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# Mitochondrial Group I Introns in Hexacorals Are Regulatory Genetic Elements

*Steinar Daae Johansen and Åse Emblem*

## Abstract

Hexacoral mitochondrial genomes are highly economically organized and vertebrate-like in size, structure, and gene content. A hallmark, however, is the presence of group I introns interrupting essential oxidative phosphorylation (OxPhos) genes. Two genes, encoding NADH dehydrogenase subunit 5 (ND5) and cytochrome c oxidase subunit I (COI), are interrupted with introns. The ND5 intron, located at position 717, is obligatory in all hexacoral specimens investigated. The ND5-717 intron is a giant-sized intron that carries several canonical OxPhos genes. Different modes of splicing appear to apply for the ND5-717 intron, including conventional *cis*-splicing, backsplicing, and *trans*-splicing. Three distinct versions of hexacoral COI introns are noted at genic positions 884, 867, and 720. The COI introns are of the mobile-type, carrying homing endonuclease genes (HEGs). Some COI-884 intron HEGs are highly expressed as in-frame COI exon fusions, while the expression of COI-867 intron HEGs appear repressed. We discuss biological roles of hexacoral mitochondrial ND5 and COI introns and suggest that the ND5-717 intron has gained new regulatory functions beyond self-splicing.

**Keywords:** backsplicing, colonial anemone, mitochondrial genome, mtDNA, mushroom corals, sea anemone, stony corals

## 1. Introduction

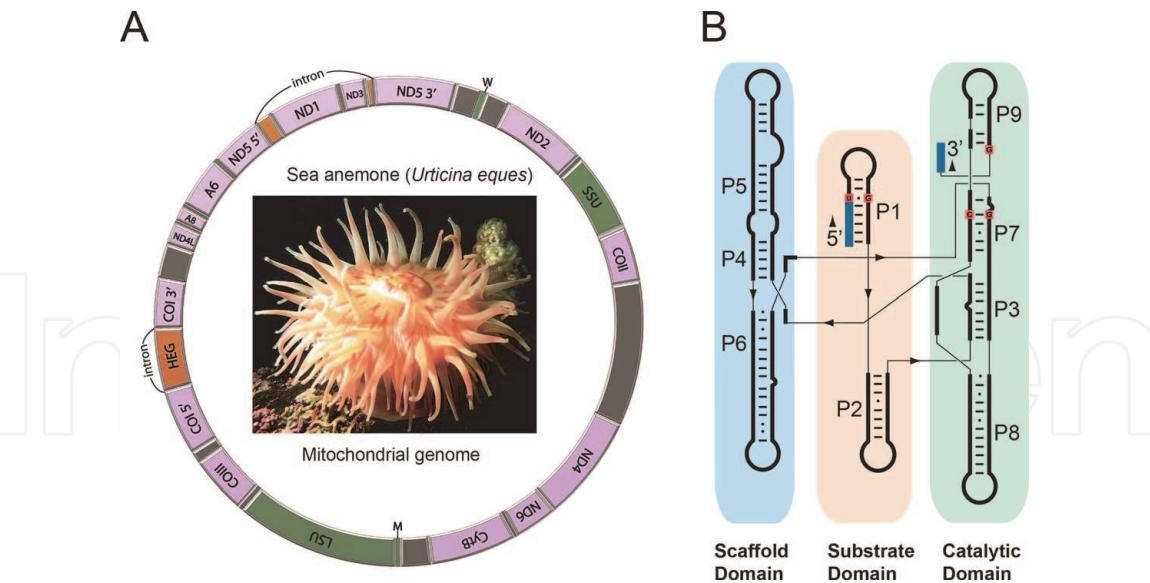
Hexacorallia (hexacorals) represents an ecologically important subclass of Anthozoa with about 4300 extant nematocyst-bearing species [1]. Well-known hexacoral orders include Actiniaria (sea anemones), Zoantharia (colonial anemones), Scleractinia (stony corals), Corallimorpharia (mushroom corals), and Antipatharia (black corals). Ceriantharia (tube anemones) was previously considered to be a hexacoral order, but recent studies suggest tube anemones to represent a distinct subclass of Anthozoa [2].

Hexacorals have a global marine distribution pattern typically recognized in tropical seas at shallow waters living in close relationships with endosymbiotic photosynthetic alga. However, coral reefs and sea anemones in deep offshore waters have more recently been investigated [3–6]. These cold-water hexacorals occur in low temperatures at high latitudes or great depths. Among the approximately 1500 stony coral species known, 50% are located in cold-water habitats [7, 8]. A common

feature among cold-water deep-sea hexacorals is that they are non-endosymbiotic in respect to the photosynthetic alga.

Mitochondria are essential organelles of animal cells, involved in processes like cell metabolism, cell signaling, and cell death [9, 10]. Hexacorals, like all other animals, contain mitochondrial genomes (mtDNAs) encoding a subset (approximately 1%) of the gene products involved in mitochondrial structure and function [11]. Complete mtDNA sequences have been determined from approximately 200 hexacoral specimens representing 133 species and 51 families from sea anemones, colonial anemones, stony corals, mushroom corals, and black corals (**Appendix Table 1**). In general, hexacoral mitochondrial genomes are vertebrate-like in size (17–22 kb), structure, and coding capacity (**Figure 1A**). The circular and economically organized mtDNA encodes the same set of 2 ribosomal RNAs and 13 hydrophobic proteins involved in the oxidative phosphorylation (OxPhos) system [11]. However, noncanonical and optional mitochondrial genes may occur in some hexacoral species [11–16]. More unusual features, however, are the highly reduced tRNA gene repertoire (only 1–2 tRNA genes) and the presence of complex group I introns [11, 17–20].

Group I introns are intervening sequences interrupting functional genes in eukaryotic (mitochondrial, chloroplast, nuclear, viral) and prokaryotic (eubacterial, archaeal, phage) genomes [21]. Like other mobile genetic elements, horizontal transfer of a group I intron can affect the host by altering the function of surrounding genes, potentially interrupting vital processes but also creating diversity and beneficial alterations. Mitochondrial group I introns in metazoans are rare and restricted to some orders within the basal phyla of Placozoa, Porifera, and Cnidaria [11, 22]. Unlike spliceosomal introns, which are abundant in the nuclear genome of eukaryotes, group I introns encode catalytic RNAs (ribozymes) with the unique



ability to self-splice as naked RNA. These introns sometimes even code for homing endonucleases, giving additional mobility to the ribozymes. The intron RNA processing reaction is catalyzed by the ribozyme, which folds into at least nine conserved paired segments (P1–P9), further organized into hallmark helical stacks named the catalytic domain, the substrate domain, and the scaffold domain (**Figure 1B**) [23–25]. Group I intron sequences are removed from precursor transcripts in a guanosine-dependent two-step transesterification reaction, leading to exon ligation and intron excision [21].

This chapter reviews recent developments in the characterization of hexacoral mitochondrial genomes with a focus on gene organization and rearrangements, complex obligatory group I introns in the NADH dehydrogenase subunit 5 (ND5) gene, and mobile-type group I introns in the cytochrome c oxidase subunit I (COI) gene.

