Naked corals: Skeleton loss in Scleractinia

Mónica Medina[†‡], Allen G. Collins[§], Tori L. Takaoka[†], Jennifer V. Kuehl[†], and Jeffrey L. Boore[†¶]

[†]Department of Evolutionary Genomics, Department of Energy Joint Genome Institute, 2800 Mitchell Drive, Walnut Creek, CA 94598; [§]National Systematics Laboratory, National Oceanic and Atmospheric Administration Fisheries Service, National Museum of Natural History, MRC 153, Smithsonian Institution, Washington, DC 20013-7012; and [¶]Department of Integrative Biology, University of California Berkeley, 3060 Valley Life Sciences Building, Berkeley, CA 94720

Communicated by James W. Valentine, University of California, Berkeley, CA, April 27, 2006 (received for review January 14, 2006)

Stony corals, which form the framework for modern reefs, are classified as Scleractinia (Cnidaria, Anthozoa, and Hexacorallia) in reference to their external aragonitic skeletons. However, persistent notions, collectively known as the ''naked coral'' hypothesis, hold that the scleractinian skeleton does not define a natural group. Three main lines of evidence have suggested that some stony corals are more closely related to one or more of the soft-bodied hexacorallian groups than they are to other scleractinians: (*i*) morphological similarities; (*ii*) lack of phylogenetic resolution in molecular analyses of scleractinians; and (*iii*) discrepancy between the commencement of a diverse scleractinian fossil record at 240 million years ago (Ma) and a molecule-based origination of at least 300 Ma. No molecular evidence has been able to clearly reveal relationships at the base of a well supported clade composed of scleractinian lineages and the nonskeletonized Corallimorpharia. We present complete mitochondrial genome data that provide strong evidence that one clade of scleractinians is more closely related to Corallimorpharia than it is to a another clade of scleractinians. Thus, the scleractinian skeleton, which we estimate to have originated between 240 and 288 Ma, was likely lost in the ancestry of Corallimorpharia. We estimate that Corallimorpharia originated between 110 and 132 Ma during the late- to mid-Cretaceous, coinciding with high levels of oceanic CO_2, which would have impacted aragonite solubility. Corallimorpharians escaped extinction from aragonite skeletal dissolution, but some modern stony corals may not have such fortunate fates under the pressure of increased anthropogenic CO_2 in the ocean.

aragonite skeleton | Corallimorpharia | mitochondrial genome evolution

The calcareous skeletons of stony corals provide the main structural framework upon which modern tropical reefs are built. Despite their classification within a single taxon, Scleractinia, there has been a long history of ideas, known collectively as the ''naked coral'' hypothesis (1), that the scleractinian skeleton may be evolutionarily ephemeral. The phylogenetic correlate to the naked coral hypothesis is that Scleractinia is not a monophyletic group. In other words, the naked coral hypothesis holds that some stony corals are more closely related to one or more of the soft-bodied hexacorallian groups (Actiniaria, Corallimorpharia, and Zoanthidea) than they are to other scleractinians (1–4). Multiple lines of evidence (from morphology, molecular phylogenetics, and the fossil record) have been used to argue for the naked coral hypothesis.

Morphological similarities between scleractinians and corallimorpharians, and to a lesser extent actiniarians, provide a line of evidence marshaled in favor of the naked coral hypothesis. In particular, the presence of paired mesenteries in all three groups has been seen as a topological arrangement difficult to understand in the absence of mineralized septa in actiniarians and corallimorpharians (5). Thus, Hand (5) concluded that scleractinians are likely ancestral (i.e., paraphyletic with respect) to both of these nonmineralized groups. Similarity in scleractinian and corallimorpharian cnidoms also has been used to argue that scleractinians have an especially close relationship to Corallimorpharia (6, 7). The cladistic analysis of Daly *et al.* (8) also identified sperm ultrastructure characteristics that may be synapomorphies for the clade uniting Scleractinia and Corallimorpharia.

