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Toward the resolution of an explosive radiation—A multilocus phylogeny of oceanic dolphins (Delphinidae)

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ABSTRACT

Oceanic dolphins (Delphinidae) are the product of a rapid radiation that yielded ~36 extant species of small to medium-sized cetaceans that first emerged in the Late Miocene. Although they are a charismatic group of organisms that have become poster children for marine conservation, many phylogenetic relationships within Delphinidae remain elusive due to the slow molecular evolution of the group and the difficulty of resolving short branches from successive cladogenic events. Here I combine existing and newly generated sequences from four mitochondrial (mt) genes and 20 nuclear (nu) genes to reconstruct a well-supported phylogenetic hypothesis for Delphinidae. This study compares maximum-likelihood and Bayesian inference methods of several data sets including mtDNA, combined nuDNA, gene trees of individual nuDNA loci, and concatenated mtDNA + nuDNA. In addition, I contrast these standard phylogenetic analyses with the species tree reconstruction method of Bayesian concordance analysis (BCA). Despite finding discordance between mtDNA and individual nuDNA loci, the concatenated matrix recovers a completely resolved and robustly supported phylogeny that is also broadly congruent with BCA trees. This study strongly supports groupings such as Delphininae, Lissodelphininae, Globicephalinae, *Sotalia* + Delphininae, *Steno* + *Orcaella* + Globicephalinae, and *Leucopleurus acutus*, *Lagenorhynchus albirostris*, and *Orcinus orca* as basal delphinid taxa.

1. Introduction

Relationships among species resulting from rapid episodes of successive cladogenesis have been notoriously difficult to disentangle (Hoelzer and Melnick, 1994; Jackson et al., 1999; Whitfield and Lockhart, 2007). This problem stems from the multiple short branch lengths between internal nodes of a phylogenetic tree, a common feature of explosive radiations. In general, short branch lengths may have had relatively little time to accumulate phylogenetically informative mutations (Allard et al., 1992). In addition, conflict between independent sources of molecular data may be a confounding factor in resolving short internodes due to the increased chance of persistence of ancestral polymorphisms across speciation events, differential lineage sorting, and introgression (Maddison, 1997). Multiple independent sources of molecular data may therefore be needed to substantiate tightly-spaced branch points and evaluate conflicting phylogenetic signals (Whitfield and Lockhart, 2007). This problem is especially problematic in cetaceans (whales and dolphins), as these species are characterized by some of the slowest rates of molecular evolution among mammals yet analyzed (Bininda-Emonds, 2007; Nabholtz et al., 2008).

The family Delphinidae (oceanic dolphins) is an example of a cetacean lineage that has experienced explosive speciation (McGowen et al., 2009; Steeman et al., 2009) and consists of approximately 36 species of small to medium-sized toothed cetaceans distributed among 17–19 genera (Jefferson et al., 2008). Oceanic dolphins are known for their large brain-to-body mass ratio, refined echolocation ability, and complex and varied societies (Marino et al., 2004; LeDuc, 2009). Delphinids also exhibit a wide range of ecological diversity, including small coastal fish-eating forms, intermediate-sized squid specialists, and larger forms that feed on other marine mammals (Slater et al., 2010). Morphological disparity among delphinids can be illustrated by well-known aquarium favorites such as the bottle-nosed dolphin (*Tursiops* spp.), killer whale (*Orcinus orca*), and pilot whale (*Globicephala* spp.). As the most abundant cetaceans, both in numbers of species and individuals, dolphins are important as marine predators and are the direct focus of international conservation and management efforts (LeDuc, 2009). Many factors have been proposed as explanations for the explosive radiation and recent evolutionary success of delphinids (e.g., brain size, echolocation, sociality), in combination with large-scale ocean restructuring and temperature fluctuations in the Late Miocene and Early Pliocene (Steeman et al., 2009). Multiple recent divergence dating analyses point to an origin of crown

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delphinids ~10–11 million years ago (Mya) (McGowen et al., 2009; Steeman et al., 2009; Xiong et al., 2009; Slater et al., 2010; Ho and Lanfear, 2010). In addition, recent studies have quantified the rapid tempo of speciation events at the base of Delphinidae, identifying one or more statistically significant positive shifts in the rate of diversification (Steeman et al., 2009; Slater et al., 2010).

Despite widespread popular and scientific interest in dolphins, many aspects of their phylogeny and evolutionary history remain unresolved. Systematic studies based on morphology have failed to agree on a consistent classification scheme for the group (Flower, 1884; True, 1889; Fraser and Purves, 1960; Kasuya, 1973; Mead, 1975; De Muizon, 1988; Perrin, 1989; Barnes, 1990; Buchholtz and Schur, 2004). A seminal phylogenetic analysis of delphinids using the mitochondrial (mt) gene cytochrome *b* (LeDuc et al., 1999) confirmed the monophyly of three major subfamilies with high support: Delphininae (bottlenose-like dolphins, ~12 spp.), Globicephalinae (blunt-headed dolphins, 6 spp.), and Lissodelphininae (piebald dolphins, 10 spp.); however, relationships among these subfamilies and among other delphinid species remain poorly resolved (LeDuc et al., 1999; May-Collado and Agnarsson, 2006).

A few recent molecular phylogenetic analyses of dolphins using mtDNA, nuclear (nu) genes, and/or nu AFLP data have added to the growing accumulation of data and made some progress in resolving relationships within the group (e.g., Harlin-Cognato and Honeycutt, 2006; Nishida et al., 2007; Onbe et al., 2007; Caballero et al., 2008; McGowen et al., 2008; Ayoub et al., 2009; Xiong et al., 2009; Kingston et al., 2009; Koito et al., 2010; Morin et al., 2010). Many of these studies concentrated on specific subfamilies or did not include critical species, and much of these data were not combined until two recent supermatrix analyses (McGowen et al., 2009; Steeman et al., 2009). The supermatrix of McGowen et al. (2009) represents the largest of these attempts, composed of 42,335 characters and sampling all extant species with available molecular data; resolution among many basal relationships improved substantially relative to previous studies, and two species were positioned as successive sister taxa to all other dolphins (*Leucopleurus acutus* and *O. orca*). However, many early branching events and relationships within subfamilies remain difficult to establish.

