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Phylogenetic Relationships in the Coral Family Acroporidae, Reassessed by Inference from Mitochondrial Genes

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ABSTRACT—Phylogenetic relationships within the dominant reef coral family Acroporidae were inferred from the mitochondrial genes cytochrome b and ATPase 6. The rate of nucleotide substitution in the genes gave proper resolution to deduce genetic relationships between the genera in this family. The molecular phylogeny divided this family into three major lineages: the genera *Astreopora, Montipora and Acropora*. The genus Anacropora was included in the same clade as the genus *Montipora*, suggesting its recent speciation from *Montipora*. The subgenus *Isopora* was significantly distant from the subgenus *Acropora*. Taken together with morphological and reproductive differences, we propose that these two subgenera be classified as independent genera. The divergence times deduced from the genetic distances were consistent with the fossil record for the major genera. The results also suggest that the extant reef corals speciated and expanded very recently, probably after the Miocene, from single lineage which survived repeated extinction by climate changes during the Cenozoic era.

INTRODUCTION

Reef-building corals play an important role in shallow tropical seas by providing an environmental base for the ecosystem. Corals in the family Acroporidae are particularly important, since they dominate the major reef assemblages in the Indo-Pacific oceans. The family Acroporidae consists of four genera, *Acropora, Montipora, Anacropora* and *Astreopora*. The genus *Acropora* is subdivided to two subgenera, *Acropora* and *Isopora*.

Many *Acropora* corals (Acroporidae) spawn gametes synchronously, in what is known as mass spawning (Harrison *et al*., 1984; Babcock *et al*., 1986; Hayashibara *et al*., 1993). This unique reproductive behavior has attracted interest in the evolution and species identity of corals, since it deals with evolutionary problems such as hybrid formation. Indeed, there is reproductive and molecular evidence shown for hybridization and sharing of a common gene pool among a number of species in the dominant genus *Acropora* (Hatta *et al*., 1999).

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The mode of sexual reproduction varies within this family (Babcock *et al*., 1986). The acroporids spawn hermaphroditic gamete bundles. The eggs and sperm within each polyp are packaged into a buoyant bundle, which is released from the polyp's mouth. The subgenus *Isopora* species is an exception, and broods planula larvae (Kojis 1986). *Montipora* produces eggs containing symbiotic algae, called zooxanthellae (Heyward and Collins 1985), whereas in the other genera zooxanthellae are incorporated after the metamorphosis of larvae to polyps. The eggs of *Astreopora* sink after separating from the buoyant egg-sperm bundles (Babcock *et al*., 1986). Reproductive manners of *Anacropora* are still unknown. It is unclear whether the different reproductive characteristics reflect phylogenetic relationships, or arose independently in unrelated groups in this family.

Most hypotheses for the evolution of the Acroporidae are based on morphological taxonomy and the fossil record, and the phylogenetic relationships within this family are still confusing. The subgenus *Isopora* is included in the genus *Acropora* by morphological classification, but this subgenus is also thought to have arisen from the genus *Astreopora* prior to the appearance of the subgenus *Acropora*, since like *Astreopora* it has a common morphological character, lack of

persistent axial corallites (Randall 1981). *Anacropora* is thought to have evolved from *Montipora* recently based on micromorphology (Ridley 1884), at the same time, similarity of overall morphology leads to an view supposing *Anacropora* as an ancestor of *Acropora* (Veron 1995). A molecular phylogenetic analysis has been applied for overall relationships within the order Scleractinia, but precise relationships within each family remain unclear (Romano and Palumbi 1996).

Mitochondrial DNA (mtDNA) is often used to infer the phylogeny of closely related taxa, such as populations within a species (Avise *et al.*, 1987), because of the high rates of nucleotide substitution, especially in mammals (Brown *et al.*, 1979). In the genus *Acropora*, however, an atypically low rate of substitution was reported for a mitochondrial gene, cytochrome *b* (Cyt b) (van Oppen *et al.*, 1999b). This feature allows mtDNA to be used to construct a molecular phylogeny of distantly related taxa in this group. In this study, we used mitochondrial genes, Cyt b and ATPase 6 (ATP6), to infer the phylogenetic relationships among the genera within the family Acroporidae.

