Phylogeny of All Major Groups of Cetaceans Based on DNA Sequences from Three Mitochondrial Genes

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Traditionally, living cetaceans (order Cetacea) are classified into two highly distinct suborders: the echolocating toothed whales, Odontoceti, and the filter-feeding baleen whales, Mysticeti. A molecular phylogeny based on 1,352 base pairs of two mitochondrial ribosomal gene segments and the mitochondrial cytochrome *b* gene for all major groups of cetaceans contradicts this long-accepted taxonomic subdivision. One group of toothed whales, the sperm whales, is more closely related to the morphologically highly divergent baleen whales than to other odontocetes. This finding suggests that the suborder Odontoceti constitutes an unnatural grouping and challenges the conventional scenario of a long, independent evolutionary history of odontocetes and mysticetes. The superfamily Delphinoidea (dolphins, porpoises, and white whales) appears to be monophyletic; the Amazon River dolphin, *Inia geoffrensis,* is its sister species. This river dolphin is genetically more divergent from the morphologically similar marine dolphins than the sperm whales are from the morphologically dissimilar baleen whales. The phylogenetic relationships among the three families of Delphinoidea remain uncertain, and we suggest that the two cladogenetic events that generated these three clades occurred within a very short period of time. Among the baleen whales, the bowhead is basal, and the gray whale is the sister species to the rorquals (family Balaenopteridae). The phylogenetic position of beaked whales (Ziphioidea) remains weakly supported by molecular data. Based on molecular clock assumptions, the mitochondrial-DNA data suggest a more recent origin of baleen whales (~25 mya) than has been previously assumed (>40 mya). This revised phylogeny has important implications for the rate and mode of evolution of morphological and physiological innovations in cetaceans.

Introduction

Whales are among the most specialized of all mammals and include the largest animals that ever lived. The movement of the ancestral cetaceans from the terrestrial to an aquatic environment involved extensive remodeling of the morphological, physiological, and behavioral systems (Barnes and Mitchell 1978; Gingerich et al. 1983; Barnes 1984a). The order Cetacea is generally considered to be a monophyletic group, although a separate origin of the two morphologically highly divergent suborders of living whales, the Odontoceti (toothed whales) and the Mysticeti (baleen whales), has been favored by others (e.g., Yablokov 1965). The origin of and evolutionary relationships among fossil and extant cetaceans are disputed (Barnes 1984a; Barnes et al. 1985; Heyning and Mead 1990; Fordyce 1992; McLeod et al.

1993; Milinkovitch et al. 1993), and the phylogenetic distinctness of the extinct suborder Archaeoceti is problematic (Fordyce 1989; Wyss 1990). The fossil record of cetaceans is incomplete and has not provided unequivocal evidence on whether the archaeocetes gave rise to one, both, or neither suborder of living whales (see, e.g., Barnes and Mitchell 1978; Barnes 1984a; Barnes et al. 1985; Fordyce 1992; McLeod et al. 1993).

Phylogenetic studies of extinct and extant cetaceans are complicated by their highly modified morphology. However, a close phylogenetic relationship between cetaceans and ungulates was first suggested more than 100 yr ago (Flower 1883) and was more recently confirmed by paleontological (Van Valen 1966; Szalay 1969; Gingerich et al. 1983, 1990; Thewissen and Hussain 1993) and molecular studies (Goodman et al. 1985; Miyamoto and Goodman 1986; McKenna 1987; Czelusniak et al. 1990; Irwin et al. 1991; Milinkovitch 1992; Milinkovitch et al. 1993). Several independent approaches support a sister-group relationship of cetaceans with artiodactyl ungulates. Accordingly, artiodactyls are more closely related to cetaceans than they are to perissodactyl ungulates (see, e.g., Czelusniak et al. 1990; Gingerich et al. 1990; Irwin et al. 1991; Milinkovitch et al. 1993).

Extant cetaceans are assigned to the suborder Mysticeti (baleen whales), which is comprised of 11 species, or to the suborder Odontoceti (toothed whales), with about 67 species. These two groups are generally considered to have diverged from the extinct suborder Archaeoceti more than 40–45 mya (see, e.g., Barnes et al. 1985; Fordyce 1992; McLeod et al. 1993). However, the paleontological evidence for this date is equivocal since the assignment of key fossils to a particular whale lineage is often uncertain (see, e.g., Marples 1956; Keyes 1973; Fordyce 1989). One of the earliest "modern" whale fossils (ZMT62) is from the early Oligocene (probably 35 my old) and has been tentatively interpreted as a primitive mysticete (Fordyce 1989). However, the specimen is very incomplete (a piece of mandible and three teeth), and, consequently, we regard it as dubious and of uncertain subordinal status (contra Novacek 1993). Fossils from lineages of the living whales are more recent (Barnes et al. 1985). The oldest known fossils of extant families, from the Oligocene-Miocene boundary (about 23 mya), are sperm whales and right whales from the same deposit (Barnes 1984*b*).

