes is compatible with the presence of echolocation abilities[1], and that low-frequency

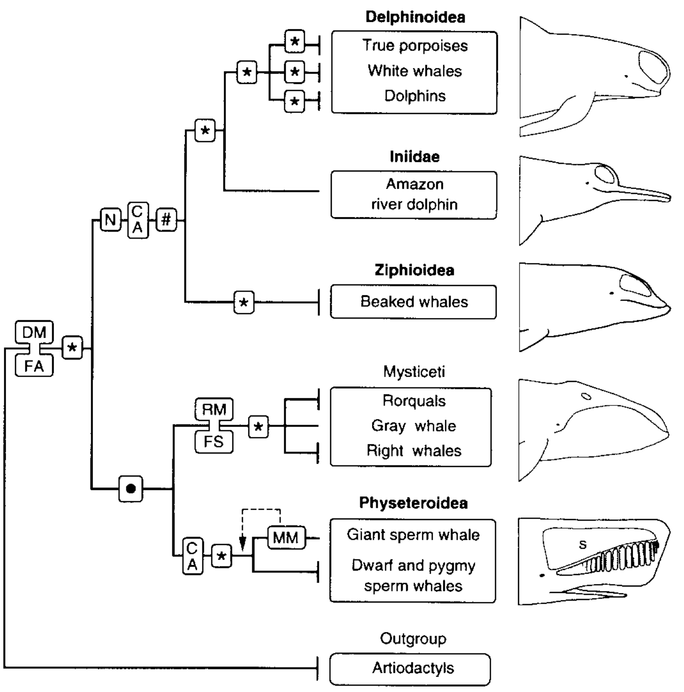


Fig. 3. Cladogram (branch lengths not proportional to time) of all extant major groups of cetaceans. The toothed whales (Odontoceti) are shown in bold. Three of the most important reevaluated morphological characters have been mapped on the phylogenetic tree: (1) the situation of the nasal passages [N, nasal passages confluent just distal to the bony nares (cf. Fig. 2e)], (2) the melon (DM, development of a melon; RM, reduction of the melon; MM, modification of the melon); and (3) facial and cranial asymmetry [FA, development of facial asymmetry (oriented to the left), concomitant to the development of the melon; FS, reversal to facial symmetry (this reversal is linked to the reduction of the melon and loss or reduction of echolocation capabilities); CA, development of cranial asymmetry (oriented to the left in response to facial asymmetry)]. Facial asymmetry is likely to have evolved further in epigenetic interaction with the development of cranial asymmetry; only one (the most parsimonious) of several possible sets of non-independent parallel developments of cranial asymmetry is shown. The asterisks represent clades consistently supported by both molecular and morphological data; the filled circle represents a clade established by the analysis of molecular data (12S and 16S mt-rDNA sequences, myoglobin amino-acid sequences, cytochrome *b* mtDNA sequences); the hash symbol represents a clade supported by morphological data and compatible with (although not strongly supported by) the molecular data; S, spermaceti organ. Since the pygmy and dwarf sperm whales also have a (small) spermaceti organ, the dashed arrow indicates where the modification of the melon occurred if the spermaceti organ (and not the junk) is homologous to the melon of other cetaceans. From top to bottom, the species are: long-finned pilot whale (*Globicephala melas*), Amazon river dolphin (*Inia geoffrensis*), Cuvier's beaked whale (*Ziphius cavirostris*), bowhead (*Balaena mysticetus*), giant sperm whale (*Physeter catodon*). The shaded areas correspond to the melon (the junk in the sperm whale). Cetaceans are not drawn to scale.

specialization of mysticete ears is likely to be a derived character in cetacean sensory evolution[36]. The vestigial melon of mysticetes might be a hint to a more-generalized paedomorphism of their facial anatomy[31]. Apparently, baleen whales are not the only cetaceans that may have experienced the regression of the melon: de Muizon[37] described a 5-million-year-old presumptive delphinoid in which telescoping of the skull had been dramatically reversed. The melon in this species must have been lost or greatly reduced[37]. Because of the high specialization of the giant sperm whale (*Physeter catodon*) facial anatomy, it is unclear what structure in this species is homologous to the melon of other cetaceans. However, based on the comparison with the facial anatomy of dwarf and pygmy sperm whales (*Kogia* species), Heyning[20] suggested the junk (a segmented tissue located below the spermaceti case and so-called because it is less rich in oil than the spermaceti itself), rather than the spermaceti organ, to be the most likely candidate.