The second set of observations used to bolster the naked coral hypothesis has been the lack of resolution in early molecular phylogenetic analyses of Scleractinia (9–13). A robust phylogeny of Hexacorallia obviously provides the most direct test of the naked coral hypothesis in any of its forms, and, in fact, more recent molecular phylogenetic analyses with greater taxon sampling across Hexacorallia have shown rather conclusively that scleractinians are more closely related to each other than any are to zoanthideans and the highly diverse actiniarians (8, 14, 15). Thus, the scleractinian skeleton has likely not been as evolutionarily ephemeral (8) as some have suggested (9). Nevertheless, molecular data have consistently confirmed the close relationship between Scleractinia and nonskeletonized Corallimorpharia (8, 12, 14, 15) evident from morphology. However, up to this point, molecular analyses have failed to provide resolution or a consistent signal at the base of the clade uniting Scleractinia and Corallimorpharia. In other words, available data are not able to discern whether corallimorpharians are naked corals.

The third line of evidence cited in favor of the naked coral hypothesis comes from the fossil record. Some 10 million years after the great Permian–Triassic extinction, Scleractinia first enters the fossil record and is represented by numerous higher taxa (1, 3). This explosive appearance postdates a molecule-based estimate of the origin of Scleractinia of at least 300 million years ago (Ma) (10), suggesting a hidden history for >60 Ma. One potential explanation for this lengthy hidden history would be that scleractinians did not possess mineralized skeletons during this time of diversification and that scleractinian skeletons must therefore have been derived independently from numerous groups of soft-bodied ancestors (1, 2, 9).

Results and Discussion

Complete mitochondrial genome comparisons from nine scleractinians, four corallimorpharians (and partial sequence for a fifth one), and six outgroups (three octocorallians, two actiniarians, and one zoanthidean), substantially clarify our understanding of scleractinian history. We confirm the existence of two major groups of Scleractinia, known as the short (robust) and long (complex) clades because of size differences in mitochondrial rDNA (9, 13) (Fig. 1). These comparisons also unambiguously indicate that the long-clade scleractinians are more closely related to corallimorpharians than they are to the short-clade scleractinians (Fig. 1). Our analysis includes both major groups of corallimorpharians (6) and suggests that one group

Conflict of interest statement: No conflicts declared.

Abbreviation: Ma, million years ago.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. DQ640646–DQ640651, DQ643965, DQ643966, and DQ643831–DQ643838).

[‡]To whom correspondence should be sent at the present address: School of Natural Sciences, University of California, P.O. Box 2039, Merced, CA 95344. E-mail: mmedina@ucmerced.edu.