## 2. Mitochondrial gene organization and expression in hexacorals

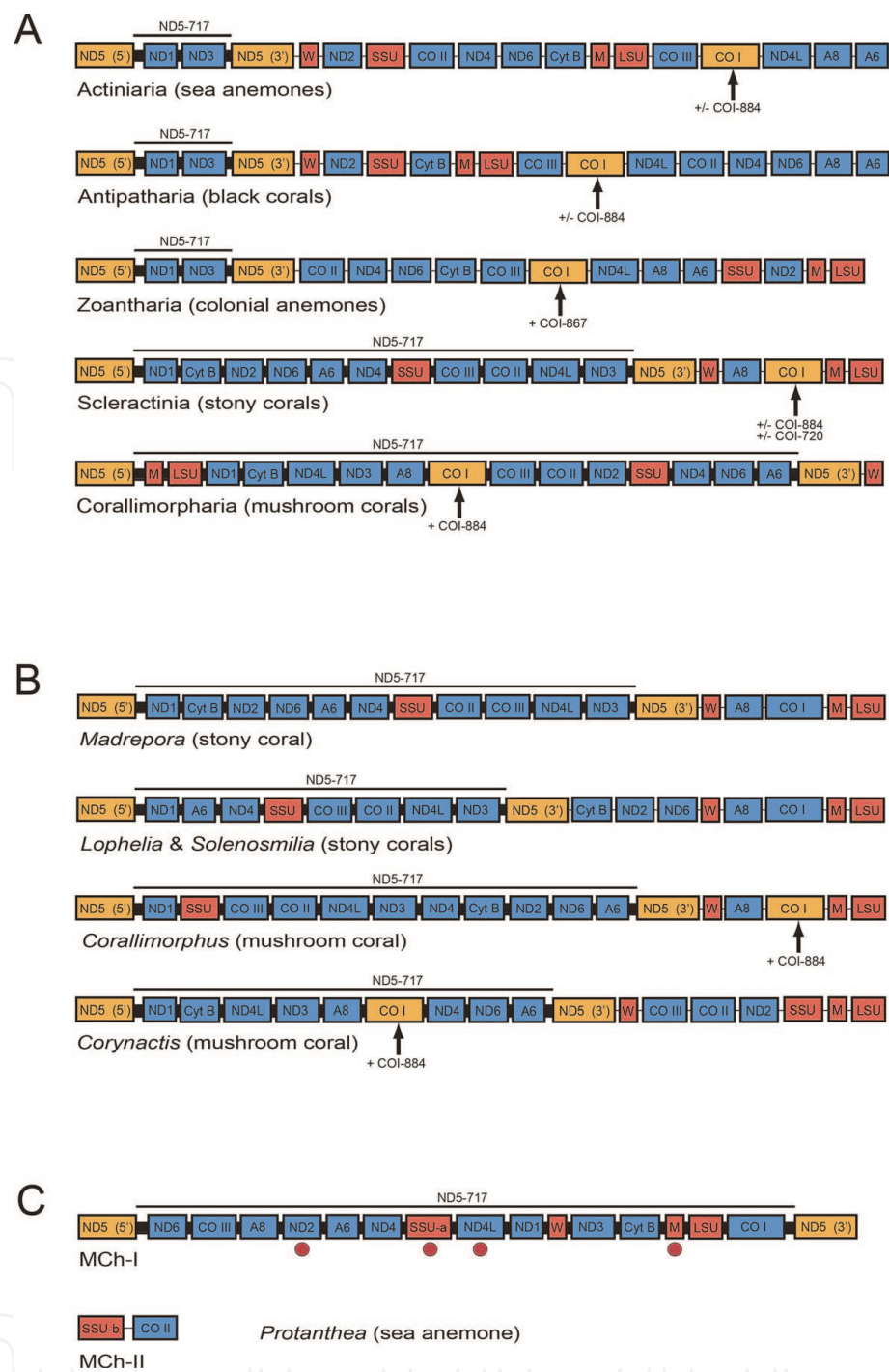
Five common features in the gene organization can be drawn from the 200 available mitochondrial genome sequences representing all five hexacoral orders (**Appendix Table 1**). (1) The 13 annotated OxPhos genes encode the same set of proteins as in vertebrate mtDNA [26], representing Complex I (ND1, 2, 3, 4, 4 L, 6, and 6), Complex III (CytB), Complex IV (COI, II, and III), and Complex V (ATPases 6 and 8). The additional approximately 70 OxPhos proteins are nuclear encoded [27]. (2) All canonical mitochondrial genes (OxPhos genes, rRNA, and tRNA genes) are encoded by the same DNA strand. (3) The tRNA gene repertoire is highly reduced, corresponding to tRNA<sup>fMet</sup> and tRNA<sup>Trp</sup> in sea anemones, stony corals, mushroom corals, and black corals, and only tRNA<sup>fMet</sup> in colonial anemones [14, 28, 29]. This indicates extensive tRNA import into mitochondria [20]. (4) The ND5 gene is split into two exons at nucleotide position 717 (human ND5 gene numbering [19]) by a group I intron found in all hexacorals studied so far (see Section 3 below). (5) The mitochondrial gene synteny appears highly conserved within, but not between, different hexacoral orders.

### 2.1 Order-specific gene organization

Each hexacoral order harbors a closely related primary mitochondrial gene organization (**Figure 2A**). This is an interesting notion since the orders have been separated from each other for 100 million years or more [28]. Stony corals and mushroom corals share some mtDNA synteny [28, 30], and similarly, some segments of synteny appear conserved between sea anemones, colonial anemones, and black corals [30]. The only mitochondrial gene synteny common to all species in all five orders is the upstream proximity of the tRNA<sup>fMet</sup> gene to the large-subunit (LSU) rRNA gene (**Figure 2**). This suggests co-expression similar to that of tRNA<sup>Val</sup> and LSU rRNA genes in vertebrate mitochondria [26]. Recent studies in human and rat conclude that the mitochondrial encoded tRNA<sup>Val</sup> has replaced the 5S rRNA and become an integrated component as a structural rRNA of the mitochondrial ribosome [31]. Thus, tRNA<sup>fMet</sup> is considered as an interesting candidate for a similar dual function in hexacorals.

Deviations from the primary order arrangements have been reported in some sea anemones, stony corals, and mushroom corals and apparently confined to non-endosymbiotic deep-water species. Among the stony corals (**Figure 2B**), *Madrepora* has a rearrangement in the COII and COIII gene order, and *Lophelia* and *Solenosmilia* have a more dramatic rearrangement involving three genes (CytB, ND2, and ND6) [19, 32]. The latter example involves a dramatic shift in the size of





**Figure 2.** Gene organization of hexacoral mitochondrial genomes. Linear presentations of circular maps. Intron-containing OxPhos genes (yellow); intron-lacking OxPhos genes (blue); structural RNA genes (red). The obligatory ND5-717 introns are indicated by black lines, and the optional COI introns by arrows. (A) Primary arrangement in the five hexacoral orders Actiniaria, Antipatharia, Zoantharia, Scleractinia, and Corallimorpharia. (B) Deviations from the primary arrangement seen in the deep-water species *Madrepora oculata*, *Lophelia pertusa*, *Solenosmilia variabilis*, *Corallimorphus profundus*, and *Corynactis californica*. (C) Deviation from the sea anemone primary arrangement seen in the deep-water *Protanthea simplex*. MCh-I and MCh-II, mitochondrial chromosome I and II. SSU-a and SSU-b, two alleles of the small subunit ribosomal RNA gene. Genes located on the opposite strand in MCh-I are indicated by red dots.

the ND5-717 intron from approximately 10 kb (primary arrangement) to 6 kb (see Section 3.1 about transfers of OxPhos genes into the intron). Two different deviations were noted in the mushroom corals *Corallimorphus* and *Corynactis* [30]. These rearrangements appear complex and involve a drastic size reduction of the ND5-717 intron from approximately 18 kb (primary arrangement) to 12 kb and 10 kb, respectively (Figure 2B).