In addition to the powerful low-frequency mysticete sounds thought to communicate information on bathymetry[38], various echolocation-like sounds have been recorded in the presence of the blue, gray, fin, humpback and minke whales (e.g. Ref. 38, and refs therein). Although the actual use of echolocation has not been demonstrated in any of these mysticetes, and although most workers agree that these sounds[38] are not related to echolocation, these behavioural data could be considered as tentatively suggesting that baleen whales do possess vestigial echolocation aptitudes for navigation and locating food (see Ref. 10). Unfortunately, experimental tests for echolocation capabilities in mysticetes are probably impractical considering the size of baleen whales and the conditions where they might indeed use echolocation (e.g. to detect fish schools or plankton aggregates, to navigate among icebergs, and so on).

Very few studies have addressed the phylogeny of cetaceans by using comprehensive morphological data sets and rigorous phylogenetic methods[39]. In extensive cladistic analyses of toothed-whale facial anatomy[20,35], many of the characters were explicitly polarized by using baleen whales as an outgroup and, consequently, the monophyly of toothed whales was constrained (cf. Box 1) and not assessed. Furthermore, among the morphological characters recruited as synapomorphies for odontocetes[20,35] are facial and cranial asymmetry (and presence of nasal sacs). However, in all sperm whales (physeteroids), these characters differ considerably from those found in all other odontocetes[20], making even the interpretation of homology doubtful. Re-evaluation of facial and cranial asymmetry is presented in Box 3.

Although phylogenetically homologous characters need not share common pathways of ontogenetic development (e.g. Ref. 40) and although even the concept of morphological homology is itself problematic[40], the ontogenetic development of the clavicle in cetaceans might produce a phylogenetically informative character. The clavicle is present as a temporary but manifest rudiment during the embryogenesis of all odontocetes[41] (although beaked whales were not investigated) except for sperm whales, where the regression of this structure has progressed further[41], as is the case for baleen whales. Accordingly, the extended regression of the clavicle rudiment might be considered a shared derived character for the (sperm whale + baleen whale) clade. More thorough examination of the ontogenetic development in cetaceans should be particularly informative for elucidating their phylogeny. For example, the ontogeny of the cranial and facial anatomy (both very different in sperm whales compared to other toothed whales), including telescoping of the skull (some features of which seem to be synapomorphies for toothed whales), needs to be investigated in all major groups of cetaceans.

How old is the baleen whale clade?

Beside the main hypothesis on the cetacean-tree topology, molecular analyses also suggested an additional, but distinct, hypothesis on the age of the lineages considered. Making the explicit assumption that the rate of the molecular clock in whale-mitochondrial-ribosomal genes is similar to that of their closest relatives (the artiodactyls), we tentatively suggested[6] that the common ancestor of sperm and baleen whales might have lived only 10–15 million years ago. Clearly, this date is an underestimate if the rate of evolution in whales is slower than in artiodactyls. Because some unambiguous sperm- and baleen-whale fossils are older than 15 million years (cf. Box 3), and because a slower rate for whale mtDNA evolution has been suggested recently[42], we attempted to calibrate the molecular clock in cetaceans by using cladogenetic events documented by fossil data[10], and obtained a divergence of a minimum of 19 million years between sperm whales and baleen whales[10]. Consequently, in conjunction with other molecular findings[43], these analyses[10] suggest that the hypothesized cladogenetic event which led to the individualization of the sperm whale and baleen whale lineages may have occurred about 25 million years ago (late Oligocene), making the baleen whales 20 million years younger than originally thought.

Box 3. Facial and cranial asymmetry

Because all asymmetrical cranial features found in living cetaceans are associated with asymmetrical facial structures[20], and because facial and cranial asymmetry seem closely correlated as important components of the echolocating system (e.g. Ref. 20), these characters might be homoplasic or ancestral for all cetaceans. For instance, Barnes[3] considers cranial asymmetry to have arisen independently in a minimum of six odontocete lineages 'because they appear to have been derived from different ancestors with symmetrical skulls or because the bones on the top of the skull are affected in different ways'. However, Heyning[20] noted that, if there is a selective advantage in asymmetry, there should be a 50% chance of skewing to the left (beginning with a symmetrical skull). Because the members of all seven odontocete families have a skull skewed to the left, the random chance that these seven events were independent would be 0.008 (Ref. 20). Considering our hypothesis of sister relationship between sperm and baleen whales, cranial asymmetry need not have evolved independently more than twice (Fig. 3): in non-sperm-whale odontocetes and in sperm whales (likelihood = 0.25).

