Mitochondrial Genome Rearrangements in the Scleractinia/Corallimorpharia Complex: Implications for Coral Phylogeny

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Abstract

Corallimorpharia is a small Order of skeleton-less animals that is closely related to the reef-building corals (Scleractinia) and of fundamental interest in the context of understanding the potential impacts of climate change in the future on coral reefs. The relationship between the nominal Orders Corallimorpharia and Scleractinia is controversial—the former is either the closest outgroup to the Scleractinia or alternatively is derived from corals via skeleton loss. This latter scenario, the "naked coral" hypothesis, is strongly supported by analyses based on mitochondrial (mt) protein sequences, whereas the former is equally strongly supported by analyses of mt nucleotide sequences. The "naked coral" hypothesis seeks to link skeleton loss in the putative ancestor of corallimorpharians with a period of elevated oceanic CO_2 during the Cretaceous, leading to the idea that these skeleton-less animals may be harbingers for the fate of coral reefs under global climate change. In an attempt to better understand their evolutionary relationships, we examined mt genome organization in a representative range (12 species, representing 3 of the 4 extant families) of corallimorpharians and compared these patterns with other Hexacorallia. The most surprising finding was that mt genome organization in *Corallimorphus profundus*, a deep-water species that is the most scleractinian-like of all corallimorpharians on the basis of morphology, was much more similar to the common scleractinian pattern than to those of other corallimorpharians. This finding is consistent with the idea that *C. profundus* represents a key position in the coral <-> corallimorpharian transition.

Key words: naked coral hypothesis, gene order, mitochondrial genome, coral evolution.

Introduction

Understanding the evolutionary history of the Scleractinia and relationships between corals and other members of the anthozoan subclass Hexacorallia should enable a better understanding of how it has been influenced by climate in the past and thus enable better predictions of the likely impacts of climate change (Romano and Palumbi 1996). Of the six Orders of hexacorals, only members of the Scleractinia develop continuous external calcified skeletons (Daly et al. 2003). The Scleractinia suddenly appear in the fossil record in the middle Triassic, about 240 Ma, but the range of morphological variation seen in the Middle Triassic fossils is comparable to that of extant scleractinians (Romano and

Palumbi 1996). Molecular phylogenies based on both mitochondrial (mt) and nuclear (nucl) genes imply a deeper divergence (~300 Ma—in the Late Carboniferous) of extant scleractinians into two major clades, the "Complexa" and the "Robusta" (Romano and Palumbi 1996; Romano and Cairns 2000; Chen et al. 2002; Le Goff-Vitry et al. 2004; Fukami et al. 2008; Barbeitos et al. 2010; Kitahara, Cairns, and Miller 2010; Kitahara, Cairns, Stolarski, et al. 2010; Kitahara, Cairns, et al. 2012; Kitahara et al. 2012; Kayal et al. 2013). By adding deep-water species to existing molecular data sets and applying an appropriately calibrated molecular clock, Stolarski et al. (2011) demonstrated that two exclusively deep-sea families, the Gardineriidae and Micrabaciidae, form a "basal" clade that diverged at around 425 Ma, prior to the Complexa/Robusta split, pushing the evolutionary origin of scleractinians deep into the Paleozoic. These results support the scenario that scleractinians are the descendants of soft-bodied (corallimorpharian-like) ancestors that survived the mass extinction at the Permian/Triassic boundary and subsequently gained the ability to deposit calcified skeletons (Stolarski et al. 2011).

The "naked coral" hypothesis, first put forward by Stanley and Fautin (2001) to explain the sudden appearance of diverse scleractinian fauna in the middle Triassic, is based on the idea that the skeleton has been an ephemeral trait during coral evolution. Under this hypothesis, the Scleractinia were skeleton-less in the early Triassic, a time when carbonate deposition was suppressed globally (Stanley 2003). Consistent with the idea of skeleton ephemerality, some coral species can undergo reversible skeleton loss under acid conditions (Fine and Tchernov 2007). Strong phylogenetic support for the "naked coral" hypothesis came from analyses based on the alignment of concatenated proteins encoded by 17 complete mt genomes from hexacorallians (Medina et al. 2006); in their analysis, scleractinians were paraphyletic, corallimorpharians being more closely related to the Complexa than are Robusta, the interpretation being that the Corallimorpharia arose by skeleton loss from a scleractinian ancestor at a time (during the mid-Cretaceous) of high oceanic CO_2 levels (Medina et al. 2006).

Although the "naked coral" scenario is supported by analyses of protein sequence data, phylogenetics based on mt nucleotide sequences instead strongly support scleractinian monophyly (Stolarski et al. 2011; Kayal et al. 2013; Kitahara et al. 2014). The fundamental disagreement between phylogenies based on nucleotide (fig. 1*A*) or amino acid (fig. 1*B*) sequence data for mt proteins stems from the fact that none of the available models for sequence evolution adequately account for the observed data (Kitahara et al. 2014). One possible explanation for this is the occurrence of a "catastrophic" event—a major and unpredictable change, such as sudden impairment of mt DNA repair processes (which are believed to be an ancestral trait within Anthozoa (Pont-Kingdon et al. 1998; Shearer et al. 2002; Brockman and McFadden 2012).

