Cytochrome *b* and Bayesian inference of whale phylogeny

Laura May-Collado [a,b,*], Ingi Agnarsson [c,d]

[a] *Department of Biological Sciences, Florida International University, 11200 SW 8th Street, Miami, FL 33199, USA*
[b] *Esuela de Biologia, Universidad de Costa Rica, Apto. 2060, San Jose, Costa Rica*
[c] *The University of British Columbia, Departments of Zoology and Botany, 3529-6270 University Blvd., Vancouver, BC., Canada V6T 1Z4*
[d] *Systematic Biology-Entomology, E-530, Smithsonian Institution, NHB-105, P.O. Box 37012, Washington, DC 20013-7012, USA*

Abstract

In the mid 1990s cytochrome *b* and other mitochondrial DNA data reinvigorated cetacean phylogenetics by proposing many novel and provocative hypotheses of cetacean relationships. These results sparked a revision and reanalysis of morphological datasets, and the collection of new nuclear DNA data from numerous loci. Some of the most controversial mitochondrial hypotheses have now become benchmark clades, corroborated with nuclear DNA and morphological data; others have been resolved in favor of more traditional views. That major conflicts in cetacean phylogeny are disappearing is encouraging. However, most recent papers aim specifically to resolve higher-level conflicts by adding characters, at the cost of densely sampling taxa to resolve lower-level relationships. No molecular study to date has included more than 33 cetaceans. More detailed molecular phylogenies will provide better tools for evolutionary studies. Until more genes are available for a high number of taxa, can we rely on readily available single gene mitochondrial data? Here, we estimate the phylogeny of 66 cetacean taxa and 24 outgroups based on Cytb sequences. We judge the reliability of our phylogeny based on the recovery of several deep-level benchmark clades. A Bayesian phylogenetic analysis recovered all benchmark clades and for the first time supported Odontoceti monophyly based exclusively on analysis of a single mitochondrial gene. The results recover the monophyly of all but one family level taxa within Cetacea, and most recently proposed super- and subfamilies. In contrast, parsimony never recovered all benchmark clades and was sensitive to a priori weighting decisions. These results provide the most detailed phylogeny of Cetacea to date and highlight the utility of both Bayesian methodology in general, and of Cytb in cetacean phylogenetics. They furthermore suggest that dense taxon sampling, like dense character sampling, can overcome problems in phylogenetic reconstruction.
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1. Introduction

Several issues of Cetacean phylogenetics have been intensely debated, as a result of independent datasets (morphology, nuclear DNA, and mitochondrial DNA) suggesting conflicting hypotheses. These debates include the phylogenetic placement of Cetacea as sister to Artiodactyla (e.g., Luckett and Hong, 1998; O'Leary and Geisler, 1999; see also Gingerich et al., 1990) or embedded within Artiodactyla, a clade called Cetartiodactyla (e.g., Arnason et al., 2004; Gatesy, 1997; Gatesy et al., 1996, 1999; Graur and Higgins, 1994; Hasegawa and Adachi, 1996; Lum et al., 2000; Matthee et al., 2001; Montgelard et al., 1997; Murphy et al., 2001; Nikaido et al., 1999; Reyes et al., 2004; Shimamura et al., 1999; Thewissen and Madar, 1999; Thewissen et al., 2001), the relationship between toothed whales and baleen whales (e.g., Cerchio and Tucker, 1998; Douzery, 1993; Geisler and Sanders, 2003; Luckett and Hong, 1998; Messenger and McGuire, 1998; Milinkovitch, 1995, 1997; Milinkovitch et al., 1993, 1994, 1995, 1996; Nikaido et al., 2001; Nishida et al., 2003), the relationships among delphinoids (e.g.,

* Corresponding author. Fax: +1 305 348 1986.
E-mail addresses: lmayc002@fiu.edu (L. May-Collado), ingi@zoology.ubc.ca (I. Agnarsson).

Milinkovitch et al., 1993; Nishida et al., 2003; Waddell et al., 2000), dolphins (e.g., Barnes et al., 1985; deMuizon, 1988; Fordyce et al., 1994; Kasuya, 1973; LeDuc et al., 1999; Mead, 1975; Perrin, 1989; Pichler et al., 2001), river dolphins (e.g., Cassens et al., 2000; Cozzuol, 1985; Flower, 1867; Hamilton et al., 2001; Nikaido et al., 2001; Simpson, 1945; Slijper, 1936; Winge, 1921; Yan et al., 2005), and porpoises (Rosel et al., 1995).

Since the mid 1990s mitochondrial DNA data have been at the forefront of advancing understanding of cetacean phylogenetics (e.g., Arnason et al., 1992, 1993, 2004; Arnason and Gullberg, 1993, 1994, 1996; Gatesy et al., 1996; Irwin and Arnason, 1994; Graur and Higgins, 1994; Milinkovitch, 1995, 1997; Milinkovitch et al., 1993, 1994; Montgelard et al., 1997; Sasaki et al., 2005), for several reasons. Mitochondrial DNA is relatively easy to amplify and sequence, it is mostly free of problems with paralogy, and it has a relatively high substitution rate and thus offers information at various phylogenetic levels (Irwin et al., 1991; Milinkovitch, 1997). Results based on mitochondrial DNA offered novel, often controversial hypotheses (e.g., Arnason and Gullberg, 1994; Irwin and Arnason, 1994; Milinkovitch, 1995; Milinkovitch et al., 1993, 1994) and sparked renewed interest in the reconstruction of the evolutionary history of whales. Some of these hypothesis such as the placement of Cetacea within Artiodactyla (Cetartiodactyla sensu Montgelard et al., 1997) (e.g., Graur and Higgins, 1994; Irwin and Arnason, 1994), and the unexpected hypothesis of the sister relationship of Cetacea and Hippopotamidae (Cetancodonta sensu Arnason et al., 2000) (see Gatesy, 1997; Irwin and Arnason, 1994; Montgelard et al., 1997) have now received support from studies based on new independent datasets. Another unexpected mitochondrial hypothesis (based on Cytb, 12S, and 16S), the placement of baleen whales within toothed whales, however, was recently resolved in a different direction. Using the entire mitochondrial genome reversed the earlier mitochondrial hypothesis and recovered the monophyly of Odontoceti (Arnason et al., 2004). These previously controversial clades can now be labeled as 'benchmark' clades, i.e., to be likely true:

