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PHYLOGENETIC RELATIONSHIPS AMONG THE DELPHINID CETACEANS BASED ON FULL CYTOCHROME *B* SEQUENCES

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Abstract

Complete cytochrome *b* gene sequences from all but one species of delphinid plus four outgroups were analyzed using parsimony, maximum likelihood, and neighbor-joining methods. The results indicate the need for systematic revision of the family; a provisional classification is presented and compared to previous studies. Among the suggested revisions are removal of *Orcinus* from the Globicephalinae, placement of *Grampus* within the Globicephalinae, removal of all *Lagenorhynchus* spp. from the Delphininae, and placement of *Sousa* in the Delphininae. The genus *Lagenorhynchus* is found to be polyphyletic. *L. albirostris* (type species for the genus) and *L. acutus* are not closely related to each other or to nominal congeners. *L. acutus* is therefore assigned to the genus *Leucopleurus*. The remaining four *Lagenorhynchus* species are closely related to *Lissodelphis* and *Cephalorhynchus* and are placed in the genus *Sagmatias*. These three genera constitute the revised Lissodelphininae. Within the Delphininae, a well-supported clade includes the two species of *Delphinus, Stenella clymene, S. frontalis, S. coeruleoalba,* and the *aduncus* form of *Tursiops truncatus*. Accepting the monophyly of this group renders the genera *Stenella* and *Tursiops* polyphyletic. Apart from this finding, phylogenetic resolution within the Delphininae was poor, so comprehensive taxonomic revision of this group awaits further study.

Key words: systematics, delphinid, mitochondrial DNA, evolution, cytochrome *b*.

The anatomy, physiology, life history, behavior, or ecology of any particular species is shaped by that species' evolutionary history. As such, adaptations are best understood in an evolutionary context, *i.e.*, their similarity to what is found in related species, their variability in space and time, and their selective advantage to the species. This context requires at least a reasonable inference regarding a taxon's phylogenetic relationships. For researchers unfamiliar with

Table 1. A classification of the family Delphinidae (Perrin 1989).

Family Delphinidae	Subfamily Lissodelphinae
Subfamily Stenoninae	*Lissodelphis borealis*
Steno bredanensis	*Lissodelphis peronii*
Sousa chinensis	Subfamily Cephalorhynchinae
Sousa teuszii	*Cephalorhynchus commersonii*
Sotalia fluviatilis	*Cephalorhynchus eutropia*
Subfamily Delphininae	*Cephalorhynchus heavisidii*
Lagenorhynchus albirostris	*Cephalorhynchus hectori*
Lagenorhynchus acutus	Subfamily Globicephalinae
Lagenorhynchus obscurus	*Peponocephala electra*
Lagenorhynchus obliquidens	*Feresa attenuata*
Lagenorhynchus cruciger	*Pseudorca crassidens*
Lagenorhynchus australis	*Orcinus orca*
Grampus griseus	*Globicephala melas*
Tursiops truncatus	*Globicephala macrorhynchus*
Stenella frontalis	Subfamily Orcaellinae
Stenella attenuata	*Orcaella brevirostris*
Stenella longirostris	
Stenella clymene	
Stenella coeruleoalba	
Delphinus delphis	
[*Delphinus capensis*]	
Lagenodelphis hosei	

the systematic literature, the context of relationships is usually derived from a particular classification (*e.g.*, closest related species are thought to be those in the same genus). However, evolutionary relationships among dolphin species (family Delphinidae) are neither well understood nor agreed upon by all systematists, and those implicit in any current classification provide but a poor framework for any such understanding. The family has had a long and complex taxonomic history, which reflects (but lags behind) the development of the field of systematics itself and is far from complete on every level—*alpha* (determining how many species there are and describing them), *beta* (inferring relationships among species), and *gamma* (understanding the ranges and patterns of intraspecific variation).

The family Delphinidae is one of three extant families (with Phocoenidae and Monodontidae) in the cetacean superfamily Delphinoidea, all of which likely arose in the mid- to late Miocene (11–12 mya, Barnes 1990). At present, the family contains 33 recognized extant species in 17 genera, making it the most speciose of the cetacean families. Ten of the genera are monotypic, and only three contain more than two species. These are *Cephalorhynchus* with four species, *Stenella* with five, and *Lagenorhynchus* with six. The 17 genera have been variously arranged in a number of subfamilies, six according to a recent classification (Table 1) (Perrin 1989). (The species and genera listed in Table 1 are identical to those found in Mead and Brownell (1993), save for the addition of *Delphinus capensis*, a species recently recognized as distinct from *D. delphis* (Heyning and Perrin 1994, Rosel *et al.* 1994).) While the various

classifications presented over the years have differed at one taxonomic level, *i.e.*, the arrangement of genera into subfamilies, the species compositions of delphinid genera have changed little from those of Flower (1884, 1885) or True (1889).

To a non-systematist, this taxonomic stability of the genera throughout the last century may be thought to have resulted from sound phylogenetic inferences about species interrelationships. That this is not always the case is widely recognized by systematists, but changes in classification have been slow in coming, even when long overdue. For example, True (1889) expressed concern about the validity of the characters used to separate the genera *Tursiops* and *Delphinus* from *Prodelphinus* (=*Stenella*) and commented that *Tursiops* and *Prodelphinus* may eventually have to be combined. Almost a century later, Perrin *et al.* (1981) indicated that there is a complex of cranial characters not shared by all of the species in the genus *Stenella*, some of which may actually be more closely related to *Tursiops* or *Delphinus* than to their congeners. Therefore, the monophyly of *Stenella* has not been and cannot be taken for granted; the genus is likely to represent an artificial assemblage of species.

Most of the previous studies of dolphin evolutionary relationships relied on examination of morphological characters, particularly of the skull. More importantly, most of this work predated modern analytical techniques, specifically cladistics, that are widely accepted by systematists today. Cladistic analysis relies solely on the presence of shared derived characters (synapomorphies) to infer recent common ancestry between taxa (Wiley 1981). Systematists have long used a similar concept, specialization, to define taxa; however, in many cases, taxon identity was based instead on convergent (homoplastic) or primitive similarities (symplesiomorphies), which do not necessarily result from recent common ancestry. In addition, the theoretical advances of cladistics have been accompanied by the development of explicit methods for coding and analyzing data. However, rigorous cladistic analyses of cetaceans using morphological data have been rare. The few methodologically sound studies conducted to date have had little bearing on dolphin interrelationships; Heyning (1989, 1997) and Messenger (1995) focused on higher-level systematics in their cetacean cladistic analyses, and Arnold and Heinsohn (1996) included only four dolphin species in their phylogenetic study of the delphinid genus *Orcaella*. At present, there have been but two attempts at cladistic analyses of the Delphinidae using morphological characters from a large number of species (de Muizon 1988, Barnes 1990), but these were somewhat cursory in that only a few characters were used, the methodologies were not described, and the monophyly of the various genera was assumed and untested. Without strong inference of generic monophyly, the arrangement of genera into subfamilies seen in these studies was a wasted exercise.

Although a comprehensive review of delphinid systematic history is beyond the scope of this study, Table 2 lists some classifications presented in recent studies. In the modern era, an influential study was that of Fraser and Purves (1960). They described evolutionary trends in the modifications of the pterygoid sinus and outer and middle ear in cetaceans and established a classifi-

Table 2. Several recent classifications of delphinids. Parentheses within subfamilies indicate closer affinities among genera as indicated by authors' phylogenies. Note that (a) does not include *Sotalia* or *Peponocephala,* (b) does not include *Lagenodelphis,* (c) does not include *Sousa,* (d) does not include numerous genera, and (f) does not list genera at all.

