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Novel Group I Introns Encoding a Putative Homing Endonuclease in the Mitochondrial *cox1* Gene of Scleractinian Corals

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Abstract. Analyses of mitochondrial sequences revealed the existence of a group I intron in the cytochrome oxidase subunit 1 (*cox1*) gene in 13 of 41 genera (20 out of 73 species) of corals conventionally assigned to the suborder Faviina. With one exception, phylogenies of the coral *cox1* gene and its intron were concordant, suggesting at most two insertions and many subsequent losses. The coral introns were inferred to encode a putative homing endonuclease with a LAGLI-DADG motif as reported for the *cox1* group I intron in the sea anemone *Metridium senile*. However, the coral and sea anemone *cox1* group I introns differed in several aspects, such as the intron insertion site and sequence length. The coral *cox1* introns most closely resemble the mitochondrial *cox1* group I introns of a sponge species, which also has the same insertion site. The coral introns are also more similar to the introns of several fungal species than to that of the sea anemone (although the insertion site differs in the fungi). This suggests either a horizontal transfer between a sponge and a coral or independent transfers from a similar fungal donor (perhaps one with an identical insertion site that has not yet been discovered). The common occurrence of this intron in corals strengthens the evidence for an elevated abundance of group I introns in the mitochondria of anthozoans.

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Introduction

The mitochondria of the subclass Anthozoa (phylum Cnidaria) have a number of unique characteristics with respect to other Metazoa. These include the existence of many noncoding regions between genes and the presence of complete stop codons in all protein-coding genes (Beagley et al. 1998; Beaton et al. 1998; Fukami and Knowlton 2005; Pont-Kingdon et al. 1995, 1998; Tseng et al. 2005; van Oppen et al. 1999a, b, 2002), very slow nucleotide mutation rates suggesting the existence of a mitochondrial mismatch repair system (Medina et al. 1999, 2006; Shearer et al. 2002; van Oppen et al. 2002), a small number of tRNA genes (Beagley et al. 1998; van Oppen et al. 2002), and the existence of group I introns (Beagley et al. 1996; Fukami and Knowlton 2005; Medina et al. 2006; Tseng et al. 2005; van Oppen et al. 2002).

Group I introns have many characteristics, such as their self-splicing mechanism and their secondary and tertiary structure, that distinguish them from other classes of introns (Burke et al. 1987; Cech 1988; Michel and Westhof 1990). Although uncommon in metazoans, they are the most widespread class of introns. They are found in plant and fungal

mitochondria (Dujon 1989) as well as in the genes of nuclei, chloroplasts, prokaryotes, and bacteriophages (Edgell et al. 2000; Kuhsel et al. 1990; Lambowitz and Belfort 1993; Michel and Westhof 1990; Reinhold-Hurek and Shub 1992; van Oppen et al. 1993).

Among the anthozoans, group I introns have been reported only in the sea anemone *Metridium senile* (Beagley et al. 1996) and in scleractinian corals, which include members of two distantly related clades, the robust and complex corals (Chen et al. 2002; Romano and Palumbi 1997; Romano and Cairns 2000). The complex corals with these introns belong to the families Acroporidae (*Acropora tenuis*, *Montipora cactus*, and *Anacropora mathami*), Agariciidae (*Agaricia humilis* and *Pavona clavus*), Poritidae (*Porites porites*), and Siderastreidae (*Siderastrea radians*) (Medina et al. 2006; Tseng et al. 2005; van Oppen et al. 2002); robust corals with these introns belong to the families Faviidae (the three members of the *Montastraea annularis* complex and *Colpophyllia natans*; note that recent molecular analyses do not support confamilial status for these corals [Fukami et al. 2004b]), Mussidae (*Mussa angulosa*), and Rhizangiidae (*Astrangia danae*) (Fukami and Knowlton 2005; Medina et al. 2006).

In both the sea anemone and the corals, one type of group I intron interrupts the NADH dehydrogenase subunit 5 (*nad5*) gene and encodes several other functional mitochondrial genes (Beagley et al. 1996; van Oppen et al. 2002). However, *M. senile* also has a cytochrome oxidase subunit I (*cox1*) group I intron of a different type; it encodes a homing endonuclease with the amino acid motif of LAGLI-DADG that could promote site-specific mobility of the intron by creating a double-stranded DNA break in the intronless allele (Belfort and Roberts 1997; Dujon 1989; Lambowitz and Belfort 1993). A fungal origin has been suggested for several group I introns of this type, including that of the sea anemone *Metridium senile* (Beagley et al. 1996; Cho et al. 1998; Vaughn et al. 1995) and the sponge *Tetilla* (Rot et al. 2006). Here we document for the first time in corals the widespread occurrence of this class of intron, which we found in the *cox1* genes of 20 species belonging to the robust clade. This intron is distinct from another observed *cox 1* group 1 intron (Medina et al. 2006; Chuang et al., in preparation) in insertion site, intron length, and DNA/amino acid sequence similarity.

Materials and Methods

Species

Most species analyzed in this study were identical to those used by Fukami et al. (2004b). However, *Plesiastrea versipora*, *Blastomussa wellsi*, and *Physogyra lichtensteini* were analyzed for the first time (two colonies each).

DNA Extraction

DNA extraction methods were the same as described by Fukami et al. (2004a,b). A small piece (1 × 1 cm) of tissue from each species was put in CHAOS solution (for details, see Fukami et al. 2004a,b), and total DNA was extracted from the solution using the phenol/chloroform extraction and ethanol precipitation method.