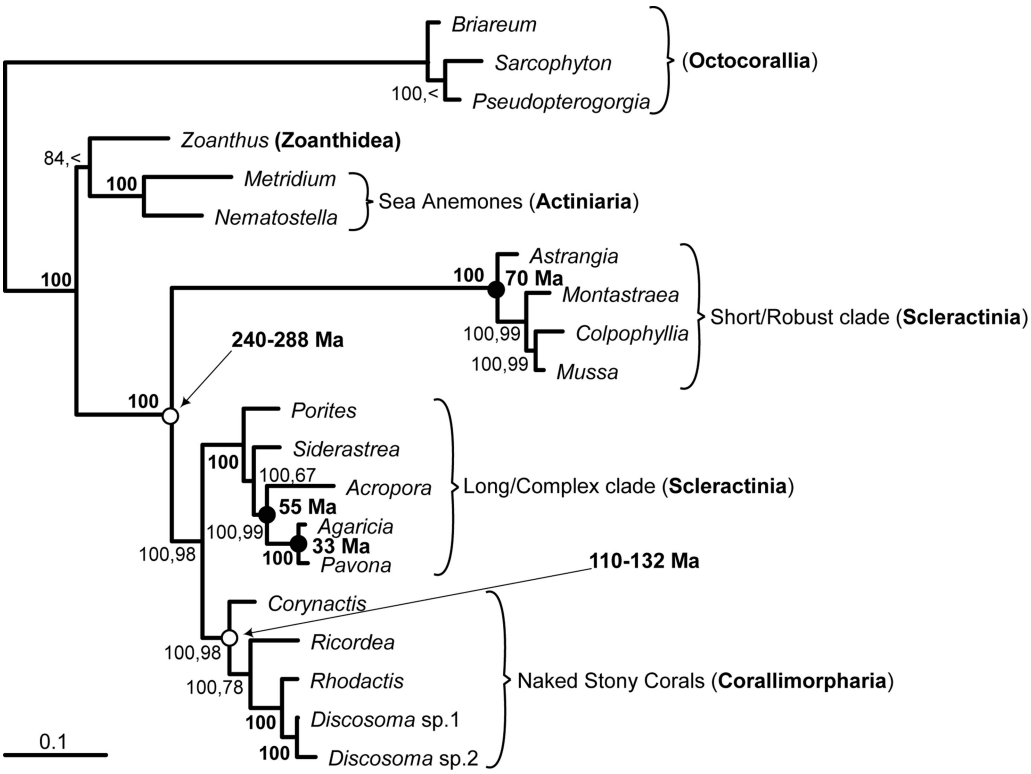

Fig. 1. Phylogenetic relationships among sampled hexacorallians. Bayesian posterior probabilities and maximum parsimony bootstrap values are shown at each node. A single 100 indicates that both values equal 100. Ranges of estimated divergence dates are shown for nodes indicated by open circles. Fixed divergence dates based on earliest fossil appearances are shown at nodes indicated by filled circles.

(Discosomatidae, represented by *Discosoma* and *Ricordea*) is derived from within the other. This outcome fits with den Hartog's (6) observation that the non-discosomatid corallimorpharians are more similar to skeletonized scleractinians. Our analysis also strongly supports Corallimorpharia as monophyletic, in agreement with assertions based on morphology (6). In light of these findings, Scleractinia should be redefined to include Corallimorpharia, as suggested by den Hartog (6), so that the former taxon refers to a clade. We also infer from our data that a calcified skeleton was likely lost during the ancestry of Corallimorpharia.

It is conceivable that the evolution of scleractinian skeletons is more complex than our inference. For instance, skeletons may have arisen independently in the two scleractinian clades. Scleractinian corals are diverse and dense taxon sampling is needed to fully investigate the history of skeletonization within Scleractinia. However, a biphyletic origin of the scleractinian skeleton seems less likely than a single origin and subsequent loss in Corallimorpharia in light of our refined estimate for the origination of Scleractinia (including Corallimorpharia) between 240 and 288 Ma. This result substantially narrows the gap between the group's first fossil appearance and its inferred origin. The earliest scleractinians appeared ≈240 Ma and were not reef-forming but were rather solitary and lacking in algal symbionts (3). Moreover, >40% of extant scleractinian diversity is represented by deep-sea forms (S. Cairns, personal communication). Thus, it seems plausible that the hidden history may represent a time when skeletonized scleractinians were rare in or absent from near-shore environments where preservation potential is enhanced.

A second point indicating skeletal loss rather than independent gains comes from our estimate for the origin of Corallimorpharia between 110 and 132 Ma. At this time, Cretaceous oceans were typified by high CO_2 levels. Such high levels would have increased the solubility of aragonite and thereby provided a selective force favoring skeletal loss (16). Cretaceous reefs were dominated by rudist bivalves rather than corals, which has been attributed to a more propitious biomineralization mechanism under less saturated water conditions (16). Experimental data on phylogenetically diverse scleractinian corals supports this notion by showing that skeletal growth is reduced when the ambient carbonate ion concentration is decreased (17–20). Therefore, our estimate for the origin of Corallimorpharia is consistent with a scenario of lower calcium carbonate saturation in the Cretaceous.