(a) Fraser and Purves (1960)
- Family Stenidae
  - *Steno, Sousa*
- Family Delphinidae
  - Subfamily Orcinae
    - *Pseudorca, Orcinus, Orcaella, Globicephala, Feresa*
  - Subfamily Lissodelphinae
    - *Lissodelphis*
  - Subfamily Cephalorhynchinae
    - *Cephalorhynchus*
  - Subfamily Delphininae
    - *Lagenorhynchus, Lagenodelphis, Grampus, Tursiops, Stenella, Delphinus*

(b) Kasuya (1973)
- Family Delphinidae
  - Subfamily Sotaliinae
    - *Sotalia, Sousa, Cephalorhynchus*
  - Subfamily Orcininae
    - *Orcinus, Pseudorca*
  - Subfamily Delphininae
    - (*Steno, Lagenorhynchus, Delphinus*), (*Lissodelphis, Stenella, Tursiops*)
  - Subfamily Globicephalinae
    - *Peponocephala, Feresa, Globicephala, Grampus*
- Family Delphinapteridae
  - Subfamily Orcaellinae
    - *Orcaella*

(c) Mead (1975)
- Family Delphinidae
  - Subfamily Steninae
    - *Steno, Sotalia*
  - Subfamily Delphininae
    - *Tursiops, Grampus, Peponocephala, Lagenorhynchus, Lagenodelphis, Stenella, Delphinus*
  - Subfamily Lissodelphinae
    - *Lissodelphis*
  - Subfamily Cephalorhynchinae
    - *Cephalorhynchus*
  - Subfamily Orcininae
    - *Orcinus, Pseudorca, Globicephala, Orcaella, Feresa*

(d) Barnes et al. (1985), Barnes (1990)
- Family Delphinidae
  - Subfamily Steninae
  - Subfamily Delphininae
    - *Tursiops, Grampus, Peponocephala, Lagenorhynchus, Lagenodelphis, Stenella, Delphinus*
  - Subfamily Lissodelphinae
  - Subfamily Cephalorhynchinae
  - Subfamily Globicephalinae
- Family Monodontidae
  - Subfamily Orcaellinae

(e) de Muizon (1988)
- Family Delphinidae
  - Subfamily Delphininae
    - (*Steno, Sotalia, Sousa*), ((*Lissodelphis, Lagenodelphis, Lagenorhynchus*), (*Grampus*, (*Tursiops*, (*Stenella, Delphinus*))))
  - Subfamily Cephalorhynchinae
    - *Cephalorhynchus*
  - Subfamily Globicephalinae
    - *Orcaella, Peponocephala*, (*Orcinus*, (*Globicephala*, (*Feresa, Pseudorca*)))

(f) Fordyce and Barnes (1994), Fordyce *et al.* (1994)
- Family Delphinidae
  - Subfamily Steninae
  - Subfamily Delphininae
  - Subfamily Globicephalinae
- Family Monodontidae
  - Subfamily Orcaellinae

cation that became the basis for comparisons in many subsequent studies. In another study, Kasuya (1973) looked at the systematic relationships of the odontocetes based on the morphology of the tympano-periotic bones. While, for both studies, the species composition of dolphin genera is the same as in Table 1, they differ from this classification and from each other in the arrangement of the genera into families and subfamilies (Table 2a, b). In each case the authors determined primitive and derived conditions for the characters under examination, yet they still described taxa in terms of overall similarity. For example, Fraser and Purves' (1960) Stenidae (= Stenoninae) is largely based on plesiomorphic (primitive) characters. In discussing his Sotaliinae, Kasuya (1973) pointed out "As this subfamily is established based on many primitive features and modification of elliptical foramen which is very variable, there remains a question on the phylogenetic uniformity of this group."

Mead (1975) examined delphinid facial anatomy and produced a classification (Table 2c) that was adapted from that of Fraser and Purves (1960). However, Mead noted that, due to the conservative nature of delphinid facial morphology, there were no characters that consistently distinguished most of the subfamilies from one another. Only one subfamily (Cephalorhynchinae), containing a single genus with four species, was unique in these characters.

Barnes (1990) ostensibly performed a cladistic analysis of a variety of cranial and postcranial characters of living and fossil cetaceans to provide a systematic context for his examination of *Tursiops*. Although the classification of Barnes (1990) and Barnes *et al.* (1985) (Table 2d) is fairly conventional, the cladogram presented in Barnes (1990) depicts a polyphyletic Delphininae in which *Tursiops* branches off from the other delphinines before the subfamily diverges from Lissodelphinae and Cephalorhynchinae.

In the phylogeny presented by de Muizon (1988, Table 2e), only 15 morphological synapomorphies were used to discern relationships among the 17 genera of delphinids (with 2 additional characters uniting the entire family), and the monophyly of genera was not tested or discussed. The analyses of Barnes (1990) and de Muizon (1988), though cladistic, thus were not very comprehensive with regard to method or content. A more recent classification (Table 2f) was presented by Fordyce and Barnes (1994) and Fordyce *et al.* (1994) as part of a review of fossil and extant cetaceans. While no phylogenetic analysis accompanied the classification, it represents yet another subfamilial arrangement of delphinid genera.

While there have been some systematic genetic studies of cetaceans that involved delphinids, none have been comprehensive with regard to the family, and there has been no proposed classification of delphinids based on genetic analysis. Previous genetic studies were limited in scope or addressed only specific questions; these studies included too few taxa to allow a family phylogeny (*e.g.*, Lint *et al.* 1990, Ohland *et al.* 1995). Others utilized types of genetic data that were either too conservative or too variable for intergeneric and interspecific comparisons or utilized inappropriate data analyses. Insight gained from these studies has thus been limited. Shimura and Numachi's (1987) allozyme study included only seven delphinid genera, and the data

were not analyzed cladistically. Jones *et al.* (1979) analyzed the amino-acid sequence of myoglobin for a number of cetaceans. Although their analysis was cladistic, only five delphinid genera were included in the study, and only three informative characters were used to determine relationships among them. Ohland *et al.* (1995) included only eight species of dolphins in their cladistic analysis of cetacean mitochondrial DNA restriction site maps. In spite of their shortcomings, these genetic studies raised questions about some of the subfamily arrangements within the family, although intrageneric relationships were largely not addressed. For example, Shimura and Numachi (1987) and Ohland *et al.* (1995) challenged the generic makeup of the subfamily Delphininae, in the former case by the placement of *Lagenorhynchus obliquidens* outside that subfamily on their dendrogram, and in the latter by the affinity shown between *Grampus griseus* and some of the Globicephalinae.

In recent years many workers have turned to DNA sequencing, most often of parts of the mitochondrial (mt) genome, to generate data for phylogenetic analysis. This approach is not without its own pitfalls (Cummings *et al.* 1995, Russo *et al.* 1996, Zhang and Hewitt 1996), but it has provided valuable insight for cetacean systematics at the higher (Milinkovitch *et al.* 1993, 1996), familial (Rosel *et al.* 1995), and species (Rosel *et al.* 1994) levels. Although these insights have not provided definitive answers in all cases, they have stimulated further genetic as well as morphological investigations (*e.g.*, Milinkovitch 1995, Heyning 1997). It is our hope that the present paper may have a similar effect on the field of delphinid systematics.

Materials and Methods

The sample set included 52 individuals representing 32 of the 33 species of delphinids (no samples of *Sousa teuszii* were available to us), as well as four additional samples from the other two delphinoid families for use as outgroups. Appendix A gives a list of samples. Fourteen species were represented by multiple samples, representing different ocean basins for 11 of those species. While the majority of tissue samples were of skin, there were two liver samples and one each of placenta and blood. Most samples were stored in a saturated salt solution with 20% dimethyl sulfoxide (DMSO). Samples not in the salt solution were frozen at −70°C. In addition, six individuals were represented by extracted DNA sent from other laboratories, and two were provided to us as PCR product. Whenever possible, dolphin species with wide geographic ranges were represented by individuals from more than one ocean basin. The list includes tissue from beachcast animals, captives, incidental fishery kills, and biopsies from free-swimming dolphins. Biopsies were taken from bow-riding dolphins by researchers using a crossbow with a modified bolt tip. Species identifications were usually made in the field by the collector, with some specimens having associated osteological material deposited in a museum. In all cases, identifications were made by experienced researchers prior to and independent of the genetic analyses.

Particular mention should be made of the identification of specimens of the

aduncus form of *Tursiops truncatus*, hereafter referred to as *T. aduncus*, since the distinctness of this form is a matter for some debate. True (1914), Ross (1977), and Ross and Cockcroft (1990) discussed differences in the cranial and external morphology between the two forms. Because the *T. aduncus* samples used here are from live captive animals, identification to type was based only on external characteristics, which included the presence of ventral spotting, length and shape of beak, and flipper size. These samples were also part of a larger set used to study worldwide genetic variation in *Tursiops* (Curry 1997).

Extraction of total genomic DNA was usually by phenol-chloroform followed by precipitation with cold ethanol (modified from Maniatis *et al.* 1982). However, for a variety of reasons including tissue conditions and post-mortem handling, some samples produced poor yields of DNA from the phenol-chloroform extraction. In these cases, other extraction methods were used, including extractions with CTAB (cetyltrimethylammonium bromide) (Winnepenninckx *et al.* 1993), QIAamp extraction kit (QIAGEN Inc., Santa Clarita, CA), and GENECLEAN for Ancient DNA (BIO 101 Inc., San Diego, CA). Dissolved DNA was run on an agarose minigel and analyzed with a spectrophotometer to determine its size and purity.