Odontoceti. Arnason et al. (2004) (mitogenomic data); Messenger and McGuire (1998) (morphology); Nishida et al. (2003) (nuclear DNA); Nikaido et al. (2001) (retroposon SINE data); O'Leary et al. (2004) (combined morphology, nuclear DNA, mitochondrial DNA, and amino acids).

Cetartiodactyla. Thewissen et al. (2001) and Boisserie et al. (2005) (morphology including fossil taxa); Arnason et al. (2004) (mitogenomic data); Matthee et al. (2001) and Murphy et al. (2001) (nuclear and mitochondrial data); Shimura et al. (1997) and Shimamura et al. (1999) (retroposon SINE data); O'Leary et al. (2004) (combined morphology, nuclear DNA, mitochondrial DNA, and amino acids).

Cetancodonta (Cetacea + Hippopotamidae). Geisler and Sanders (2003) and Boisserie et al. (2005) (morphology including fossils); Gatesy et al. (1999) (nuclear and mitochondrial data); Lum et al. (2000) (retroposon SINE data); Arnason et al. (2000, 2004) (mitogenomic data); O'Leary et al. (2004) (combined morphology, nuclear DNA, mitochondrial DNA, and amino acids).

Some long standing debates are thus all but resolved: our understanding of deeper level cetacean phylogeny has grown strong. However, the strong focus of most recent studies, aiming specifically to resolve these higher level conflicts by adding mostly characters rather than taxa, has left our understanding of lower level relationships among whale species lagging behind. Mitogenomic data, for example, is available only for 16 cetacean species, and no molecular study to date has included more than 33 cetaceans. It seems timely to focus on more detailed (genus, and species level) molecular phylogenies. These will provide better tools for detailed evolutionary studies, and are necessary to test existing morphological phylogenetic hypotheses, and current cetacean classification. Furthermore, adding taxa, as adding characters, can be an efficient way of overcoming phylogenetic uncertainty (Graybeal, 1998; Hillis, 1996, 1998; Hillis et al., 2003; Pollock et al., 2002; Zwickl and Hillis, 2002; but see Miller and Hormiga, 2004; Rosenberg and Kumar, 2001, 2003; Rokas and Caroll, 2005). Obviously, combining multiple lines of evidence is beneficial to any phylogenetic problem. The study of O'Leary et al. (2004) is an excellent example of how seemingly incongruent data subsets can, when combined, yield a globally robust (and credible) result. However, until more genes are available for a high number of cetacean taxa, can we rely on readily available single gene mitochondrial data? Here, we estimate the phylogeny of 66 cetaceans taxa representing 63 species, and 24 outgroups based on Cytb sequences from GenBank. This data matrix approximately doubles the taxon sampling of the most complete previous molecular study on cetacean phylogenetic relationships. We chose Cytb as it is available for more species than any other gene, and as it is a protein coding gene where alignment is trivial; in contrast many portions of the mitochondrial genome are notoriously difficult to align (e.g., Cerchio and Tucker, 1998; Messenger and McGuire, 1998).

We judge the reliability of our phylogeny based on the recovery of the previously mentioned benchmark clades, in addition to the less controversial clades Perissodactyla, Euungulata (sensu Waddell et al., 2001; Perissodactyla + Cetartiodactyla), Cetacea, and Mysticeti. Because Cytb is thought to be most reliable at lower taxonomic levels (due to high substitution rates), recovering 'known' deeper clades gives credibility to these new findings which have not been addressed by studies using few taxa. We compare the performance of Bayesian analyses versus parsimony under four different models, and briefly examine the sensitivity of the results to taxon sampling. We use our results to discuss agreement and remaining conflict in cetacean phylogenetics, and provide comments on current classification.

2. Materials and methods

Cytochrome data was compiled from GenBank for 66 cetaceans representing 63 species (see Table 1 for accession

numbers). Most previous mitochondrial DNA studies have included relatively few outgroups. For a stronger test of Cetartiodactyla monophyly and deeper level relationships we sampled 24 outgroup taxa using the recent mammalian phylogeny of Murphy et al. (2001) as a guide to outgroup choice. Murphy et al.'s (2001) phylogeny, based on 18 gene segments, suggested the following relationships: (Carnivora (Perissodactyla + Cetartiodactyla)). Outgroups therefore include non-cetacean cetartiodactylans (16 species), Perissodactyla (six species), and two carnivores chosen as primary outgroups on which the preferred tree is rooted (Table 1). To minimize potential missing data problems in an already difficult phylogenetic problem, we chose to exclude cetacean taxa when the following two conditions applied: (1) only small partial Cytb sequences were available (less than 50% of the entire sequence), and (2) congeners with longer sequences were already present in the matrix.

The molecular matrices were matched and aligned using the Needleman–Wunsch algorithm (gap cost = 10, mismatch = 1) in MacClade 4.07 (Maddison and Maddison, 2003). As Cytb is a protein coding gene, the alignment of the Cytb sequences was unambiguous without any gaps.