This study complements existing data with new sequences from three mt genes (*12S, 16S* and *COII*) and 20 nu markers (*ACTA2, AMBN, ATP7A, BTN1A1, CASB, CAT, CHRNA1, GBA, LALBA, MAS1, MC1R, MCPH1, OR1I1, PKDREJ, PRM1, RAG1, SPTBN1, STAT5, SWSOPN1, TSHB*) to examine the effect of their addition in resolving delphinid relationships. All together, a total of 19,567 characters were assembled, and 393 new sequences were generated from 27 out of 36 species in all 19 genera, representing all major lineages of oceanic dolphins. To my knowledge, this study represents the greatest amount of sequence data assembled to date to specifically address the species-level phylogeny of the group. Here I compare resolution of delphinid relationships using independent gene trees, concatentation, and species tree reconstruction methods using Bayesian Analysis of Concordant Knots (BUCKy), and employ these results in interpreting patterns and conflicts concerning the evolution of Delphinidae.

2. Methods

2.1. Taxa sampled and PCR amplification

DNA samples were provided by P. Morin, A. Dizon and K. Robertson (SWFSC: Southwest Fisheries Science Center, NOAA, La Jolla, CA), G. Amato (NYZS: New York Zoological Society), H. Rosenbaum (HR; Wildlife Conservation Society, Bronx, NY), M. Stanhope (MS; Cornell University), and M. Milinkovitch (MM; University of Geneva). Species for which we obtained sequence data are shown in Table 1, and all genes used in this study are listed in Table 2. Primers for PCR amplification and sequencing are cataloged in Supplemental Table 1. Sequences were amplified using published protocols from the citations in Supplemental Table 1 or the procedures outlined in Deméré et al. (2008). Polymorphisms were treated as ambiguous nucleotides using the appropriate IUPAC nucleotide code. Possibly due to the degraded nature of many of the samples, I was unable to amplify all 20 nu loci in 7 out of 27 delphinid species. These taxa included *Globicephala melas*, *Stenella frontalis*, *Orcaella brevirostris*, and *Orcaella heinsohni*, as well as *Lagenorhynchus albirostris, Lissodelphis borealis,* and *Lagenodelphis hosei*, which were missing only one gene each (*STAT5*, *MC1R*, and *TSHB* respectively). All sequences were deposited in GenBank (JF504709–JF504738, JF504740–JF504760, JF504762–JF504779, JF504781–JF504808, JF504810–JF504951, JF504953–JF504966, JF504968–JF504974, JF504976–JF505108).

2.2. Alignment and phylogenetic analysis

In this study, one sequence from each species was sampled for each locus. The closely-related species *O. brevirostris* and *O. heinsohni* were combined to form the operational taxonomic unit (OTU) *Orcaella* sp. Both species of *Orcaella* originally were considered one species until very recently, and their close relationship is well established (Beasley et al., 2005). *Kogia breviceps TSHB* and *Kogia sima GBA* were included with sequence data from *Physeter macrocephalus*; all three taxa are included within the highly-supported clade Physeteroidea (McGowen et al., 2009; Steeman et al., 2009). For all sampled loci, I included additional sequence data from GenBank for all available delphinid and outgroup species; GenBank accession numbers are listed in Supplemental Appendix 1. In addition to new data as described above, I also included sequences from the mt gene cytochrome *b*, as well as data from the nu gene *MCPH1* (McGowen et al., 2011) in both gene tree and concatenated analyses. All genes were aligned individually using Clustal X (Thompson et al., 1994) with a gap-opening penalty of 10 and gap-extension penalty of 1. Nu genes included exons, introns, pseudogenes, and non-coding flanking regions (Table 2); all indels within exons were constrained to occur as in-frame triplets. All nucleotide positions were included in analyses. Indels (insertions and deletions) were coded for each gene in SeqState (Müller, 2005) using the simple-gap coding method of Simmons and Ochoterena (2000). Twenty-three matrices were assembled: one matrix of mtDNA data only, 20 separate matrices for each nu locus, one concatenated matrix of all nuDNA data, and one concatenated matrix of both mt and nu data.

Markov chain Monte Carlo (MCMC) Bayesian analyses were conducted for each data set using default parameters and four simultaneous chains (three "heated", one "cold") in MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) via the Cyberinfrastructure for Phylogenetic Research (CIPRES) Portal (www.phylo.org). Mitochondrial sequence data were analyzed using four separate partitions: three for each codon position of mt protein-coding genes and one partition for rDNA. Sequence data from each individual nuDNA data set were analyzed using a maximum of four partitions: three for each codon position (if applicable) and one partition for any intronic sequence (if applicable). The concatenated nu data also were analyzed using the same four partitions. For the total mt + nu concatenated matrix, three different partitioning schemes were conducted for sequence data: (1) 2 partitions; mt and nu, (2) 8 partitions; mt protein-coding genes by codon position, nu protein-coding genes by codon position, mt rDNA, and nu intronic DNA, (3) 57 partitions; as in 8-partitioning scheme, but codon positions are also partitioned by gene. Gaps (if present) were treated as a separate partition, and the binary model of character evolution was implemented for this partition of data (Ronquist et al., 2005). For DNA sequence alignments, MrModeltest