Amplification of the cytochrome *b* gene was performed with the GeneAMP kit (Perkin Elmer Cetus) on a Perkin Elmer 9600 Thermocycler using flanking primers on the transfer RNA (tRNA) genes on either side of cytochrome *b*. The L-strand primer was on tRNA glutamine and the H-strand primer on tRNA threonine. The former primer (L14724; 5′-tgacttgaaraaccaycgttg-3′) was from Palumbi *et al.* (1991), and the latter (5′-ccttttccggtttacaagac-3′) was designed from published tRNA threonine gene sequences (Southern *et al.* 1988). PCR conditions were as follows: 35 cycles, each consisting of 45 sec at 94°C, 60 sec at 48°C, and 90 sec at 72°C. Final extension was 3 min at 72°C. Mg^{++} concentration was 1.5 mM.

Both strands of the entire cytochrome *b* gene were sequenced using standard protocols (Applied Biosystems Inc.) for dye-labelled terminators and cycle sequencing. The sequencing reaction products were run on an Applied Biosystems Inc. model 373 automated sequencer. Seven sequencing primers were used to sequence the gene, two of which were the same primers used for PCR reactions. Other sequencing primers used were H15387 (Rosel *et al.* 1994, modified from Irwin *et al.* 1991) (5′-gaatgggattatgtctatgt-3′), H15149 (Kocher *et al.* 1989) (5′-cagaatgatatttgtcctca-3′), and three additional primers designed by the authors. These were 5′-taacagtcatagcyactgcatt-3′ (L15129), 5′-accctactagcattaaccctatt-3′ (L15480), and 5′-ctggtttgatgtgtgcaggggtg-3′ (H15535). Base numbers for PCR and sequencing primers are according to the numbering of Anderson *et al.* (1981). The sequences from the different reactions were compared and edited using SeqEd v. 1.0.3 (Applied Biosystems Inc. 1992).

Sequence statistics were generated using Molecular Evolutionary Genetics Analysis (MEGA) version 1.0 (Kumar *et al.* 1993), Phylogenetic Analysis Using Parsimony (PAUP) version 3.1.1 (Swofford 1993), and MacClade version 3.05 (Maddison and Maddison 1995). Parsimony analysis was conducted using

the computer program PAUP 3.1.1 (Swofford 1993). Due to the large number of taxa in the dataset and the associated computational burden, exhaustive and branch-and-bound parsimony searches were not conducted. To reduce the risk of the heuristic searches being caught in local minima, random addition of sequences was conducted (10 replicates) for each search. A search of 100 random addition replicates was conducted in some cases to test the efficacy of the 10-replicate search setting. No additional trees were found in those tests. Branch swapping was by the tree-bisection-reconnection method (Swofford 1993). One thousand bootstrap replicates were run for the unweighted parsimony analysis, with clades that appeared in >50% of the bootstrap replicates being retained in the bootstrap consensus tree. In addition to unweighted parsimony analysis, weighted parsimony analyses were conducted using 10:1 transversion:transition weighting, expected:observed ratio (EOR) weighting of susbstitution types (Finnerty and Block 1995), 3:11:1 weighting of codon positions, a combination of EOR and codon position weighting, and successive (or *a posteriori*) reweighting of the dataset (Farris 1969), using the results from unweighted searches and each of the weighting schemes as separate starting points for reweighting. The 3:11:1 codon position weighting is an approximation of the inverse ratio of the numbers of variable sites for the respective codon positions.

MEGA 1.0 (Kumar *et al.* 1993) was also used to generate a neighbor-joining (NJ) bootstrap tree (Saitou and Nei 1987), using Kimura 2-parameter distances (Kimura 1981) between sequences and 400 replicates. The bootstrap confidence limits (BCL) calculated by MEGA represent the frequencies that nodes from the original NJ tree appear in the bootstrap replicates. Maximum likelihood analysis (Felsenstein 1981) was conducted using fastDNAml version 1.1 (Olsen *et al.* 1994), set to use the observed base composition, do local rearrangements after the addition of each taxon, and do global rearrangements of the final tree. Due to the sensitivity of the analysis to initial order of taxa, the large number of taxa, and the difficulty in testing the likelihood of all trees, ten searches were performed with different seeds for the random addition of sequences. This was performed with both a 12:1 and the default 2:1 transition : transversion ratio weighting.

Results

All sequences were of equal length; no insertions or deletions were observed. The cytochrome *b* gene proved fairly conservative in this set of taxa. The percent differences between conspecific sequences from different ocean basins ranged from 0.2% (*Grampus griseus*) to 1.8% (*Stenella attenuata*). (The sequences of *Tursiops truncatus* and *T. aduncus* ranged up to 4.5% different, and that of the riverine and coastal forms of *Orcaella brevirostris* differed by 5.3%; see discussion below.) Interspecific differences between other delphinids ranged from 0.8% (*Globicephala melas—G. macrorhynchus*) to 10.0% (*Pseudorca crassidens—Tursiops truncatus* (Gulf of Mexico sample)). The differences between delphinid sequences and those of phocoenid and monodontid outgroups ranged

from 11.2% (*Monodon monoceros–Lagenorhynchus australis*) to 15.1% (*Phocoena phocoena–Stenella coeruleoalba* (Mediterranean Sea sample)).

Of the 1,140 bp sequenced, 343 were informative sites, and 289 were informative within the family Delphinidae. However, for those 289, the majority, 212, were 3rd-codon-position sites, with 1st and 2nd positions represented by 63 and 14 informative sites, respectively. Only 26 sites demonstrated informative transversional differences (1, 1, and 24 for the three respective codon positions).

The vast majority of base substitutions were silent changes. Of the 380 amino acids in the cytochrome *b* protein sequence predicted by the DNA data, only 55 were variable among the delphinids in the dataset, and only 33 of these were informative. Furthermore, since 25 of these were merely autapomorphies that join two sequences from the same species, only eight amino acid changes could be used to infer interspecific relationships. Clearly, whatever phylogenetic signal that was contained in the dataset was largely from silent substitutions.

Unweighted parsimony analysis produced 792 most parsimonious (MP) trees of 1,249 steps (uninformative sites excluded). The Ti:Tv ratio for the trees was approximately 12:1, and C-T transitions were over three times more common than A-G transitions. None of the topologies produced from weighted parsimony analyses differed from the unweighted trees with regard to the findings discussed below and will not be discussed further. The unweighted parsimony bootstrap consensus tree is shown in Figure 1.

Below we discuss some of the well-supported findings from the unweighted parsimony analysis. Maximum likelihood (ML) and neighbor-joining (NJ) analyses of the data produced the same results with regard to the main findings, with some minor differences in tree topologies. This also applied to trees with likelihoods less than, but not significantly less than, the ML tree. While each of these methods, along with parsimony and weighted parsimony, was used for the same purpose (*i.e.*, phylogenetic inference), their relative performances could vary with the characteristics of the dataset (*e.g.*, equal *vs.* unequal substitution rates—Felsenstein 1988, Hillis *et al.* 1994), and discrepancies between their results in some situations may provide insight into evolutionary processes. However, the similarity of these results from the various methods indicates that the analysis of our dataset is fairly straightforward. In the interest of brevity, only the unweighted parsimony consensus tree (Fig. 1) is depicted, with bootstrap values of both parsimony and NJ analyses given. When the differences in topologies resulting from the various analyses are germane to the primary conclusions, they are discussed in the text.

For ease of examination, the tree depicted was simplified, in that conspecific sequences, with three exceptions, were lumped into single species names because their monophyly was well supported. The number of included sequences is indicated in parentheses. One exception is the *Delphinus* clade, for reasons discussed below. Also considered separately and discussed are *Tursiops truncatus* and *T. aduncus,* which, although previously lumped together under *T. truncatus*,