Given the intractability of coral/corallimorph relationships using conventional molecular phylogenetics, we explored the informativeness of mt genome architecture in this context. mt gene rearrangements occur relatively infrequently and have proven useful in resolving evolutionary relationships, both shallow and deep, across a broad range of organisms (e.g., Gai et al. 2008; Brockman and McFadden 2012; Kilpert et al. 2012). This study is based on the complete mt genomes of a total of 12 corallimorpharians (8 of which are novel), representing 3 of 4 currently described families (Daly et al. 2007; Fautin et al. 2007), and 32 scleractinians, and includes both the early diverging coral *Gardineria hawaiiensis* (Stolarski et al. 2011), and corallimorpharian, *Corallimorphus profundus*, which is considered to be the most coral-like of corallimorpharians based on morphological grounds (Moseley 1877; den Hartog 1980; Riemann-Zürneck and Iken 2003). The results indicate that, by contrast with the Scleractinia, extensive rearrangements of the mt genome have occurred within Corallimorpharia. The most surprising finding, however, was that the mt genome of *C. profundus* is scleractinian-like, and is organized very differently to those of all other corallimorpharians for which data are available. Both nucleotide and amino acid sequenced-based phylogenetics unequivocally place *C. profundus* as an early diverging corallimorpharian, indicating that this organism most closely reflects the coral <-> corallimorpharian transition.

Materials and Methods

DNA Extraction, Polymerase Chain Reaction, Long Polymerase Chain Reaction, Cloning, and Sequencing

Genomic DNA was extracted from corallimorpharian samples that had been preserved in 95% (V/W) ethanol following Chen et al. (2002)—sampling information is summarized in table 1. Long-range polymerase chain reaction (L-PCR; Cheng et al. 1994) was used to amplify large (6–9 kb) and overlapping fragments covering the entire mt genomes of corallimorpharians and corals. For each species, either two- or three-specific primer pairs were designed on the basis of previously available partial sequence data for of *rns*, *rnl*, and *COI* (Folmer et al. 1994; Romano and Palumbi 1997; Chen and Yu 2000; Lin et al. 2011) (supplementary table S1, Supplementary Material online). Reactions were set up in a total volume of 50 µl: 10× LA PCR buffer, 2.5 mM $MgCl_2$, 2.5 mM of each dNTP, 2.5 units of *TaKaRa La Taq*, 0.5 µm of each primer, and approximately 0.5 µg of genomic DNA. The L-PCR conditions were slightly modified from those recommended by the polymerase manufacturer as follows: 94 °C for 1 min, then 30 cycles of 10 s at 98 °C, 45 s at 62–63 °C, 14.25 min at 68 °C, and 10 min at 72 °C. PCR products were recovered from the agarose gel using the TOPO XL gel purification

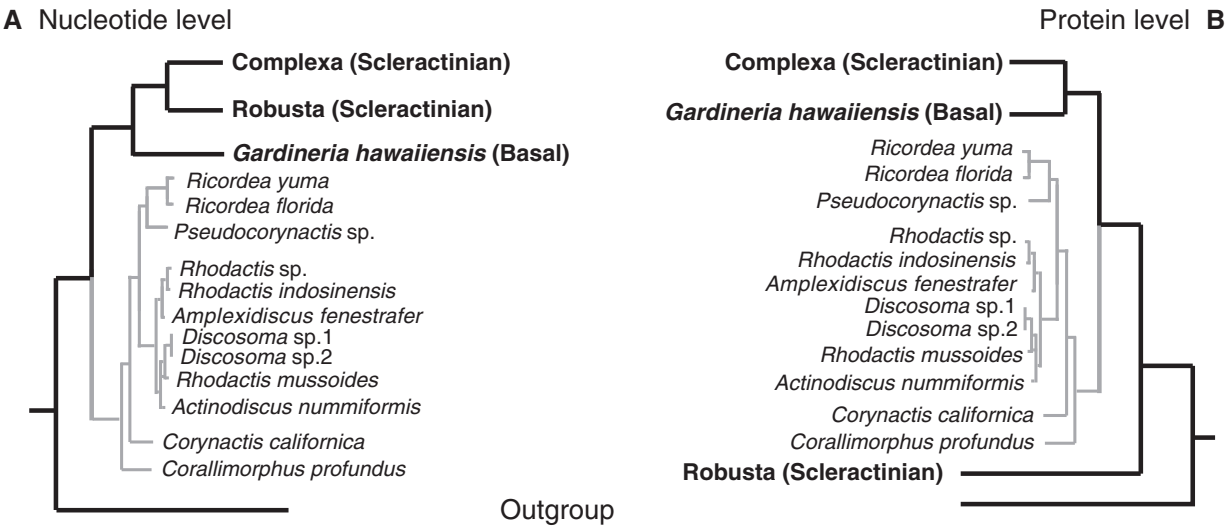

Fig. 1.—Alternative phylogenetic hypotheses for relationships between Scleractinia and Corallimorpharia based on mt genome nucleotide sequences (*A*) or the amino acid sequences of the proteins that they encode (*B*). The trees were modified from Kitahara et al. (2014). Note that, for both (*A*) and (*B*) scenarios, support for the node separating Corallimorpharia from Scleractinia (the root of the gray part of the tree) was over 97% under both maximum-likelihood analysis and Bayesian inference.

method, cloned into a pCR-XL-PCR vector system using topoisomerase I (Invitrogen), and transformed into *Escherichia coli* (Top10) by electroporation. The nucleotide sequences were determined for complementary strains of two to six clones from each sample using primer walking on the same PCR product by an ABI 377 Genetic Analyzer (Applied Biosystems). The M13 forward and reverse primers were used to obtain the initial sequences from the ends of each insertion. The consensus sequences from three sequenced clones were present for each species.