The data were analyzed using Bayesian, and parsimony methods. The appropriate model for the Bayesian analyses was selected with Modeltest (Posada and Crandall, 1998, 2001), using the AIC criterion (Posada and Buckley, 2004) with a parsimony tree chosen as the basis for Modeltest. The best model was GTR+Γ+I (Rodríguez et al., 1990; Yang, 1994). Estimates for the model parameters ($-\ln L = 23900.7090$, $K = 10$, base frequency A = 0.368, C = 0.400, G = 0.0518, and T = 0.1802).

Bayesian analysis was performed using MrBayes V3.0 (Huelsenbeck and Ronchist, 2001) with the following settings. The maximum likelihood model employed six substitution types ("nst = 6"), with base frequencies estimated from the data. As substitution frequencies differ starkly between first, second and third positions in Cytb (Irwin et al., 1991), each codon position was treated separately (substitution rate partitioning) (charset 1st_pos = 1-1140\3; charset 2nd_pos = 2-1140\3; charset 3rd_pos = 3-1140\3; partition bycodon = 3: 1st_pos; 2nd_pos; 3rd_pos; set partition = bycodon). Rate variation across sites was modeled using a γ-distribution (rates = "invgamma"). The Markov chain Monte Carlo search was run with four chains for 5,000,000 generations (repeated three times), sampling the Markov chain every 1000 generations, and the sample points of the first 70,000 generations were discarded as "burn-in," after which the chain reached stationarity.

Parsimony analyses were done in PAUP* (Swofford, 2001) and NONA (Goloboff, 1993) through the WINCLADA shell (Nixon, 2002). In each of the analyses, heuristic searches were done with 1000 random stepwise additions, and subtree-pruning and regrafting branch swapping algorithm (chosen arbitrarily). As transitions (Ti) are much more common than transversions (Tv) and different codon positions show different levels of Ti saturations (third position showing the highest), we used some of the many weightings schemes suggested by previous authors. In addition to equal weights (Tv = Ti = all positions = 1), down weighting transitions (Ti = 0, Tv = 1, as suggested by Milinkovitch et al., 1996), (Ti = 1, Tv = 3 as suggested by Milinkovitch et al., 1994; see Messenger and McGuire, 1998), unequal codon weighting (4:17:1 as suggested by Arnason and Gullberg, 1994).

Node support for the parsimony analyses was estimated using Bootstrapping (Felseinstein, 1985). Each analysis was run for 200 Bootstrap replicates, with 10 random addition sequences, and holding a maximum of 100 trees, per replicate.

To examine the effect of sparse taxon sampling on the Bayesian analysis (numerous previous studies have analyzed smaller Cytb datasets using parsimony) we analyzed two, rather arbitrarily chosen subset of the data. First, we pruned the dataset to contain a comparable taxon sampling to that of Messenger and McGuire (1998)—subsample in Table 1; second, we used the pruned ingroup dataset, but added all the outgroups from the main data matrix (subsample, plus outgroups in Table).

3. Results

3.1. Bayesian analysis

The Bayesian analysis recovered all seven benchmark clades (Table 2). Support for five of the benchmark clades is high (100 posterior probabilities) but rather low for Cetancodonta (79) and marginal for the monophyly of Odontoceti (67) (Fig. 1, Table 2). The analysis also recovered all but one family level, and most sub- and super-family level cetacean taxa (Fig. 1, for posterior probability values for each clade, see Fig. 2). The results thus broadly corroborate current cetacean classification, while also pointing to some lower-level groups that may need redefinition.

3.2. Pruned Bayesian analyses

The Bayesian analysis of pruned matrix I (see Table 1) was broadly congruent with the parsimony analysis of Messenger and McGuire (1998) based on a similar taxon sampling, rejecting Odontoceti monophyly. When all outgroups of the main matrix were added (subsample matrix II, see Table 1), however all the benchmark clades were again recovered (Table 2).

3.3. Parsimony analyses

The parsimony analyses all recovered Perissodactyla, Cetancodonta, Cetacea, and Mysticeti, with variable support (Table 2). Euungulata was recovered with high support by three out of the four analyses, but not under

Table 1
Species used in the analyses with respective GenBank accession numbers of Cytb sequences

Species	Accession #	Subsample 1	Subsample 2
Carnivora			
Canis familiaris	AY729880		X
Panthera leo	AF053052		X
Euungulata			
Perissodactyla			
Dicerorhinus sumatrensis	AJ245723		X
D. bicornis	X56283		X
Equus caballus	AY515162		X
Equus grevyi	X56282		X
Tapirus indicus	AF145734		X
T. terrestris	AF056030		X
Cetartiodactyla			
Alces alces	M98484		X
Antilocapra americana	AF091629	X	X
Antilope cervicapra	AF022058		X
Babyrousa babyrussa	Z50106		X
Bos taurus	AB090987	X	X
Camelus dromedarius	X56281	X	X
Cephalophus zebra	AF153903		X
Giraffa camelopardalis	X56287		X
Lama lama	U06429		X
Moschus leucogaster	AF026889		X
Odocoileus hemionus	X56291		X
Oryx gazella	AF249973		X
Ovis aries	AB006800		X
Pecari tajacu	X56296	X	X
Sus barbatus	Z50107		X
Cetancodonta			
Hippopotamus amphibius	Y08813		
Cetacea			
Mysticeti			
Balaena glacialis	X75587		
Balaena mysticetus	U13125	X	X
Balaenoptera bonaerensis	X75581	X	X
B. borealis	X75582		
B. edeni	X75583		
Capera marginata	X75586		
Eschrichtius robustus	X75585	X	X
Megaptera novaeangliae	X75584	X	X
Odontoceti			
Physeteroidea			
Physeteridae			
Physeter macrocephalus (catodon)	X75589	X	X
Kogidae			
Kogia breviceps	U72040	X	X
Kogia simus	AF304072	X	X
Ziphoidea			
Ziphiidae			
Berardius bairdii	X92541		
Hyperoodon planifrons	AY579560		
H. ampullatus	AY579558		
Indopacetus pacificus	AY162441		
Mesoplodon densirostris	X92536	X	X
M. bidens	X92538	X	X
Tasmacetus shepherdi	AF334484		
Ziphius cavirostris	AF304075	X	X
Platanistoidea			
Platanistidae			
Platanista gangetica	AF304070		
Platanista minor	X92543		