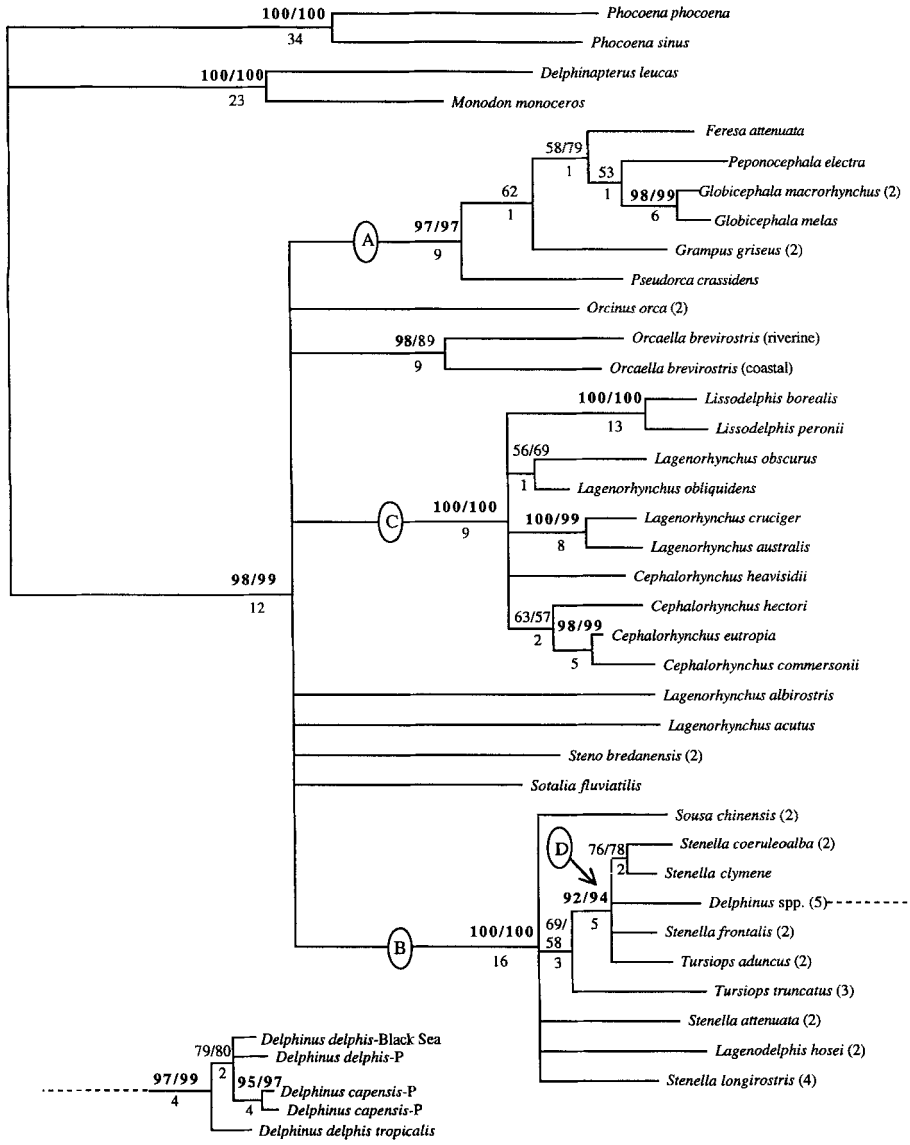

Figure 1. Bootstrap consensus tree from unweighted parsimony analysis. Numbers above nodes are bootstrap values from MP/NJ analyses; those below are Bremer indices (Bremer 1988). Single bootstrap values are from MP analysis and occur where the MP and NJ topologies differ. Numbers in parentheses after species names represent number of included sequences for species represented by multiple samples. Unless depicted otherwise, bootstrap values for monophyletic species are >95% and are not given in figure. This topology is identical to the strict consensus of all MP trees, except that in the latter, the genus *Lissodelphis* was basal within its subfamily clade. Branch lengths are proportional to amount of genetic change and were calculated along the strict consensus tree by PAUP (Swofford 1993).

are believed by many investigators to be distinct. The last exception is the two sequences of *Orcaella brevirostris*, also discussed in the text.

Discussion

A number of results of the present analyses are congruent with current, morphology-based classifications. These include the grouping of the blackfish (*Pseudorca, Peponocephala, Feresa,* and *Globicephala*), the close affinity of *Stenella* spp. with *Tursiops* and *Delphinus*, and the monophyly of the delphinoid families. These family monophylies were maintained even with the addition of *Inia, Kogia,* and *Mesoplodon* species as more distant cetacean outgroups (data not shown). While many of the other indicated relationships conflict with current or past classifications, it should be remembered that they do not conflict with any extensive morphological cladistic analysis, as this has yet to be done for delphinids. We indicate in the following discussion how many extra steps are required in the MP tree to retain some of the relationships that are implied by the current taxonomy. It is not our primary purpose to present a definitive family revision, but to indicate the need for further genetic and morphological systematic research. Suggested taxonomic changes, although tentative and provisional, represent a balance between correcting mistakes in the present classification and avoiding the introduction of new ones.

Subfamilies

While the overall resolution of the phylogeny is somewhat low, some conclusions can be drawn regarding the arrangement of genera into subfamilies. Three large groups of species are well supported by the analyses. The revised subfamily Globicephalinae (blackfish, node "A" in Fig. 1) is similar to that shown in Table 1, save for the inclusion of *Grampus*, which was formerly placed in the Delphininae, and the exclusion of *Orcinus*. *Grampus* has been taxonomically associated with various blackfish before at the generic, subfamilial, or tribal level (Gray 1866*a*, Gervais 1855, Gill 1872, Guérin 1874, Winge 1942, Kasuya 1973, Ohland *et al.* 1995), although most recent studies have included it in the Delphininae (*e.g.*, Fraser and Purves 1960, Barnes 1990) based on the morphology of the pterygoid sinuses. In the present analysis, inclusion of *Grampus* in the Delphininae requires 18 additional steps in the phylogeny. *Orcinus*, which is often associated with at least some of the blackfish (Gray 1864, 1868, Gervais 1855, Slijper 1938, Winge 1942, Kasuya 1973, Barnes 1990), has only a tenuous link to that group in the present analysis and will be discussed later.

Another well-supported group is the Delphininae (node "B" in Fig. 1), although not as currently defined in the literature. Besides the removal of *Grampus* mentioned above, none of the species currently placed in the genus *Lagenorhynchus* shows close relationships to the remaining delphinines (*contra* Burmeister 1864, Gray 1871, Flower 1885, Fraser and Purves 1960, Mead 1975, Barnes 1990). This result was partially forecast by Shimura and Nu-

machi (1987) and Ohland *et al.* (1995) with genetic data. Unexpectedly, *Sousa chinensis* is well integrated into the delphinine clade, which also includes *Stenella, Tursiops, Delphinus,* and *Lagenodelphis*. *Sousa*, showing some primitive morphological characteristics, is usually associated with *Steno* and/or *Sotalia* by non-cladistic analysis, often being combined with the latter genus (Flower 1885, True 1889, Hershkovitz 1966). Although we could not find any reference that explicitly associates *Sousa* with the delphinines independent of *Sotalia* and/or *Steno*, Arnold and Heinsohn (1996) did note a more derived condition in *Sousa* morphology than had been recognized by previous workers, even noting derived similarities of that genus to *Tursiops* and *Stenella*. Removal of *Sousa* from the Delphininae and associating it with *Steno* and *Sotalia* requires 29 additional steps in the MP phylogeny.

The last major species group well supported by the present study (node "C" in Fig. 1) contains the genera *Cephalorhynchus*, *Lissodelphis*, and four of the six species of *Lagenorhynchus* (*L. obscurus, L. obliquidens, L. australis,* and *L. cruciger*). As mentioned above, this clustering of *Lagenorhynchus* species apart from the delphinines is somewhat novel. *Lagenorhynchus, Lissodelphis,* and *Cephalorhynchus* have been allied at times in the past, although not as an exclusive group (*e.g.*, Winge's (1942) Lagenorhynchi also included, besides those three genera, *Feresa* and *Tursiops*). *Cephalorhynchus* and *Lissodelphis* have been most often placed in their own separate subfamilies since the work of Fraser and Purves (1960), an arrangement that needs revision according to the present analysis. The type species of *Lagenorhynchus* (*L. albirostris*) does not belong in this group, and *Lissodelphis* Gloger 1841 has priority over *Cephalorhynchus* Gray 1846 or any of the generic synonyms for these four *Lagenorhynchus* species. Therefore, it seems appropriate to refer to this clade as the subfamily Lissodelphininae. Since the present analysis does not clearly resolve relationships among these 10 species, it is best to continue for now to recognize the generic distinction of *Cephalorhynchus* and *Lissodelphis* from each other and from these four species of *Lagenorhynchus*.