Polymerase Chain Reaction (PCR)

In a preliminary study, we determined the entire DNA sequence of *cox1* from several species in the suborder Faviina and found the intron in the middle of *cox1* in some species. Subsequently, for most species, only half of *cox1*, including the intron region, was amplified using the following primers: MCOIF (5′ TCT ACA AAT CAT AAA GAC ATA GG 3′) and MCOIR (5′ GAG AAA TTA TAC CAA AAC CAG G 3′) (see Fukami et al. 2004a). The protocol for amplifications of *cox1* with the intron region was 94°C for 120 s for preheating, followed by 30 cycles at 94°C for 45 s, 55°C for 60 s, and 68°C for 4 min, with extension for 20 s for each cycle. The amplified fragment was separated by agarose gel electrophoresis and purified using Gene Clean (Promega). The recovered fragment was cloned with the pGEM-T System (Promega) and sequenced using the Dye Terminator Kit (Amersham Bioscience) for both strands. For the intron sequences, the following internal primers were used to determine DNA sequences: Mcox1F4 (5′ TGT TAG CGG GTG CAA TTA CT 3′), cox1_intF (5′ GCC GAC GAG GTC TTT TAA AAG TA 3′), and cox1_intR (5′ TAA CCA TCT GCA TCT AAA AAC CC 3′). For the three genera analyzed for the first time (see above), DNA sequences of two mitochondrial genes, cytochrome oxidase b (*cob*) and *cox1*, were determined as described by Fukami et al. (2004b).

RNA and DNA Analyses

The secondary structure of the group I intron was estimated manually using previously published folding data (Bhattacharya et al. 1994; Michel and Westhof 1990) and current understanding of the group I intron recognition process (Cech et al. 1994; Lisacek et al. 1994) and, also, by comparison with conserved regions (such as P, Q, R, and S) of the group I intron of the mitochondrial *cox1* gene of *Penicillium marneffei* (Woo et al. 2003), whose DNA sequence has a high similarity to the coral *cox1* intron (see Results). For less conserved regions, the RNA mfold server (http://bioinfo.rpi.edu/applications/mfold/ma/form1.cgi [Zuker 2003]) was used. Sequence analysis was performed with Sequencher version 4.0 (Gene Codes Corp., Inc.). Open reading frames (ORFs) were translated in DNASIS version 2 (Hitachi Software Engineering) using the *Acropora tenuis* genetic code (van Oppen et al. 2002). PAUP 4.0b10 (Swofford 2002) was used to construct phylogenetic trees of DNA sequences using the neighbor-joining (NJ) and maximum likelihood (ML) methods. Topologies obtained by both methods were very similar, and only the ML trees are shown in this paper. Heuristic searches used TBR branch swapping and 10 random additions of taxa based on either the K81uf model with gamma parameter and proportion of invariable positions (for combined *cob* and *cox1*) (for details see Fukami et al. 2004b) or the HYK model with gamma parameter (for *cox1* alone and for the *cox1* intron). To find an appropriate model of evolution, Modeltest (Posada and Crandall 1988) was used. Indels (only for the *cox1* intron) were deleted from the analyses, although the topology was largely unchanged with indels included. The final data set contained 630 (*cox1*) and 1053 (*cox1* intron without indels) characters, including 58 and 51 (64 with sponge) parsimony informative sites for *cox1* and the *cox1* intron, respectively. Bootstrap values were estimated by the ML method with 100 bootstraps and the NJ method with 1000 bootstraps using the models above.

RT-PCR Analysis

To test the function of the *cox1* intron and to determine the splicing site, RT-PCR was carried out. Total RNA was extracted from the tissue of *Diploastrea* using TRIzol reagent following a protocol recommended by the manufacturer (Invitrogen). The total purified RNA was treated with *DNaseI* and then reverse transcribed into cDNA using Superscript III (Invitrogen). The mitochondrial *cox1* fragment was amplified using super *Taq* DNA polymerase (Protech) with the forward primer Mcox1F4 (5' TGT TAG CGG GTG CAA TTA CT 3') and the reverse primer McoxIRR (5' CAA TAT CAA GAG AAC TAT TTG C 3'), which were designed based on the *cox1*-coding regions outside of the *cox1* intron of *Diploastrea*. Amplification was carried out in a PC-9606 thermal sequencer (Corbett Research) using the following thermal cycle: 1 cycle at 94°C for 5 min, then 35 cycles at 94°C for 30 s, 46°C for 30 s, and 72°C for 1 min. The PCR products were checked by 1.2% TBE agarose electrophoresis, purified and subcloned into pCRII-TOPO vectors (Invitrogen), and then transformed into *Escherichia coli* TOP10 cells. They were sequenced on both strands by using T7 and SP6 primers to confirm that the PCR products were the *cox1* gene of *Diploastrea*.

Sources of Gene Sequences

Coral sequences resulting from this study are deposited in DDBJ with accession numbers AB289561 for *cox1* (*P. versipora*), AB289562–AB289563 for *cox1* with *cox1* group I introns (*B. wellsi* and *P. lichtensteini*,), AB289564–AB289566 for *cob* (*P. versipora*, *B. wellsi*, and *P. lichtensteini*), and AB289567–AB289584 for *cox1* group I introns (other species). Names and GenBank accession numbers of the *cox1* group I introns for other organisms used in our analyses are as follows: *Tetilla* sp. (Porifera) *cox1*-intron, AM076987 (Rot et al. 2006); *Smittium culisetae* (Fungi) *cox1*-intron4, AY863213 (Seif et al. 2005); *Penicillium marneffei* (Fungi) *cox1*-I3, AY347307 (Woo et al. 2003); *Schizosaccharomyces octosporus* (Fungi) *cox1*-I3, AF275271 (Bullerwell et al. 2003); and *Metridium senile* (Cnidaria) *cox1* intron, U36783 (Beagley et al. 1996).