The classification of cetaceans into odontocetes and mysticetes is based on morphological differences that include the presence of teeth and baleen, respectively. Some extinct mysticete whales, however, were toothed and did not possess baleen. In addition, some living odontocete whales lack teeth (e.g., the females in ziphiids and narwhal), while baleen whale embryos exhibit vestigial teeth. Clearly, teeth are an ancestral characteristic for all whales and therefore are not phylogenetically informative for subordinal classification. The lack of definitive morphological characters and the paucity and usually poor quality of key cetacean fossils make the assignment of these specimens to the mysticete, odontocete or archaeocete lineages challenging (see, e.g., Fordyce 1989).

Character-based phylogenies including most major lineages of living cetaceans are rare (Milinkovitch et al. 1993). Here, we present an extensive molecular-phylogenetic hypothesis for all major groups of cetaceans, including the "boto" or Amazon river dolphin (*Inia geoffrensis*), the largest of the platanistoid dolphins. The analyses are based on three mitochondrial gene segments, the efficacy of which has been well documented for molecular-phylogenetic reconstruction in mammals (see, e.g., Kocher et al. 1989; Mindell and Honeycutt 1990; Miyamoto et al. 1990; Irwin et al. 1991; Allard et al. 1992; Gatesy et al. 1992). *Via* the polymerase chain reaction (PCR; Saiki et al. 1988), we previously amplified and directly sequenced portions of the two mitochondrial ribosomal genes (12S, 395 base pairs and 16S, 533 base pairs) for 16 species of cetaceans and several outgroup

taxa (Milinkovitch et al. 1993). Here, we present a larger data set that includes, for the same genes, five additional key species of cetaceans and three artiodactyl species as outgroups. These eight species have been chosen to break up long branches on the tree and hence to facilitate proper polarization of the characters. In addition, we determined the DNA sequences of portions of the cytochrome *b* gene (424 base pairs including 22 base pairs of the adjacent tRNA-Glu) for the same 21 cetacean species and three outgroups.

Material and Methods

We determined DNA sequences from the species listed in the Appendix. Tissue samples were obtained from delphinaria or from stranded or by-catch animals. DNA was extracted from blood, skin, spleen, or liver tissue from frozen or DMSO-preserved specimens. The primers used for PCR amplification and direct sequencing (Kocher et al. 1989) of part of the 12S gene were modified L1091 and H1478 (Kocher et al. 1989) primers. Primers for the 16S gene were 16sar-L and 16sbr-H (Palumbi et al. 1991), and for cytochrome *b* were modified L14724 (Pääbo 1990) (5′-TGACATGAA-AAAYCAYCGTTG) and H15149 (Kocher et al. 1989) (5′-CCCTCAGAATGATATYTGTCCTCA). Details of the protocol have been reported previously (Kocher et al. 1989; Palumbi et al. 1991). The DNA sequences were determined with an automatic sequencer (Applied Biosystems 373A) following the manufacturer's protocols. In all cases, both strands were sequenced.