Our data also reveal unusual patterns in the evolution of mitochondrial genomes. Anthozoan genomes are quite divergent from bilaterian metazoan genomes because the former lack most tRNAs (21), possess introns (22–24), evolve more slowly (25, 26), and some have *MutS* (a DNA repair gene in bacteria) (27). All scleractinian corals examined have a uniform mitochondrial gene order, suggesting that this represents the ancestral condition for the clade Scleractinia (Fig. 2). Corallimorpharian mitochondrial gene orders, therefore, appear to be derived from this condition (Fig. 2). The gene order obtained for three corallimorpharians, *Discosoma* sp., *Ricordea florida*, and *Rhodactis* sp., is uniform, whereas the partial sequence we derived from *Corynactis californica* indicates that it has a different gene order. Given that *Corynactis* appears to be the sister lineage to all other sampled corallimorpharians, it is not yet possible to infer the precise history of gene order rearrangements within this group. Given the modest diversity (some 30–40 species) of Corallimorpharia, such an understanding appears to be tractable. Available data from other metazoan mitochondrial genomes clearly shows that there is no molecular clock of gene-order evolution and that there are long periods of stasis followed by

EVOLUTION

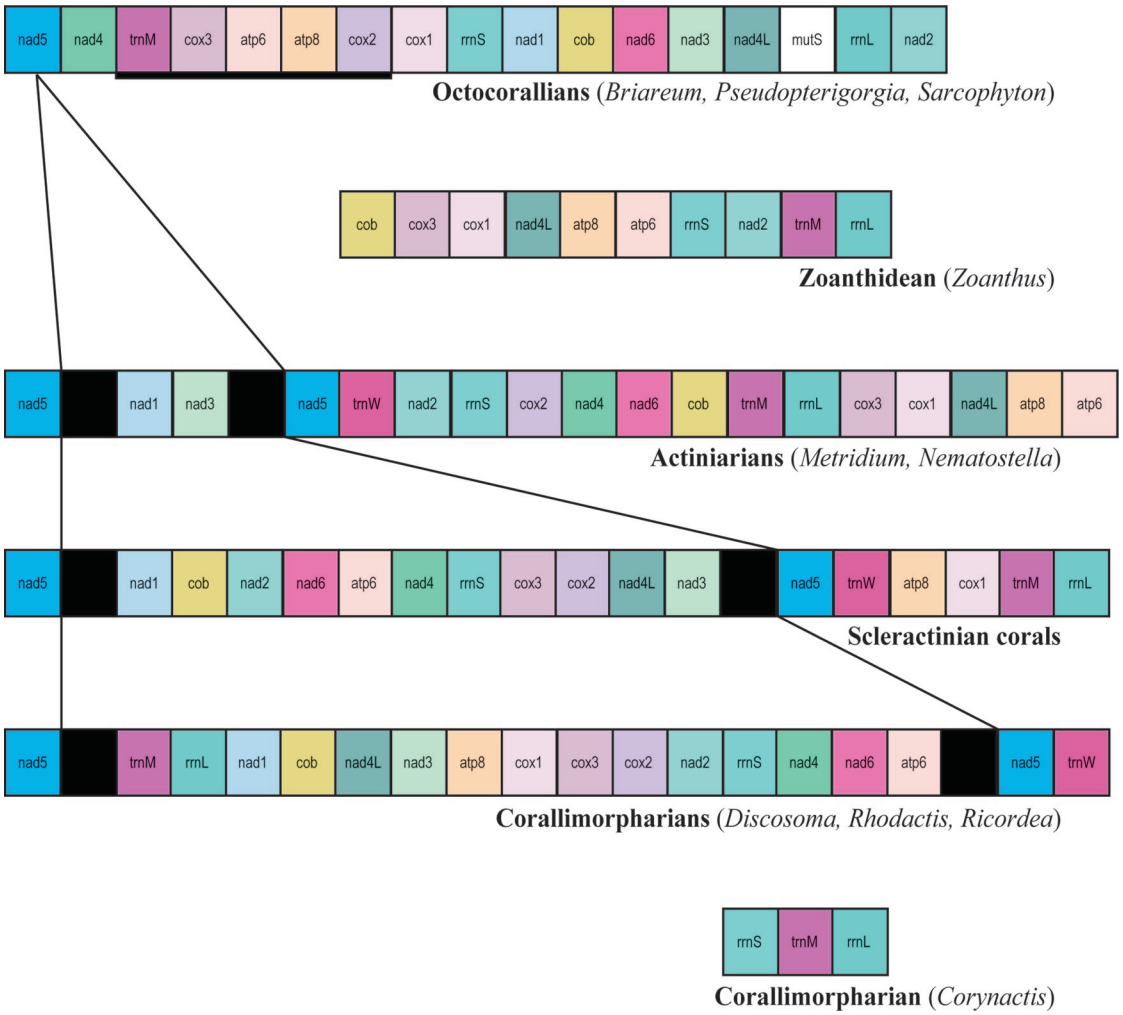

Fig. 2. Linearized mitochondrial gene orders for each group. The boxes for *trnM* and *trnW* represent the methione and tryptophan tRNAs. Black boxes represent noncoding regions of the *nad5* intron. Lines connecting the different genomes highlight intron expansion in the different anthozoan genomes. The black bar at the bottom of the octocorallian genomes represents the opposite transcriptional orientation of that region in the genome.

rapid events of gene rearrangements (reviewed in ref. 28). The evolution of highly rearranged mitochondrial genomes in corallimorpharians after diverging from a scleractinian ancestor is one more piece of evidence supporting this observation.

An atypical feature of the mitochondrial molecule in anthozoans and scleractinians, in particular, when compared with other metazoans is an apparent trend for the expansion of the group I intron within the *nad5* gene. The case is most extreme in some of the corallimorpharians in which most of the genes are located inside this intron, to the exclusion of the tryptophan tRNA (Fig. 2). Group I introns are