Genome Annotation and Sequence Analysis

Sequences were verified and assembled using SeqManII (DNAstar v5.0) or Sequencher v4.8 (Gene Codes Corporation) and then analyzed in Vector NTI v9.0 (InforMax). Open-reading frames (ORFs) of length more than 50 (amino acids) were translated using National Center for Biotechnology Information translation table 4 and compared with the databases using BlastX (Gish and States 1993). No novel ORFs were identified on this basis. MEGA v5.0 (Tamura et al. 2011) with a weighted matrix of Clustal W (Thompson et al. 1994) was used to align the identical putative ORFs and rRNA genes with previously published data. The 5′- and 3′-ends of the rRNA genes were predicted using the program SINA on the Silva ribosomal RNA database site (www.arb-silva.de/, last accessed February 1, 2014) using the default settings (Pruesse et al. 2012). tRNAs were predicted using tRNAscan-SE search server v1.21 (Lowe and Eddy 1997). rRNA loci were identified on the basis of sequence similarity. Finally, Vector NTI v9.0 was used to generate maps of the mt genomes based on the assembled sequence data.

Gene Order Phylogeny

The double cut and join (DCJ) distance metric (Yancopoulos et al. 2005), implemented in GRAPPA (Moret et al. 2002; Zhang et al. 2009), was used to calculate the pairwise DCJ and breakpoint distances (BPDs) from the gene order data and to generate pairwise distance matrixes. Gene order phylogenies (DCJ and BPD) were estimated with FastME (Desper and Gascuel 2002).

Because gene order is a single character with multiple states (Shi et al. 2010), bootstrapping is not applicable, hence the reliability of each branch was estimated by applying a jackknife resampling technique that in each iteration randomly removed 25% of the initial orthologous gene sets. Note that, because the data set consisted of only 13 protein-coding genes, higher removal rates (e.g., 50%) are unable to resolve the tree branching order. Jackknifing was used to generate 1,000 matrices, which were imported into FastME and used to obtain 1,000 DCJ- and BPD-based trees. Finally, the CONSENSE program in the PHYLIP software package (Felsenstein 1989) was used to calculate majority-rule consensus trees with percent values at each node. Each value represents the percentage of trees supporting a clade defined by a node.

Results

Characteristics of mt Genomes of Corallimorpharians and *Gardineria hawaiiensis*

The molecular characteristics of the mt genomes of a representative range (8) of corallimorpharians and the "basal" scleractinian *G. hawaiiensis* are summarized in table 1, along

Table 1

Characteristics of the mt Genomes of Corallimorpharians, Scleractinians, and Other Anthozoans