For each DNA segment, sequences from all taxa were aligned using the multiple-alignment program MALIGN (Wheeler and Gladstein 1993). Indels (insertions and deletions) were not detected in cytochrome *b*, and large differences in parameter settings did not significantly influence the alignment of the ribosomal gene sequences. Data were analyzed by neighbor-joining (NJ) (Saitou and Nei 1987), maximum parsimony (MP) (Swofford 1993), and maximum-likelihood (ML) (Felsenstein 1981) methods, and the robustness of the phylogenetic hypotheses was tested by bootstrapping (Felsenstein 1985). Previous phylogenetic studies including more distantly related orders of mammals have demonstrated that artiodactyls are the sister group to cetaceans (Czelusniak et al. 1990; Irwin et al. 1991; Milinkovitch et al. 1993); we therefore used three very divergent artiodactyl species as outgroups to root the phylogenetic trees. The three gene sequences were analyzed individually as well as combined for a total of 1352 bp in each of the 21 whale species and three outgroup species. The following options were used in all parsimony analyses (Swofford 1993): heuristic search, MULPARS option in effect, MAXTREES = 200, and TBR branch swapping. In addition to unweighted
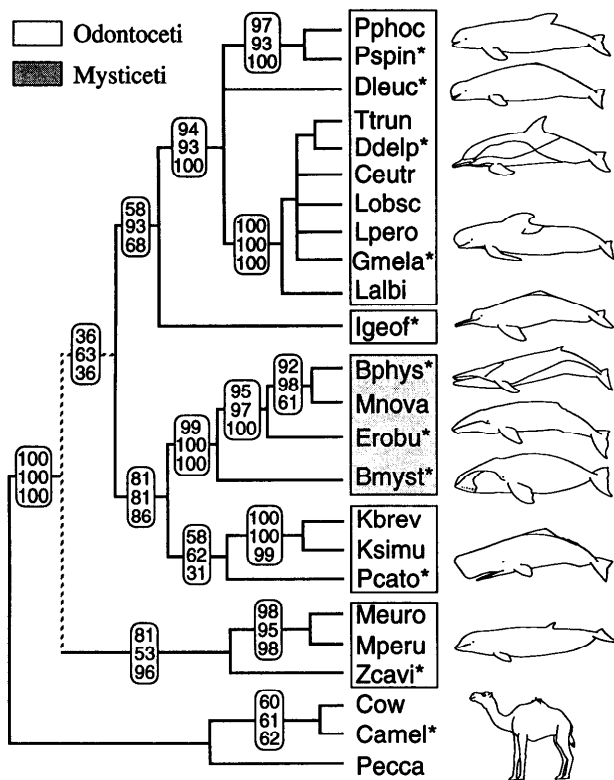
FIG. 1.—Majority-rule bootstrap consensus tree based on the combined three gene fragments for the 21 cetacean species and three artiodactyl outgroups. The outgroup taxa for the phylogenetic analyses were Camel, *Camelus dromedarius* (dromedary camel) (our cytochrome *b* differs by one nonsynonymous substitution from the sequence of Irwin et al. 1991); Cow, *Bos taurus* (cow) (sequences from Anderson et al. 1982); and Pecca, *Tayassu tajacu* (collared peccary) (for cytochrome *b*, from Irwin et al. 1991). Other species abbreviations are provided in the Appendix. Numbers indicate bootstrap values. The top values correspond to an unweighted MP (Swofford 1993) bootstrap analysis (200 replications), and the middle values refer to a MP (Swofford 1993) bootstrap analysis (100 replications) in which Tv are weighted three times more than Ti except for cytochrome *b* where only Tv in third positions of all codons and in first positions of leucine codons were considered. The bottom values correspond to the majority consensus tree among the 100 NJ trees produced from the 100 ML-distance matrices generated after bootstrap resampling of the original data set (Seqboot, Dnadist, Neighbor; Felsenstein 1993). The dotted line emphasizes the unstable placement of beaked whales. An asterisk indicates the species drawn (cetaceans are not drawn to scale).

acter state. For cytochrome *b*, in addition to unweighted searches, only transversions in third positions of all codons and in first positions of leucine codons were examined (Irwin et al. 1991). Similar Ti:Tv ratios were tested in the maximum-likelihood analyses.

Results and Discussion

The present analyses comprise all major lineages of extant cetaceans, including a river dolphin (family Iniidae, superfamily Platanistoidea), and resolve several open questions in cetacean systematics and evolution (figs. 1 and 2). Figure 1 summarizes the bootstrap analyses combining the three gene fragments. An MP analysis (Swofford 1993) with no differential weighting of genes, positions, Ti, or Tv produced one shortest tree (length = 1503, CI = 0.49, CI excluding uninformative characters = 0.42, number of informative characters = 361) congruent with the tree in figure 1 except for the beaked whales, which were positioned as the sister group of the Delphinoidea + Inia clade. Constraining Odontoceti monophyly produced three cladograms eight steps longer (length = 1511) than the shortest tree. All of the 156 trees less than 1509 steps long (= maximum 5 steps longer than the shortest tree) contain a Physeteroidea + Mysticeti clade. Among the 556 trees less than 1511 steps long (= maximum 7 steps longer than the shortest tree): 539 trees (= 97%) contain a Physeteroidea + Mysticeti
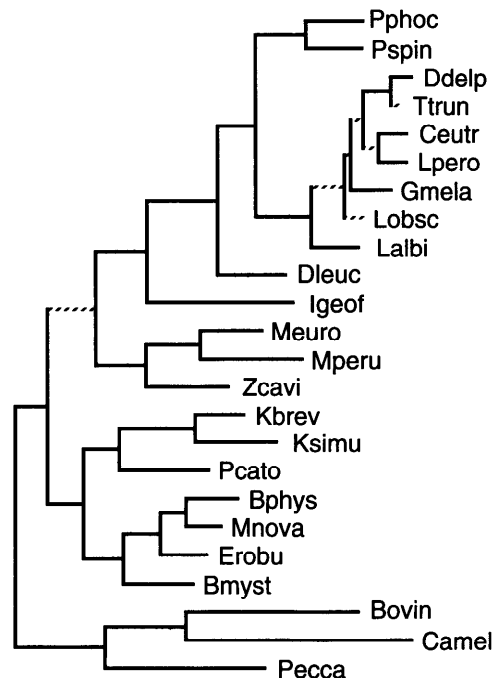