Table 1 (*continued*)

Species	Accession #	Subsample 1	Subsample 2
Inoidea			
Pontoporidae			
Pontoporia blainvelli	AF334488		
Iniidae			
Inia geoffrensis boliviensis	AF334487	X	X
Inia geoffrensis geoffrensis	AF334485		
Inia geoffrensis humboldtiana	AF521110		
Lipotoidea			
Lipotidae			
Lipotes vexillifer	AF304071		
Delphinoidea			
Monodontidae			
Delphinapterus leucas	U72037	X	X
Monodon monocerus	U72038		
Phocoenidae			
Australophocoena dioptrica	U09681		
Neophocaena phocaenoides	AF334489		
Phocoena phocoena	U72039	X	X
P. sinus	AF084051		
P. spinipinnis	U09676	X	X
Phocoenoides dalli	U09679		
Delphinidae			
Cephalorhynchus commersonii	AF084073		
C. eutropia	AF084072	X	X
C. hectori	AF084071		
C. heavisidii	AF084070		
Delphinus delphis	AF084085	X	X
D. capensis	AF084087		
D. tropicalis	AF084088		
Feresa attenuata	AF084052		
Globicephala macrorhynchus	AF084055		
G. melas	AF084056	X	X
Grampus griseus	AF084059		
Lagenorhynchus acutus	AF084075		
L. australis	AF084069		
L. cruciger	AF084068		
L. obliquidens	AF084067	X	X
L. obscurus	AY257161	X	X
Lagenodelphis hosei	AF084099		
Lissodelphis borealis	AF084064		
L. peronii	AF084065	X	X
Orcinus orca	AF084061		
Orcaella brevirostris	AF084063		
Pseudorca crassidens	AF084057		
Stenella attenuata	AF084096		
S. clymene	AF084083		
S. coeruleoalba	AF084082		
S. frontalis	AF084090		
S. longirostris	AF084103		
Sousa chinensis	AF084080		
Sotalia fluviatilis	AF304067		
Steno bredanensis	AF084077		
Tursiops truncatus	AF084095	X	X

In subsample 1 species were included to mirror taxon selection in Messenger and McGuire (1998) molecular analyses except for the outgroup species *Tragulus napu*. Furthermore, the following cetacean species were replaced by close relatives: *Mesoplodon europaeus* and *M. peruvianus* by *M. bidens* and *M. densirostris*, *Lagenorhynchus albirostris* by *L. obliquidens*, and *Balaenoptera physalus* by *B. bonaerensis*. In subsample 2 the same species were included as in subsample 1 plus all outgroups from this study.

the 4:17:1 weighting scheme. None of the parsimony analyses unambiguously recovered Cetartiodactyla or Odontoceti. Under equal weights, the majority of the most parsimonous trees supported Odontoceti monophyly while the strict consensus collapses Mysticeti, *Kogia*,

Table 2
Posterior probabilities and bootstrap values for all benchmark clades

Model	Benchmark clades						
	Euungulata	Perissodactyla	Cetartiodactyla	Cetancodonta	Cetacea	Mysticeti	Odontoceti
Bayesian GTR+Γ+I	100	100	100	79	100	100	67
Subsample Bayesian	n/a	n/a	n/a	n/a	100	98	NO
Subsample w/outgr.	100	100	100	71	100	99	78
Equally weighted MP	100	100	NO	<50	100	100	NO
MP (3:1)	100	100	NO	100	100	100	NO
MP (17:4:1)	NO	100	NO	<50	100	100	<50
MP (1:0)	88	91	NO	<50	97	100	NO

Physeter, and Ziphidae + *Platanista*, into a pentachotomy with the remaining cetaceans. Cetartiodactyla is not recovered under equal weights, due to the placement of *Camelus* + *Lama* basal to Perissodactyla. Ignoring transitions altogether (see Milinkovitch et al., 1996) was similar to the equal weights analysis, although the strict consensus is less resolved, with the same pentachotomy formed at the base of Cetacea. Weighting transversions three times transitions (see Messenger and McGuire, 1998; Milinkovitch et al., 1994) placed Mysticeti sister to Ziphidae, in turn sister to sperm whales (Table 2).

Apart from benchmark clades, most analyses broadly agreed on the monophyly of superfamily, family, and subfamily level taxa within Cetacea. All cetacean families are supported with the exception of Balaenopteridae which consistently contained Eschrichtiidae Subfamilies within Phocoenidae and Ziphidae were furthermore contradicted by all analyses. Our results support the transfer of *Lagenorhynchus acutus* to *Leucopleurus* (as suggested by LeDuc et al., 1999; LeDuc, 2002), and in our preferred phylogeny (Figs. 1 and 2) this taxon is place sister to Delphinae plus Stenoninae. The following genera are not monophyletic according to our results: *Lagenorhynchus* (even after excluding *L. acutus*), *Stenella*, *Phocoena*, and *Balaenoptera*.

