Novel Phylogeny of Whales Revisited but Not Revised

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Molecular phylogenetic data, if analyzed correctly, have the demonstrated capacity to resolve evolutionary relationships (i.e., the topology of phylogenetic trees) with confidence and can additionally suggest ages of lineages if assumptions about the rate of accumulation of mutations are accepted. Based on detailed maximum-parsimony, neighbor-joining, and maximum-likelihood analyses of 930 base pairs (bp) of mitochondrial DNA (mtDNA) from 16 cetacean species (of 13 genera) and four outgroups, we (Milinkovitch et al. 1993) published a novel phylogenetic hypothesis which is in conflict with the classic division (Barnes et al. 1985) of living cetaceans into toothed whales and baleen whales. Our major finding was the suggestion that the suborder Odontoceti (toothed whales) is paraphyletic and, more specifically, that the sperm whales are more closely related to baleen whales (suborder Mysticeti) than to any other group of toothed whales. Further support for the (sperm whale + baleen whale) relationship derives from cladistic analyses of myoglobin amino-acid sequences (McKenna 1987; Czelusniak et al. 1990; Milinkovitch et al. 1993). Importantly, the sister-group relationship between sperm whales and baleen whales was recently reaffirmed by an expanded phylogenetic analysis (Milinkovitch et al. 1994) of 1,352 bp of mtDNA from 21 cetacean species (including a river dolphin and representatives of three baleen whale families) and several artiodactyl outgroup species. Independently, based on the analysis of 657 bp of the 16S mitochondrial gene from three cetacean species, Árnason et al. (1993) presented a neighbor-joining tree where the sperm whale is more closely related to the fin whale than to the only dolphin species included in that analysis. Although that result supports our hypothesis (Milinkovitch et al. 1993) of toothed whale paraphyly, Árnason et al. (1993) and Árnason and Gullberg (1994) concluded that it must be artifactual.

Based on a new data set of complete cytochrome *b* DNA sequences (1,140 bp) from 14 cetacean species (of 7 genera) and 1 outgroup, Árnason and Gullberg

(1994) assert that our study (Milinkovitch et al. 1993) is incorrect. Although, they (Árnason and Gullberg 1994) also show that toothed whales are paraphyletic, they claim, however (contradicting their earlier study; Árnason et al. 1993), that *dolphins* (rather than sperm whales, as suggested by us; Milinkovitch et al. 1993) are the sister group of baleen whales. We seriously doubt the correctness and robustness of this result because the bootstrap value (Felsenstein 1985) supporting this hypothesis is alarmingly low (52%); only a single and inappropriate (see below) weighting scheme for the parsimony analysis was used; only one of many available ungulate cytochrome *b* outgroups (Irwin et al. 1991) was used.

It is entirely surprising that Árnason and Gullberg (1994) claim that our study implied that baleen whales are paraphyletic. Indeed, Árnason and Gullberg (1994) allege that we (Milinkovitch et al. 1993) suggested a close relationship between sperm whales and *rorquals* (baleen whale family Balaenopteridae) to the exclusion of other baleen whale families. Our paper did not (and could not) address this point since, in addition to various toothed-whale species, it exclusively included balaenopterid–baleen whale species which we clearly assumed to represent the whole suborder Mysticeti (it is conceptually inappropriate to assert relationships between three or more taxa when data from one of them are not included in the analyses). Furthermore, our extended phylogenetic analyses (Milinkovitch et al. 1994) incorporate sequences from representatives of additional baleen whale families and support both the monophyly of baleen whales and the sister relationship between sperm whales and baleen whales. In order to test the robustness of Árnason and Gullberg' hypothesis of a sister relationship between non-sperm-whale odontocetes and baleen whales, we submitted their cytochrome *b* data to reanalyses.

It is well understood that, in the mitochondrial genome, transversion substitutions (Tv) accumulate more slowly over time than transitions (Ti) and hence are more reliable phylogenetic markers. Multiple substitutions (e.g., back mutations) in Ti will quickly accumulate with increasing sequence divergence, creating noise and concealing the phylogenetic signal (DeSalle et al. 1987; Meyer and Wilson 1990; Irwin et al. 1991; Hillis et al. 1994). In the cytochrome *b* data of Árnason and Gullberg (1994), we find Ti substitutions to accumulate linearly only up to a level of about 12% sequence divergence