Lagenorhynchus

In his commentary on *Lagenorhynchus*, Fraser (1966) pointed out some of the systematic problems presented by the genus. However, he excluded the well-studied North Atlantic species *L. albirostris* and *L. acutus* from the discussion, concentrating on distinctions among the remaining four species. Apart from indicating the dubiousness of including *L. obscurus* in the genus, the integrity of the genus as a whole was not questioned by Fraser. Our results indicate that the genus, as currently recognized, is an artificial grouping, a result in agreement with Cipriano (1997), who also analyzed mtDNA sequences. Eighteen extra steps are required in the unweighted tree to create a monophyletic *Lagenorhynchus*, a clade that received zero bootstrap support. The 100% (MP and NJ) bootstrap support for the Lissodelphininae itself is enough to break up this genus. Specifically, *L. australis*, *L. obscurus*, *L. cruciger,* and *L. obliquidens* form a group closely related to *Cephalorhynchus* and *Lissodelphis*. This

is concordant with the relationship between *Lagenorhynchus obscurus* and *C. heavisidii* in Ohland *et al.*'s (1995) MP tree based on restriction-site mapping of the mitochondrial genome (although the relationship was not well supported in their bootstrap analysis). As for the remaining two species, except for appearing as distant sister taxa in some of the MP trees, *L. acutus* and *L. albirostris* do not show particularly close affinity to each other in this study (36% MP bootstrap support and 8.7% sequence divergence). Furthermore, neither shows close affinity to any other species (≥7.5% difference from all other species for both). Unless further research indicates that the mitochondrial phylogeny is in error, placing these species in distinct genera is justified. In fact, they may warrant their own separate subfamilies, but that conclusion would be premature based on the present data. It is best to leave their subfamily placement unresolved for the present. As the type species for the genus is *L. albirostris* Gray 1846 (Hershkovitz 1966), the generic name remains with that species. The next available generic name for *L. acutus* is *Leucopleurus*, first proposed as a subgenus of *Lagenorhynchus* by Gray (1866*b*). The other four species of *Lagenorhynchus* are clearly unrelated to *L. acutus* and *L. albirostris*, belonging solidly in the revised Lissodelphininae (joined by node "C"). Although their monophyly was not supported, we provisionally retain their status as congeners. The next available generic name for any of the four *Lagenorhynchus* species in the Lissodelphininae is *Sagmatias* Cope 1866 (type species *Sagmatias amblodon* =*Lagenorhynchus australis*).

Delphinus *and the Sister-species "Problem"*

Rosel *et al.* (1994) demonstrated species-level genetic differences between *Delphinus delphis* and *D. capensis*, the short-beaked and long-beaked common dolphins, respectively, including fixed amino-acid differences in cytochrome *b* sequences. Our common-dolphin sequences, consisting of two samples of each species plus one sample of the West Pacific/Indian Ocean *tropicalis* form, are consistent with those fixed differences. However, at first glance, their species recognition appears to be contradicted by our analyses, since the two *D. capensis* sequences are nested within the two *D. delphis* sequences in the phylogeny (Fig. 1, inset). (The relationships of the *tropicalis* form are discussed below.) In other words, the two species are not reciprocally monophyletic. This implies that some *D. delphis* are cladistically just as closely related to *D. capensis* as they are to other *D. delphis*. However, this is likely due to lineage sorting (Pamilo and Nei 1988, Doyle 1992), where the distribution of cytochrome *b* lineages in the current species reflects the pattern of variation in their common ancestor more than their divergence since speciation. This results in the paraphyly of *D. delphis* on the present gene tree, in spite of reproductive isolation between the two forms. This also illustrates a shortcoming of using reciprocal monophyly as a criterion for establishing sister populations as separate species. In spite of good genetic and morphological evidence of reproductive isolation between sympatric morphotypes of the common dolphin in the eastern Pacific (Rosel *et al.* 1994, Heyning and Perrin 1994), the cytochrome *b* topology

suggests conspecificity by a criterion of reciprocal monophyly. This situation also illustrates the difference between biological species, which Rosel *et al.* (1994) and Heyning and Perrin (1994) demonstrated as present in the eastern Pacific, and phylogenetic species (*i.e.*, reciprocally monophyletic), which are not yet established in *Delphinus* for cytochrome *b*. An alternative explanation, that the Black Sea common dolphins represent yet another species, would require much more data to be accepted.

In spite of the recent advances in the systematic study of the *Delphinus* complex, there is still much that remains unanswered. In particular, the status and relationships of the Indo-Pacific *tropicalis* form need investigation. Heyning and Perrin (1994) noted that its tooth counts and rostral length/zygomatic width ratio are above the range of the long-beaked common dolphin, *D. capensis*, and that its pigmentation pattern most closely resembles that species as well. Based on measurements of Indian Ocean common dolphin specimens, they speculated that either (1) *D. capensis* and *D. tropicalis* are distinct species that both occur in the Indian Ocean, or (2) *D. capensis* rostral length increases clinally in the Indian Ocean, reaching its maximum in the *tropicalis* form. Their sample size from that ocean was too small to test either hypothesis. In the present study, the *tropicalis* sequence occupies a basal position in the *Delphinus* complex (Fig. 1, inset), supported by the 79% (MP) and 80% (NJ) bootstrap values that join the *D. capensis* and *D. delphis* sequences together. This may seem to favor the idea that *D. tropicalis* represents a distinct species, since it does not show particular affinity to *D. capensis*. However, neither of the *D. capensis* samples included here come from the Indian Ocean, or even from adjacent parts of the Atlantic or Pacific, and so would not be expected to be congruent with any possible morphological intergradation. In this sense, the species status of the *tropicalis* form is tied to the relationship between eastern Pacific and Indian Ocean populations of *D. capensis*. If future genetic and morphological data indicate that Indian Ocean *D. capensis* and *tropicalis* are conspecific, this raises the possibility that the long-beaked form in the eastern Pacific represents yet another species.

Stenella

The genus *Stenella* was originally described (Gray 1866*b*) as a subgenus of *Steno* with *S. attenuata* as its sole member. At that time, other species currently assigned to *Stenella* were placed in other genera (*Clymene* and *Delphinus*). The five species currently included in *Stenella* (and synonyms thereof) later coalesced into the genera *Clymenia* and *Prodelphinus* (Gray 1868 and Flower 1885, respectively), although these also included some species of what are now *Lagenorhynchus* and *Lissodelphis*. Oliver (1922) elevated *Stenella* to full generic rank and demonstrated its priority, although some later authors (*e.g.*, Winge 1942) still used *Prodelphinus* and included other species ("probably including '*Sotalia*'," *op. cit.*) within it. In addition to these problems, complicated synonymies and a lack of understanding of intraspecific variation led to more confusion regarding the number of valid species in the genus (*e.g.*, Hershkovitz (1966)

recognized eight species). The situation has been clarified in recent years (Perrin *et al.* 1981, 1987), and five species are currently recognized. In spite of the progress in defining the number of species, the cohesiveness of the genus has recently been questioned (Perrin *et al.* 1981, 1987; Perrin and Hohn 1994). The present study strongly indicates that the genus is indeed an artificial assemblage, with some member species more closely related to *Tursiops, Delphinus, Sousa*, or *Lagenodelphis* than to nominal congeners. Thirteen extra steps are required on the unweighted parsimony tree to create a monophyletic *Stenella*, which received zero bootstrap support. Even without considering other nodes, the 92% and 94% (MP and NJ, respectively) bootstrap supports for the node ("D" in Fig. 1) that joins *Stenella coeruleoalba, S. clymene,* and *S. frontalis* to *Delphinus* spp. and *Tursiops aduncus* are enough to render the genus polyphyletic. As mentioned in the beginning of this section, the conflict of these results with the current classification does not necessarily indicate a conflict with a possible cladistic analysis of morphological data. However, it does appear to be incongruent with some published observations. In noncladistic examinations of cranial characters (Perrin *et al.* 1981) and pigmentation patterns (Perrin 1997) in delphinines, similarities between *Delphinus, S. coeruleoalba, S. clymene, S. longirostris,* and *Lagenodelphis,* exclusive of *Tursiops, S. frontalis,* and *S. attenuata* were noted. However, the existence of two clades consisting of these respective species groups is not supported by the present analysis, requiring 14 more steps in the unweighted MP analysis.

Tursiops

In most current classifications, the many nominate species of bottlenose dolphins are generally considered forms of a single species, *Tursiops truncatus.* While full species status for the Indo-Pacific *Tursiops aduncus* has been proposed in recent years (Hershkovitz 1966, Ross 1977), Ross and Cockcroft (1990) suggested that it be considered a subspecies of *T. truncatus* in which adults have ventral spotting. Apart from the question of species status for *T. aduncus*, the polyphyly of the genus is a novel finding. The node discussed above that rendered *Stenella* polyphyletic bestows the same fate on *Tursiops. T. aduncus* is more closely related to other species in the node "D" clade than it is to *T. truncatus.* Five extra steps are needed to make *Tursiops* monophyletic in the MP tree. While this number may seem small, the bootstrap support for such an arrangement was nil. Furthermore, seven unambiguous characters supported the node "D" clade, but none supported *Tursiops* monophyly. It is worth noting that Curry (1997), using d-loop sequences, also found *T. aduncus* haplotypes represented in her sample set to be distinct from those of *T. truncatus.* The differences between the d-loop clades included a fixed insertion/deletion and two nearly-fixed base substitutions. Unlike this study, her larger sample set included animals of both types from the Indian Ocean, indicating concordance between haplotypes and morphotypes within an ocean basin and reproductive isolation. Barring the existence of an as yet unsampled hybrid zone, the present

polyphyly would likely persist even with the addition of more cytochrome *b* sequences.