FIG. 2.—ML tree derived from the 12S + 16S data set. The dotted lines correspond to branches for which the lower approximate confidence limit (DNAML 3.52c; Felsenstein 1993) reaches zero.

searches, we performed MP analyses (Swofford 1993) in which sequence divergences were corrected for multiple substitutions according to the observed threefold higher frequency of transitions (Ti) over transversions (Tv) (Milinkovitch et al. 1993). A similar Ti:Tv ratio for the same genes was detected among some groups of ungulates (Miyamoto et al. 1990). Indels were coded as single characters, irrespective of their length, and were weighted as transversions. When indels of different length overlapped, each size class was considered a different char-

clade, and 16 trees contain a *Physeter catodon* + Mysticeti clade. In addition, we performed parsimony analyses with no weight and with Ti:Tv ratios of 1:3 and 0:1 on the individual 12S and 16S and on the 12S and 16S combined. None of the most parsimonious solutions or bootstrap consensus trees contained a monophyletic odontocete group, and this monophyly was violated by a Physeteroidea + Mysticeti clade in all instances (except for the transversion-only analysis on the individual 12S, in which the relationships between Physeteroidea and Mysticeti were unresolved). The unweighted bootstrap parsimony analysis on the combined data set yielded 0.5% support for the monophyly of odontocetes, while weighted (Ti/Tv = 1/3) bootstrap parsimony and NJ analyses yielded 0.0% support.

It is well-known that the MP method can be positively misleading when the evolutionary rate differs substantially among lineages (Felsenstein 1978). Therefore, we used the ML method (DNAML 3.52c; Felsenstein 1993), which is known to be very robust against violation of the constant rate (Hasegawa et al. 1991). The ML analysis (with Ti/Tv = 1/3) on the combined total (12S + 16S + cytochrome *b*) data set produced a single tree compatible with that in figure 1. The same analysis on the combined 12S + 16S data set yielded one tree shown in figure 2.

NJ analyses were performed on the combined 12S + 16S data set and on the combined total data set using distance matrices calculated with Kimura-2-parameters and ML models (Felsenstein 1993). All four NJ trees obtained were congruent with the tree in figure 1.

In all analyses (MP, ML, and NJ), most relationships are stable and occur in the 50% majority rule bootstrap consensus trees (fig. 1). The order Cetacea and each of its families and superfamilies are monophyletic. The confirmation of these traditional groupings produces confidence in the reliability of the phylogenetic information contained in these DNA sequences.

We find strong support for the monophyly of the superfamily Delphinoidea and of the families Delphinidae (the dolphins) and Phocoenidae (the true porpoises) (fig. 1). Within the Delphinoidea, the phylogenetic relationship among the three families (Delphinidae, Phocoenidae and Monodontidae [or white whales]) is ambiguous (fig. 1) since the three possible groupings are almost equally supported for most variations of the analyses. The position of the Monodontidae was already debated (see, e.g., Lint et al. 1990), and this question will need to be investigated further with the inclusion of the second monodontid species, the narwhal (*Monodon monoceros*). Interestingly, in a cladistic analysis of morphological characters, Heyning (1989) failed to resolve this trichotomy and noted that the Delphinoidea

are relatively conservative in general morphology, which makes an unequivocal phylogenetic analysis difficult. We hypothesize that the two cladogenetic events, which led to the individualization of the three families, occurred within a very short period of time. Although the relationships within the other Delphinidae remain largely unresolved (at the 50% majority-rule bootstrap level), we find strong support (within the confines of this study) for the sister group relationship of the currently monotypic genera *Delphinus* (*delphis*) and *Tursiops* (*truncatus*) (figs. 1 and 2). This result is consistent with the morphology-based classification of the two genera in the same subfamily, Delphininae (Barnes 1990).

The Amazon River dolphin, *Inia geoffrensis* (the only platanistoid species examined in this study), is strongly suggested to be the sister species to the superfamily Delphinoidea (figs. 1 and 2). This hypothesis is supported by some morphological analyses (Heyning 1989; Heyning and Mead 1990) and by myoglobin data (McKenna 1987).