(fig. 1), after which they become saturated. In contrast, Tv substitutions appear to accumulate linearly well above 12% sequence divergence (fig. 1). In all sequence comparisons between baleen whales and any of the dolphins, sperm whale, and the cow (the only outgroup used in their DNA sequence analysis), divergence values are always above 14%, which indicates that Tv rather than Ti are more reliable phylogenetic markers to establish relationships among these groups. Therefore, Árnason and Gullberg (1994) incorrectly considered Ti and Tv to be equally informative by using a weighting scheme based on codon position only. Parsimony (Swofford 1993) and neighbor-joining (Saitou and Nei 1987) reanalyses of their data using only Tv substitutions result in high bootstrap (Felsenstein 1985) support for our (Milinkovitch et al. 1993) phylogenetic hypothesis (fig. 2) rather than for the one reported by Árnason and Gullberg (1994). Moreover, additional parsimony analyses downweighting Ti (but including both types of substitutions) and/or with the inclusion of several artiodactyl outgroups and a maximum-likelihood analysis (Felsenstein 1991) also yielded unambiguous support for our (Milinkovitch et al. 1993) topology (fig. 2). The only major result from Árnason and Gullberg (1994) confirmed by the reanalyses of their cytochrome *b* sequences is the monophyly of mysticetes, which validates our previously untested (Milinkovitch et al. 1993) and now tested (Milinkovitch et al. 1994) assumption. When the data are analyzed in a way which reflects better the underlying evolutionary processes, molecular systematic studies of cetaceans converge to the same topology (Milinkovitch et al. 1993, 1994, this paper; McKenna 1987).

Tree topology and the calibration of molecular clocks are two separate issues. Since artiodactyls appear to be the sister group of whales, we (Milinkovitch et al. 1993) made the *prima facie* assumption that the ticking rate of the molecular clock in whale mitochondrial ribosomal genes is similar to that of ungulates (for which
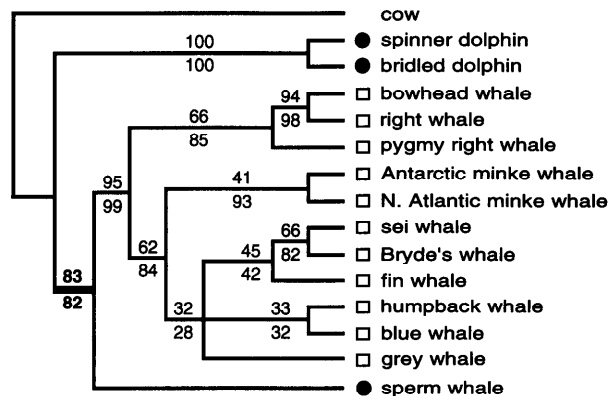
FIG. 2.—Majority-rule bootstrap consensus tree based on Tv only. Numbers above/below branches indicate bootstrap (Felsenstein 1985) values (500 replicates) from parsimony (Swofford 1993)/neighbor-joining (Saitou and Nei 1987) analyses. The reanalyses of cytochrome *b* data from Árnason and Gullberg (1994) unambiguously support our (Milinkovitch et al. 1993, 1994) hypothesis of a sister group relationship (defined by the bold branch on the tree) of *sperm whales* and baleen whales over the hypothesis of the sister group relationship of *dolphins* and baleen whales (Árnason and Gullberg 1994). The odontocetes (forming a paraphyletic group) and the mysticetes are indicated with black circles and open squares, respectively.

a reliable fossil record allowed the calibration of that clock). Based on this reasonable and explicit assumption, we (Milinkovitch et al. 1993) suggested that the common ancestor of sperm whales and baleen whales might have lived only 15 million years ago. However, the existence of fossils (Barnes 1984) (which we were unaware of) that date back to about 23 million years and that are assigned to the sperm and baleen whale lineages suggests that the molecular clock in whales ticks at a slower rate than that of ungulates. Since the publication of our original study (Milinkovitch et al. 1993), a slower rate for whale-mtDNA evolution has indeed been suggested (Martin and Palumbi 1993).

In summary, the phylogenetic split between sperm whales and baleen whales is probably somewhat older than our initial estimate, but the likelihood of the reality of their sister group relationship (Milinkovitch et al. 1993, 1994) remains high. Our hypothesis is supported rather than challenged by the data from Árnason and Gullberg (1994).

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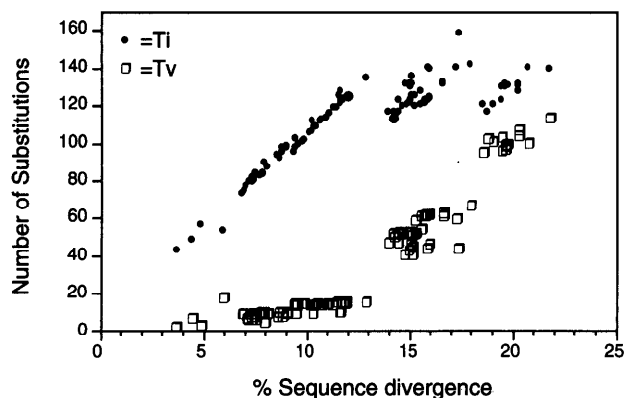BARNES, L. G., D. P. DOMING, and C. E. RAY. 1985. Status

FIG. 1.—Accumulation of transitions (Ti) and transversions (Tv) with increasing sequence divergence in cytochrome *b* data of Árnason and Gullberg (1994). Ti accumulate linearly only up to about 12% sequence divergence, while Tv do well above this value.

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