MATERIALS AND METHODS

Specimens and species identification

Fifteen species belonging to 4 genera in the family Acroporidae were analyzed. Two *Anacropora* species and *Acropora (Isopora) parifera* were collected around Ishigaki Island (24N, 124E), Okinawa, Japan, and the other 12 species were collected around Akajima Island (30N, 123E), Okinawa, Japan. Species identification was based on Veron and Wallace (1984) and Veron (1986). The two *Anacropora* species could not be identified, and were designated *Anacropora* sp.1 and sp.2. Among the mass spawning species in the genus *Acropora*, the following four species were studied: *A. digitifera*, *A. florida*, *A. gemmifera* and *A. nasuta*. Small pieces of colonies were collected, dipped in guanidine solution (Sargent *et al.*, 1986), and stored at room temperature. As a source of mitochondrial DNA for analysis of the entire mitochondrial genome, eggs of *A. nasuta* were obtained at Akajima in the manner described previously (Hatta *et al.*, 1999), and dissolved in lysis buffer (100mM Tris-Cl pH8, 10mM EDTA, 1%SDS) containing 100μg/ml proteinase K.

DNA extraction, PCR amplification, and sequencing

Total DNA was extracted from the tissue stored solution in guanidine solution and lysis buffer by conventional phenol/chloroform extraction and ethanol precipitation.

The entire mitochondrial (mt) genome in DNA extracted from A. nasuta eggs, except for a part of the 16S ribosomal DNA (rDNA), was amplified by PCR using the primer 5'-GACAGTGAGACCCTCGTGACACCATTCATA-3' and 5'-GACTGCCAGGGGGAAACCTAGAGCAGACAC-3', which were designed from partial sequences of the 16S rDNA of the genus *Acropora* (Romano and Palumbi 1996). Ten PCR cycles were performed at 94°C for 15 sec, 60°C for 30 sec, 68°C for 4 min, followed by 20 cycles at 94°C for 15 sec, 60°C for 30 sec, 68°C for 4 min with extension of 20 sec for each cycle, using Expand HiFi polymerase mix (Roche). The amplified fragment was separated by agarose gel electrophoresis, and purified using a GeneClean kit (BIO 101). The recovered fragment was digested with *Eco*RV, *Hae*III or *Sau*3AI, cloned in pBluescript, and sequenced. The DNA sequences were compared with the sea anemone mtDNA sequences (Beagley *et al.*, 1998).

The Cyt b gene was amplified by PCR using the primers described by van Oppen *et al.* (1999b). The ATP6 gene was amplified using primers 5'-ATGAGCGGTGCTTATTTTGATCAAT-3' and 5'-CTAATGTAATACAATTGTATCCGCC-3', which were designed from the *A. nasuta* ATP6 gene sequence. The PCR conditions were 40 cycles of 94°C for 30 sec, 60°C for 45 sec, 72°C for 45 sec, using Expand HiFi polymerase mix (Roche). The amplified products were separated by agarose gel electrophoresis, purified using GeneClean (BIO 101), and subjected to direct sequencing.

The DNA sequences of both strands were determined using a Dye Terminator Cycle Sequencing kit (ABI). The following additional primers designed from the *A. nasuta* sequences were used to sequence the Cyt b and ATP6 genes: for ATP6 5'-GCCAAGTGGCGCTCCCTTG-3' and 5'-CAAGGGAGCGCCACTTGGC-3' for ATP6, and for Cyt b 5'-CATGCTAATGGGGCTTCT-3', 5'-TCTGGGCTATGTGCTACC-3', 5'-GACGATGTGGTATTTCAT-3', 5'-TTGGGCGATCCAGAAAAT-3', 5'-AGAAGCCCCATTAGCATG-3', 5'-CTCAGGCTGAATGTGCAC-3' and 5'-AGAAGAACAAAATTCAC-3'. The DNA sequences are available in DDBJ under accession numbers AB033171 - 033200.

Molecular Phylogenetic analysis

Sequences composed of 1061 bases for Cyt b and 649 bases for ATP6 were aligned manually, and used for phylogenetic analysis using the software programs ODEN (Ina 1994) and CLUSTAL W available in DDBJ, and PHYLIP package (Felsenstein 1990). The genetic distances were calculated using a Kimura's two-parameter model (Kimura 1980). Phylogenetic trees were constructed using the neighbor-joining method (NJ; Saitou and Nei 1987) to infer genetic relationships, and the unweighted pair-group method (UPGMA; Sneath and Sokal 1973) to estimate divergence times. The bootstrap analysis was replicated 1,000 times. The view of Wells (1956) was used to give divergence times based on the fossil record.

RESULTS

Isolation and structure analysis of mitochondrial genes

Fig.1 shows the gene organization of *Acropora nasuta* mitochondrial genome. The complete DNA sequences were determined for 8 genes and 1 non-coding region, and partial sequences for 10 genes. The full length of the mtDNA was about 16 kilobase pairs (kbp), and the distance between the identified genes were measured using the lengths of the PCR fragments between the genes. The gene organization in *A. nasuta* is identical to that in A. tenuis, although that was determined for only a half (van Oppen *et al.*, 1999a), and very different from that in the sea anemone (Beagley *et al.*, 1998). No histories of inversion or translocation between the two taxa could be identified.