The monophyly of the suborder Mysticeti and the relationships among its members are strongly supported (fig. 1). The bowhead (family Balaenidae) is the most basal baleen whale, and the gray whale (family Eschrichtiidae) is the sister group to the family Balaenopteridae (the rorquals). This result seems to break up the unresolved trichotomy (between *Megaptera*, *Eschrichtius*, and *Balaenoptera*) observed in a molecular analysis based on satellite-DNA sequences (Arnason et al. 1992); however, further analyses including more species of rorquals will be necessary to firmly resolve this issue.

Our molecular data support the monophyly of the beaked whales (superfamily Ziphioidea) and the sperm whales (superfamily Physeteroidea). Among members of the Physeteroidea, the placement of both the dwarf and pygmy sperm whales as the sister group to the third species, the giant sperm whale (figs. 1 and 2), is consistent with traditional morphological classification (Barnes et al. 1985; Heyning 1989). Because the mean Tv divergence is always higher between *Kogia* and *Physeter* than between the three delphinoid families, we agree with Barnes et al. (1985) in the placement of *Kogia* and *Physeter* in two different families (Kogiidae and Physeteridae, respectively).

Based on morphological data, the exact placement of beaked whales within Cetacea remains uncertain (Barnes 1984a; Heyning 1989). Some authors classify the beaked whales as the sister group to sperm whales in the superfamily Physeteroidea (see, e.g., in Barnes 1984a). As in our previous analysis (Milinkovitch et al. 1993), we find weak support for the placement of the ziphioids as the sister group to all other whales (fig. 1). However, in the present analyses, the position of beaked

whales was unstable since, depending on the method or the variations of analyses, this group is positioned at the base of cetaceans (fig. 1), at the base of the Delphinoidea + *Inia* clade (fig. 2), or with the sperm + baleen whale group. This last grouping is also weakly favored by myoglobin data (Milinkovitch et al. 1993) and possibly by one morphological trait, the throat grooves, found only in sperm, baleen and beaked whales. However, a cladistic analysis of facial anatomy in cetaceans (Heyning 1989) supports the placement of beaked whales as the sister group to the Delphinoidea + *Inia* clade, and in our analysis this grouping is supported by a bootstrap value (22%, unweighted search) similar to the 36% support for the placement of the beaked whales as the sister group to all other whales. Because none of the alternative hypotheses is strongly supported, we regard this question as unresolved.

The inclusion of several new key species and an additional mitochondrial-gene fragment supports the previous surprising outcome of molecular-phylogenetic analyses of cetaceans (Milinkovitch et al. 1993): the apparent sister-group relationship between sperm whales and baleen whales. This suggests that sperm whales are more closely related to baleen whales than they are to any other group of toothed whales (figs. 1 and 2). This phylogenetic hypothesis conflicts with the traditional division of cetaceans into the two suborders Odontoceti (toothed whales) and Mysticeti (baleen whales) (see, e.g., Barnes et al. 1985; Arnason et al. 1992; McLeod et al. 1993) and suggests that the toothed whales are paraphyletic. Further support for the sperm + baleen whale relationship derives from phylogenetic analyses of myoglobin amino-acid sequences (McKenna 1987; Milinkovitch et al. 1993) and from a recent morphogenetic study (Klima 1990). Previous analyses of cetacean relationships based on myoglobin and hemoglobin amino acid sequences yielded equivocal results but hinted that toothed whales might not constitute a monophyletic group (Czelusniak et al. 1990). Based on a 16S mitochondrial gene fragment (657 base pairs from only three species of cetaceans), Arnason et al. (1993) presented a NJ tree in which the sperm whale is more closely related to the fin whale than to the only dolphin species included in that analysis. Although that result supports our hypothesis (Milinkovitch et al. 1993) of toothed whale paraphyly, the authors summarily concluded that it must be artifactual.

In addition, based on a parsimony analysis of complete cytochrome *b* DNA sequences of seven cetacean genera, Arnason and Gullberg (1994) recently challenged our hypothesis (Milinkovitch et al. 1993) of a sister relationship between sperm whales and baleen whales. Indeed, their single parsimony analysis, with transitions and transversions equally weighted, supports (with a low bootstrap value of 52%) a sister relationship between baleen whales *and dolphins*. However, we have demonstrated (Milinkovitch et al., submitted) that transition substitutions are saturated in Arnason and Gullberg's cytochrome *b* data set (while transversion substitutions are not): for all sequence comparisons (1) between baleen whales and any of the three species of toothed whales included in their data set and (2) between cetaceans and the cow (the only outgroup included in their analysis). Consequently, the transition substitutions need to be down-weighted (see, e.g., Irwin et al. 1991), to substantially improve the performance of the parsimony analysis (Hillis et al. 1994). Maximum parsimony and NJ reanalyses (Milinkovitch et al., submitted) of Arnason and Gullberg's (1994) data resulted in high bootstrap support (83%) for our hypothesis of sister relationship between sperm whales and baleen whales (Milinkovitch et al. 1993) when only transversions were considered. Additional analyses of the same data set (MP analyses [Swofford 1993] with transition substitutions down-weighted but not excluded, and an ML analysis [DNAML 3.52c; Felsenstein 1993]) also yielded (Milinkovitch et al., submitted) unambiguous support for our (Milinkovitch et al. 1993) topology rather than for the one reported by Arnason and Gullberg (1994).