known to be acquired often by horizontal transfer, which could lead to multiple acquisitions in related lineages (reviewed in ref. 29). However, our data from multiple mitochondrial genomes (from actiniarians to corallimorpharians) suggest a single gain of the *nad5* intron in hexacorallians, as previously hypothesized (22). These genomes share the same *nad5* intron insertion site and share conserved sequence motifs on both the 5′ and 3′ ends of the noncoding intronic region. It seems that once this intron was acquired by hexacorallians, there may have been a tendency for the intron to gain genes from the rest of the genome undergoing major size expansion, although the partial information for *Zoanthus* prevents us from inferring if this might also be the case for that lineage. The rearrangements observed within the corallimorpharians for which we have a complete sequence seem to have occurred in a systematic fashion in sets of two genes (Fig. 3). Only in one case is there an inversion in gene order of the two genes involved in one of these sets (*rrnS* and *nad4*). It is possible that these dual rearrangements are induced during the processing of the *nad5* intron. The molecular mechanisms that cause this unusual pattern, however, remain unclear at this point. Finally, some hexacorallian genomes also have acquired a group I intron in the *cox1* gene (data not shown), an event that has occurred multiple times. Thus, intron gains appear to be a common trend within Hexacorallia.

Our findings represent strong evidence supporting the evolution of corallimorpharians from scleractinians, raising important evolutionary questions, such as the role of molecular mechanisms of biomineralization in organisms that have lost a skeleton. More importantly, the world's oceans are presently experiencing an increase in CO_2 concentrations that is similar to what occurred in the Cretaceous when multiple calcifying scleractinians went extinct (3, 16). Current observations show a steady increase in $CaCO_3$ undersaturation, which, under projected carbon cycle models, will have dramatic impacts in shallow marine biomineralization, in particular on the more soluble aragonitic forms, such as reef coral skeletons (17, 18). Although Cretaceous scleractinians, one of which gave rise to corallimorpharians, were able to adapt to higher CO_2 levels in the ocean, it is not clear how many modern coral lineages have the potential to adapt similarly.