Nucleotide substitution and genetic distance

Table 1 shows the pairwise genetic distances calculated from the Cyt b and ATP6 sequences between all 15 species. The rate of substitution within the genus *Montipora* was similar to the rate among the mass spawning species in the subgenus *Acropora*. There were no significant differences observed in the GC content and codon usage among all species. The ratio of nonsynonymous and synonymous substitution rates were 0.08−0.12. Transition was more than 70% of the nucleotide substitution.

The substitution rates at the 3rd codon position of Cyt b and ATP6 for 8 species of *Acropora* were compared with the rate of an intron or the 3rd codon position of coding regions of the mini-collagen nuclear gene (Hatta *et al.*, 1999) (Table 2). Among the four mass spawning species, the genetic distances

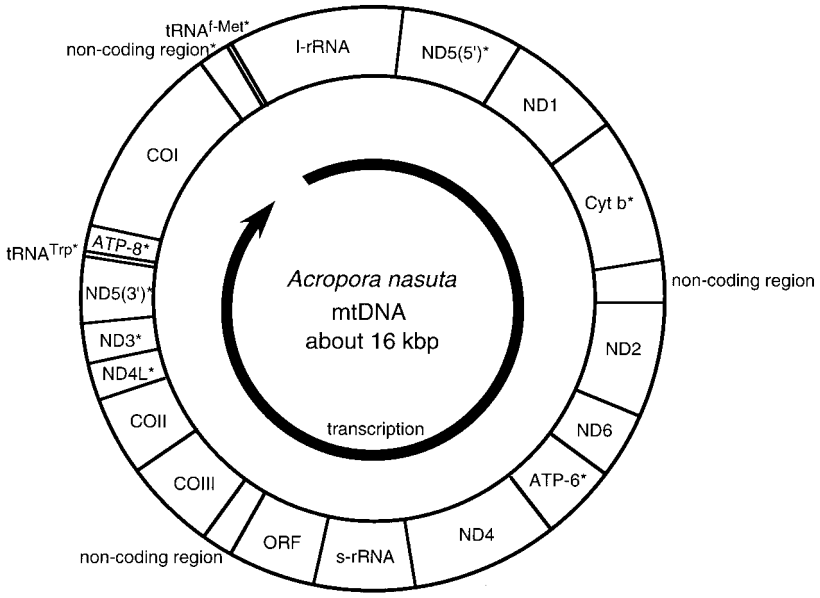

Fig. 1. Gene map of the *Acropora nasuta* mtDNA molecule. Asterisks represent genes which were determined for the full sequences. The arrow indicates the direction of transcription.

Table 1. Genetic distances between species in the family Acroporidae. Values above the diagonal show distances of Cyt b, and below ATP6.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. *Astreopora myriophthalma*	—	6.03	6.24	6.03	6.14	6.35	6.13	7.94	7.94	8.46	8.46	8.46	8.46	8.36	8.36
2. *Montipora aequituberculata*	5.45	—	0.38	0.38	0.47	0.47	0.47	4.09	4.29	5.72	5.72	5.10	5.10	5.00	5.00
3. *Montipora digitata*	5.61	0.77	—	0.38	0.28	0.47	0.47	4.49	4.70	5.92	5.93	5.30	5.30	5.20	5.20
4. *Montipora efforescens*	6.13	0.93	0.77	—	0.28	0.47	0.28	4.49	4.70	5.92	5.93	5.51	5.51	5.41	5.41
5. *Montipora altasepta*	5.78	0.93	0.77	1.08	—	0.38	0.38	4.60	4.80	5.82	5.62	5.41	5.41	5.31	5.31
6. *Anacropora* sp. 1	5.12	0.31	0.77	0.93	0.93	—	0.38	4.60	4.80	6.24	6.03	5.62	5.62	5.52	5.52
7. *Anacropora* sp. 2	5.29	0.46	0.93	1.08	1.08	0.15	—	4.59	4.80	6.03	6.03	5.61	5.61	5.51	5.51
8. *Isopora brueggemanni*	4.96	4.79	4.96	5.46	5.13	4.62	4.79	—	0.19	2.21	2.21	1.82	1.82	1.72	1.72
9. *Isopora palifera*	4.96	4.79	4.96	5.46	5.13	4.62	4.79	0.00	—	2.21	2.21	1.82	1.82	1.72	1.72
10. *Acropora donei*	4.96	4.46	4.62	5.13	4.79	4.29	4.46	0.62	0.62	—	0.57	0.85	0.95	0.86	0.86
11. *Acropora tenuis*	4.96	4.46	4.62	5.13	4.79	4.29	4.46	0.62	0.62	0.00	—	0.95	0.95	0.86	0.86
12. *Acropora florida*	4.96	4.46	4.62	5.13	4.79	4.29	4.46	0.62	0.62	0.00	0.00	—	0.19	0.09	0.09
13. *Acropora gemmifera*	5.13	4.62	4.79	5.29	4.96	4.46	4.62	0.77	0.77	0.15	0.15	0.15	—	0.09	0.09
14. *Acropora digitifera*	5.29	4.78	4.95	5.46	5.12	4.62	4.78	0.93	0.93	0.31	0.31	0.31	0.15	—	0.00
15. *Acropora nasuta*	4.96	4.46	4.62	5.13	4.79	4.29	4.46	0.62	0.62	0.00	0.00	0.00	0.15	0.31	—