Results

Characteristics of the Coral cox1 Intron

The group I intron in *cox1* was observed in 13 of 41 genera (20 of 73 species, including 3 members of the *Montastraea annularis* complex) in the Indo-Pacific, but not in any Atlantic corals (Fig. 1). The insertion sites of these introns into *cox1*, which were determined from the DNA sequence obtained by RT-PCR (Fig. 2), were identical in all species analyzed (Fig. 3). The coral insertion sites were the same as in a Mediterranean sponge *Tetilla* sp. (Rot et al. 2006), but they differed by eight nucleotides from the insertion sites of some fungi and were also distinct from that of the sea anemone *Metridium senile* (Table 1). Total lengths of the DNA sequences of the coral *cox1* introns were 1077–1129 bases (Fig. 3), and these introns contained the regions P1 through P10 (except P2) and core regions P, Q, R, and S (Fig. 4); these characteristics were similar to those of the intron of the sponge *Tetilla* sp. (Rot et al. 2006). The data from RT-PCR showed that the splicing of these introns was functional (Fig. 2). These data are consistent with characteristics of group I introns. The open reading frame (containing 310 to 317 amino acids in

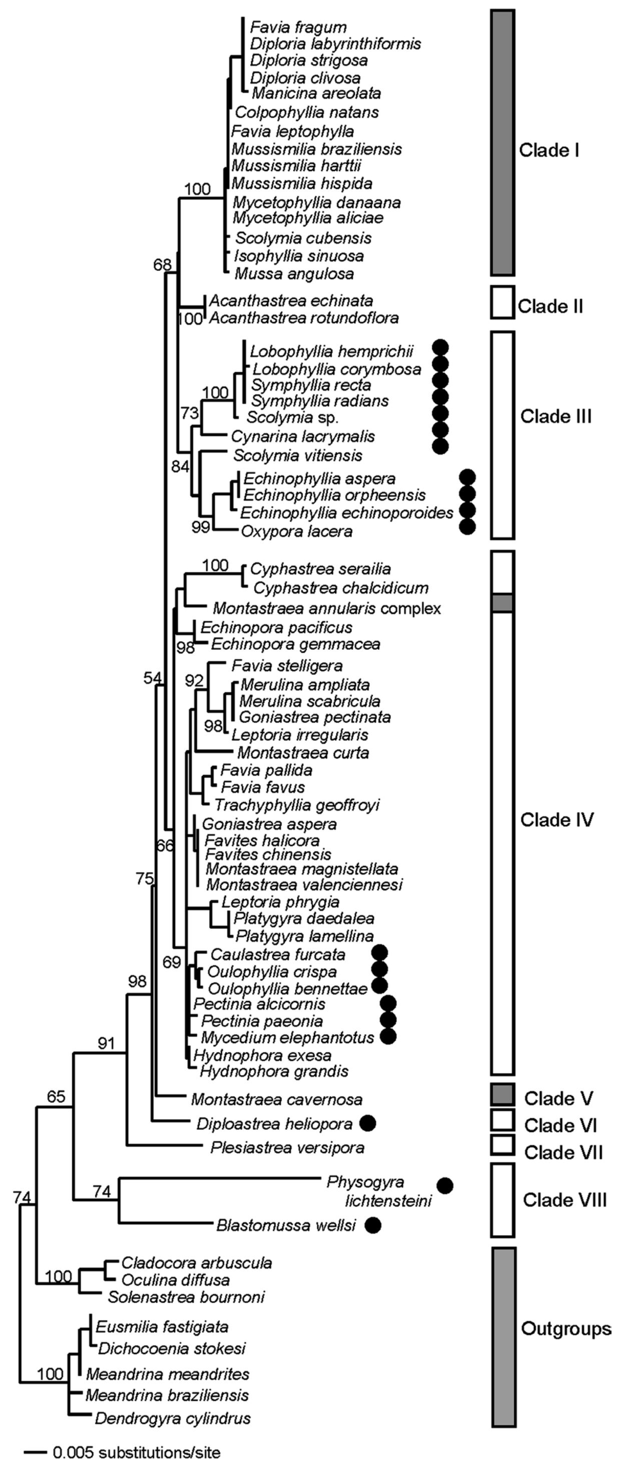

Fig. 1. Phylogenetic relationships of the scleractinian corals based on the *cox1* and *cob* genes with ML analysis. Bootstrap values (estimated by NJ; ML bootstrap values were similar) are shown only on the main branches. Corals from the Indo-Pacific are indicated by white bars, and corals from the Atlantic are indicated by gray bars. Species with a *cox1* group I intron are shown by black circles.

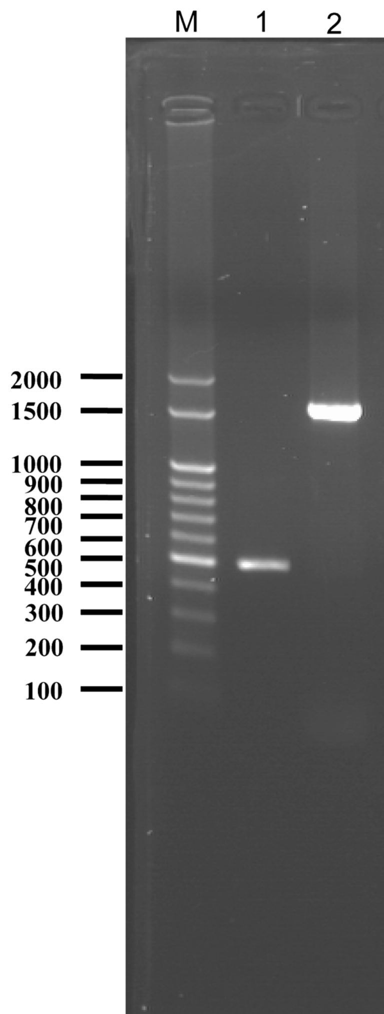

Fig. 2. RT-PCR analysis of *Diploastrea cox1* group I intron. Lane 1, amplification product (0.5 kb) from RT-PCR using the RT product of *Diploastrea* as a template. Lane 2, amplification product (1.5 kb) from PCR using genomic DNA of *Diploastrea* as a template. Lane M, 100-bp DNA molecular marker.