However, there is an even more likely scenario for the evolution of the left-oriented cranial asymmetry in odontocetes[31] (see photograph). In the context of the classical hypothesis of toothed-whale monophyly, Heyning[20] suggested that odontocete cranial asymmetry might have been produced in response to the changes in the soft anatomy. Therefore, it is reasonable to assume[31] that the 'echolocation-linked' asymmetry of soft facial anatomy (not observable on fossil specimens) started to develop in the ancestor of all extant cetaceans, and, by chance, was oriented to the left. In other words, left-oriented facial asymmetry might be, for all toothed whales, an ancestral character that was lost or greatly reduced (along with the melon and echolocation abilities) in baleen whales (cf. Fig. 3). Accordingly, cranial asymmetry would be a concomitant (hence, violating independence of characters) of facial asymmetry and would have been developed independently in two (possibly up to four) odontocete lineages (Fig. 3). This scenario[31] of secondary parallel acquisition of cranial asymmetry would explain why different groups of toothed whales (e.g. sperm, beaked and other toothed whales) have significantly different patterns of cranial asymmetry[3,20].

Indeed, because mysticetes are believed to have evolved from archaeocetes (e.g. Ref. 21), it is classically considered that the mysticete lineage originated about 45 million years ago (e.g. Fig. 3.1 in Ref. 21) although Fordyce[22,44] recently reduced this figure to the Eocene/Oligocene boundary (34 million years ago). I suggest that even this latter date is still not firmly established by the available paleontological data since it is based on highly incomplete and/or damaged (hence not directly comparable) fossil specimens (cf. Box 2).

Although calibration of molecular clocks (suggesting that baleen whales originated only c.25 million years ago[10,43]) are not clearly contradicted by available paleontological data, it is important to emphasize that these calibrations are necessarily approximate since, among other reasons[31], some of the branches on the tree are longer than others[10]. Obviously, the sister relationship between sperm whales and baleen whales constitutes the core of the new molecular argument[6,10] and the dating of the nodes would be better addressed with new, key, fossil specimens.

Conclusions

The evolution of filter-feeding was a key element in the origin of mysticetes, as this remarkable innovation allowed the exploitation of a new food resource. The strong selective factors for refining the ability to filter-feed explain the rapid morphological evolution of baleen whales. It is likely that morphological characters linked to filter-feeding evolved quickly as they appeared, making middle-Miocene mysticetes very similar to extant species. In addition, the spectacular transformations (allowing efficient baleen filter-feeding) of the early mysticete skull could explain the dramatic reduction of incompatible (but otherwise advantageous) morphological structures such as the melon.

Although all the speculations and re-interpretations presented here must be tested by morphological cladistic analysis, the existing morphological analyses are, at best, too ambiguous to reject the provocative hypothesis of sister relationship between sperm and baleen whales[6,10,26]. Furthermore, because most morphological phylogenetic inferences in cetaceans have been based on overall similarity rather than on the use of cladistically informative characters, paraphyletic groupings (cf. Box 1) are probably common throughout all levels (from genera to suborders) of the traditional classification.

While recent reevaluations of relevant morphological characters[2,8,9,15,16] have dramatically improved our understanding of the cetacean ancestors spectacular transition from land to sea, such key fossil specimens are not available for investigating the not-less remarkable evolution of the major groups of cetaceans. Consequently, the molecular findings[6,10,26], based on extensive cladistic and statistical analyses of multiple gene fragments (12S and 16S mt-rDNA, cytochrome *b* mtDNA, and myoglobin amino acid sequences), constitute, so far, the most supported hypothesis for cetacean phylogeny.

Although practical and theoretical reasons (reviewed in Ref. 45) make the mitochondrial genome a molecule of choice in phylogenetic analyses, one could hypothesize that mtDNA provided a biased description of the phylogeny of whales. Therefore, additional molecular data (especially nuclear markers), as well as extensive morphological character-based analyses including all extinct and extant available taxa, are needed to address this major molecular–morphology conflict and resolve further the evolutionary relationships among the major groups of cetaceans. However, for both the molecular and morphological approaches, such a task is necessarily difficult, because the internal branches defining these relationships are probably short and, hence, are unlikely to bear numerous synapomorphies.

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