Our results might warrant a reclassification of the order Cetacea. The taxonomic rank of baleen whales would need to be lowered from a subordinal level (Milinkovitch et al. 1993) if one subscribes to the cladistic view that groups of organisms of equal taxonomic ranks (i.e., the suborders Mysticeti and Odontoceti) must each include an ancestor and all of its descendants. For example, one might consider the designation of three superfamilies: the Delphinoidea (including the Iniidae, Monodontidae, Delphinidae, and Phocoenidae), the Ziphioidea (consisting of the beaked whales), and a new group composed of the baleen whales (of the previous suborder Mysticeti) and the sperm whales.

The molecular rate of evolution might be much slower in baleen whales than in toothed whales; therefore, one could argue that the sister relationship between sperm and baleen whales is an artifact due to very different rates of evolution among cetaceans. This is unlikely for two reasons. First, the mean Tv divergence (for the 12S + 16S data set) between the outgroups and the cetaceans are 4.4%, 4.4%, 4.4%, 5.0%, 4.0%, 4.3%, and 4.6% for the porpoises, dolphins, beluga, *Inia*, beaked whales, sperm whales, and baleen whales, respectively. Second, the ML method is very robust against the violation of the constant rate (Hasegawa et al. 1991) and our ML analyses supported the same sister relationship between sperm and baleen whales.

Our molecular data strongly suggest that the Amazon river dolphin is genetically more divergent from the morphologically similar marine dolphins than the sperm whales are from the morphologically dissimilar baleen whales. Indeed, for the three gene fragments analyzed, the mean sequence divergence (Tv + Ti and Tv only) between *Inia* and the morphologically similar delphinoids is always higher than that between sperm whales and the morphologically highly divergent baleen whales. Because this high divergence value is only in part due to a high number of autapomorphies in the branch leading to *Inia*, we suggest that the Iniidae separated from the delphinoid clade at approximately the same time as the sperm and baleen whales diverged from each other. The important question of the monophyly or polyphyly of the biogeographically widely separated (two species live in South America, two on the Indian subcontinent, and one in China) and highly endangered extant Platanistoidea (five species in four genera and four families) must await further study.

The results of our molecular phylogenetic study suggest that the mode and rate of morphological evolution in cetaceans have proceeded differently than previously assumed. The speed of morphological and behavioral divergences would appear to have been rapid for the baleen whales compared to other cetaceans. For example, active echolocation is believed to occur in all toothed whales but was supposedly never developed in baleen whales (see, e.g., Barnes 1984a, 1990; McLeod et al. 1993). If our phylogenetic hypothesis is correct, we suggest that echolocation capabilities (and other behavioral, physiological, and morphological adaptations related to the acquisition of food) have been present in the ancestor of all extant whales and have been secondarily lost in baleen whales. This is not inconsistent with paleontological data, as there is good morphological evidence that early odontocetes and perhaps even archaeocetes had the ability to echolocate (Barnes and Mitchell 1978; Barnes 1984a). In support of our hypothesis, a vestigial, but manifest, melon (this adipose acoustic lens is located in the forehead of odontocetes, is part of the echolocation system, and is generally considered a synapomorphy for all toothed whales) has been described in mysticetes (Heyning and Mead 1990). In addition, behavioral data (references in Beamish 1978; Amato 1993) suggest that baleen whales have vestigial echolocation aptitudes for navigation and food finding. The evolution of filter feeding, which allowed the exploitation of a new food resource (Fordyce 1984), was a critical component in the origin of mysticetes, and we hypothesize that this strong selective factor explains the rapid morphological evolution of baleen whales.