In their revision of the spotted dolphins, Perrin *et al.* (1987) noted similarities between *T. truncatus* and the spotted dolphins (*Stenella attenuata* and *S. frontalis*), particularly that *S. frontalis* is in many characteristics intermediate between *S. attenuata* and *T. truncatus*. Although their association of the three species is not supported here (see above), they noted that, for *S. frontalis*, "the similarity to *T. truncatus* is greatest for the spotted form of that species . . ." (*i.e.*, *T. aduncus*). In the present analysis, the resolution within the node "D" clade that includes *T. aduncus* and *S. frontalis* is low. However, given the noted morphological similarities, a working hypothesis could be proposed that the Indo-Pacific *T. aduncus* and the Atlantic *S. frontalis* represent sister species. Furthermore, it may also be suggested that *T. truncatus* is the sister taxon to the entire clade defined by node "D." Although the bootstrap support for this relationship is low (69% and 58% for MP and NJ, respectively), it does appear consistently in the results of all the present analyses (*i.e.*, ML and weighted MP trees, as well as Fig. 1).

Delphinine Taxonomy

With the polyphyly of both *Stenella* and *Tursiops* strongly indicated, it seems that a taxonomic revision of the subfamily Delphininae is due. To retain monophyly of both of these genera requires 16 additional steps. However, the relationships among the species in these genera cannot be resolved independent of the genera *Delphinus*, *Lagenodelphis,* and *Sousa*. Apart from the few critical nodes discussed above, resolution within the group is relatively poor. For example, most sub-ML trees (but not significantly worse trees) differed from the ML tree and from each other in relationships within the "D" clade and in the arrangement of delphinine species above *Tursiops truncatus* (*i.e.*, *Stenella longirostris, S. attenuata, Lagenodelphis hosei,* and *Sousa chinensis*). This is paralleled by the poor resolution of the MP bootstrap tree (Fig. 1) and the low BCL values on the NJ tree (54%–56%, not shown in Fig. 1) regarding these relationships. One possible taxonomic arrangement is to place all the species grouped by node "D" in the genus *Delphinus*, the name with priority. Outside of this clade, *Lagenodelphis* remains valid and *T. truncatus, Sousa chinensis,* and *Stenella attenuata* can remain in their respective genera (being type species). However, *Stenella longirostris* would then need to be placed in another genus. The next available genus name, *Micropia*, was first used by Gray (1868) as a subgenus of *Clymenia*, with *Clymenia stenorhyncha* (= *S. longirostris*) as its type species (Hershkovitz 1966). Alternatively, the entire subfamily could be relegated to *Delphinus*. However, given the lack of resolution in the present study, both of these revisions would be premature. To avoid replacing one erroneous classification with another, it seems best to take a conservative approach, retaining generic names while recognizing *T. aduncus* as a species and acknowledging that further morphological and genetic work is imperative.

Other Relationships

A few additional, albeit less well-supported, relationships on the cytochrome *b* trees deserve mention. The pairing of *Steno* and *Sotalia* as sister taxa appears in the majority of the MP trees (539), as well as in the results of ML and NJ analyses. When not paired (the rest of the MP and a few sub-ML trees), neither species shows affinity to other taxa in the dataset. The terminal branches leading to *Steno* and *Sotalia* are long relative to the internal branch joining them, and bootstrap support for the clade is low (41% and 48% for MP and NJ bootstrap analyses, respectively). While their sister relationship seems in agreement with earlier morphological studies (*e.g.*, Fraser and Purves 1960), the non-inclusion of *Sousa* in the clade is novel. Accepting for the moment the sister relationship of *Steno* and *Sotalia*, we provisionally retain them in the revised subfamily Stenoninae. Further studies are needed to determine if this relationship is distant enough to warrant their separation into different subfamilies.

A similar situation is seen with *Orcinus* and *Orcaella*, which consistently form a clade in unweighted parsimony (486 of the MP trees), NJ, and ML analyses. The relationship is relatively distant and bootstrap support is low (45% and 67% for MP and NJ bootstrap, respectively) but still stronger than the 9% MP bootstrap linking *Orcinus* to the Globicephalinae exclusive of *Orcaella*. Given that, we tentatively propose to separate *Orcinus* from the blackfish and combine it with *Orcaella* in the subfamily Orcininae. As with Stenoninae above, further work may demonstrate that the erection of different subfamilies for these two genera is appropriate. Deeper relationships of this clade are even more tenuous. NJ and ML analyses consistently support a sister relationship between this pair and the Globicephalinae, although it receives only 12% MP and 24% NJ bootstrap support. On the other hand, there is little resolution of the deeper relationships of these two groups in the set of MP trees; they appear in different combinations with other lineages.

A related issue involves the inclusion of *Orcaella* in the Delphinidae. Considered by some to be more closely related to the monodontid genus *Delphinapterus* (Kasuya 1973, Barnes *et al.* 1985, Pilleri *et al.* 1989, Fordyce and Barnes 1994, Fordyce *et al.* 1994), its status as a delphinid has been supported by recent molecular (Lint *et al.* 1990, Grétarsdóttir and Árnason 1992) and morphological (Heyning 1989, Arnold and Heinsohn 1996) studies. Obviously, the placement of *Orcaella* within the Delphinidae is strongly supported here.

Of particular interest is the high degree of genetic differentiation between the two samples of *Orcaella brevirostris*. The 5.3% difference between them is greater than that seen in all the interspecific and intergeneric comparisons within the Lissodelphininae and in most of the same types of comparisons within the Globicephalinae and Delphininae. However, one should exercise caution in interpreting this level of difference. Good species may be genetically very close and some populations of conspecifics quite divergent, and single sequences are not sufficient evidence from which to draw conclusions about

species status of sister taxa. In the rest of the present dataset, there is overlap in the levels of intraspecific (*e.g., Tursiops truncatus* and *Stenella attenuata*) and interspecific (*e.g.,* between species of *Lissodelphis* or *Cephalorhynchus*) variation. To examine sister populations for species-level differences, one needs to consider the pattern of variation within the taxon as a whole, as represented by a large sample set from throughout the geographic range. While suggesting separate species status for the two forms of *Orcaella* based on degree of difference is premature and perhaps inappropriate, further investigation of the geographic pattern of variation in this taxon is obviously warranted. The samples of *Orcaella* used in the present study are from coastal Australia and from the Mekong River in Laos. Marsh *et al.* (1989) reviewed the species' taxonomic history, which included proposed recognition of the freshwater form as a separate species. The current consensus is recognition of only one species. The status of the freshwater form may therefore deserve reconsideration. On the other hand, given the species' propensity for shallow water and the few deep water distributional breaks within its range (India to eastern Australia) (Stacey and Leatherwood, in press), it is possible that the genetic differentiation is primarily between Australia/New Guinea and mainland Asia/Indonesia/Philippines, rather than between riverine and coastal habitats. Of course, more extensive sampling is required to address such questions. If the pattern of variation is shown to be more complex, or even if no geographic pattern is found, the high degree of mitochondrial sequence variation itself is worth investigating.

A few points can be made regarding the relationships within the subfamilies Globicephalinae and Lissodelphininae. Within the Globicephalinae, nodes have low bootstrap support save for the one joining the two species of *Globicephala*. However, a pattern of relationships in the clade (*Feresa* (*Peponocephala, Globicephala*)) appears consistently in all trees from all analyses, including the sub-ML trees examined. The relative positions of *Pseudorca* and *Grampus* outside this group varies between NJ and the other analyses.

Within the Lissodelphininae, the monophyly of *Lissodelphis* is well supported (bootstrap values of 100%), that of *Cephalorhynchus* less so (41% and 30% for MP and NJ, respectively), and that of *Sagmatias* virtually not at all (<5% MP). The pairing of *Sagmatias cruciger* and *S. australis* (*Lagenorhynchus cruciger* and *L. australis* auctorum), on the other hand, is well supported (bootstrap values of 100% and 99%). In addition, NJ and ML analyses favor this species pair as sister to *Cephalorhynchus*, although the evidence can scarcely be considered strong (29% NJ bootstrap). If further evidence were to favor this arrangement, the genus *Sagmatias* as defined here would then be polyphyletic.