of the intron varied from 0.98 to 6.09%, which presumably correspond to the neutral substitution rate in the nuclear genome. The substitution rates at the 3rd codon position of the mini-collagen gene was 0–6.47%. On the other hand, the genetic distances between the mt genes was small: 0% in Cyt b and 0–0.46% in ATP6. Even between the subgenera *Isopora* and *Acropora*, the values were approximately 3 times smaller in Cyt b and 10 times smaller in ATP6 than in the nuclear intron and coding regions. There was no obvious bias of the GC content, 37% in average at the 3rd codon position of the mt genes, whereas 42% at the 3rd codon position of the mini-collagen gene and 34% in the intron.

Phylogenetic analysis

Phylogenetic trees are shown in Fig. 2. *Astreopora* was used as an outgroup according to the fossil record that *Astreopora* occurred first in this family (Wells 1956). Branching of *Astreopora* became the most outside when sea anemone (Beagley *et al.*, 1998) was used as an outgroup in Cyt b analysis (data not shown). All of the trees suggested that this family is divided in to three major lineages, although the branch length of the *Astreopora* lineage in the ATP6 tree became shorter than in the Cyt b tree (Fig. 2A, B). The topology of the tree produced when the Cyt b and ATP6 sequences were combined together and subjected to analysis (Fig. 2C),

Table 2. Comparison of genetic distances of mitochondrial genes and a nuclear gene. Only the 3rd codon position was used for Cyt b and ATP6. *Acropora donei* and *A. tenuis* spawn a few hours earlier than mass spawning species. *A. florida*, *A. gemmifera*, *A. digitifera* and *A. nasuta* take part in mass spawning events.

(A) 3rd codon position of Cyt b

Species	1	2	3	4	5	6	7
1. *Acropora florida*							
2. *Acropora gemmifera*	0.00						
3. *Acropora digitifera*	0.00	0.00					
4. *Acropora nasuta*	0.00	0.00	0.00				
5. *Acropora donei*	2.01	2.01	2.01	2.01			
6. *Acropora tenuis*	1.73	1.73	1.73	1.73	0.85		
7. *Isopora brueggemanni*	3.50	3.50	3.50	3.50	4.40	4.41	
8. *Isopora palifera*	3.81	3.81	3.81	3.81	4.71	4.42	2.84

(B) 3rd codon position of ATP6

Species	1	2	3	4	5	6	7
1. *Acropora florida*							
2. *Acropora gemmifera*	0.46						
3. *Acropora digitifera*	0.46	0.00					
4. *Acropora nasuta*	0.00	0.46	0.46				
5. *Acropora donei*	0.00	0.46	0.46	0.00			
6. *Acropora tenuis*	0.00	0.46	0.46	0.00	0.00		
7. *Isopora brueggemanni*	0.93	1.40	1.40	0.93	0.93	0.93	
8. *Isopora palifera*	0.93	1.40	1.40	0.93	0.93	0.93	0.00

(C) Intron region of a nuclear gene, mini-collagen

Species	1	2	3	4	5	6	7
1. *Acropora florida*							
2. *Acropora gemmifera*	0.98						
3. *Acropora digitifera*	5.57	4.53					
4. *Acropora nasuta*	6.09	5.04	2.00				
5. *Acropora donei*	8.81	8.83	7.73	8.85			
6. *Acropora tenuis*	8.26	8.27	7.18	8.29	1.49		
7. *Isopora brueggemanni*	13.32	13.34	11.60	12.77	14.58	13.97	
8. *Isopora palifera*	13.92	13.94	12.18	13.36	14.58	14.58	0.98