20 species) always contained the LAGLI-DADG sequence characteristic of this family of the group I intron-encoded, site-specific homing endonucleases (Fig. 3).

All *cox1* introns in the corals were conserved and easily aligned (Fig. 3). The base composition of the introns and base composition of the ORFs within the introns (in parentheses) was 33.9% (34.7%) A, 13.6% (12.5%) C, 22.0% (21.5%) G, and 30.5% (31.3%) T, whereas that of the *cox1* gene was 21.1% A, 16.2% C, 21.0% G, and 41.7% T. No statistically significant difference in base composition was found between *cox1* and the intron ($p = 0.20$, $\chi^2 = 5.28$ [4.97 for ORF], df = 3).

Four types of sequences based on indel differences (that in each case also affect amino acid translation relative to the other types) were observed (Fig. 3). Type 1 (found in *Diploastrea heliopora, Blastomussa wellsi*, *Physogyra lichtensteini, Oxypora lacera*, *Cynarina lacrymalis*, and *Scolymia vitiensis*) is likely to be ancestral (see the following section). Relative to Type 1, Type 2 (observed in *Echinophyllia aspera, E. orpheensis, E. echinoporoides*, *Symphyllia recta, S. radians*, *Lobophyllia hemprichii*, and *L. corymbosa*) has one deletion of T at position 77. Type 3 (observed in one unidentified species of *Scolymia*) has an insertion at positions 83–123, which is likely to be a duplication of positions 43–82 (boxed sequence of T3 in Fig. 3). Type 4 (observed in *Caulastrea furcata*, *Oulophyllia bennettae*, *Oulophyllia crispa, Mycedium elephantotus*, *Pectinia alcicornis*, and *P. paeonia)* has an insertion at positions 170–219, which is likely to be a duplication of positions 220–291 (boxed sequence of T4 in Fig. 3). In addition, Type 1 has a start codon at positions 58–60, whereas Types 2–4 have a start codon at positions 36–38.

The entire *cox1* intron from *Diploastrea heliopora* (1076 bases) was submitted as a query to BLAST (Altschul et al. 1997) and FASTA (Pearson and Lipman 1988) analyses in the DDBJ database (www.ddbj.nig.ac.jp); we used the results from the FASTA search because the BLAST search retrieved only very short sequences. The *cox1* introns of *D. heliopora* had the highest similarity to the sponge mitochondrial *cox1* putative group I intron in *Tetilla* sp. (78.8% in 684 bp; expectation = 1.1e-49), followed by the fungal mitochondrial *cox1* group I introns in *Smittium culisetae* (58.0% in 785 bp; expectation = 3e-15), *Penicillium marneffei* (58.1% in 757 bp; expectation = 9.3e-14), and *Schizosaccharomyces octosporus* (56.9% in 706 bp; expectation = 1.7e-09). Notably, the similarity to the *cox1* intron of the sea anemone *Metridium* was much lower (45% in 837 bp estimated using DNASIS). The sponge mitochondrial *cox1* intron was very similar to the coral *cox1* intron at the DNA level across the entire intron (Fig. 3), whereas the DNA intron sequences of the fungi and sea anemone were too divergent to be aligned with coral *cox1* intron at several points. However, large parts of the amino acid sequences of the intron-encoded endonuclease are conserved among scleractinians, the sea anemone, the sponge, and the fungi, allowing us to align them using amino acid sequences for these regions (Fig. 5). In particular, parts of the LAGLI-DADG motif were highly conserved for all (although the *cox1* intron from the sea anemone had a large deletion).

Relationship Between Phylogenies of Corals and Their cox1 *Introns*

Phylogenetic analysis of coral DNA sequences of two combined mitochondrial genes (*cox1* and *cob*; 1395 bases) yielded a tree with eight clades (in addition to two outgroups) (Fig. 1). Clades I–VI were apparent in an earlier analysis of the Faviina (Fukami et al.