Order	Scleractinian Clades	Species	Total Length (bp)	Nucleotide (%)		Gene Size (bp)																			Species Collection Site and GenBank No.
				A+T	C+G	atp6	atp8	cob	COI	COI intron	COII	COIII	nd1	nd2	nd3	nd4	nd4l	nd5	nd6	rnl	rns	trnM	trnW	IGS (length)	
Corallimorpharia		*Actinodiscus nummiformis*	20,922	60.9	39	699	210	1,161	1,581	1,209	756	789	984	1,098	357	1,476	300	1,839	612	2,350	1,304	71	70	3,032	Wanlitung, Taiwan
		Amplexidiscus fenestrafer	20,188	61	39	699	210	1,161	1,561	1,207	756	789	984	1,098	357	1,476	300	1,839	612	2,349	1,304	71	70	2,729	Taioshi, Taiwan
		Corallimorphus profundus[a]	20,488	60.3	39.6	699	237	1,149	1,734	1,183	744	789	984	1,098	357	1,521	300	1,839	630	2,434	1,253	71	70	3,396	Southern Ocean
		Corynactis californica[a]	20,632	60.2	39.8	699	219	1,179	1,602	1,266	765	789	984	1,098	357	1,479	300	1,839	612	2,552	1,256	71	69	2,906	California, USA
		Discosoma sp. 1	20,908	61	38.9	699	210	1,161	1,581	1,208	756	789	984	1,098	357	1,476	300	1,839	615	2,340	1,224	71	70	4,284	NC_008071
		Discosoma sp. 2	20,912	61	38.9	699	210	1,161	1,581	1,207	756	789	984	1,098	357	1,476	300	1,839	615	2,342	1,068	71	70	4,289	NC_008072
		Pseudocorynactis sp.	21,239	60.9	39	699	213	1,230	1,575	1,178	756	789	984	1,098	357	1,476	300	1,839	612	2,537	1,223	71	70	3,177	Birch Aquarium at SIO
		Rhodactis indosinesis	20,092	60.9	39.1	699	210	1,161	1,581	1,205	756	789	984	1,098	357	1,476	300	1,839	612	2,350	1,303	71	71	2,624	Wanlitung, Taiwan
		R. mussoides	20,826	61	39	699	210	1,161	1,581	1,207	756	789	984	1,098	357	1,476	300	1,839	615	2,355	1,304	71	70	3,007	Taioshi, Taiwan
		Rhodactis sp.	20,093	61	39	699	210	1,161	1,581	1,207	756	789	984	1,098	357	1,413	300	1,839	612	2,348	1,240	71	70	3,358	NC_008158
		Ricordea florida	21,376	62.1	37.9	699	210	1,140	1,623	1,180	756	789	987	1,098	357	1,476	300	1,839	606	2,447	1,218	71	70	4,510	NC_008159
		Ri. yuma	22,015	62.4	37.6	699	213	1,140	1,599	1,199	756	789	984	1,098	357	1,476	300	1,839	606	2,444	1,262	71	70	5,148	Wanlitung, Taiwan
Scleractinia	Basal	*Gardineria hawaiiensis*[a]	19,429	60.3	39.7	699	264	1,197	1,584	1,136	738	789	981	1,098	357	1,452	300	1,836	615	2,400	1,159	71	70	2,278	New Caledonia
	Complex	*Acropora tenuis*	18,338	62.1	37.9	699	219	1,155	1,602		744	780	984	1,098	357	1,476	300	1,836	594	2,261	1,176	71	70	3,615	NC_003522
		Agaricia humilis	18,735	59.6	40.4	699	196	1,152	1,581		744	789	984	1,098	357	1,479	300	1,836	594	1,577	1,136	70	69	4,773	NC_008160
		Alveopora sp.	18,146	62.2	37.8	699	237	1,158	1,602		744	789	984	1,098	357	1,476	300	1,836	594	2,261	1,125	71	70	3,444	KJ634271
		Anacropora matthai	17,888	61.6	38.4	699	219	1,158	1,602		744	789	984	1,098	357	1,476	300	1,836	594	2,261	1,174	71	70	3,155	NC_006898
		Astreopora explanata	18,106	62.2	37.8	699	219	1,146	1,587		744	789	984	1,098	357	1,476	300	1,836	594	2,243	1,176	71	70	3,416	KJ634269
		Astreopora myriophthalma	18,106	62.1	37.8	699	219	1,146	1,587		744	789	984	1,098	357	1,476	300	1,836	594	2,244	1,176	71	70	3,415	KJ634272
		Euphyllia ancora	18,875	62.3	37.8	699	219	1,155	2,301		744	789	984	1,098	357	1,476	300	1,863	594	2,308	1,177	71	70	3,369	NC_015641
		Fungiacyathus stephanus[a]	19,381	62.2	37.8	699	231	1,161	1,629	962	744	789	984	1,098	357	1,476	300	1,839	594	2,366	1,114	71	70	3,596	NC_015640
		Goniopora columna	18,766	62.9	37.1	699	216	1,164	1,652	947	744	789	984	1,098	357	1,476	300	1,836	594	2,227	1,029	69	70	3,214	NC_015643
		Isopora palifera	18,725	61.7	38.2	699	219	1,158	1,602		744	789	984	1,098	357	1,476	300	1,836	594	2,259	1,175	71	70	3,993	KJ634270
		Isopora togianensis	18,637	61.8	38.2	699	219	1,158	1,602		744	789	984	1,098	357	1,476	300	1,836	594	2,259	1,177	71	70	3,903	KJ634268
		Montipora cactus	17,887	61.6	38.4	699	219	1,158	1,602		744	789	984	1,098	357	1,476	300	1,836	594	2,266	1,172	71	70	3,151	NC_006902
		Pavona clavus	18,315	59.5	40.5	699	219	1,152	1,581		744	789	984	1,098	357	1,476	300	1,836	606	2,299	1,169	70	69	3,566	NC_008165
		Porites okinawanesis	18,647	63.8	36.2	699	216	1,161	1,531	966	744	789	984	1,098	357	1,476	300	1,836	594	2,301	1,029	71	70	3,124	NC_15644
		Porites porites	18,648	63.7	36.2	699	216	1,161	1,578	966	744	789	984	1,098	357	1,476	300	1,836	594	2,271	1,060	71	70	3,077	NC_008166
		Siderastrea radians	19,387	63.1	36.9	699	234	1,155	1,584	989	744	789	984	1,098	357	1,476	300	1,836	594	2,242	1,296	71	70	3,568	NC_008167
	Robust	*Astrangia* sp.	14,853	68.1	31.9	678	198	1,140	1,551		685	780	948	1,092	342	1,440	300	1,812	561	1,178	532	72	70	1,474	NC_008161
		Colpophyllia natans	16,906	66.4	33.5	678	198	1,140	1,566		685	780	948	1,104	342	1,440	300	1,815	561	1,885	1,012	72	70	2,310	NC_008162
		Lophelia pertusa[a]	16,150	65.1	34.9	699	159	1,161	1,566		618	780	948	1,092	345	1,446	300	1,836	507	1,829	907	71	70	1,816	NC_015143
		Madracis mirabilis	16,951	68.4	31.7	678	224	1,140	1,587		759	780	978	1,092	345	1,446	300	1,815	564	1,937	910	71	70	2,255	NC_011160
		Madrepora oculata[a]	15,839	69.6	30.3	681	198	1,140	1,560		792	780	948	1,092	345	1,446	300	1,815	567	1,998	1,163	71	70	873	JX_236041
		Montastraea annularis	16,138	66.4	33.5	678	198	1,140	1,578		708	780	948	1,287	342	1,440	300	1,815	561	1,973	903	73	69	1,345	NC_007224
		Montastraea faveolata	16,138	66.4	33.6	678	198	1,140	1,578		708	780	948	1,287	342	1,440	300	1,815	561	1,973	903	72	69	1,346	NC_007226
		Montastraea franksi	16,137	66.4	33.6	678	198	1,140	1,578		708	780	948	1,287	342	1,440	300	1,815	561	1,973	903	72	69	1,345	NC_007225
		Mussa angulosa	17,245	66.3	33.7	678	198	1,140	1,575		685	780	948	1,104	342	1,440	300	1,815	561	550	695	72	70	4,292	NC_008163
		Pocillopora eydouxi	17,422	69.8	30.1	678	213	1,140	1,550		801	780	978	1,308	345	1,491	300	1,839	564	1,917	909	71	70	2,468	NC_009798
		Polycyathus chaishanensis	15,357	70.9	29.1	678	198	1,140	1,574		708	780	948	1,092	342	1,440	300	1,812	561	1,893	905	72	70	844	NC_015642
		Seriatopora caliendrum	17,010	69.7	30.3	678	237	1,140	1,548		759	780	978	1,092	345	1,446	300	1,839	564	1,902	916	71	70	2,345	NC_010245
		S. hystrix	17,059	69.9	30.2	678	237	1,140	1,548		759	780	978	1,092	345	1,446	300	1,839	564	1,904	916	71	70	2,392	NC_010244
		Stylophora pistilata	17,177	70.2	29.9	678	249	1,140	1,548		837	780	978	1,092	345	1,446	300	1,839	564	1,936	914	71	70	2,390	NC_011162