(D) 3rd codon position of exson region of a nuclear gene, mini-collagen

Species	1	2	3	4	5
1. *Acropora florida*					
2. *Acropora gemmifera*	0.00				
3. *Acropora digitifera*	5.32	5.32			
4. *Acropora nasuta*	6.47	6.47	3.61		
5. *Acropora donei*	2.70	2.70	4.90	5.72	
6. *Acropora tenuis*	2.70	2.70	4.90	5.72	0.00

was the same as that of the Cyt b tree. The inconsistency of the ATP6 phylogeny with the others may be due to short DNA sequences.

In all of the trees, the two species from the genus *Anacropora* were included in the clade with the *Montipora* species. Low bootstrap values for the *Anacropora* branches suggest that the *Anacropora* species are not distinguished from *Montipora* genetically. The relationships within this *Montipora* group could not be resolved because of short genetic distances.

The subgenus *Isopora* formed a side branch in the *Acropora* lineage. The genetic distance between the subgenera *Isopora* and *Acropora* was significant (99–100% bootstrap values), while the relationships within the subgenus *Acropora* appeared to be very close. In Fig. 2A and 2C, A. donei and *A. tenuis*, which spawn 2–3 hours earlier than mass spawning (Hayashibara *et al*., 1993; Hatta *et al*., 1999), formed a cluster separate from the other mass spawning acroporids. This pattern is consistent with the tree inferred from the mini-collagen nuclear gene (Hatta *et al*., 1999).

In addition to the NJ method, these results were reproduced by the maximum-likelihood method too, and we obtained the same genetic phylogeny (data not shown).