Limitations of the Study

It is obvious that the present analysis does not resolve all relationships among the delphinids. Although the four key nodes in Figure 1 ("A–D") have numerous taxonomic implications, the analyses fail to resolve many of the interrelationships among closely related species. In some cases, for example

Stenella and the subfamily Delphininae, we can only state that the present classification is in error but can say little regarding any proposed revisions. At the other end of the spectrum, little can be said about deeper relationships within the family either. If one considers the Globicephalinae, Lissodelphininae, and Delphininae as three well-defined monophyletic clades, then the family Delphinidae can be regarded as having nine primary extant lineages (these three with *Orcinus, Orcaella, Steno, Sotalia, Lagenorhynchus,* and *Leucopleurus*). Apart from two weakly supported clades (Orcininae and Stenoninae), there is no resolution of relationships among these nine lineages. They appear in many combinations among the MP, NJ, ML, and weighted parsimony trees. The uncertainty regarding the true relationships at both levels stems from the relative amounts of phylogenetic signal (or lack thereof) and noise contained in the dataset. This can be addressed in the future by increasing the dataset by size (more bp sequenced) and by type (*i.e.*, using faster evolving markers for closely related species), or by arguing for the greater reliability of the results of a particular analysis (*e.g.*, ML or a particular weighting scheme). Since none of the present analyses strongly supported (by bootstrap) any clade that was not also strongly supported in the others, the last stratagem would not have changed any of our conclusions. It should also be noted that bootstrap values may not be good indicators of phylogenetic accuracy if the variability of the taxa being studied is not adequately represented or if an inappropriate weighting scheme is applied (Philippe and Douzery 1994, Milinkovitch *et al.* 1996). However, given the completeness of the present delphinid dataset and the congruence of results with different weighting schemes, this concern is minimal here.

When a phylogeny conflicts with the another phylogeny, the simplest and most likely explanation is that one of them is in error due to homoplasy in the underlying dataset. Ideally, in the present study, these conflicts would be based on comparisons of our gene tree to other gene trees, or to purported species trees. However, since neither other gene trees nor species trees are currently available for delphinids, we are by default left with comparisons to relationships implied by current taxonomy. Although genetic or morphological homoplasy is still the simplest explanation of observed discrepancies, below we discuss some alternative mechanisms that could lead to our gene tree being in conflict with morphological characters. It should be noted that there is as yet no evidence for these other mechanisms operating in the delphinids; they are only presented as possibilities for consideration.

Even for the well-supported clades in the present phylogeny, there are caveats attached to their reliability as indicators of the true phylogeny. For one, the cytochrome *b* phylogeny can not necessarily be taken as reflecting the true mitochondrial phylogeny. Some studies (Cummings *et al.* 1995, Russo *et al.* 1996) have indicated that single mitochondrial gene trees can conflict with each other in spite of the genes' linkage, and then of course some would have to conflict with the true species tree. Even within a single mitochondrial gene, the bases (as phylogenetic characters) are not independently inherited (al-

though they may be mutationally independent), and therefore the analyses are essentially of a single multistate character, the sequence (Doyle 1992).

If one does accept the cytochrome *b* tree as a good representation of the mitochondrial phylogeny, other problems can arise that relate to the genome's maternal inheritance and non-recombination (Doyle 1992). For example, as discussed above, lineage sorting may have played a role in determining the pattern of phylogenetic relationships among sequences of *Delphinus* and the apparent paraphyly of *D. delphis*. Although biological species will become monophyletic given enough time, the effects of lineage sorting can persist in the topology of a given gene tree, even among deeper branches.

Another factor that could confound the analysis is the possibility of introgressive hybridization. In addition to numerous cases of dolphin hybridization in captivity, there have been reported cases of wild hybrids between *Grampus* and *Tursiops* (Fraser 1940), and between *Lagenorhynchus obscurus* and *Delphinus capensis* (Reyes 1996). It is noteworthy that the parent species in each of these cases are not closely related according to the present analysis. Although there is no information on the frequency or fertility of wild dolphin hybrids, the possibility of their influence on the resulting gene tree cannot be ruled out without more data. This influence could be dramatic if the parent species were not closely related. If hybridization and introgression did occur, the mt gene tree would then be an incomplete representation of the species tree, and the true species tree would be reticulate (see Maddison 1997).

Perrin *et al.* (1981) discussed the similarities among *Stenella clymene*, *S. coeruleoalba*, and *S. longirostris*. Externally and behaviorally, *S. clymene* resembles *S. longirostris*. Cranially, it closely resembles *S. coeruleoalba*, albeit smaller. Perrin *et al.* (1981) hypothesized that the three species are closely related and that based on similarities within the group, *S. clymene* is more closely related to *S. longirostris* than to *S. coeruleoalba*. In the present analysis, however, *S. clymene* and *S. coeruleoalba* are close sister taxa and both are fairly distant from *S. longirostris* (though still in the same subfamily). This raises the possibility of past hybridization or even of a hybrid origin of *S. clymene,* with *S. longirostris* and *S. coeruleoalba* as the parent species. For *S. clymene*, nuclear genetic data, ideally including that from paternally inherited Y-chromosome genes, are needed to allow examination of the possible role of hybridization in dolphin evolution.

On another level, mitochondrial phylogenetic studies can be misled by the presence of nuclear copies of the mt genes (Zhang and Hewitt 1996). These non-transcribed copies act as pseudogenes, presumably evolving at different rates and under different selective pressures, and have been detected in a variety of mammal species. Their presence can confound the sequencing and data analysis. While we did not test specifically for the presence of nuclear copies of cytochrome *b*, none of the characteristic indicators listed by Zhang and Hewitt (1996) are apparent in the dataset (multiple PCR bands, ambiguous sequences, frameshifts, insertions or deletions, inappropriate stop codons, or radically different sequences).

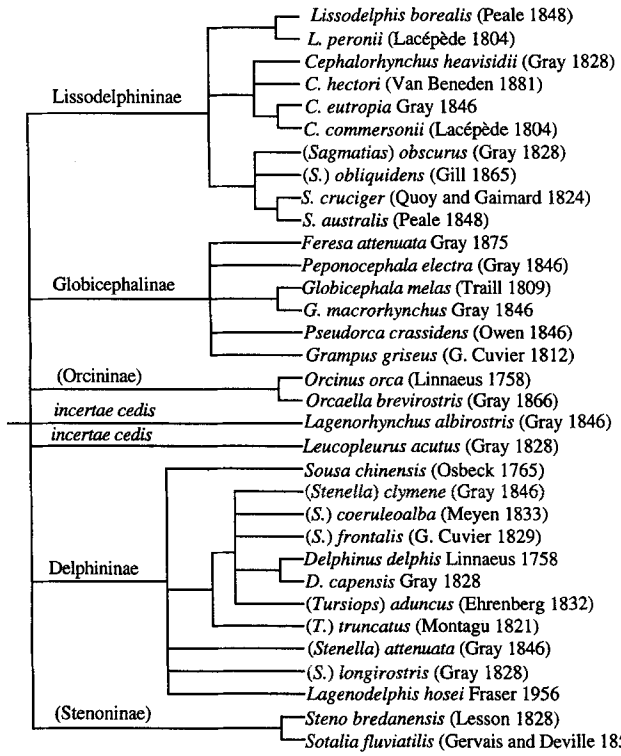

Figure 2. Systematic revision of Delphinidae based on cytochrome *b* analyses. Proposed nomenclatural changes are incorporated (see text). Taxa in parentheses are to be considered either definitely (*e.g.*, delphinine genera) or possibly (*e.g.*, *Sagmatias,* Stenoninae, and Orcininae) in need of further revision. The figure does not include the unsampled species (*Sousa teuszii)* or divergent forms whose species status was suggested but not established by the present dataset (*e.g.*, the *tropicalis* form of *Delphinus* or the two samples of *Orcaella*).

A Tentative Classification of Delphinidae

A conservative representation of the relationships inferred from the cytochrome *b* dataset is shown by Figure 2, which reflects the suggested revisions and some of the uncertainty of the present results. We emphasize that this is not offered as a definitive classification but rather as a classification that is supported by cytochrome *b* gene sequences. Currently recognized taxa are left intact if the analyses did not strongly support sundering them. Therefore, some (*e.g., Cephalorhynchus*) are still recognized despite low bootstrap support. In other cases, new taxa are established in spite of low bootstrap support if the balance of evidence favors the arrangement. For example, although the bootstrap value for the subfamily Orcininae is low, it is still much greater than the support for including *Orcinus* in the Globicephalinae or for associating either of the orcinine genera with any other taxon. A more definitive classification must await comprehensive cladistic analysis of morphology in the

group and confirmation of the genetic results through analyses of independent genes.

Comments on Morphological Characters

If one accepts all or part of the present mt phylogeny as an approximation of the true evolutionary relationships among delphinids, it may be useful, in the context of this phylogeny, to reconsider some of the morphological characters that formed the basis of past classifications. In fact, providing a framework and an impetus for the reinterpretation of morphological characters is one of the most important aspects of molecular phylogenetics and has been applied to higher level cetacean systematics (Milinkovitch 1995, Shimamura *et al.* 1997, Milinkovitch and Thewissen 1997, Gatesy 1997, Heyning 1997).