The most dramatic mitochondrial genome rearrangement is seen in the deep-water sea anemone *Protanthea* [16]. Here, the 21 kb mtDNA is arranged along two circular mitochondrial chromosomes, MCh-I and MCh-II (**Figure 2C**). The mitochondrial gene order is heavily scrambled compared to the primary sea anemone arrangement. Different from all other hexacorals, genes at MCh-I are coded on both DNA strands. The ND5-717 intron size was increased from approximately 2 kb (primary sea anemone arrangement) to 15 kb in *Protanthea* (**Appendix Table 1**). Interestingly, the smaller MCh-II encodes the mitochondrial COII and one allele of the small subunit (SSU) rRNA. Phylogenetic analysis indicates that MCh-II is horizontally transferred into *Protanthea* from a distantly related sea anemone [16]. Not all deep-water hexacorals have mtDNA rearrangements. The *Relicanthus* sea anemone, sampled at a depth of 2500 m, [4] harbors the primary arrangement [33]. Similarly, *Bolocera* specimen samples at 40 m (Atlantic Ocean) [12] and at 1100 m (Pacific Ocean) [5] contain the same primary sea anemone arrangement.

## 2.2 Mitochondrial RNA in hexacorals

Mitochondrial RNAs have been investigated in a few hexacoral species representing sea anemones, colonial anemones, and mushroom corals [6, 12, 14–16]. RNAseq data were obtained from 454 pyrosequencing and Ion Torrent PGM sequencing. Several general features are noted: (1) ribosomal RNA constituted more than 90% of the reads and is found to be at least 10–20 times more abundant than most OxPhos gene transcripts; (2) all the conventional genes were transcribed, and the Complex IV OxPhos genes appeared most expressed; (3) group I introns were perfectly spliced out from ND5 and COI mRNA precursors; (4) COI-884 intron splicing appeared more efficient than that of the ND5-717 intron, suggesting intron retention of ND5 mRNA [16]; and (5) noncanonical mitochondrial genes, such as the intron-encoded HEG and non-annotated open reading frames (ORFs), were clearly expressed. One of these ORFs, corresponding to a 306-amino-acid unknown protein in the mushroom coral *Amplexidiscus*, was highly expressed and located at the opposite strand compared to canonical OxPhos genes [16].

## 3. An obligatory group I intron in the ND5 gene

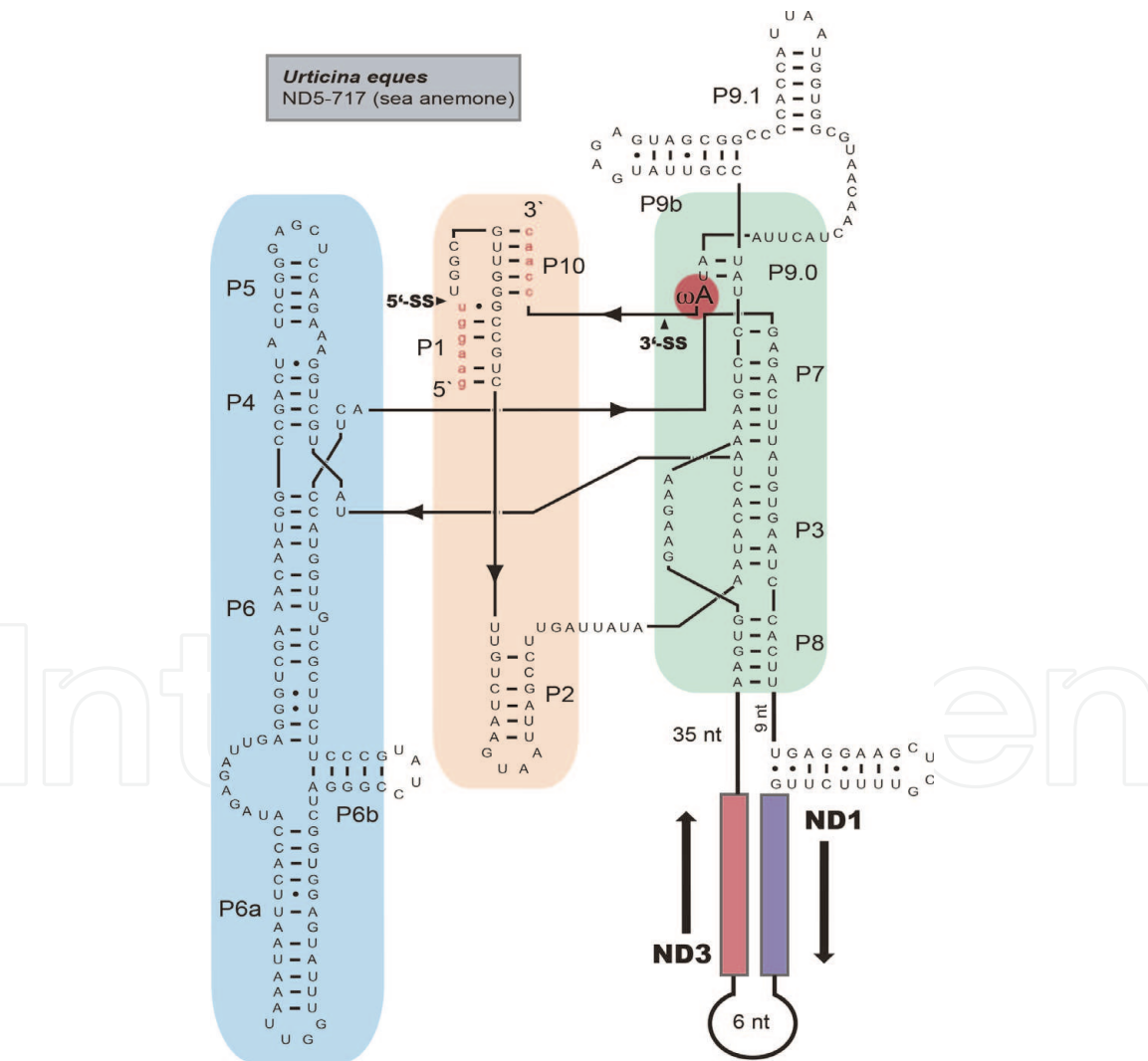
All hexacoral mitochondrial genomes harbor ND5-717 introns (**Appendix Table 1**), making this group I intron an obligatory feature. Evolutionary analyses of ND5-717 introns have previously been performed and show a strict vertical inheritance pattern and a fungal origin [19]. Homologous group I introns at the ND5 insertion site 717 are frequently noted in the fungi Ascomycota, Basidiomycota, and Zygomycota [34], which include mobile-type versions with HEGs [35, 36]. This supports an ancient transfer with a subsequent progression into an obligatory strict vertical inherited intron. Interestingly, HEG-containing ND5-717 was also reported in the mitochondrial genome of choanoflagellates, species considered as the animal ancestors [37].

### 3.1 The ND5-717 intron is a giant group I intron

Phylogenetic analysis supports the early version of hexacoral ND5-717 introns to harbor two OxPhos genes (ND1 and ND3) in P8 [19]. This ancient organization is represented by sea anemones, colonial anemones, and black corals (**Figure 2A**). Insertions of ORFs into loop regions are a common feature in group I introns, and engulfing these compulsory genes might be a strategy for the intron in becoming

essential to the host genome. RNA secondary structure folding of the ND5-717 ribozyme reveals that the catalytically important  $\omega$ G (last nucleotide of the intron) is replaced by  $\omega$ A (**Figure 3**). This replacement is likely to have a dramatic effect on intron biology, leading to host-factor dependent splicing and inhibition of 3' hydrolysis-dependent intron RNA circularization [38].