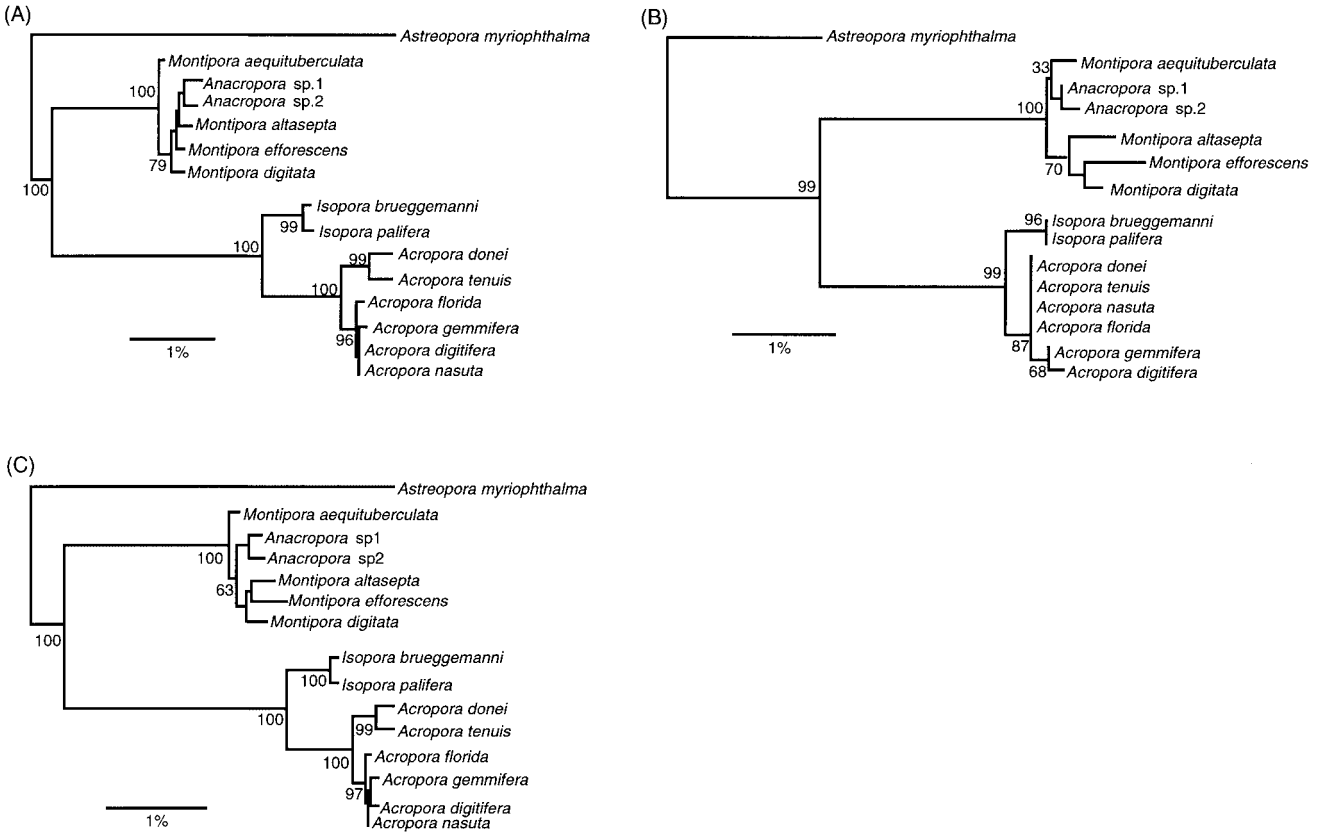

Fig. 2. Molecular phylogenetic trees of (A) Cyt b, (B) ATP6 and (C) Cyt b+ATP6, constructed by the NJ method. Astreopora was used as an outgroup. Scale bars represent 1% genetic distance. Percent values of 1,000 bootstrap replicates are shown for major branches.

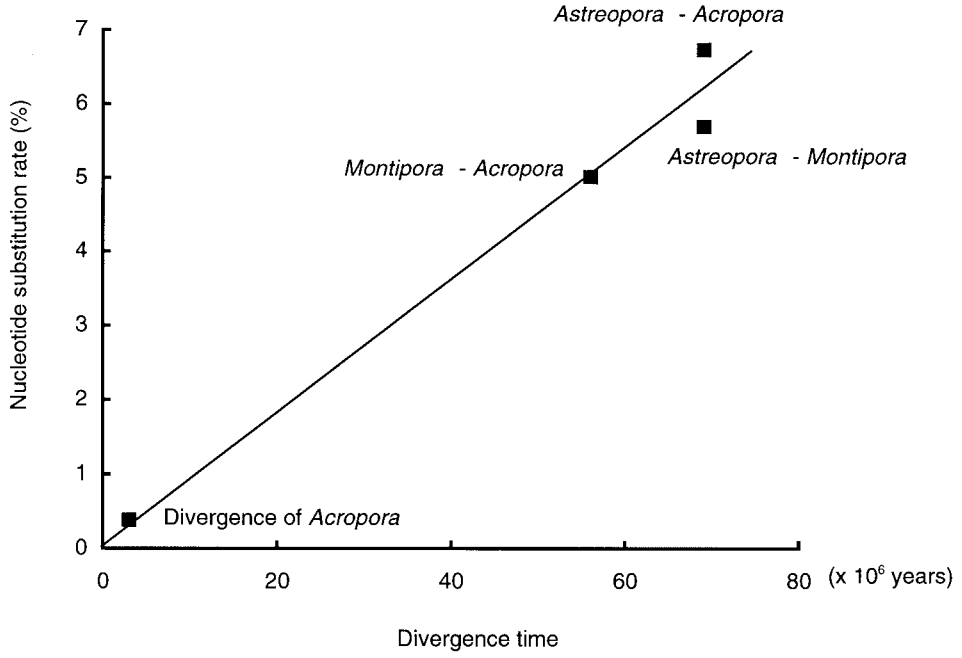

Fig. 3. The relationship between the nucleotide substitution rates and divergence times. The nucleotide substitution rate of the combined sequences of Cyt b and ATP6 was calculated for each pair of species, and an average value between two taxa was plotted on the divergence time estimated by the fossil record. Standard deviation was less than 0.1. *Anacropora* and Isopora were excluded from this comparison since fossil has not been found.