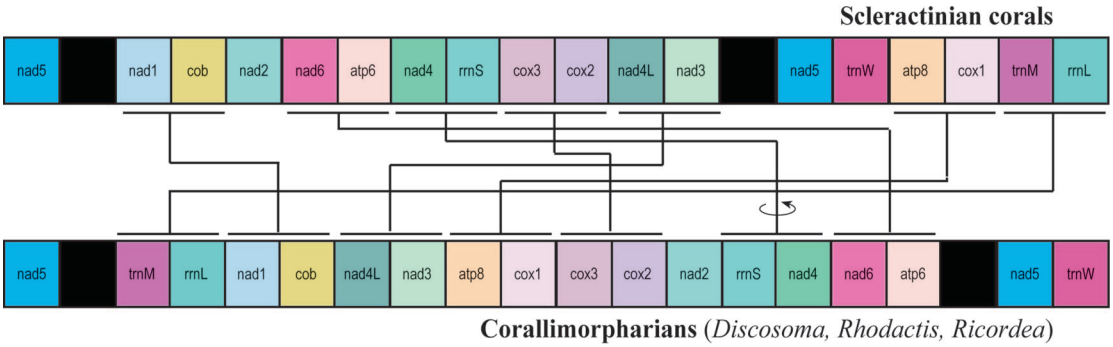

Fig. 3. Pairwise comparison of gene rearrangements between the scleractinian and corallimorpharian gene orders. Black bars highlighting seven pairs of genes in the two genomes are connected by lines indicating the relative positional rearrangement of each pair. In one case only the rearrangement involved an inversion.

Materials and Methods

DNA Extraction and Amplification. Scleractinian total DNA was extracted by using a DNeasy kit (Qiagen, Valencia, CA). Sample information is available as Table 1, which is published as supporting information on the PNAS web site. Scleractinian coral samples were lawfully collected and exported after local regulations and reported under the appropriate authorities either as larvae, under permit FKNMS-2002-2006 in the Florida Keys to Alina Szmant, or as small fragments to prevent colony destruction under a Convention of International Trade in Endangered Species of Wild Fauna and Flora Permit SEX/A-130-2003 issued to Peter Glynn in the Republic of Panama and the Bahamas (Bahamas Department of Fisheries permit issued to Howard Lasker). All collectors were familiar with the systematics of the specimens collected guaranteeing accurate identifications. Mitochondrial DNA was amplified in approximately two halves by long PCR with universal hexacorallian primers from the 12S (*rrnS*) and 16S (*rrnL*) genes. In several cases, one half was obtained with the hexacorallian primers and the second half was amplified with species-specific primers (Table 2, which is published as supporting information on the PNAS web site).

Cloning and Sequencing. Long PCR products were randomly sheared in a HydroShear (GeneMachines, San Carlos, CA), blunt-end-repaired enzymatically, size-selected on an agarose gel (1.5 kb), and ligated into pUC vector. Ligated DNA was transformed into *Escherichia coli* DH10b to create plasmid libraries. The clones were then plated and grown overnight, and individual clones were picked into 10% glycerol stock plates. The plates were sequenced by automated technology as follows: rolling circle amplification of glycerol stock plates with a TempliPhi DNA amplification kit (Epicentre Biotechnologies, Madison, WI) was used to create a template for sequencing. Standard M13 primers were used for forward and reverse reactions. DNA was cleaned by using solid-phase reversible immobilization before capillary sequencing (catalog no. 3730, Applied Biosystems).

Genome Assembly and Annotation. Base calls were made with PHRED, assemblies were generated with PHRAP, and the consensus sequence was called in CONSED (30, 31). Consensus sequences were then annotated with DOGMA (32). The genetic code for BLASTX was set to four-mold mitochondria (identical to the cnidarian mitochondrial genetic code), the percent identity cutoff for protein-coding genes and RNAs was set to 40, the *E* value was 1×10^{-5}. The DNA sequences are available at the National Center for Biotechnology Information (GenBank accession nos. DQ640646–DQ640651, DQ643965, DQ643966, and DQ643831–DQ643838).