4. Discussion

4.1. Recovery of benchmark clades

Many recent cetacean phylogenetic studies include relatively few taxa (exceptions include Arnason and Gullberg, 1996; Hamilton et al., 2001; LeDuc et al., 1999; LeDuc, 2002; Messenger and McGuire, 1998), in part due to a focus on generating more characters to resolve higher level phylogenetics (see e.g., Arnason et al., 2004; Lum et al., 2000; Nikaido et al., 2001; Nishida et al., 2003). While addressing crucial questions and providing the backbone for lower level phylogenies, such studies have limited utility for classification, and for comparative evolutionary studies. In some cases sparse taxon sampling may also confound the results (Hillis et al., 2003). Of course, taxon sampling is usually simply constrained by the availability of character data, but for some reason many studies have opted to include only one, or a few outgroup taxa, even if many are available. Outgroup choice may have marked impact on any phyloge-

netic analysis (see e.g., Adachi and Hasegawa, 1995; Milinkovitch and Lyons-Weiler, 1997).

Here, we have extensively sampled cetacean taxa, and outgroups, to provide a more detailed phylogenetic hypothesis than previous studies. We analyzed the data using Bayesian methods, increasingly popular in molecular phylogenetics, but hitherto little used in cetacean studies (but see e.g., Yan et al., 2005), in addition to parsimony under various previously proposed weighting schemes.

Given the relatively few characters we certainly acknowledge the limitations of our study, and we did not expect robust clade support, especially for deeper level clades that have been consistently contradicted by previous Cytb analyses. However, we set up to test the reliability and sensitivity of our extended Cytb phylogeny based on the recovery of deep level benchmark clades (Euungulata, Perissodactyla, Cetartiodactyla, Cetancodonta, Cetacea, Mysticeti, and Odontoceti). Our study finds: (1) Bayesian phylogenetic methods outperformed parsimony under various models; (2) increased taxon sampling, in particular outgroup sampling (Table 2) increased congruence with other datasets, e.g., for the first time some of our analyses support Odontoceti monophyly based on Cytb data alone.

We find that as long as outgroup taxon sampling was extensive, Bayesian analyses of Cytb recovered all the a priori identified benchmark clades. When only a few outgroups were chosen, however, the Bayesian analysis negated Odontoceti monophyly (Table 2), as have many previous parsimony analyses of mitochondrial DNA. Furthermore, in almost every detailed comparison possible our results mirror the findings O'Leary et al. (2004), the most 'character-complete' (but including relatively few cetacean taxa) analysis to date (37,000 characters from morphology, SINE, and 51 gene fragments). This result gives credibility to our findings, including previously untested lower level clades.

The low support for Odontoceti is unsurprising given previous analysis of Cytb, and the finding of Arnason et al. (2004) that explosive radiation took place early in the evolutionary history of whales, with little time to accumulate synapomorphies for major lineages such as Odontoceti. The parsimony analyses likewise recover the benchmark clades Perissodactyla, Cetancodonta, Cetacea, and Mysticeti, but support for Cetartiodactyla and Odontoceti was highly sensitive to a priori character weighting schemes. Using the Arnason and Gullberg (1994) codon weighting scheme (4:17:1), a
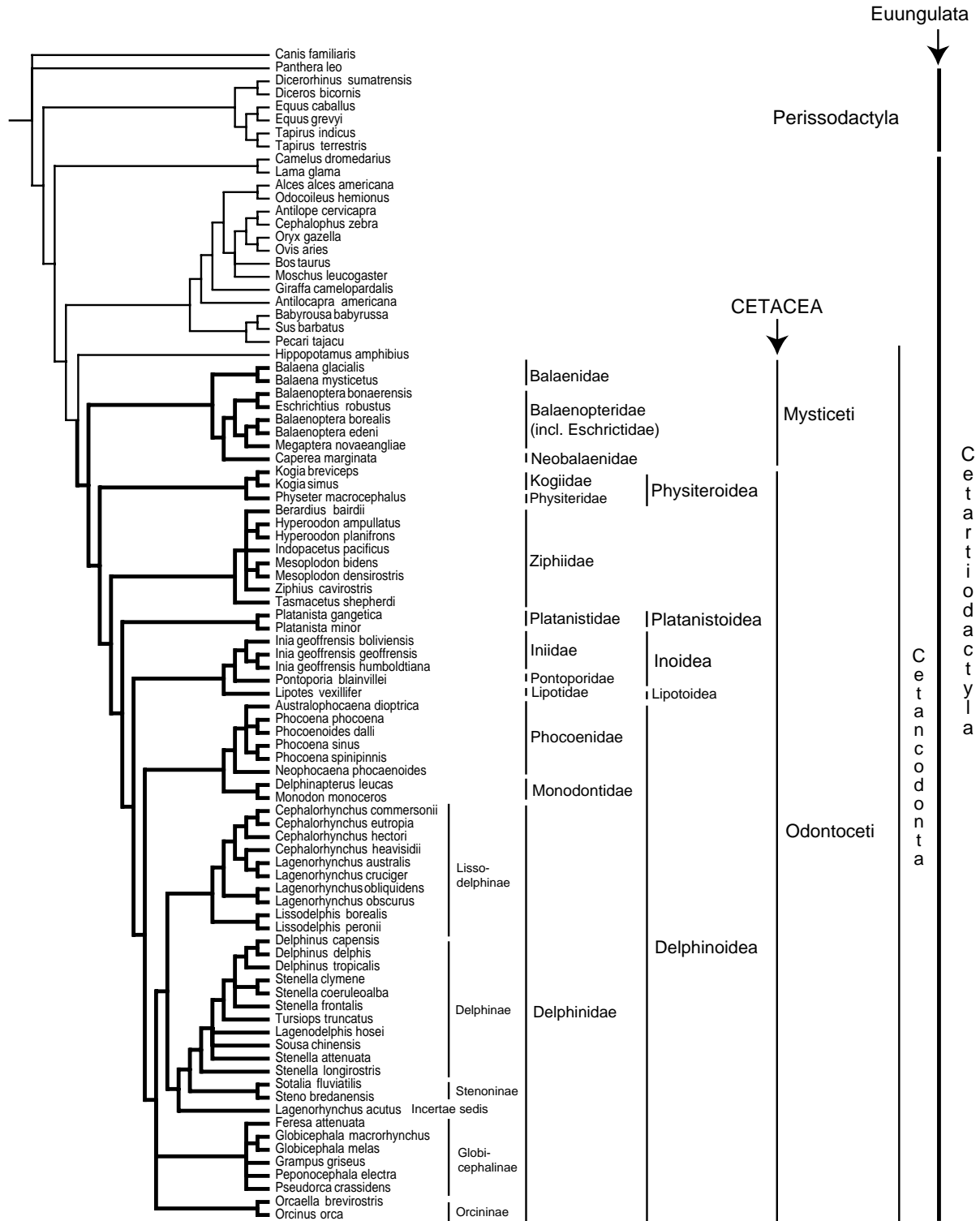
Fig. 1. The preferred phylogenetic hypothesis based on the Bayesian analysis of 63 cetacean species and 24 outgroups. Major groups are labeled according to recent literature, Cetacea is indicated by bold branches on the cladogram. For posterior probability support values, see Fig. 2.