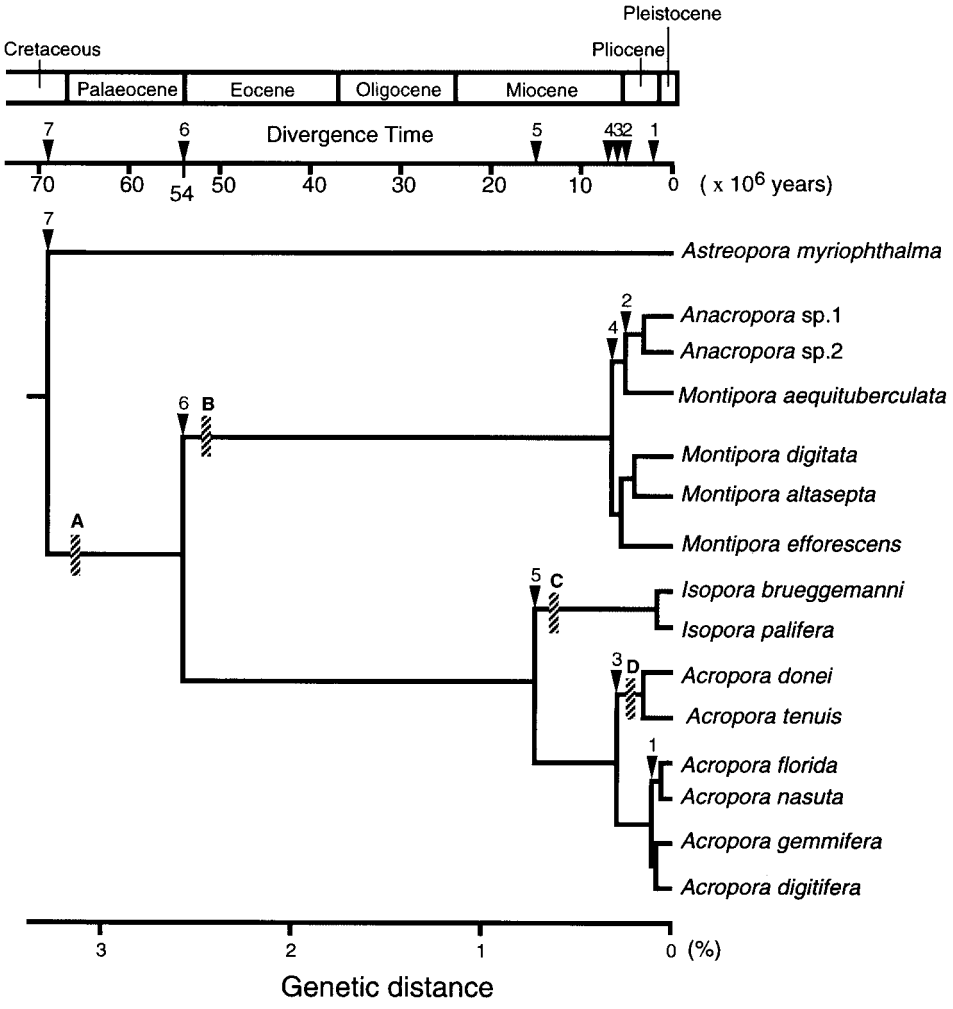

Fig. 4. Molecular phylogram constructed by the UPGMA method using the combined sequences of Cyt b and ATP6. Geological intervals are shown in the upper bar. Arrowheads with numbers indicate the divergence of lineage and corresponding time points. Shaded bar represent occurence of novel reproductive behavior, A: buoyant eggs, B: incorporation of algae into eggs, C: brooding of larvae, D: spawning at early hr.

Estimation of divergence time

In Fig. 3, genetic distances were plotted on divergence times assigned by fossil records as follows. Fossils show that *Montipora* and *Acropora* first appeared during the Eocene (37–54 million years ago; MYA) (Wells 1956), implying that the two genera diverged 54 MYA. *Astreopora* appeared in the late Cretaceous (about 70 MYA, Wells 1956). Mass spawning acroporids began diversification 2 MYA (Veron 1995). The genetic distances showed a linear correlation to the divergence times.

Figure 4 shows the molecular phylogram constructed by the UPGMA method. The divergence times were adjusted using the correlation of the genetic distances and the fossil records mentioned above (Fig. 4, arrowhead 1, 6, 7). According to the phylogram, *Anacropora* appeared 5 MYA (Fig. 4, arrowhead 2) and *Isopora* 15 MYA (Fig. 4, arrowhead 5), although no fossils of either taxa have been found. The diversification of *Montipora* was deduced to have occurred 6.6 MYA (Fig. 4, arrowhead 4), and the lineage of the mass spawning acroporids and early-hour spawners also diverged at about the same time (Fig. 4, arrowhead 3).

DISCUSSION

Features of mitochondrial genes in Acroporidae

In the class Anthozoa, the complete mtDNA sequences have been determined for the sea anemone (Beagley *et al.*, 1998) and the octocoral (Pont-Kingdon *et al.*, 1998). The molecular phylogeny indicates that the Acroporidae corals are more closely related to the sea anemone than to the octocoral (data not shown), which is consistent with their classification into the subclasses Hexaradiata and Octoradiata. However, the gene organization of *Acropora nasuta* and the sea anemone is very different. Recombination with resulting rearrangement of the genes might have occurred frequently in mitochondria along the Anthozoa lineage.