Phylogenetic Analysis. Amino acid alignments were generated with CLUSTALX for all protein encoding genes. The octocorallians (Cnidaria, Anthozoa, and Alcyonaria) *Sarcophyton glaucum*, *Pseudopterogorgia bipinnata*, and *Briareum asbestinum* were used as the outgroup to the hexacorallians. Regions of unambiguous alignment were determined with GBLOCKS (33) and excluded from further analysis. Sites with gaps were allowed to be included as long as half the taxa were not represented by a gap. Alignments were subsequently concatenated into a single file for phylogenetic analysis. We performed maximum parsimony analysis in PAUP* (with 100 random additions, tree bisection–reconnection, and 10,000 bootstrap replicates) (34) and Bayesian analysis in MRBAYES 3.1 with the following settings: prior, mixed amino acid models; likelihood settings, invariants and gamma; Markov chain Monte Carlo, 2 million generations; printfreq, 1,000; samplefreq, 1,000; and burnin, 500) (35).

Molecular Dating. The Bayesian tree with branch lengths was used in R8S (36) to estimate divergence times for Corallimorpharia and Scleractinia. The three dates used as calibration points were those that we considered most reliable from the fossil record: the first appearances of the genera *Pavona* (33 Ma), *Acropora* (55 Ma), and *Astrangia* (70 Ma). We chose the first two points because the part of the tree that contained *Pavona* and *Acropora* was well supported in both the Bayesian and maximum parsimony analyses. We chose the *Astrangia* point because it was the most basal lineage in the short clade in our analyses. Using these dates as fixed values or using upper and lower date boundaries (minimum and maximum age constraints at these nodes) yielded similar results. We also constrained the minimum age of Scleractinia to the first appearance in the fossil record, but this date was estimated otherwise. When we used the first two dates from the long clade, we obtained divergence time estimates for Corallimorpharia and Scleractinia of 110 and 240 Ma, respectively. When we also included the short clade date, we obtained divergence time estimates for Corallimorpharia and Scleractinia of 132 and 288 Ma, respectively. We obtained similar results by either assuming a molecular clock or using penalized likelihood (36) with low smoothing values.

We thank J. Maté, J. Jara, A. Szmant, B. Mason, and R. Carter for collecting tissue/gamete samples; P. Francino and P. Dehal for feedback on phylogenetic analysis; Y. Vallès and J. Manilay for providing insight into intron evolution; M. Sanderson for advise on the use of R8S; S. Cairns for reading the manuscript; N. Budd for confirming the earliest occurrences of certain coral lineages; D. Fautin and M. Daly for critical comments on the manuscript; Jim Valentine for editing the manuscript; and Y. Vallès for help with figure design. This work was supported by the U.S. Department of Energy's Office of Science Biological and Environ-

mental Research Program, the University of California, and the Lawrence Berkeley National Laboratory under Contract DE-AC02-05CH11231 and National Science Foundation Grant OCE 0313708 (to M.M).