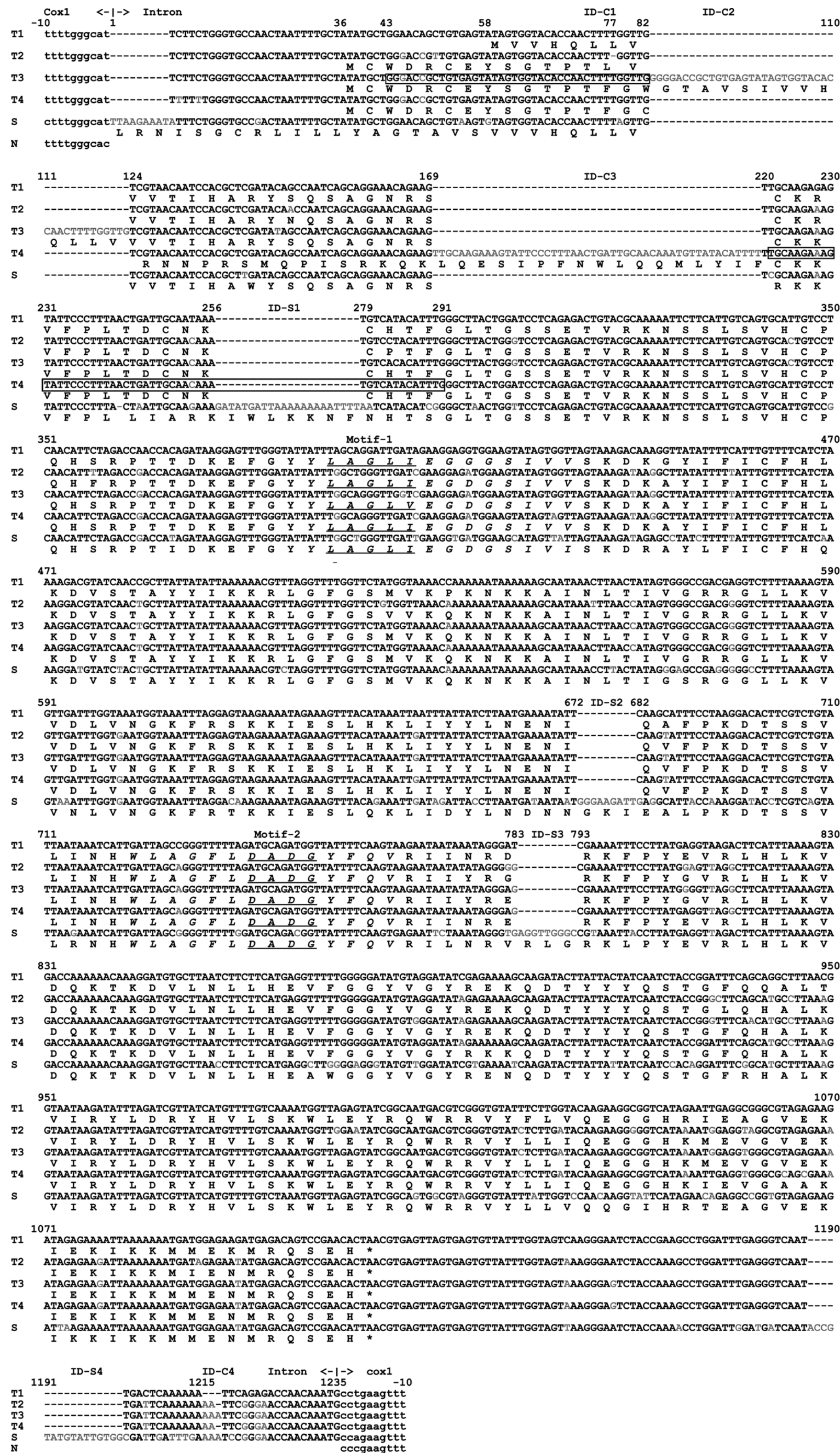

Fig. 3. Alignments of DNA sequences and amino acids of the *cox1* group I intron in four scleractinian corals and the sponge *Tetilla* (AM076987 [Rot et al. 2006]). Four types of indels are mentioned in the text: Type 1 (T1) shown here is from *Diploastrea heliopora*, Type 2 (T2) is from *Echinophyllia aspera*, Type 3 (T3) is from *Scolymia* sp., and Type 4 (T4) is from *Caulastrea furcata*. N (shown at beginning and end) is an example of the DNA sequence of the *cox1* gene without the intron (*Montastraea annularis* from Fukami and Knowlton [2005]). Nucleotide differences among these DNA sequences are shown in gray. ID indicates indel, and C and S refer to coral and sponge DNA sequences, respectively (e.g., first indel for coral, ID-C1, and first indel for sponge, ID-S1). DNA sequences of the *cox1* gene are represented by lowercase letters and the group I intron sequences are capitalized. Regions in boxes show possible repeat sequences of ID-C2 and ID-C3. Gaps are shown by dashes. Parts of the LAGLI-DADG motif, which is specific to group I introns, are shown in italics, with underlining for the core parts.

2004b), and the three taxa analyzed for the first time (*Blastomussa wellsi, Plesiastrea versipora*, and *Physogyra lichtensteini*) form two additional clades (VII and VIII). The relationship of *Blastomussa* (conventionally assigned to the Mussidae) and *Physogyra* (conventionally assigned to the Euphyllidae) and the separation of *Plesiastrea* from other conventionally defined faviids (Fig. 1) were somewhat unexpected, but substantial incongruities between earlier taxonomic groupings based on