This study is the first to accurately compare the mitochondrial and nuclear DNA substitution rates in Cnidaria. When the 3rd codon position of the mt genes is examined, the substitution rate was 3–10 times lower than the rate in an intron

or the 3rd codon position of coding regions of a nuclear single copy gene (Table 2). No obvious biases were found in the GC content and substitution patterns between the mt genes and the nuclear locus. In a comparison with the internal transcribed spacer (ITS) of the nuclear ribosomal DNA, van Oppen *et al*., (1999b) concluded that the substitution rate of Cyt b is atypically low in *Acropora*. However, analysis using the ITS includes a serious bias, since the ITS is hypervariable corresponding to a presumed recombination hotspot in *Acropora* (Fukami *et al*., in preparation). The low substitution rate of Cyt b is not atypical even though it is significantly lower than nuclear genes.

Phylogenetic relationships of genera in the Acroporidae

An important finding of this study is the close relationship between the genera *Montipora* and *Anacropora* (Fig. 2). Ridley (1884) postulated that *Anacropora* recently speciated from *Montipora*, based on micromorphology characteristics; on the other hand, Veron (1995) proposed that *Anacropora* gave rise to *Acropora*, based on macromorphology. Our molecular phylogeny suggests that *Anacropora* diverged from *Montipora* very recently. *Anacropora* species are found in non-reef environments (Veron 1995), and inhabit quieter environments than most *Montipora* and *Acropora* in Okinawa (unpublished observation by the authors). *Anacropora* may have speciated by occupying niches in different environments or at different depths.

A second important finding is the significant distance between the subgenera *Isopora* and *Acropora*, which are related nevertheless (Fig. 2). *Isopora* arose from the *Acropora* lineage, however, it is clearly separated from the cluster of *Acropora* species in the phylogenetic trees. *Isopora* is morphologically distinguished from *Acropora* (Randall 1981), and broods planula larvae (Kojis 1986), while the other genera in the family Acroporidae spawn gametes. Combining our molecular phylogeny with the morphological and reproductive differences, we propose that *Acropora* and *Isopora* be classified as separate genera.

This study is the first report describing genetic relationships within the coral family Acroporidae, and helps to clarify the phylogenetic relationships in this family, which have been confused.

Divergence of reproductive behavior

Alteration of the reproductive feature are indicated in the phylogram in Fig. 4. Since the majority of species in the three major lineage, *Astreopora*, *Montipora* and *Acropora*, participate in mass spawning in the Akajima region (Hayashibara *et al*., 1993), mass spawning is thought to be the ancestral reproductive behavior in the family Acroporidae. The sinking eggs of *Astreopora* are common characteristics seen in some of the *Pocillopora* species in the family Pocilloporidae (Kinzie 1993), implying an ancestral feature. Buoyant eggs can be thought as a derivative feature which arose in the lineage before branching of *Acropora* and *Montipora* (Fig.4, bar A). Buoyancy of eggs might contribute for higher chance of fertilization and dispersal to lead present prosperity of the two genera, *Acropora* and *Montipora*. The transmission of symbiotic algae into unfertilized eggs is another novelty that arose in *Montipora* (Fig.4, bar B). *Anacropora* seems to be derived from *Montipora*, and it will be interesting to determine whether *Anacropora* eggs contain symbiotic algae. In the family Acroporidae, brooding of larvae only occurred in *Isopora* (Fig.4, bar C), which evolved from the *Acropora* lineage. Spawning at early hours arose recently in *Acropora* (Fig.4, bar D). We believe that the different reproductive systems evolved independently in different lineage in the family Acroporidae.

Evolutionary history of the Acroporidae

The molecular phylogeny of the mt genes corresponds well to the divergence times deduced from the fossil record (Fig. 3, 4). The results suggest that the present species in the genus *Acropora* are monophyletic and the genus began to diverge after the middle Miocene (Fig. 4), and the subgenus *Acropora* radiated just after the Pliocene. Speciation and diversification must have proceeded very rapidly in the subgenus *Acropora*, since it contains more than 150 extant species with quite varied morphology. Many other acroporids, which are found as fossils in deposits from the Eocene to Miocene, have become extinct. The genus *Montipora* has a similar evolutionary history. Although fossil *Montipora* are recorded from the Eocene, the existing *Montipora* species are monophyletic (Fig. 4). Their ancestor likely survived the catastrophic extinction at the Miocene-Pliocene boundary and diverged to give rise to the present species.

Climate changes, such as glacial cycles, have led repeated rounds of mass extinction and expansion from small populations of a few surviving species in a variety of organisms. Reef-building corals are no exception. The existing reef corals appeared to have spread throughout the oceans very rapidly in a short period of geological time. Our molecular phylogeny matches the geological history well, and supports this evolutionary history for corals in the Acroporidae.

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