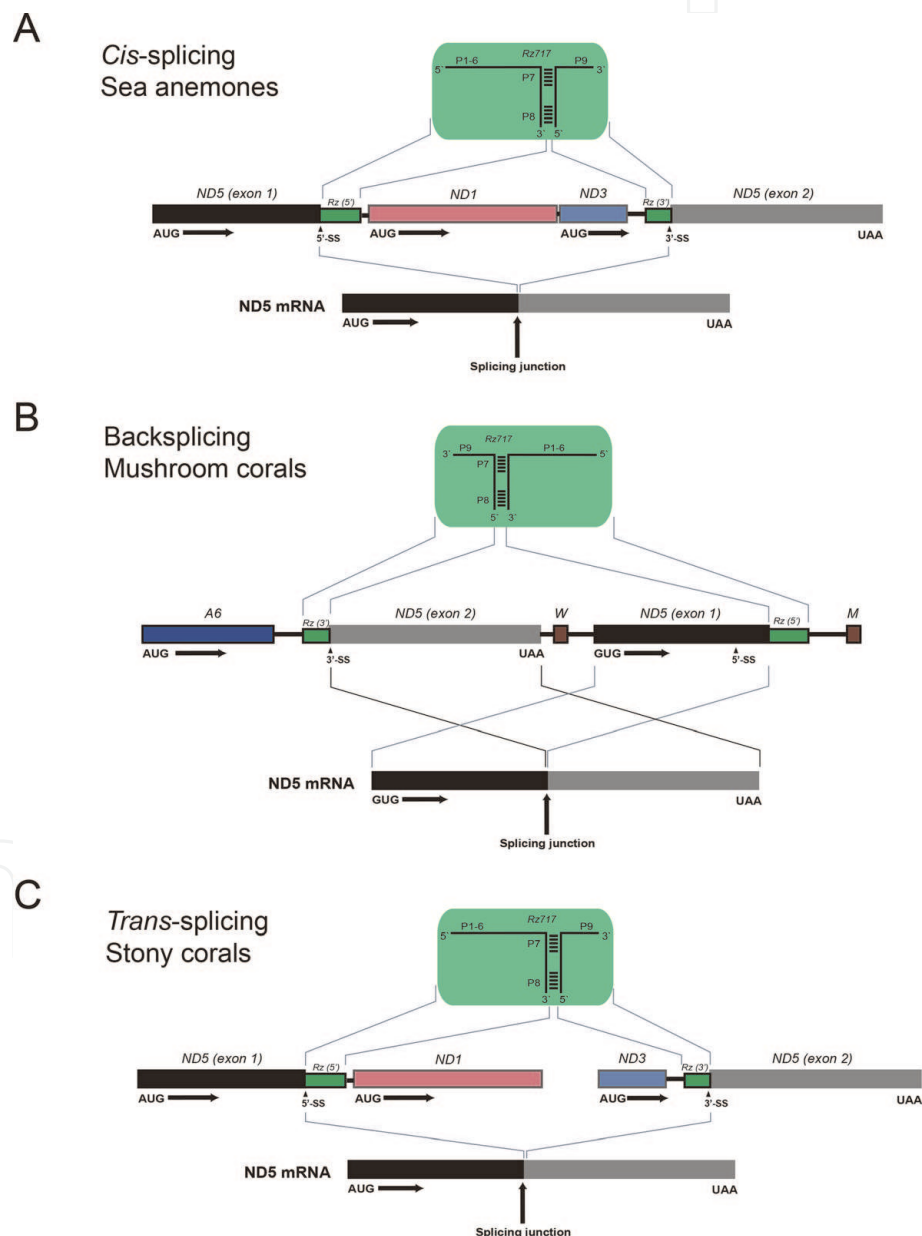
In some hexacoral orders, mitochondrial genome rearrangements resulted in additional transfers of canonical genes into the P8 segment. In stony corals two versions of 6 and 11 genes are intron-located (**Figure 2A and B**). Furthermore, it was noted that robust-clade species have developed a highly compact ribozyme core compared to complex-clade species (and all other hexacorals) [19]. The most complex ND5-717 introns are found in mushroom corals and in the *Protanthea* sea anemone [6, 16, 28, 30]. Whereas three versions of 9, 11, and 15 intron-located genes are noted in mushroom corals, 14 genes are present in P8 of *Protanthea* (**Figure 2B and C**). The ND5-717 intron in mushroom corals represent the largest group I intron known to date with an approximate size of 19 kb.



**Figure 3.** Structure diagram of *Urticina eques* ND5-717 group I intron. Conserved helical segments (P1–P10) are indicated, and flanking ND5 exon sequences are shown in lowercase letters. The three helical stacks, named scaffold domain, substrate domain, and catalytic domain, are indicated by blue, yellow, and green boxes, respectively. The last nucleotide of the intron ( $\omega$ ), which is considered as a universally conserved guanosine ( $\omega$ G) in group I intron, is  $\omega$ A in hexacoral ND5-717 introns (red circle). The P8 segment harbors the two OxPhos genes ND1 and ND3.

3.2 Unconventional splicing of ND5-717 introns

Mitochondrial RNA sequencing reveals perfectly ligated ND5 mRNA exons in sea anemones [12, 15], colonial anemones [14], and mushroom corals [16], which support a biological splicing activity of ND5-717 introns. In the mushroom corals *Ricordea* and *Amplexodiscus*, the splicing efficiency of the ND5-717 intron was reported to be about 10% of that of the COI-884 intron located in the same mitochondrial genome [16]. The complex ND5-717 intron contains 2–15 mitochondrial genes within P8 that challenges its mode of splicing. The shortest forms of ND5-717 introns (approximately 1.6–2.4 kb) detected in sea anemones, colonial anemones, and black corals are likely to be excised by conventional group I intron *cis*-splicing from one single precursor RNA (Figure 4A).



**Figure 4.** Different modes of ND5-717 intron splicing. A schematic group I ribozyme (RZ717; green box) is indicated above each precursor map, and ligated ND5 mRNA is shown below. Splice sites (5'SS and 3'SS), initiation codons (AUG/GUG), and stop codons (UAA) are indicated. (A) *Cis*-splicing performed from a single precursor RNA where both ND5 exons are in a conventional order (exon 1-exon 2). (B) *Backsplicing* performed from a single precursor RNA where both ND5 exons are in a non-conventional order (exon 2-exon 1). (C) *Trans-splicing* performed from two separate precursor RNAs, each containing one ND5 exon.



The longest forms of ND5-717 introns (approximately 15–19 kb), present in mushroom corals [28, 30] and the deep-water *Protanthea* sea anemone [6], contain almost the entire mitochondrial genome within P8. Recently, experimental support of intron removal by backsplicing in mushroom corals was reported [16]. It was found that the primary ND5 transcript contains a permuted exon arrangement where exon 2 is followed by exon 1 (**Figure 4B**). Correct ND5 exon ligation was achieved by involving a circular exon-containing RNA intermediate, which is a hallmark of intron backsplicing [16]. This is the first example of a natural group I intron removed by backsplicing and may explain why some hexacorals tolerate giant ND5-717 group I introns.

How the ND5-717 introns in stony corals are removed from their precursors by splicing is currently not known. These introns (sizes from approximately 6–12 kb) [19, 39] may be too large and complex to be removed by conventional *cis*-splicing, and the ND5 exons may be too distant apart for backsplicing. Thus, a more plausible alternative is *trans*-splicing that generates a ligated ND5 mRNA from two separate precursor RNAs (**Figure 4C**). An interesting notion is that group I intron *trans*-splicing has been reported in mitochondrial transcripts of placozoan animals [40].

#### 4. Mobile-type group I introns in the COI gene

The gene encoding COI is a frequent host of group I introns in hexacoral mitochondrial genomes. Of the total 133 species inspected (**Appendix Table 1**), about 50% harbor an intron insertion. COI introns are present in all five hexacoral orders, but at different distribution patterns.