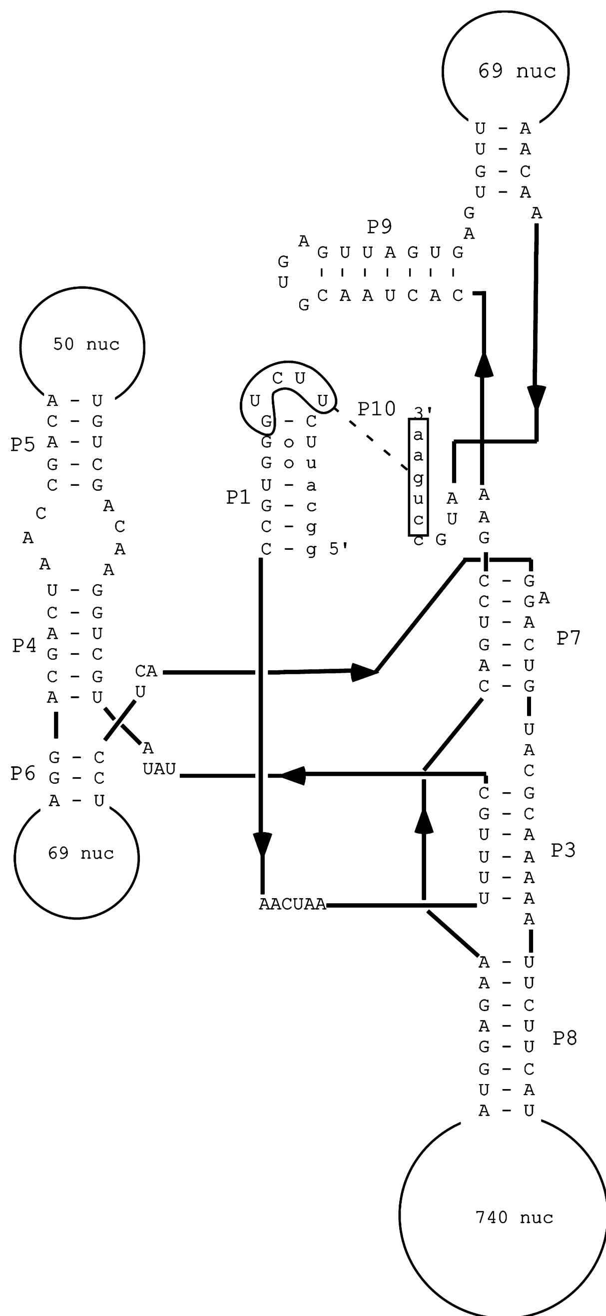

Fig. 4. Predicted secondary structure of the *cox1* group I intron of *Diploastrea heliopora*.

morphology and recent phylogenies based on DNA sequences are common (e.g., Fukami et al. 2004b).

Thirteen Indo-Pacific coral genera from four different lineages (clades III, IV, VI, VIII) contained the *cox1* intron (Fig. 1). In general, the phylogeny of the corals based on either the *cox1* + *cob* DNA sequences (Fig. 1) or the *cox1* sequences alone (in both cases excluding the *cox1* introns) and the phylogeny of the *cox1* intron sequences showed similar topologies (Fig. 6). There was, however, one point of discordance involving the most basal corals containing the intron. The intron of *Diploastrea* was genetically very distant from the introns of all other species, whereas *Blastomussa* and *Physogyra* were basal with respect to *Diploastrea* in the coral phylogeny.

Discussion

In this study, *cox1* introns were observed in 20 out of 73 species belonging to four phylogenetically distant coral lineages (Fig. 1). This might suggest several independent insertions of the intron into *cox1* from one or more donors or, alternatively, a single insertion followed by multiple losses. However, comparison of phylogenetic relationships between the coral *cox1* genes and their introns showed broadly similar topologies (Fig. 6). This suggests that the *cox1* intron was acquired in an ancestral coral, maintained in some species by vertical inheritance, but lost in many genera. Interestingly, the type 2 intron (one deletion of T: indel-1 in Fig. 3) seems to have appeared twice—in the *Echinophyllia* species group and the *Lobophyllia*/*Symphyllia* species group (Fig. 6B). However, in the case of three basal corals (*Diploastrea*, *Blastomussa*, and *Physogyra*), topologies of the *cox1* gene and *cox1* introns were quite different (Fig. 6).

There are two possible explanations for this discordance. First, the intron in *Diploastrea* may have been acquired independently following loss of the intron originally acquired by the common ancestor of *Diploastrea*, *Physogyra* and *Blastomussa*. Alternatively, it is possible that an accelerated rate of evolution in *cox1* in the latter two species has resulted in an inaccurate estimation of the phylogenetic relationships among the corals, caused perhaps by the phenomenon of long branch attraction (Felsenstein 1978). However, the distant relationship of *Blastomussa* and *Physogyra* is also supported by analysis of tubulin genes (unpublished data), suggesting that the coral phylogeny presented here is correct. Hence the most plausible scenario is that *Diploastrea* lost the original intron and experienced a second and independent insertion.

Regardless of the details of the history of this intron in corals, it seems highly unlikely that a single origin occurred in the common ancestor of corals and sea anemones. The *cox1* group I introns of corals and sea anemones differ in several aspects: (i) the length of the *cox1* group I intron of *M. senile* (672 bp for DNA sequences and 223 amino acids [Beagley et al. 1996]) is much shorter than that of corals (about 1100 bp and 310 amino acids; see also Fig. 5); (ii) the insertion site in *M. senile* differs from that observed in corals (Table 1); and (iii) the DNA sequence similarity of the *cox1* group I intron ORF between *M. senile* and the corals is low (45%), apart from the characteristic

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Diploastrea heliopora             SSLSVHCPQHSRPTTDKEFGYYLAGLIEGGGSIVVSKDKGYIFICFHLKDVSTAYYIKKRLGFGSMVKPKNKKAINLTIVG
Tetilla sp.                       .......................................RA......Q....................Q..........GS
Smittium culisetae                .TI.D.V.I.KK.LN.ND...........D...--N.INP..S...YEL.TPL......KI.Y.TIL.I...R...YHLRH
Penicillium marneffei             KPI.N.VSK.LK.LN.EQL.H......D.D.YF---SKIPQLV.A.SSP.AFL...L.EK.VYANVK.V...NVYL.VVSK
Schizosaccharomyces octosporus    KLV.E.PKK.VK.MN.T..........D.D.HF----S.SGVE.A..SA.HLL.H...T...H.KISEV.G.N.IV.RF-.
S. japonicus                      -FKPAKL.KLIL.KN.I...H......D.D.HF---SS--RFS...NIA.SHL...LQR.....YVY.V.D.N.V.FTVSD
Metridium senile                  --------------------QW.I..V.SQ.-------------.LS-------------------.AR.SHNTEE.SLSV