(continued)

Table 1 Continued

Order	Scleractinian Clades	Species	Total Length (bp)	Nucleotide (%)		Gene Size (bp)																	Species Collection Site and GenBank No.
				A + T	C + G	atp6	atp8	cob	COI	COI intron	COII	COIII	nd1	nd2	nd3	nd4	nd4l	nd5	nd6	ml	rns	tmM	trnW	IGS (length)	
Other Anthozoa		*Chrysopathes formosa*	18,398	60.5	39.6	714	213	1,143	1,593		750	750	984	1,146	357	1,476	300	1,851	633	2,588	1,168	71	70	2,591	NC_008411
		Savalia savaglia	20,764	51.7	48.3	699	219	1,161	1,521	1,239	753	789	990	1,158	357	1,515	300	1,848	666	2,644	1,197	71		3,637	NC_008827
		Nematostella sp.[a]	16,389	60.9	39.1	699	231	1,179	1,587		744	789	984	1,110	357	1,476	300	1,816	600	602	693	71	70	3,081	NC_008164
		Metridium senile[a]	17,443	61.8	38.1	690	219	1,182	1,593	853	747	789	1,005	1,158	357	1,476	300	1,803	609	2,189	1,082	71	70	2,103	NC_000933
		Briareum asbestinum	18,632	62.9	37.1	708	218	1,143	1,582		762	786	972	1,164	354	1,449	294	1,818	558	2,224	581	71		882	DQ_640649
		Pseudopterogorgia bipinnata	18,733	62.7	37.3	708	219	1,144	1,597		762	786	972	1,093	354	1,449	294	1,818	558	2,211	924	71		815	DQ_640646

NOTE.—Sources of publically available data and collection sites in the case new sequences are also listed. IGS here refers to the total length (bp) of IGSs in each of the mt genomes. Data for the octocoral mtMuts gene are not included. NC_008158 rns gene is 1,239 bp based on the analyses in this study.

[a]Azooxanthellate species.

with the publically available data for hexacorallians (42 species). All the corallimorpharian and scleractinian mt genomes, both those determined in this study and previous work, encode 13 protein-coding genes, 2 tRNA genes (*trnM* and *trnW*; but note that *Seriatopora* spp. have a duplicated *trnW*), the small (*rns*) and large (*rnl*) subunit ribosomal DNA genes, and a *COI* group I intron. Corallimorpharian mt genomes range in size from 20,093 bp in *Rhodactis* sp. to 22,015 bp in *Ricordea yuma* and are significantly larger than those of both Complexa and Robusta corals due not only to the presence of *COI* group I intron (table 1) but also to differences in size of the intergenic spacers (IGSs) between the three lineages (supplementary fig. S1, Supplementary Material online). In fact, the mt genome architectures of the Corallimorpharia are less dense than those of Scleractinia; mt genome size correlates with the total size of the IGS ($r^2 = 0.5371$, $P < 0.001$; supplementary fig. S2, Supplementary Material online). Corallimorpharian mt genomes are characterized by the genes being discrete (i.e., nonoverlapping), whereas this is quite rare in the Scleractinia, where this in shown by only 2 (the complex corals, *Siderastrea* sp. and *Fungiacyathus stephanus*) of the 29 species for which data are available.

The mt genomes of scleractinians are smaller than those of corallimorpharians, but the size (19,429 bp) reported here for that of *G. hawaiiensis* is the largest known for a scleractinian. Two cases of gene overlap were observed in the *G. hawaiiensis* mt genome; *ND4* and *rns* loci overlap by 1 bp, and *ATP8* and *COI* overlap by 18 bp.