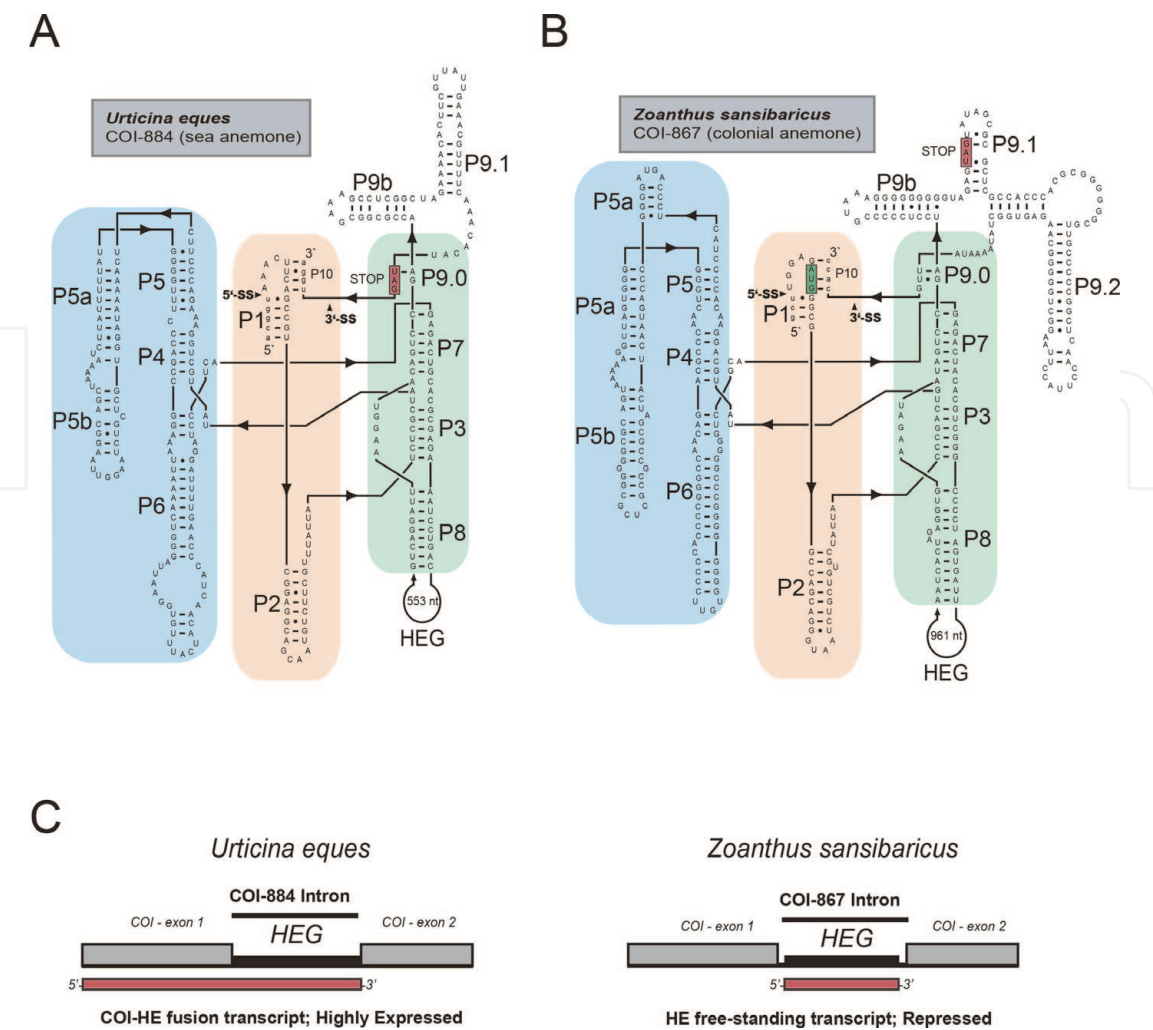
##### 4.1 Three different insertion sites in the COI gene

The COI gene is interrupted by group I introns at three genic positions, where each intron site represents a unique evolutionary history [14, 41]. The intron insertion sites correspond to positions 720, 867, and 884 (human COI gene numbering [19]). The COI-884 introns are widespread in hexacorals, present in most investigated species of sea anemones, mushroom corals, and black corals, as well as a few stony corals (**Appendix Table 1**) [12, 41, 42]. Colonial anemones harbor COI-867 introns [14], and some Indo-Pacific stony coral species contain COI-720 introns [41, 43]. It appears that hexacorals are infected at least three times by COI introns or that this mitochondrial gene is subjected to recurrent group I intron invasion and extinction.

COI introns at different insertion sites are distinct in their ribozyme secondary structure, exemplified by the *Urticina* sea anemone and *Zoanthus* colonial anemone introns COI-884 and COI-867, respectively (**Figure 5A and B**). A common feature, however, is the large insertion within helical segment P8 harboring a HEG that codes for a homing endonuclease of the LAGLIDADG family. These HEGs extend beyond P8 and into the ribozyme domains [12, 14, 15, 43]. Thus, COI-720, COI-867, and COI-884 intron sequences possess dual coding potentials of catalytic RNAs and homing endonucleases. This integration of the endonuclease into the ribozyme core structure ties the two elements closer together, making the endonuclease less prone to degradation.

##### 4.2 Expression of intron-encoded homing endonucleases

Mobile-type introns, like the hexacoral mitochondrial COI introns, promote homing into cognate intron-less alleles by gene conversion [44, 45]. Intron homing



**Figure 5.**  
*COI introns and HEG expression strategy. (A) Secondary structure diagram of the sea anemone Urticina eques COI-884 group I intron. The conserved paired segments of the catalytic core (P1–P10) are shown, and flanking COI exon sequences are in lowercase letters. The P8 extension containing the HEG is indicated. Note that the HEG stop codon (UAG; red box) refers to the last three nucleotides of the intron. The three helical stacks are indicated by blue, yellow, and green boxes. (B) Secondary structure diagram of the colonial anemone Zoanthus sansibaricus COI-867 group I intron. The P1–P10 core segments are shown, and the P8 extension containing the HEG is indicated. Flanking COI exon sequences are in lowercase letters. Note the HEG initiation codon (AUG; green box) and stop codon (UAG; red box) are located in the 5' end and 3' end, respectively, of the intron sequence. The three helical stacks are indicated by blue, yellow, and green boxes. (C) Organization of homing endonuclease transcripts from the COI-884 intron (Urticina eques; left) and the COI-867 intron (Zoanthus sansibaricus; right). While the COI-884 intron transcript is in-frame with the COI exon 1 and highly expressed, the COI-867 intron transcript is freestanding within the intron and repressed. HE, homing endonuclease.*

is initiated by a DNA double-strand break catalyzed by the intron-encoded homing endonuclease. Expression of HEGs has been studied in COI introns of sea anemones, colonial anemones, and mushroom corals [12, 14–16]. Two main versions were noted, leading to either highly expressed or repressed HEGs (**Figure 5C**).

(1) The most successful mode of expression is the in-frame COI-HEG fusion strategy. The HEG, which covers most of the intron sequences (including the ribozyme encoded parts), is fused in-frame with the 5' COI exon. Highly expressed in-frame HEGs are observed in the sea anemones *Urticina* and *Bolocera* [12], and similar in-frame organizations appear common in other sea anemones such as *Isosicyonis*, *Phymanthus*, *Actinia*, and *Stichodactyla* ([15, 46, 47]; our unpublished results). A COI fusion strategy for intron HEG expression in mitochondria, however, is not unique to sea anemones since several fungi are using this approach [44, 48].

(2) Truncated in-frame fusions or freestanding intron HEGs result in

significant lower expressions. This is observed for COI-884 introns of *Hormathia* and *Anemonia* sea anemones [12, 15], COI-884 introns of mushroom corals [16], and COI-867 introns of colonial anemones [14].