Diploastrea heliopora             RRGL--LKVVDLVNGKFR-SKKIESLHKLI-YYLNENI----QAFPKDT---SSVLINHWLAGFLDADGYFQVRII-NR-D
Tetilla sp.                       .G..--....N.......-T.....Q...-D...D.NGKI-E.L....---....R................L-..-V
Smittium culisetae                KD.I--IRLI..I...L.-TN..IDWNTY.IHKI..KYDKTYFKYNI.N---.NLKN.Y.....T.S..S..IKT.-K.NN
Penicillium marneffe              KE.I--LK.IN.I...I.LEHRYDQIINY.INNDKYKDININFTLNT-----.NDFS.......T.G..S..IK..-...-T
Schizosaccharomyces octosporus    LD.T--KE.IT.I.N...THHRF.QIE.NLFTKPSFSNFCNNLDLSL.K---.ENLD.Y..S..S...AS..IK.L-K.-S
S. japonicus                      .L.I--EI.IS.I.....THS.FKQ.TR.LEA-PKF.RFSKTIKLSLNKGTSPKDFD.Y.....S...AS..IKLV-K.-S
Metridium senile                  S.P.KDTQ.LYYMK.L.G-YGHVKL.TTAKY.VA.KSLL----MNVLPL--KC.GDGAR...LV.GE.V.I.SLKH.P.-N

Diploastrea heliopora             ---RKFPYEVRLHLKVDQKTKDVLNLLHEVFGGYVGYREKQDTYYYQSTGFQQALTVIRYLDRY--HVLS-K-WLEYRQWR
Tetilla sp.                       RLG.........................AW........N...........RH..K........--....-.-........
Smittium culisetae                ---K.NN..I..SYQI....DHI..Q.K.L.S..L.F.S.LN...F.TVS.SS.YK..K...N.--.L..S.-Y.N.LK..
Penicillium marneffe              ---TRVRP.I..NYQIEHRKDLI.KKIKTFL..NI...KS......G..S.GS.KN..Q.F.E.--.LQ.R.-Y.S.LR..
Schizosaccharomyces octosporus    ---LT-RT.....NYQ....YDYL.K.IQKEL..NI.H.KS......G..S.VN.VK..K.F.K.--.LM.T.-LIS.LR..
S. japonicus                      ---AS-RT.....NYQ.....DYL.CMIKNL......H.GS.NTA..G..SYTS.DK.VK.F.H.--.M..T.-FIN.L...
Metridium senile                  ---GPKKKN.SFSLIMS.VEGV..KPFL.KL..SMSKN..GG.FKWVMQEKGDLIRIV.LFKK.--SLRTK.-SVDFLK.C
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Fig. 5. Partial amino acid alignments of the mitochondrial *cox1* group I intron among coral and other species (see Materials and Methods for the sequence sources for the latter). Positions are shown for the coral *Diploastrea heliopora* (positions 53–276 of 310 amino acids; formatted as 53–276/310 in the following), for the sponge *Tetilla* sp. (79–307/342), for the fungal species *Smittium culisetae* (15–242/279), *Penicillium marneffei* (36–258/294), *Schizosaccharomyces octosporus* (64–263/305), *S. japonicus* (25–250/293), and for the sea anemone *Metridium senile* (21–196/223). Dots indicate identical amino acids versus coral alignments, and dashes indicate deletions. Regions in boxes show highly conserved areas, and the region in the dashed box shows a variable area. The LAGLI-DADG motifs are shown in boldface for the coral sequence.

LAGLI-DADG motif (about 66% against the motif-1 of corals at the amino acid level) and the P, Q, R, and S core regions (75%–93% at the DNA level). In contrast, similarity of the entire *cox1* DNA sequence between *M. senile* and corals was high (about 80%). The absence of this type of intron from eight other more distantly related coral families in both the robust and the complex clades (unpublished data, based on the same region of *cox1*) also supports the idea that the *cox1* intron was acquired by a basal member of the lineage analyzed here in detail (conventionally referred to as the suborder Faviina) but that sea anemones and corals acquired these introns independently. This conclusion is also supported by the fact that DNA sequences of the coral introns are much more similar to the *cox1* intron of the sponge *Tetilla* sp. (78.8% [Rot et al. 2006]), as well as to some fungal introns (57%–58% with the mitochondrial *cox1* introns of *Smittium culisetae*, *Penicillium marnefferi*, and *Schizosaccharomyces octosporus* [Bullerwell et al. 2003; Seif et al. 2005; Woo et al. 2003]) than to the intron in the sea anemone *M. senile* (45% [Beagley et al. 1996]).

What do these data suggest about the likely source of the coral introns? Although Beagley et al. (1996) suggested that prime donor candidates for the sea anemone *cox1* intron would be ancestors of the endosymbiotic dinoflagellate algae, the existence of a group I intron with homing endonucleases with the LAGLI-DADG motif is unknown in any dinoflagellates (including *Symbiodinium*) based on sequences available in GenBank. Because the sponge intron is the most similar in sequence to the coral introns and is the only intron reported to date with the same insertion site as the coral introns, one might conclude that sponges and corals share a common ancestral insertion early in the history of the Metazoa with subsequent losses. However, only one of eight sponges, representing a diverse array of higher sponge taxa, possessed the intron (Rot et al. 2006) and the taxonomic distribution of the coral intron is also limited (see above).

Alternatively, corals and sponges may have independently acquired their introns from fungi (fungal donors have been suggested for the plant *Peperomia* and the sponge *Tetilla* [Adams et al. 1998; Rot et al. 2006; Vaughn et al. 1995]) or from each other (sponge to coral, or vice versa). Because sponges live surrounding and sometimes covering living corals (e.g., López-Victoria and Zea 2004; Schönberg and Wilkinson 2001) and fungi live in the skeletons of corals (e.g., Bentis et al. 2000; Le Campion-Alsumard et al. 1995; Raghukumar and Raghukumar 1991), such horizontal transfers are not unanticipated. The substantially closer relationship of sponge and coral introns suggests a direct transfer between them, but some as yet unstudied fungi may contain introns that are more similar to the coral and sponge introns than those from fungi studied to date.