Gene Order and Rearrangements

The organization of the mt genomes of hexacorallian anthozoans is summarized as linear maps in figure 2 and potential rearrangement mechanisms discussed below. As in the Scleractinia, there is a canonical corallimorpharian gene arrangement (CII), but these two patterns are clearly distinct. Ten of 12 corallimorpharian mt genomes exhibited an identical gene arrangement (referred to as Type CII in fig. 3), the exceptions being those of *Corynactis californica* (Type CI) and *C. profundus* (Type CIII). In the Scleractinia, 27 of the 29 complete mt genomes have identical gene order, but again two cases of rearrangement are known (fig. 2). However, although noncanonical gene arrangements have been observed in both Corallimorpharia and Scleractinia, those in the latter involve relatively small changes (i.e., can be explained by single rearrangement events), the rearrangements within Corallimorpharia are much more extensive (fig. 2). At least four rearrangement events are required for the transition between Type CII and Type CI, up to six rearrangement events were identified between Type CII and Type CIII. In the case of scleractinians, far fewer rearrangement events can explain the two deviations from the canonical pattern (Type SII), which *G. hawaiiensis* shares with most of the

Fig. 2.—Linear maps showing mt genome architecture in Corallimorpharia, Scleractinia, and other members of the anthozoan subclass Hexacorallia. Names of each Order are indicated in bold. The arrow indicates the direction of transcription. The positions of the 5′- and 3′-ends of the *ND5* intron are indicated by black squares. Corresponding blocks of genes are marked with color; for clarity, lines showing how genes or gene blocks differ in organization between the mt genomes are shown for only the Scleractinia. Note the relatively small number of rearrangements required to account for genome organization between the scleractinians and *Corallimorphus* compared with the large number of rearrangements that appear to have occurred in the corallimorpharians.

Scleractinia. *Madrepora oculata* (Type SIII) differs from the SII pattern only in having the order of the *COII–COIII* genes changed, whereas in *Lophelia pertusa* (Type SI), a block of genes (*COB-ND2-ND6*) has been rearranged (Type SI). The most surprising finding was that, in terms of gene organization, the mt genome of the deep sea corallimorph *C. profundus* (Type CIII) was more similar to the canonical scleractinian organization (Type SII) than it was to other corallimorpharians. Only two rearrangements of blocks of genes are required to explain the SII–CIII transition (fig. 2). Thus, although *Corallimorphus* is unquestionably a corallimorpharian in terms of the sequences of mt genes, the organization of those genes is scleractinian-like, implying that it might represent a key transitional state.

Among metazoans, one unique characteristic of the mt genomes of hexacorallians is the presence of a self-splicing intron within the *ND5* gene that contains a number of complete genes. In the case of the Zoanthidea, Antipatharia, and Actiniaria for which data are available, only two genes, *ND1* and *ND3*, are contained in the *ND5* intron, whereas in the Type CII, all of the genes (including *trnM*, but excluding *trnW*) are contained in the *ND5* intron. In the Type CI pattern, nine protein-encoding genes are located in the *ND5* intron, whereas in Types CIII, SII, and SIII, the same ten protein-coding genes and rns are contained in the *ND5* intron. In Type SI, the number of genes within the *ND5* intron is reduced to 8 due to a rearrangement event between Type SI and these two types of mt genomes in the scleractinians (fig. 2).

Discussion

The most surprising finding of this study was that the mt genome of the deep-sea corallimorpharian, *C. profundus*, more closely resembles scleractinians in gene organization than it does other corallimorpharians (fig. 3*A* and *B*). Although molecular phylogenetic analyses based on nucleotide or amino acid sequence data for mt proteins yield