## 5. Concluding remarks

A hallmark of hexacoral mitochondrial genomes is the presence of self-catalytic group I introns. What is the biological role of these mitochondrial introns—are they purely selfish genetic elements, or could they have gained new regulatory functions beyond self-splicing? Current knowledge suggests a fungal origin of the hexacoral introns [19, 34, 49]. The group I introns in the COI gene encode LAGLIDADG-type homing endonucleases, consistent with intron mobility between cognate intron-less alleles [12, 45]. The hexacoral COI introns appear gained and lost in multiple cycles during the last 0.5 billion years [42], which supports a selfish intron behavior.

The ND5-717 intron is apparently obligatory in hexacoral mitochondrial genomes, making this genetic element an interesting candidate in gene regulation. Similar obligatory group I introns have been noted in the chloroplast tRNA<sup>Leu</sup> gene of all green plants and in the nuclear LSU rRNA gene of all Physarales myxomycetes [50, 51]. These obligatory mitochondrial, chloroplast, and nuclear introns are considered domesticated group I introns that may have gained new host-specific functions beyond self-splicing [21, 25]. The mitochondrial ND5 mRNA stability has a key role in respiratory control in higher animals; it is tightly regulated and contains m<sup>1</sup>A base modification [52–54]. Intron retention of ND5 mRNA was recently reported in mushroom corals [16], suggesting possible host regulatory functions in hexacorals. Thus, further investigations on hexacoral mitochondrial intron functions and biological roles are needed and highly welcome.

## Acknowledgements

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## Conflict of interest

The authors declare that they have no conflict of interest.

## A. Appendix

In January 2020 about 200 hexacoral mitochondrial genomes have been completely, or nearly completely, sequenced. These mitochondrial genomes represent all 5 hexacoral orders, 51 families, 77 genera, and 133 distinct species. All specimens (100%) harbor ND5-717 and approximately 50% harbor COI introns. Key features are summarized in **Appendix Table 1**.

Species	Family	Accession no	Mt size <sup>1</sup>	ND5 intron (size) <sup>2</sup>	COI intron (size) <sup>3</sup>
<b>A: Sea anemones (Order Actiniaria)</b>					
<i>Synhalcurias elegans</i>	Actiniaridae	KR051009	P11,445 bp	ND5+ (1635 bp)	COI–
<i>Actinia equina</i>	Actiniidae	MH545699	C20,690 bp	ND5+ (2170 bp)	884_COI+ (857 bp)
<i>Actinia tenebrosa</i>	Actiniidae	MK291977	C20,691 bp	ND5+ (2170 bp)	884_COI+ (854 bp)
<i>Anemonia majano</i>	Actiniidae	KY860670	C19,545 bp	ND5+ (1679 bp)	884_COI+ (853 bp)
<i>Anemonia sulcata</i>	Actiniidae	MN011067	C20,390 bp	ND5+ (1725 bp)	884_COI+ (1053 bp)
<i>Anemonia viridis</i>	Actiniidae	KY860669	C20,108 bp	ND5+ (1726 bp)	884_COI+ (853 bp)
<i>Anthopleura midori</i>	Actiniidae	KT989511	C20,039 bp	ND5+ (1714 bp)	884_COI+ (854 bp)
<i>Bolocera tuediae</i>	Actiniidae	HG423145	C19,143 bp	ND5+ (2055 bp)	884_COI+ (853 bp)
<i>Bolocera</i> sp.	Actiniidae	KU507297	C19,463 bp	ND5+ (2397 bp)	884_COI+ (854 bp)
<i>Entacmaea quadricolor</i>	Actiniidae	MN066616	C20,960 bp	ND5+ (2052 bp)	884_COI+ (853 bp)
<i>Epiactis japonica</i>	Actiniidae	MN076184	C18,835 bp	ND5+ (1681 bp)	884_COI+ (853 bp)
<i>Epiactis prolifera</i>	Actiniidae	Ref. [33]	C19,752 bp	ND5+ (1737 bp)	884_COI+ (853 bp)
<i>Isosicyonis striata</i>	Actiniidae	KR051006	C19,001 bp	ND5+ (1695 bp)	884_COI+ (853 bp)
<i>Urticina eques</i>	Actiniidae	HG423144	C20,458 bp	ND5+ (1681 bp)	884_COI+ (850 bp)
<i>Antholoba achates</i>	Actinostolidae	KR051002	C17,816 bp	ND5+ (1884 bp)	884_COI+ (853 bp)
<i>Stomphia selaginella</i>	Actinostolidae	Ref. [33]	C18,349 bp	ND5+ (1784 bp)	884_COI+ (829 bp)
<i>Aiptasia pulchella</i> <sup>4</sup>	Aiptasiidae	HG423147	C19,791 bp	ND5+ (1730 bp)	884_COI+ (847 bp)
<i>Aiptasia pulchella</i> <sup>4</sup>	Aiptasiidae	HG423148	C19,790 bp	ND5+ (1730 bp)	884_COI+ (847 bp)
<i>Bartholomea annulata</i>	Aiptasiidae	MN066614	C19,615 bp	ND5+ (1754 bp)	884_COI+ (847 bp)
<i>Alicia sansibarensis</i>	Aliciidae	KR051001	C19,575 bp	ND5+ (2158 bp)	COI–
<i>Relicanthus daphneae</i>	Boloceroididae	MK947129	C17,727 bp	ND5+ (1721 bp)	884_COI+ (926 bp)



Species	Family	Accession no	Mt size <sup>1</sup>	ND5 intron (size) <sup>2</sup>	COI intron (size) <sup>3</sup>
<i>Edwardsia gilbertensis</i>	Edwardsiidae	MN066615	P17,661 bp	ND5+ (1604 bp)	COI–
<i>Edwardsia timida</i>	Edwardsiidae	Ref. [33]	C18,683 bp	ND5+ (1622 bp)	COI–
<i>Nematostella</i> sp.	Edwardsiidae	DQ643835	C16,389 bp	ND5+ (1620 bp)	COI–
<i>Protanthea simplex</i>	Gonactiniidae	MH500774/75	C21,326 bp	ND5+ (15,262 bp)	COI–
<i>Halcampoides purpurea</i>	Halcampoidisae	KR051003	C18,038 bp	ND5+ (1648 bp)	884_COI+ (856 bp)
<i>Halcurias pilatus</i>	Halcuriidae	KR051004	P10,972 bp	ND5+ (1635 bp)	COI–
<i>Haloclava producta</i>	Haloclavidae	MN076185	P17,416 bp	ND5+ (1681 bp)	884_COI+ (853 bp)
<i>Hormathia digitata</i>	Hormathiidae	HG423146	C18,754 bp	ND5+ (1681 bp)	884_COI+ (853 bp)
<i>Liponema brevicorne</i>	Liponematidae	MN076188	C19,143 bp	ND5+ (2055 bp)	884_COI+ (853 bp)
<i>Metridium senile</i>	Metridiidae	HG423143	C17,444 bp	ND5+ (1681 bp)	884_COI+ (853 bp)
<i>Metridium senile</i>	Metridiidae	AF000023	C17,743 bp	ND5+ (1681 bp)	884_COI+ (853 bp)
<i>Phymanthus crucifer</i>	Phymanthidae	KR051007	C19,727 bp	ND5+ (1911 bp)	884_COI+ (865 bp)
<i>Sagartia ornata</i>	Sagartiidae	KR051008	C17,446 bp	ND5+ (1671 bp)	884_COI+ (853 bp)
<i>Heteractis aurora</i>	Stichodactylidae	MN076186	C19,999 bp	ND5+ (1737 bp)	884_COI+ (853 bp)
<i>Heteractis crista</i>	Stichodactylidae	MN076187	C18,835 bp	ND5+ (1681 bp)	884_COI+ (853 bp)
<i>Stichodactyla helianthus</i>	Stichodactylidae	Ref. [33]	C19,551 bp	ND5+ (1681 bp)	884_COI+ (866 bp)
<i>Stichodactyla helianthus</i>	Stichodactylidae	Unpublished <sup>5</sup>	C18,999 bp	ND5+ (1680 bp)	884_COI+ (865 bp)
<i>Stichodactyla meretensii</i>	Stichodactylidae	Ref. [33]	C18,849 bp	ND5+ (1681 bp)	884_COI+ (866 bp)
<b>B: Colonial anemones (Order Zoantharia)</b>					
<i>Savalia savaglia</i>	Parazoanthidae	DQ825686	C20,764 bp	ND5+ (2052 bp)	867_COI+ (1238 bp)
<i>Palythoa heliodiscus</i>	Sphenopidae	KY888673	C20,841 bp	ND5+ (2077 bp)	887_COI+ (1276 bp)
<i>Zoanthus sansibaricus</i>	Zoanthidae	KY888672	C20,972 bp	ND5+ (2096 bp)	867_COI+ (1327 bp)