relatively strong support is found for Odontoceti monophyly. This is an interesting example of how dense taxon sampling can impact the phylogenetic signal. Arnason and Gullberg (1994) used this weighting scheme in an Cytb analysis of 14 cetacean species and one outgroup (cow) suggested the placement of Mysticeti within Odontoceti.

Because Bayesian analyses allows for an objective way of weighting characters (Felsenstein, 1981) and because it recovers all the benchmark clades supported by other independent data (e.g., Arnason et al., 2000, 2004; Messenger and McGuire, 1998; Nikaido et al., 2001; O'Leary et al., 2004) we favor the Bayesian hypothesis. As for other clades,
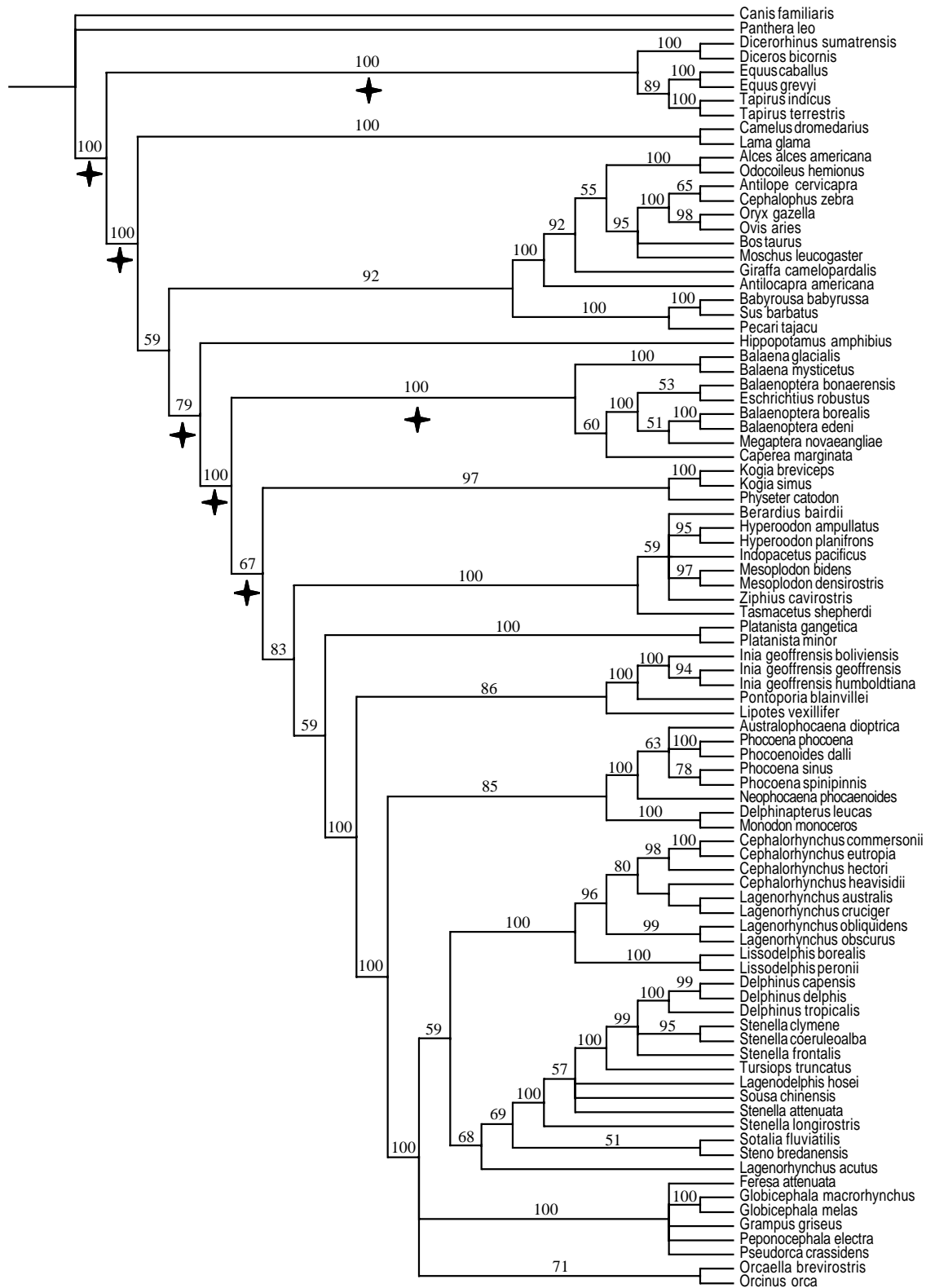
Fig. 2. The preferred hypothesis showing posterior probabilities (numbers above branches) from the Bayesian analysis. Stars below branches indicate benchmark clades.