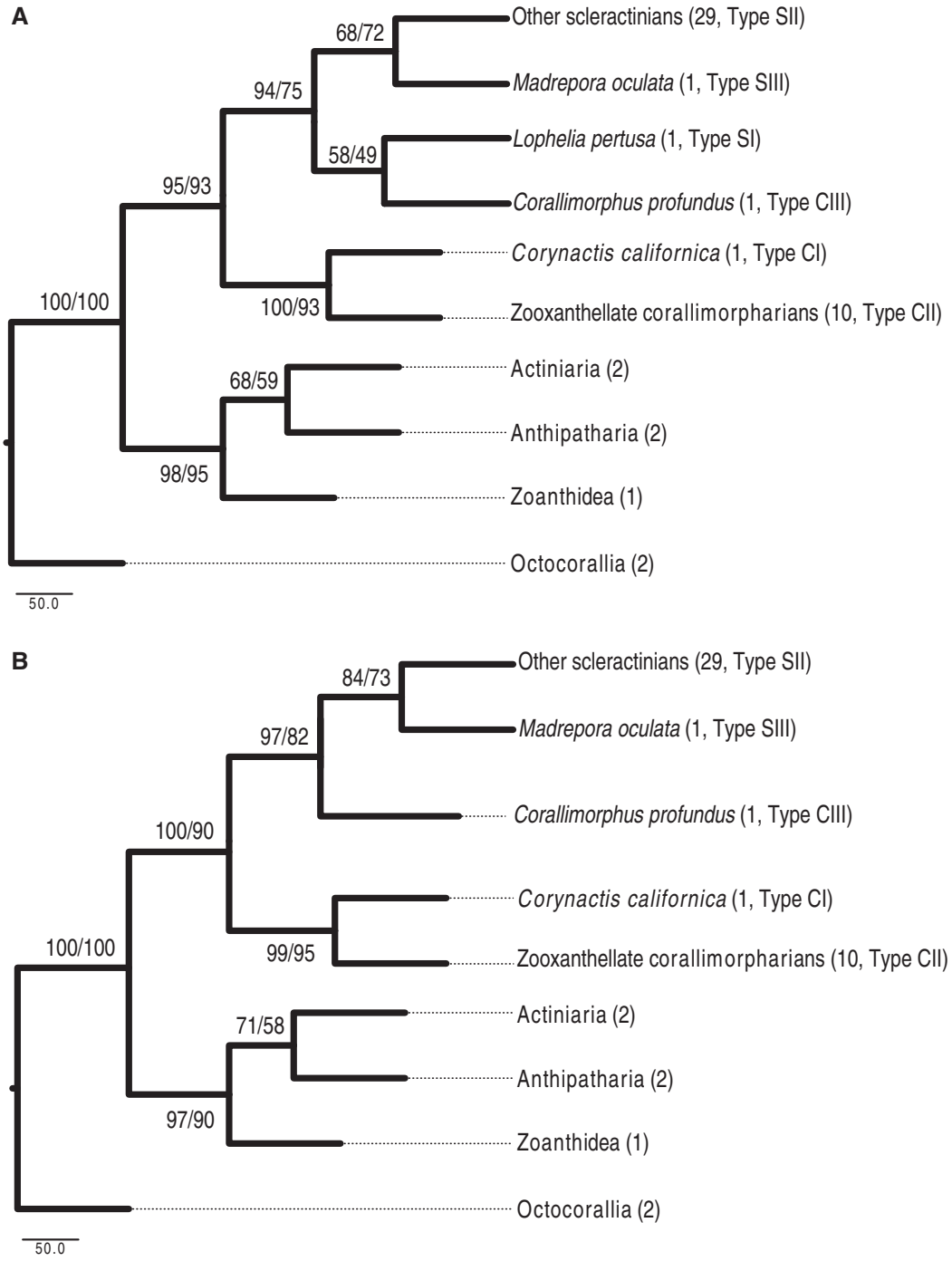

Fig. 3.—mt gene order phylogeny of anthozoans. The trees shown are majority-rule cladograms generated using the CONSENSE program in PHYLIP (Felsenstein 1989). The numbers shown at the nodes indicate the percentages of 1,000 jackknife analyses supporting the topology shown in breakpoint and DCJ analyses, respectively. Numbers of species exhibiting the gene arrangement shown are indicated in parentheses. (*A*) Gene order phylogeny with *Lophelia* included. (*B*) Gene order phylogeny with *Lophelia* excluded. Note the weak support for the *Lophelia*/*Corallimorphus* clade in (*A*).

fundamentally different results with respect to the relationship between the "complex" and "robust" scleractinian clades, there is no disagreement concerning the monophyly of the Corallimorpharia nor about the early divergence of *Corallimorphus* within that clade (fig. 1; Kitahara et al. 2014). On morphological grounds, *Corallimorphus* is also considered the most coral like of corallimorpharians (Moseley 1877; den Hartog 1980; Riemann-Zürneck and Iken 2003).

Several authors (den Hartog 1980; Owens 1984; Cairns 1989, 1990; Fautin and Lowenstein 1992) have pointed out the level of similarity between *Corallimorphus* and members of the scleractinian family Micrabaciidae, which are characterized by a reduced skeleton, the fleshy polyp totally investing the rudimentary corallum. Molecular clock estimates imply that the micrabaciids and gardineriids diverged from the scleractinian lineage in the mid-Paleozoic, well prior to the Robusta/Complexa split (Stolarski et al. 2011). The similarity between the earliest diverging members of both the Scleractinia and Corallimorpharia in terms of both morphology and mt genome architecture (fig. 2) implies that *Corallimorphus* occupies a key position in the corallimorpharian <-> scleractinian transition. *Corallimorphus* therefore diverged either close to the point of the scleractinian/corallimorpharian divergence (under scleractinian monophyly) or at the point of skeleton loss (under the "naked coral" scenario).

If we accept that the organization of the mt genome in *Corallimorphus* most closely reflects the ancestral pattern (figs. 1 and 4), then extensive reorganizations are required to generate the consensus corallimorpharian architecture (CII in fig. 2) and that seen in *Corynactis*; in contrast, the rearrangements documented to date within Scleractinia require far fewer steps. In the case of *Lophelia*, the presence of a 67 bp direct repeat comprising the 3′-end of the *ND1* and 5′-end of *COB* genes (Emblem et al. 2011) implies that the likely mechanism of reorganization was tandem duplication and random loss (Moritz et al. 1987; Zhang 2003), which may also account for the *COII–COIII* inversion seen in *Madrepora* (Lin et al. 2012). We were unable to identify signatures of duplication-mediated rearrangement in corallimorpharians; however, neither are there obvious examples of inversion of segments of the mt