Species	Family	Accession no	Mt size <sup>1</sup>	ND5 intron (size) <sup>2</sup>	COI intron (size) <sup>3</sup>
<b>C: Mushroom corals (Order Corallimorpharia)</b>					
<i>Corallimorphus profundus</i>	Corallimorphidae	KP938440	C20,488 bp	ND5+ (12,389 bp)	884_COI+ (1182 bp)
<i>Corynactis californica</i>	Corallimorphidae	KP938436	C20,715 bp	ND5+ (10,531 bp)	884_COI+ (1265 bp)
<i>Pseudocorynactis</i> sp.	Corallimorphidae	KP938437	C21,239 bp	ND5+ (18,840 bp)	884_COI+ (1177 bp)
<i>Amplexidiscus fenestrafer</i>	Discosomatidae	MH308002	C20,054 bp	ND5+ (17,960 bp)	884_COI+ (1206 bp)
<i>Amplexidiscus fenestrafer</i>	Discosomatidae	KP938435	C20,188 bp	ND5+ (18,094 bp)	884_COI+ (1206 bp)
<i>Discosoma nummiforme</i>	Discosomatidae	KP938434	C20,925 bp	ND5+ (18,791 bp)	884_COI+ (1208 bp)
<i>Discosoma</i> sp.	Discosomatidae	DQ643965	C20,908 bp	ND5+ (18,803 bp)	884_COI+ (1207 bp)
<i>Discosoma</i> sp.	Discosomatidae	DQ643966	C20,912 bp	ND5+ (19,807 bp)	884_COI+ (1206 bp)
<i>Discosoma</i> sp.	Discosomatidae	MH308003	C20,288 bp	ND5+ (18,196 bp)	884_COI+ (1206 bp)
<i>Rhodactis indosinensis</i>	Discosomatidae	KP938438	C20,100 bp	ND5+ (18,013 bp)	884_COI+ (1204 bp)
<i>Rhodactis mussoioides</i>	Discosomatidae	KP938439	C20,826 bp	ND5+ (18,721 bp)	884_COI+ (1206 bp)
<i>Rhodactis</i> sp.	Discosomatidae	DQ640647	C20,093 bp	ND5+ (18,001 bp)	884_COI+ (1206 bp)
<i>Ricordea florida</i>	Ricordeidae	DQ640648	C21,376 bp	ND5+ (19,247 bp)	884_COI+ (1176 bp)
<i>Ricordea yuma</i>	Ricordeidae	MH308004	C21,430 bp	ND5+ (19,301 bp)	884_COI+ (1198 bp)
<i>Ricordea yuma</i>	Ricordeidae	MH308005	C21,566 bp	ND5+ (19,437 bp)	884_COI+ (1198 bp)
<i>Ricordea yuma</i>	Ricordeidae	KP938441	C22,015 bp	ND5+ (19,886 bp)	884_COI+ (1198 bp)
<b>D: Black corals (Order Antipatharia)</b>					
<i>Cirrhipathes lutkeni</i> <sup>6</sup>	Antipathidae	JX023266	C20,448 bp	ND5+ (2062 bp)	884_COI+ (1439 bp)
<i>Myriopathes japonica</i>	Antipathidae	JX456459	C17,733 bp	ND5+ (1699 bp)	884_COI+ (924 bp)
<i>Chrysopathes formosa</i>	Cladopathidae	DQ304771	C18,398 bp	ND5+ (1932 bp)	COI–

Species	Family	Accession no	Mt size <sup>1</sup>	ND5 intron (size) <sup>2</sup>	COI intron (size) <sup>3</sup>
<b>E: Stony corals (Order Scleractinia)</b>					
<i>Complex clade</i>					
<i>Acropora aculeus</i>	Acroporidae	KT001202	C18,528 bp	ND5+ (12,116 bp)	COI–
<i>Acropora acuminata</i>	Acroporidae	LC201815	C18,586 bp	ND5+ (12,175 bp)	COI–
<i>Acropora aspera</i>	Acroporidae	KF448532	C18,479 bp	ND5+ (12,070 bp)	COI–
<i>Acropora austera</i>	Acroporidae	LC201816	C18,346 bp	ND5+ (11,937 bp)	COI–
<i>Acropora awi</i>	Acroporidae	LC201849	C18,478 bp	ND5+ (12,070 bp)	COI–
<i>Acropora awi</i>	Acroporidae	LC201850	C18,479 bp	ND5+ (12,070 bp)	COI–
<i>Acropora awi</i>	Acroporidae	LC201851	C18,479 bp	ND5+ (12,070 bp)	COI–
<i>Acropora awi</i>	Acroporidae	LC201852	C18,479 bp	ND5+ (12,070 bp)	COI–
<i>Acropora awi</i>	Acroporidae	LC201853	C18,479 bp	ND5+ (12,070 bp)	COI–
<i>Acropora awi</i>	Acroporidae	LC201854	C18,479 bp	ND5+ (12,070 bp)	COI–
<i>Acropora awi</i>	Acroporidae	LC201855	C18,479 bp	ND5+ (12,070 bp)	COI–
<i>Acropora carduus</i>	Acroporidae	LC201813	C18,373 bp	ND5+ (11,964 bp)	COI–
<i>Acropora carduus</i>	Acroporidae	LC201814	C18,372 bp	ND5+ (11,963 bp)	COI–
<i>Acropora cytherea</i>	Acroporidae	LC201817	C18,568 bp	ND5+ (12,158 bp)	COI–
<i>Acropora cytherea</i>	Acroporidae	LC201818	C18,567 bp	ND5+ (12,157 bp)	COI–
<i>Acropora cytherea</i>	Acroporidae	LC201819	C18,568 bp	ND5+ (12,158 bp)	COI–
<i>Acropora digitifera</i>	Acroporidae	KF448535	C18,479 bp	ND5+ (12,070 bp)	COI–
<i>Acropora divaricata</i>	Acroporidae	KF448537	C18,481 bp	ND5+ (12,072 bp)	COI–
<i>Acropora echinata</i>	Acroporidae	LC201820	C18,480 bp	ND5+ (12,071 bp)	COI–
<i>Acropora echinata</i>	Acroporidae	LC201821	C18,480 bp	ND5+ (12,071 bp)	COI–