Cranial morphology has played a large role in dolphin systematics at all taxonomic levels. In examining variability of cranial characters within and among *Stenella* species, Perrin (1975) noted that those characters associated with feeding seemed to be the most evolutionarily plastic, with aspects of the braincase being most conservative and those involved in sound production/reception being intermediate. Although they may be more prone to homoplasy, characters of the feeding apparatus have been prominent in dolphin systematic studies. They are useful in differentiating closely related species, but they may have less reliability in diagnosing deeper relationships, such as subfamily placement of a genus, and perhaps the establishment of a genus as well. This limitation has not always been recognized, and such characters (*e.g.*, the length and shape of the rostrum, number of teeth, overall skull shape, length of mandibular symphysis, crowding of mandibular foramina at the symphysis) were used in the past to help diagnose the genera *Stenella* and *Lagenorhynchus* and to justify the latter's inclusion in the subfamily Delphininae (by its similarities to *Stenella* and *Tursiops* for those characters).

More recently, presumably more conservative characters, such as aspects of the pterygoid sinus system (Fraser and Purves 1960, de Muizon 1988) or of the tympano-periotic bones (Kasuya 1973) have been used to diagnose subfamilies. Aside from the fact that these studies conflicted with each other, the data represent multiple characters from functionally integrated systems. The assumption of character independence for phylogenetic analysis may therefore have been violated. However, correlation *vs.* independence of characters was rarely considered. Agreement between several characters of the pterygoid sinus system, for example, in inferring species relationships may result from correlations due to functional and evolutionary constraints; what appears to be several characters may actually be a single complex character. This correlation does not negate the phylogenetic usefulness of the character(s), but it should be considered when drawing conclusions about degree of support for a taxon or the possibility of homoplasy.

In addition, the polarization of morphological characters was usually either not considered or not used in a cladistic sense. Few authors discussed the primitive and advanced state of rostrum length, for example. This relates to

the fact that most past studies utilized similarities, largely without regard to whether they were primitive (symplesiomorphies), convergent (homoplasies), or truly synapomorphic. A proper cladistic analysis of tympano-periotic bone characters might produce quite different results from those presented by Kasuya (1973). Similarly, the analyses of Fraser and Purves (1960) and de Muizon (1988), which both drew heavily on characters of the pterygoid sinus system, led to quite different conclusions about dolphin relationships. This was to be expected, since only the latter study attempted a cladistic analysis.

For a final illustration, it would be useful to examine the morphological bases for the dolphin genera *Stenella, Delphinus,* and *Tursiops*. As implied by the present study, the interrelationships of the various species of *Stenella* cannot be determined without consideration of the species of *Tursiops* and *Delphinus* (and *Lagenodelphis* and *Sousa* as well). Among the morphological characters used in the past to diagnose the genus *Stenella* (as well as *Tursiops*) are tooth size and number, absence of palatal grooves, rostrum length and shape, number of vertebrae, and length of mandibular symphysis (Gray 1866*b*, Flower 1884, True 1889). In most classifications, the absence of palatal grooves is used to distinguish *Stenella* from *Delphinus*, while the elongate rostrum, and tooth and vertebral numbers differentiate it from *Tursiops* (which also lacks palatal grooves). Apart from the evolutionary plasticity of the feeding apparatus (*i.e.*, rostrum length and tooth number), there are other problems with these characters. The presence or absence of palatal grooves is a recurring character in the history of delphinine taxonomy, with its presence used to separate *Delphinus* from other genera. However, the grooves are absent in non-delphinine dolphins, and so their absence (a symplesiomorphy) should not be used to diagnose genera, as has been done for *Tursiops* and *Stenella*. Regarding the apomorphic state (presence of grooves), Perrin *et al.* (1981) noted that deep grooves are present in *Lagenodelphis* as well as *Delphinus*, and that shallower grooves are present in *Stenella clymene, S. longirostris,* and *S. coeruleoalba.* In fact, the presence of grooves in some specimens of *Stenella* species led earlier workers to place them in *Delphinus* (*e.g.*, *Delphinus roseiventris* (=*Stenella longirostris*) in True (1889)). Although *Delphinus* as used here is monophyletic, the present mt phylogeny would lead one to conclude that palatal grooves have evolved more than once in different delphinine lineages, or that character reversals occurred in others. To his credit, True (1889) recognized the uncertainty of the palatal groove character, as well as of vertebral and tooth numbers for separating *Tursiops* from *Prodelphinus* (=*Stenella*). Furthermore, Rice (1998) states, in regards to *Stenella*, "There appear to be no plausible synapomorphies that would unite all of the species included herein . . .." As soon as one recognizes the polyphyly of *Stenella*, the morphological boundaries of *Tursiops*, *Lagenodelphis,* and perhaps *Delphinus* become less well defined.

In summary, some delphinid genera and subfamilies have been established on the basis of hypervariable and/or improperly analyzed morphological characters. It is beyond the scope of this study to reanalyze the data, but we can perhaps point out that an abundance of morphological data has already been

collected and deserves proper reanalysis. Until then, no suite of morphological characters should be dismissed offhandedly.

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Appendix A

Samples used in this study. Institutional abbreviations are as follows: SWFSC = Southwest Fisheries Science Center; LACM = Los Angeles County Museum, USNM = United States National Museum; ITESM = Instituto Tecnológico Estudios Superiores de Monterey.

Sample identification	SWFSC catalog no.	Collection locality	museum/field no.
Cephalorhynchus commersonii	z00040	Sea World, CA, USA	Sea World 8337
C. heavisidii	z07320	South Africa	PBB 9622
C. hectori	z03746	New Zealand	
C. eutropia	z02317	Chile	JAO 086
Delphinapterus leucas	z00801	AK, USA	LDL-19-88
Delphinus capensis	z00202	CA, USA	CAMMS 91-10
D. capensis	z03829	CA, USA	LACM 91367
D. delphis	z00174	Black Sea	
D. delphis	z04038	eastern tropical Pacific	RAM0010
D. delphis (cf. *tropicalis*)	z04523	Indian Ocean	BAL950719.01
Feresa attenuata	z02605	Philippines	WFP758
Globicephala melas	z00532	Newfoundland, Canada	STR02247
G. macrorhynchus	z00085	eastern tropical Pacific	DSJ900927.03
G. macrorhynchus	z00482	NC, USA	USNM 550310
Grampus griseus	z00141	CA, USA	EKL0009
G. griseus	z00483	NC, USA	USNM 550383
Lagenodelphis hosei	z00453	eastern tropical Pacific	USNM 500355
L. hosei	z02507	FL, USA	MM9407
Lagenorhynchus acutus	z00512	NE Atlantic	USNM 571395
L. albirostris	z00481	Newfoundland, Canada	USNM 550223
L. australis	z04926	South America	RNP 1476
L. cruciger	z05104	Southern Ocean	96J/IWC
L. obliquidens	z00001	CA, USA	LACM 86019
L. obscurus	z02319	Peru	KVW1834
Lissodelphis borealis	z00176	CA, USA	LACM 86066
L. peronii	z02316	Peru	KVW1857
Monodon monoceros	z02094	NW Territories, Canada	MMAB87-01
Orcaella brevirostris	z02907	Queensland, Australia	
O. brevirostris	z07205	Laos	95-3-LAO
Orcinus orca	z00476	Newfoundland, Canada	USNM 504925
O. orca	z02808	Bering Island, Russia	
Phocoena phocoena	z00032	CA, USA	LML-84-5
P. sinus	z01654	Gulf of California, Mexico	ITESM920.24
Peponocephala electra	z00545	Sea Life Park, HI, USA	
Pseudorca crassidens	z00022	Sea Life Park, HI, USA	
Sotalia fluviatilis	z00455	South America	USNM 504316
Sousa chinensis	z04955	Hong Kong	SC 95-0404
S. chinensis	z04960	Natal, South Africa	
Stenella attenuata	z00591	eastern tropical Pacific	DXB 172
S. attenuata	z00608	Gulf of Mexico	920601.01
S. clymene	z04185	FL, USA	MML-9510A
S. coeruleoalba	z00023	HI, USA	LBC-86-03
S. coeruleoalba	z00251	Mediterranean Sea	STE 8D1
S. frontalis	z00470	NC, USA	USNM 504736
S. frontalis	z00491	ME, USA	USNM 550751

Appendix A
Continued.

S. longirostris	z00594	Gulf of Mexico	920509.01
S. longirostris	z02060	eastern tropical Pacific	SWL369
S. longirostris	z02603	Philippines	WFP748
S. longirostris	z00375	Timor Sea	TH15
Steno bredanensis	z00459	VA, USA	USNM 504488
S. bredanensis	z01316	eastern tropical Pacific	RCR150
Tursiops truncatus	z00508	NE Atlantic Ocean	USNM 571382
T. truncatus	z00639	Gulf of Mexico	
T. truncatus	z01300	CA, USA	WXC0083
T. aduncus	z04117	South Africa	BN4
T. aduncus	z04172	Jakarta, Indonesia	OPL 940801