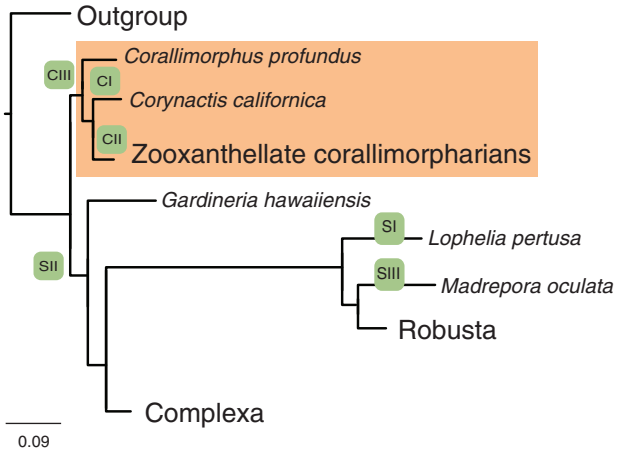

Fig. 4.—Hypothetical scheme for the evolution of mt genome architecture in the Scleractinia and Corallimorpharia. The scheme is based on the phylogenetic tree shown as figure 5 in Kitahara et al. (2014), with patterns of gene organization (numbered as in fig. 2) indicated in green boxes.

genome in this Order. Rather, extensive segmental reorganization without inversion has occurred within Corallimorpharia, possibly facilitated by the less compact nature of the mt genomes (reviewed in Boore and Brown 1998). This contrasts markedly with the situation in octocorals, where many successive inversion events explain the observed diversity of mt gene organization (Brockman and McFadden 2012).

Can comparisons of mt genome organization resolve the question of coral monophyly? Although the data presented here are consistent with monophyly of the Scleractinia, they do not exclude the possibility of an origin for corallimorpharians within the coral clade. Phylogenetic analyses based on gene order (fig. 3*A* and *B*) were ambiguous. Although both AA- and nt-based molecular phylogenetic analyses unambiguously support monophyly of the Corallimorpharia, the gene order analysis (fig. 3*A* and *B*) did not. We interpret the grouping of *Lophelia* and *Corallimorphus* in this analysis as an artifact resulting from superficial similarities in gene organization in these two organisms; although gene order is similar, the sequences of those genes are highly divergent. The idea that the grouping of *L. pertusa* with *C. profundus* is artifactual is supported by the relatively low DCJ and BPD confidence values (58/49) associated with this node (i.e., well below the 85% confidence interval recommended by Shi et al. 2010). When *L. pertusa* was removed from the analysis, the overall DCJ and BPD statistic performances at the nodes of Corallimorpharia and Scleractinia increased, particularly for the node of *C. profundus* and Scleractinia/*M. oculata*, where support increased from 94/75 to 97/82 (fig. 3).

The mt genomes of the Robusta differ from both corallimorpharians and all other corals in several characteristics. First, within the larger Scleractinia/Corallimorpharia clade, the Robusta have the most compact mt genomes (size range 14,853–17,422 bp) as a consequence of having in general shorter intergenic regions and the largest number of overlapping gene pairs (three to six cases of overlaps). In contrast, corallimorpharians have the largest mt genomes (size range 20,092–22,015 bp), longer intergenic regions, and no cases of overlapping genes, with complex corals intermediate in these characteristics (genome sizes 17,887–19,387 bp; 0–2 overlapping gene pairs—most frequently a single case of overlapping genes). Second, the Robusta differ in structural comparisons of the *ND5* group I intron (Emblem et al. 2011) as well as in molecular phylogenetics based on this feature. A group I intron interrupts the *ND5* gene of all hexacorallians examined to date; these introns typically come and go during evolution but that in hexacorallians contains a variable number of genes and has become an essential feature. The hexacorallian *ND5* intron has been "captured" in the sense that it is now dependent on host-derived factors for splicing, as indicated by the substitution of the ωG (the last nucleotide of the intron) by ωA (reviewed in Nielsen and Johansen 2009; Emblem et al. 2011). Although these characteristics are common across the

coral-corallimorpharian clade, the *ND5* introns of robust corals have a more compact core and overlapping intron and *ND5*-coding sequences (Emblem et al. 2011). In some robust corals, ωA is replaced by ωC, indicating a higher level of dependency on host factors for processing and thus greater integration of intron and host. These qualitative factors, as well as molecular phylogenetics of the *ND5* intron sequences, are most parsimoniously accommodated by scleractinian monophyly (Emblem et al. 2011). Third, of the three lineages, the mt genomes of Robusta have the highest (A+T) content and most constrained codon usage, one obvious consequence of which is that phenylalanine is overrepresented in the proteins that they encode, suggesting that mt DNA repair may be reduced in the Robusta (Kitahara et al. 2014).

The features outlined above, in which the Robusta differ from complex corals and corallimorphs, are derived characteristics—they serve to resolve the robust corals but do not unambiguously identify the sister group. Scleractinian monophyly explains all of the data most parsimoniously, but the alternative cannot yet be ruled out. The mt genome has been exhaustively mined for answers, but these must likely wait for the availability of appropriate nuclear markers.

Supplementary Material

Supplementary table S1 and figures S1 and S2 are available at *Genome Biology and Evolution* online (http://www.gbe.oxfordjournals.org/).

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