most of the analyses showed remarkable congruence with previous phylogenies based on nuDNA, morphology, and mtDNA data (e.g., Cassens et al., 2000; Hamilton et al., 2001; LeDuc et al., 1999; Messenger and McGuire, 1998; Rosel et al., 1995; Rychel et al., 2004; Sasaki et al., 2005; Waddell et al., 2000). Below we briefly review the implications of our results to lower level cetacean phylogenetics and classification.

4.2. Monophyly and placement of Mysticeti (baleen whales)

The monophyly of baleen whales is virtually uncontroversial (see e.g., Sasaki et al., 2005). However, their placement has been debated. Based on mitochondrial data Milinkovitch et al. (1993, 1994, 1995, 1996) suggested that baleen whales were sister to sperm whales (Physeteroidea), Verma et al. (2004) placed them sister to Platanistidae, while Arnason and Gullberg (1994) based on Cytb placed baleen whales sister to dolphins (however, with very few taxa presented). These hypotheses have remained contradicted by both morphological and nuclear data, which agree on the sister relationship of monophyletic Odontoceti and Mysticeti. Our phylogenetic results agree with morphological and nuclear DNA data (e.g., O'Leary et al., 2004), echoing a new mitogenomic study by Arnason et al. (2004).

Within Mysticeti, we found support for the monophyly of Balaenidae, and the placement of Neobalaenidae sister to (extended) Balaenopteridae. However, *Eschrichtius robustus* consistently nested within Balaenopteridae, rendering the latter paraphyletic as found by Rychel et al. (2004), O'Leary et al. (2004), and Sasaki et al. (2005).

4.3. Monophyly of Odontoceti (toothed whales)

Odontoceti is one of our benchmark clades, and was supported by the Bayesian analysis and one of the parsimony analyses. The recovery of this clade shows that with sufficient taxa mitochondrial phylogenies can be reliable. Within Odontoceti the superfamilies Delphinoidea, Physeteroidea, and Inoidea were monophyletic, and also all family level taxa (Fig. 1).

4.3.1. Delphinoids

All analyses agree on the monophyly of Delphinoidea and monophyly of each of the delphinoid families Monodontidae, Phocoenidae, and Delphinidae, and all subfamilies within Delphinidae. Our results strongly support the relationship Delphinidae (Monodonotidae + Phocoenidae). Waddell et al. (2000) found the same relationships with nuclear genes and Nishida et al. (2003) with SRY (sex determining region of the Y chromosome) gene. Our findings contradict the division of Phocoenidae into two subfamilies Phocoeninae and Phocoenoidinae. The porpoises *Australophoceona dioptrica* and *Phoecoenoides dalli*, rather nested within Phocoeninae, and *Neophocaena phocaenoides* is basal to all the porpoises. As Rosel et al. (1995) suggested, *Australophocoena* should be returned to *Phocoena* where it was originally placed by Lahille (1912), and *Phocoenoies dalli* classification needs further analysis.

LeDuc et al. (1999) and LeDuc (2002) proposed a new classification for Delphinidae based on Cytb data. Unsurprisingly, our results largely agree. *Stenella* and *Lagenorhynchus* are paraphyletic and both need revision. *Grampus griseus* nested within the subfamily Globicephalinae in all our analyses (see also Kasuya, 1973), rather than within Delphininae as previously suggested (Barnes et al.,

1985; deMuizon, 1988; Mead, 1975; Perrin, 1989). Orcininae (*Orcinus orca* + *Orcaella brevirostris*), separate from Globicephalinae is supported. *Sousa chinensis* groups within the subfamily Delphininae and not with Stenoninae. Furthermore, our results show a monophyletic Lissodelphininae including *Cephalorhynchus* spp., *Lissodelphis* spp., and *Lagenorhynchus australis*, *L. cruciger*, *L. obliquidens* and *L. obscurus*. As suggested by previous studies *Lagenorhynchus* is not monophyletic. Our results support LeDuc et al. (1999) in transferring *Lagenorhynchus acutus* to *Leucopleurus*, but its phylogenetic position also requires the creation of a new subfamily, likely also including *L. albirostris*. LeDuc et al. (1999) and LeDuc (2002) suggested returning these four species to the genus *Sagmatias* (type species *L. cruciger*), however, in our analyses *L. cruciger* and *L. australis* are nested within *Cephalorhynchus*. Thus, it may be simplest to transfer the *L. cruciger* and *L. australis* to *Cephalorhynchus*, while retaining *L. obliquidens* and *L. obscurus* in *Lagenorhynchus*. Interestingly, the placement of *L. australis* within *Cephalorhynchus* is supported by acoustic data. Uniquely among dolphins, *L. australis*, and the four *Cephalorhynchus* species do not whistle (Schevill and Watkins, 1971). There is not published data on the acoustic behavior of *L. cruciger*.