Unlike the situation with the *cox1* group I intron, the *nad5* group I intron in the sea anemone *Metridium* and some corals does appear to represent a single transfer event. Beagley et al. (1996) searched for *cox1* and *nad5* introns in several cnidarians (Anthozoa [sea anemone, hard corals, soft corals], Hydozoa,

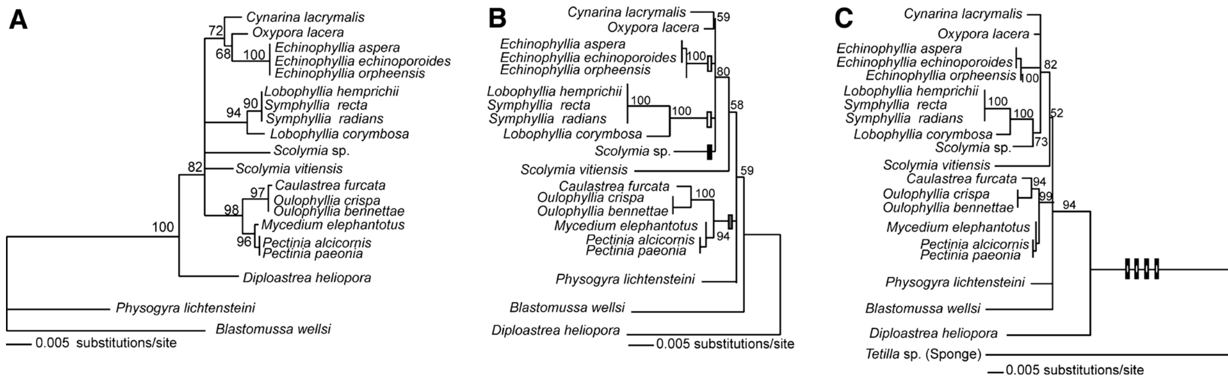

Fig. 6. Comparison of phylogenetic trees constructed using sequences of the coral *cox1* gene (**A**) versus the coral *cox1* group I intron (**B**) (see legend to Fig. 1 for methodological details). The topology in B was largely the same when the sponge *Tetilla* intron DNA sequence was used as an outgroup (**C**). White bars show Type 2 introns, the black bar shows a Type 3 intron, the gray bar shows a Type 4 intron (for the positions of indels, see Fig. 3), and the cluster of bars indicates indels that distinguish coral and sponge introns.

Table 1. Insertion sites (indicated by ˆ) of *cox1* group I introns with the LAGLI-DADG motif for corals, a sponge, the three fungi with intron sequences most closely matching coral sequences, and a sea anemone

	Partial DNA/amino acid sequence of *cox1*
Coral	
Diploastrea heliopora	..TTATTTTGGTTTTTTGGGCATˆCCTGAAGT TTATATTTTAATTTTGCCT..
	L F W F F G H ˆ P E V V I L I L P
Sponge	
Tetilla sp.	..TTATTTTGATTCTTTGGGCATˆCCTGAAGT TTATGTGCTGATTTTACCT..
	L F W F F G H ˆ P E V Y V L I L P
Fungi	
Smittium culisetae	..GATTATGGGTTCTTTGGT*CATCCCGAGGTˆTTATATTATAATTTTACCT..
	L F W F F G * H P E Vˆ V I I I L P
Penicillium marneffei	..CTTTTCTGATTCTTTGGA CATCCAGAGGTˆTTATATTTTAATTATACCT..
	L F W F F G H P E Vˆ Y I L I I P
Schizosaccharomyces octosporus	..TTATTCTGGTTCTTTGGT*CATCCGGAGGTˆTTACATTCTAATTATGCCT..
	L F W F F G * H P E Vˆ Y I L I M P
Sea anemone	
Metridium senile	..LFWFFGHPEVYILILP..(41 aa)..MFTˆVGMDVDTR..

Note. See Materials and Methods for the sources of these sequences. Asterisks indicate the insertion sites of other kinds of group I introns.

Scyphozoa, and Cubozoa) but only found these introns in the sea anemone. More recently, however, several studies found a group I intron in *nad5* in 11 genera from nine families (Fukami and Knowlton 2005; Medina et al. 2006; Tseng et al. 2005; van Oppen et al. 2002), all of which had the same insertion position as in the sea anemone but with different numbers of other coding genes inside of the intron. Our preliminary search for *nad5* introns, which was done by sizing using PCR and partial sequencing, also revealed the existence of the *nad5* intron (with the same size as in other corals previously reported) in all specimens in this study (data not shown). These findings support the suggestion by van Oppen et al. (2002) that the *nad5* group I intron was acquired by a common ancestor of the Scleractinia and Actiniaria. The observed differences in the evolutionary patterns of *nad*5 and *cox1* introns may be caused by a higher mobility of the *cox1* intron associated with its LAGLI-DADG homing endonuclease (Bell-Pedersen et al. 1990; Loizos et al. 1994) and by the fact that functional mitochondrial genes (e.g., *nad1*, *cob*, *nad2*, *rns* for *Acropora tenuis* [van Oppen et al. 2000]) are encoded within the *nad5* intron, which might reduce its mobility.

The reason for the higher prevalence of introns in anthozoan mitochondrial genomes compared to other metazoans remains unclear but may be related to another unusual aspect of anthozoan mitochondrial genomes, namely, their very slow rates of molecular evolution (Medina et al. 1999; Shearer et al. 2002; van Oppen et al. 1999a). For example, introns might be more abundant as a consequence of slow rates of molecular evolution by virtue of lower rates of elimination once inserted. The evolution of anthozoan mitochondrial introns deserves further study as more mitochondrial DNA sequences become available.

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