Because it implies a significant revision of cetacean evolution, our molecular study also suggests the need to reevaluate the existing morphological and paleontological analyses. For example, in an extensive cladistic analysis of facial anatomy, Heyning (1989) polarized most of the characters by using baleen whales as outgroup and, consequently, constrained monophyly of toothed whales (contra Novacek 1993). In addition, the number of blowhole(s) is generally considered an unambiguous character supporting the monophyly of toothed whales. Indeed, the typical condition in odontocetes is the presence of two nasal passages that become confluent just distal to the bony nares (Heyning 1989; Heyning and Mead 1990); a single tube leads from this point to the unique blowhole. On the other hand, mysticetes have two blowholes since the nasal passages are separate tubes that extend to the external nares. However, the sperm whale, the only odontocete with this condition, has a sigmoidally shaped blowhole that is made up by two nasal tubes that remain distinct from the bony nares to the top of the head (Heyning 1989; Heyning and Mead 1990). Rare examples of adult sperm whales with two distinct blowholes have also been reported (Heyning 1989). In the pygmy sperm whales (Kogiidae), the nasal passages remain discrete tubes until just proximal to the blowhole (Heyning 1989). Consequently, the number of blowhole(s) is a very ambiguous character that should not be used as a synapomorphy for toothed whales (contra Heyning 1989; contra Heyning and Mead 1990). Most other morphological features recruited for the monophyly of odontocetes (facial and cranial asymmetry, presence of nasal sacs, etc.) seem closely correlated as components of the advanced echolocating system and hence might be subject to homoplasy. For instance, Barnes (1984a, 1990) considers cranial asymmetry to have independently arisen several times within the odontocetes because several toothed whale lineages appear to have evolved from different ancestors with symmetrical skulls.

Heyning (1989) noted that the facial anatomy and the cranial asymmetry of sperm whales are highly specialized and significantly different from the pattern found in all other odontocetes, which makes interpretation of homology difficult. Consequently, the question is which, if any, of the facial structures of Physeteridae are strictly homologous to those found in other odontocetes.

Schlötterer et al. (1991) found surprisingly low amounts of sequence divergence (3.2%) in DNA sequences of four nuclear simple-sequence loci between the odontocetes and mysticetes—a very low divergence for an estimated split of more than 40 mya (Barnes et al. 1985; Arnason et al. 1992; Fordyce 1992; McLeod et al. 1993). They (Schlötterer et al. 1991) suggested that either whale DNA evolves at the slowest reported substitution rate for neutral nucleotide positions, or a reas-

sessment of the age of modern whales is warranted. Our mitochondrial-DNA data are consistent with these nuclear-DNA data and suggest further that baleen whales may not have experienced a long independent history from all toothed whales. The transversion divergence rate for mitochondrial ribosomal genes of ungulates has been calibrated to be about 0.14% per million years (Allard et al. 1992). We find a 1.4% transversion divergence between sperm and baleen whales. If cetaceans have a similar molecular-divergence rate as ungulates, then the split between sperm whales and baleen whales might be approximately 10 my old (Milinkovitch et al. 1993). However, Martin and Palumbi (1993) suggested that the mitochondrial mutation rate in whales might be lower than in ungulates. To test this hypothesis, we can attempt to calibrate the molecular clock in cetaceans by using cladogenetic events documented by fossil data. The 12S + 16S substitution mean divergence and the cytochrome b third-position Tv mean divergence are 4.46% and 3.38%, respectively, for the delphinoid interfamily relationships, and 7.19% and 5.80%, respectively, for the sperm whales + baleen whales comparisons. Since the oldest Phocoenidae, Delphinidae and Monodontidae, have been dated to the late Miocene (Barnes 1984a, 1990; Barnes et al. 1985 [possibly 11 my old]), this corresponds to a maximum rate (because the actual splits of these three taxa might be older) of 0.41% and 0.31% per million years and a minimum 18- to 19-mya divergence between sperm whales and baleen whales. Even though this calibration confirms the suggested (Martin and Palumbi 1993) lower rate of evolution in cetaceans than in ungulates, it supports a ~25-my-old separation (Schlötterer et al. 1991; this study) between odontocetes and mysticetes. We consider that this date is not in conflict with paleontological records since the oldest unambiguous sperm whale (*Diaphorocetus poucheti*) and baleen whale (right whale, *Morenocetus parvus*) are from the same 23-my-old deposits in Argentina (Barnes 1984a, 1984b; McLeod et al. 1993).

Several authors (Benham 1937, 1942; Marples 1956; Keyes 1973; Whitmore and Sanders 1977; Fordyce 1989; Mitchell 1989) described fossil specimens as being archaic mysticetes from the Oligocene (24–38 mya). However, these specimens are either highly incomplete, damaged and hence ambiguous to interpret, or from the very late Oligocene (~25 mya). Part of the problem is that phylogenetic interpretations have often been made from not directly comparable parts of skeletons; nonetheless, homology statements and polarization of the morphological characters are problematic even in well-preserved fossils. Furthermore, archaeocetes constitute a "wastebasket" group (Fordyce 1984, 1992; Wyss 1990) defined as the Cetacea lacking the derived characters of

Mysticeti or Odontoceti, which might explain some of the disagreement among morphologists on the assignment of particular extinct genera to odontocetes, mysticetes, or archaeocetes. In addition, many of these problematic fossils (see, e.g., Mchedlidze 1976) could represent extinct parallel lineages that are not direct ancestors of any modern cetacean family.