1. Stanley, G. D., Jr. (2003) *Earth Sci. Rev.* **60,** 195–225.
2. Stanley, G. D., Jr. & Fautin, D. G. (2001) *Science* **291,** 1913–1914.
3. Veron, J. E. N. (1995) *Corals in Space and Time: Biogeography and Evolution of the Scleractinia* (Cornell Univ. Press, Ithaca, NY).
4. Wells, J. W. (1956) in *Treatise on Invertebrate Paleontology*, ed. Moore, R. C. (Univ. Kansas, Lawrence, KS), pp. F328–F444.
5. Hand, C. (1966) in *The Cnidarians and Their Evolution*, ed. Rees, W. J. (Academic, New York), pp. 135–146.
6. Hartog, J. C. (1980) *Zool. Verhand.* **176,** 1–83.
7. Pires, D. O. & Castro, C. B. (1997) *Proc. 8th Int. Coral Reef Symp.* **2,** 1581–1586.
8. Daly, M., Fautin, D. G. & Cappola, V. A. (2003) *Zool. J. Linn. Soc.* **139,** 419–437.
9. Romano, S. L. & Cairns, S. D. (2000) *Bull. Mar. Sci.* **67,** 1043–1068.
10. Romano, S. L. & Palumbi, S. R. (1996) *Science* **271,** 640–642.
11. Romano, S. L. & Palumbi, S. R. (1997) *J. Mol. Evol.* **45,** 397–411.
12. Chen, C. A., Odorico, D. M., Lohuis, M. T., Veron, J. E. N. & Miller, D. J. (1995) *Mol. Phyl. Evol.* **4,** 175–183.
13. Chen, C. A., Wallace, C. C. & Wolstenholme, J. (2002) *Mol. Phyl. Evol.* **23,** 137–149.
14. Bernston, E. A., France, S. C. & Maullineaux, L. S. (1999) *Mol. Phyl. Evol.* **13,** 417–433.
15. Won, J. H., Rho, B. J. & Song, J. I. (2001) *Coral Reefs* **20,** 39–50.
16. Buddemeier, R. W. & Fautin, D. G. (1996) *Bull. l'Inst. Océanogr. Monaco* **14,** 23–30.
17. Feely, R. A., Sabine, C. L., Lee, K., Berelson, W., Kleypas, J., Fabry, V. J. & Millero, F. J. (2004) *Science* **305,** 362–366.
18. Kleypas, J. A., Buddemeier, R. W., Archer, D., Gattuso, J. P., Langdon, C. & Opdyke, B. N. (1999) *Science* **284,** 118–120.
19. Langdon, C. (2000) *Proc. 9th Int. Coral Reef Symp.*, 1091–1098.
20. Marubini, F., Ferrier-Pages, C. & Cuif, J. P. (2003) *Proc. Biol. Sci.* **270,** 179–184.
21. Beagley, C. T., Macfarlane, J. L., Pont-Kingdon, G. A., Okimoto, R., Okada, N. A. & Wolstenholme, D. R. (1995) in *Progress in Cell Research: Symposium on Thirty Years of Progress in Mitochondrial Bioenergetics and Molecular Biology*, eds. Palmieri, F., Papa, S., Saccone, C. & Gadaleta, N. (Elsevier, Amsterdam), pp. 141–153.
22. Beagley, C. T., Okada, N. A. & Wolstenholme, D. R. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 5619–5623.
23. Beagley, C. T., Okimoto, R. & Wolstenholme, D. R. (1998) *Genetics* **148,** 1091–1108.
24. van Oppen, M. J. H., Catmull, J., McDonald, B. J., Hislop, N. R., Hagerman, P. J. & Miller, D. J. (2002) *J. Mol. Evol.* **55,** 1–13.
25. van Oppen, M., Willis, B. L. & Miller, D. J. (1999) *Proc. R. Soc. London B* **266,** 179–183.
26. Fukami, H. & Knowlton, N. (2005) *Coral Reefs* **24,** 410–417.
27. Pont-Kingdon, G. A., Okada, N. A., Macfarlane, J. L., Beagley, C. T., Watkins-Sims, C. D., Cavalier-Smith, T., Clark-Walker, G. D. & Wolstenholme, D. R. (1998) *J. Mol. Evol.* **46,** 419–431.
28. Boore, J. (1999) *Nucleic Acids Res.* **27,** 1767–1780.
29. Haugen, P., Simon, D. M. & Bhattacharya, D. (2005) *Trends Genet.* **21,** 111–119.
30. Ewing, B. & Green, P. (1998) *Genome Res.* **8,** 186–194.
31. Ewing, B., Hillier, L., Wendl, M. C. & Green, P. (1998) *Genome Res.* **8,** 175–185.
32. Wyman, S. K., Jansen, R. K. & Boore, J. L. (2004) *Bioinformatics* **20,** 3252–3255.
33. Castresana, J. (2000) *Mol. Biol. Evol.* **17,** 540–552.
34. Swofford, D. L. (1997) PAUP*: *Phylogenetic Analysis Using Parsimony (and Other Methods)* (Sinauer, Sunderland, MA).
35. Ronquist, F. & Huelsenbeck, J. P. (2003) *Bioinformatics* **19,** 1572–1574.
36. Sanderson, M. J. (2002) *Mol. Biol. Evol.* **19,** 101–109.