4.3.2. River Dolphins

Our results agree with most molecular and recent morphological studies that river dolphins are polyphyletic, and do not offer unambiguous support for the infraorder Delphinida (containing Delphinoidea, Lipotidae, Iniidae, Pontoporidae, and Platanistidae). As suggested by most studies Platanistidae does not group with other river dolphins, but is here the most basal family of Delphinida (e.g., Cassens et al., 2000; Hamilton et al., 2001; Messenger and McGuire, 1998; Yan et al., 2005). Note that a recent study based on nuDNA and Cytb placed Platanistidae sister to Mysticeti, although with little support (Verma et al., 2004). *Platanista* is the only surviving genus of the superfamily Platanistoidea which contains the extinct marine families Prosqualodontidae, Dalpiazinidae, Waipatiidae, Squalodontidae, and Squalodelphinidae, in addition to Platanistidae (deMuizon, 2002). Although, paleontologists agree that *Platanista* is a close relative of the family Squalodelphinidae (Heyning, 2002) new paleontological data points to *Lipotes vexillifer* and *Inia geoffrensis* as its closest relatives (Geisler and Sanders, 2003).

Geisler and Sanders (2003) suggest a single ecological shift to riverine habitats in the 'river dolphins,' instead of two as argued by other authors (e.g., Cassens et al., 2000; Hamilton et al., 2001; Nikaido et al., 2001). Our results indicate two to three shifts in the 'river dolphins.' An unambiguous one in *Platanista*, and either one in *Inia* and another in *Lipotes*, or a single origin in the node leading to Inoidea plus Lipotoidea with a reversal in *Pontoporia*. In addition, populations of *Sotalia fluviatilis*, *O. brevirostris* (LeDuc et al., 1999), and the porpoise *N. phocaenoides*, independently shifted to a riverine habitat.

Previously the three 'river dolphin' genera were placed in a single family Iniidae (Heyning, 1989) or two families Pontoporidae (*Pontoporia* and *Lipotes*) and Innidae (*Inia*) (Fordyce et al., 1994). Our phylogenetic results agree with the classification of the three genera into three families as suggested by Fordyce and deMuizon (2001) with the following relationship ((Pontoporidae + Iniidae) + Lipotidae). This arrangement is supported by both morphology and molecular data (e.g., Cassens et al., 2000; Hamilton et al., 2001; Messenger and McGuire, 1998; Nikaido et al., 2001; Yan et al., 2005; Yang and Zhou, 1999). Furthermore, the relationship of *Inia* subspecies is unsurprisingly identical to that found by Hamilton et al. (2001) (*I. g. humboldtiana* + *I. g. geoffrensis* + *I. g. boliviensis*).

4.3.3. Beaked and sperm whales

Our results support the superfamily Physeteroidea which includes the families Kogiidae and Physeteridae, whereas ziphiids interrelationships were largely unresolved. The molecular work of Dalebout et al. (2004) calls for a revision of Ziphiidae and Mead (2002) proposed the subfamilies Ziphininae (*Berardius* spp., *Tasmacetus shepherdi*, and *Ziphius cavirostris*) and Hyperoodontinae (*Mesoplodon* spp. and *Hyperoodon* spp). Our analyses all indicate *Tasmacetus shepherdi* sister to all other ziphiids. To date, most cetacean phylogenies have not aimed at solving ziphiid species relationships, and thus their relationships are largely unknown Since low level taxonomic relationships were fairly well supported in other groups of toothed whale, Cytb seems promising in providing future insights in the evolutionary relationships of ziphiids. Physeteroids and ziphiids are the most basal toothed whales. Both groups show a clear reduction in dentition, in physeteroids teeth are only present in the lower jaw, and in most ziphiid species, teeth are reduced or absent in both jaws, with the exception of males that have two prominent teeth in the lower jaw (Mead, 2002).

It is interesting to notice that *T. sheperdi* is basal in our ziphiid phylogeny and it is the only beaked whale with full dentition in both jaws. Although this particular relationship is weakly supported, it hints that the loss of teeth may be convergent in Physeteroidea, and within Ziphiidae.

4.4. Concluding remarks

Substitution saturation imposes limitation on Cytb (and other mitochondrial data) for deeper level phylogenetics, and may lead to misleading results (Irwin et al., 1991; Springer et al., 2001). Furthermore, many studies have shown that single gene analyses rarely agree with global optima (e.g., O'Leary et al., 2004). However, our results show that by densely sampling taxa, especially outgroup taxa, and using appropriate methods of analysis with realistic models of evolution, this problem may be reduced, and in this particular example, mostly overcome. Low-level phylogenies are essential for classification and as a tool for comparative evolutionary (and ecological) studies. In this context 'single gene' phylogenies may be of great value (as long as they are 'reality checked') as relatively many species can be included, offering more detailed phylogenies than currently possible with phylogenies based on multiple genes and morphology. Ultimately, of course, a major goal of phylogenetics is a phylogeny of life (i.e., many taxa), based on multiple lines of evidence (many characters of many types). However, when phylogenies based on relatively few characters can be judged reliable based on external evidence (taxonomic congruence with other phylogenies using many characters, but few taxa), they seem like very promising and useful 'first guess' hypotheses. The evolution of sexual dimorphism, echolocation, social behavior, and whistles and other communicative signals, and major ecological shifts (e.g., transition to fresh water) are among the numerous interesting questions in cetacean biology that this phylogeny can help answer.

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