Less ambiguous is the 25-my-old *Aetiocetus cotylalveus* (Aetiocetidae; Emlong 1966), a particularly important fossil specimen possessing teeth. Several characters of the well-preserved skull suggest that this species belong to the early mysticete lineage (see, e.g., McLeod et al. 1993). Mchedlidze (1976) has referred two other late-Oligocene genera (*Mirocetus* and *Ferecetotherium*) to the Aetiocetidae mysticetes, but, interestingly, Barnes (1984a) and Fordyce (1992) reinterpreted the latter as a sperm whale, and Barnes (personal communication) noted that early sperm whales and late-Oligocene Aetiocetidae had the same dental formula. We suggest that *Aetiocetus* and *Ferecetotherium* correspond to early baleen whales and sperm whales, respectively, just after the two lineages diverged in late Oligocene (~25 mya).

Although the existing fossil record does not contradict our hypothesis of a ~25-my-old origin of the sperm and baleen whale lineages, it is conceivable that the rate of DNA evolution in cetaceans may have been even slower. Indeed, the three gene fragments we used are evolving at different rates (e.g., the cytochrome b third positions are evolving at least 4–7 times faster than the 12S + 16S sequences), and they give different dates for the sperm whale–baleen whale divergence. The calibration of the mean nucleotide divergence is necessarily approximate, as some of the branches on the tree are longer than others. Therefore, new key fossil specimens would be necessary for dating the nodes more precisely.

Nonetheless, the three gene fragments are consistent with each other in supporting the sister relationship between sperm whales and baleen whales. Consequently, even if the baleen and sperm whale lineages originated more than 40 mya (the "classical" view; see, e.g., Fordyce 1992; Arnason et al. 1993), we consider that the most important part of our hypothesis is the branching order of the tree rather than the dates on the nodes. Indeed, if our suggested sister relationship of sperm whales and baleen whales is correct (regardless to the date of this divergence), the mode of evolution of morphological, physiological, and behavioral innovations in cetaceans will have to be significantly reevaluated.

Sequence Availability

The sequences reported in this article have been deposited in GenBank data base under accession numbers U13079–U13146.

For the fin whale: the 16S sequence is from Arnason et al. (1991); in our 12S sequence, one position differs from Arnason et al. (1991).

Acknowledgments

APPENDIX
Traditional Classification of the Order Cetacea

Suborder Archaeoceti (extinct)
Suborder Odontoceti (toothed whales)
    Superfamily Delphinoidea
        Family Phocoenidae (true porpoises)
            Pphoc, *Phocoena phocoena* (harbor porpoise)
            Pspin, *Phocoena spinipinnis* (Burmeister's porpoise)
        Family Monodontidae (white whales)
            Dleuc, *Delphinapterus leucas* (beluga)
        Family Delphinidae (dolphins)
            Ttrun, *Tursiops truncatus* (bottlenose dolphin)
            Ddelp, *Delphinus delphis* (common dolphin)
            Ceutr, *Cephalorhynchus eutropia* (black dolphin)
            Lobsc, *Lagenorhynchus obscurus* (dusky dolphin)
            Lalbi, *Lagenorhynchus albirostris* (white-beaked dolphin)
            Lpero, *Lissodelphis peronii* (southern right whale dolphin)
            Gmela, *Globicephala melas* (long-finned pilot whale)
    Superfamily Platanistoidea (river dolphins)
        Family Iniidae
            Igeof, *Inia geoffrensis* (Amazon river dolphin)
    Superfamily Ziphioidea (beaked whales)
        Family Ziphiidae
            Meuro, *Mesoplodon europaeus* (Gervais' beaked whale)
            Mperu, *Mesoplodon peruvianus* (Peruvian beaked whale)
            Zcavi, *Ziphius cavirostris* (Cuvier's beaked whale)

    Superfamily Physeteroidea (sperm whales)
        Family Kogiidae
            Kbrev, *Kogia breviceps* (pygmy sperm whale)
            Ksimu, *Kogia simus* (dwarf sperm whale)
        Family Physeteridae
            Pcato, *Physeter catodon* (sperm whale)
Suborder Mysticeti (baleen whales)
    Family Balaenopteridae (rorquals)
        Bphys, *Balaenoptera physalus* (fin whale)
        Mnova, *Megaptera novaeangliae* (humpback whale)
    Family Eschrichtiidae
        Erobu, *Eschrichtius robustus* (gray whale)
    Family Balaenidae (right whales)
        Bmyst, *Balaena mysticetus* (bowhead)

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