Species	Family	Accession no	Mt size <sup>1</sup>	ND5 intron (size) <sup>2</sup>	COI intron (size) <sup>3</sup>
<i>Acropora echinata</i>	Acroporidae	LC201822	C18,480 bp	ND5+ (12,071 bp)	COI–
<i>Acropora echinata</i>	Acroporidae	LC201823	C18,480 bp	ND5+ (12,071 bp)	COI–
<i>Acropora echinata</i>	Acroporidae	LC201824	C18,480 bp	ND5+ (12,071 bp)	COI–
<i>Acropora echinata</i>	Acroporidae	LC201825	C18,480 bp	ND5+ (12,071 bp)	COI–
<i>Acropora echinata</i>	Acroporidae	LC201826	C18,482 bp	ND5+ (12,071 bp)	COI–
<i>Acropora echinata</i>	Acroporidae	LC201834	C18,481 bp	ND5+ (12,072 bp)	COI–
<i>Acropora echinata</i>	Acroporidae	LC201835	C18,368 bp	ND5+ (11,959 bp)	COI–
<i>Acropora echinata</i>	Acroporidae	LC201836	C18,482 bp	ND5+ (12,071 bp)	COI–
<i>Acropora echinata</i>	Acroporidae	LC201837	C18,368 bp	ND5+ (11, 959 bp)	COI–
<i>Acropora echinata</i>	Acroporidae	LC201838	C18,480 bp	ND5+ (12,071 bp)	COI–
<i>Acropora echinata</i>	Acroporidae	LC201839	C18,482 bp	ND5+ (12,073 bp)	COI–
<i>Acropora echinata</i>	Acroporidae	LC201840	C18,480 bp	ND5+ (12,071 bp)	COI–
<i>Acropora echinata</i>	Acroporidae	LC201841	C18,367 bp	ND5+ (11,958 bp)	COI–
<i>Acropora florida</i>	Acroporidae	KF448533	C18,365 bp	ND5+ (11,956 bp)	COI–
<i>Acropora florida</i>	Acroporidae	LC201827	C18,365 bp	ND5+ (11,956 bp)	COI–
<i>Acropora grandis</i>	Acroporidae	LC201828	C18,479 bp	ND5+ (12,070 bp)	COI–
<i>Acropora horrida</i>	Acroporidae	KF448530	C18,480 bp	ND5+ (12,071 bp)	COI–
<i>Acropora humilis</i>	Acroporidae	KF448528	C18,479 bp	ND5+ (12,070 bp)	COI–
<i>Acropora hyacinthus</i>	Acroporidae	KF448531	C18,566 bp	ND5+ (12,157 bp)	COI–
<i>Acropora hyacinthus</i>	Acroporidae	LC201829	C18,567 bp	ND5+ (12,157 bp)	COI–
<i>Acropora hyacinthus</i>	Acroporidae	LC201830	C18,567 bp	ND5+ (12,157 bp)	COI–
<i>Acropora hyacinthus</i>	Acroporidae	LC201831	C18,567 bp	ND5+ (12,157 bp)	COI–



Species	Family	Accession no	Mt size <sup>1</sup>	ND5 intron (size) <sup>2</sup>	COI intron (size) <sup>3</sup>
<i>Acropora hyacinthus</i>	Acroporidae	LC201832	C18,568 bp	ND5+ (12,158 bp)	COI–
<i>Acropora intermedia</i>	Acroporidae	LC201833	C18,479 bp	ND5+ (12,070 bp)	COI–
<i>Acropora microphthalma</i>	Acroporidae	LC201842	C18,479 bp	ND5+ (12,070 bp)	COI–
<i>Acropora microphthalma</i>	Acroporidae	LC201843	C18,481 bp	ND5+ (12,072 bp)	COI–
<i>Acropora muricata</i>	Acroporidae	KF448529	C18,481 bp	ND5+ (12,072 bp)	COI–
<i>Acropora muricata</i>	Acroporidae	LC201844	C18,480 bp	ND5+ (12,071 bp)	COI–
<i>Acropora nasuta</i>	Acroporidae	KF448536	C18,481 bp	ND5+ (12,072 bp)	COI–
<i>Acropora nasuta</i>	Acroporidae	LC201845	C18,374 bp	ND5+ (11,965 bp)	COI–
<i>Acropora nasuta</i>	Acroporidae	LC201846	C18,484 bp	ND5+ (12,074 bp)	COI–
<i>Acropora robusta</i>	Acroporidae	KF448538	C18,480 bp	ND5+ (12,071 bp)	COI–
<i>Acropora selago</i>	Acroporidae	LC201847	C18,482 bp	ND5+ (12,073 bp)	COI–
<i>Acropora selago</i>	Acroporidae	LC201848	C18,480 bp	ND5+ (12,071 bp)	COI–
<i>Acropora tenuis</i>	Acroporidae	AF338425	C18,338 bp	ND5+ (11,928 bp)	COI–
<i>Acropora tenuis</i>	Acroporidae	LC201856	C18,342 bp	ND5+ (11,933 bp)	COI–
<i>Acropora tenuis</i>	Acroporidae	LC201857	C18,342 bp	ND5+ (11,933 bp)	COI–
<i>Acropora tenuis</i>	Acroporidae	LC201858	C18,343 bp	ND5+ (11,934 bp)	COI–
<i>Acropora tenuis</i>	Acroporidae	LC201859	C18,342 bp	ND5+ (11,933 bp)	COI–
<i>Acropora tenuis</i>	Acroporidae	LC201860	C18,342 bp	ND5+ (11,933 bp)	COI–
<i>Acropora tenuis</i>	Acroporidae	LC201861	C18,342 bp	ND5+ (11,933 bp)	COI–
<i>Acropora tenuis</i>	Acroporidae	LC201862	C18,343 bp	ND5+ (11,934 bp)	COI–
<i>Acropora tenuis</i>	Acroporidae	LC201863	C18,342 bp	ND5+ (11,933 bp)	COI–
<i>Acropora tenuis</i>	Acroporidae	LC201864	C18,342 bp	ND5+ (11,933 bp)	COI–

Species	Family	Accession no	Mt size <sup>1</sup>	ND5 intron (size) <sup>2</sup>	COI intron (size) <sup>3</sup>
<i>Acropora tenuis</i>	Acroporidae	LC201865	C18,342 bp	ND5+ (11,933 bp)	COI–
<i>Acropora tenuis</i>	Acroporidae	LC201866	C18,342 bp	ND5+ (11,933 bp)	COI–
<i>Acropora tenuis</i>	Acroporidae	LC201867	C18,342 bp	ND5+ (11,933 bp)	COI–
<i>Acropora tenuis</i>	Acroporidae	LC201868	C18,342 bp	ND5+ (11,933 bp)	COI–
<i>Acropora tenuis</i>	Acroporidae	LC201869	C18,341 bp	ND5+ (11,933 bp)	COI–
<i>Acropora tenuis</i>	Acroporidae	LC201870	C18,342 bp	ND5+ (11,933 bp)	COI–
<i>Acropora valida</i>	Acroporidae	MH141598	C18,385 bp	ND5+ (11,976 bp)	COI–
<i>Acropora yongei</i>	Acroporidae	KF448534	C18,342 bp	ND5+ (11,933 bp)	COI–
<i>Anacropora matthai</i>	Acroporidae	AY903295	C17,888 bp	ND5+ (11,492 bp)	COI–
<i>Astreopora explanata</i>	Acroporidae	KJ634269	C18,106 bp	ND5+ (11,795 bp)	COI–
<i>Astreopora myriophthalma</i>	Acroporidae	KJ634272	C18,106 bp	ND5+ (11,795 bp)	COI–
<i>Montipora cactus</i>	Acroporidae	AY903296	C17,887 bp	ND5+ (11,485 bp)	COI–
<i>Montipora aequituberculata</i>	Acroporidae	KU762339	C17,886 bp	ND5+ (11,488 bp)	COI–
<i>Montipora efflorescens</i>	Acroporidae	MG851914	C17,886 bp	ND5+ (11,491 bp)	COI–
<i>Agaricia fragilis</i>	Agariciidae	KM051016	C18,667 bp	ND5+ (11,525 bp)	COI–
<i>Agaricia humilis</i>	Agariciidae	DQ643831	C18,735 bp	ND5+ (11,536 bp)	COI–
<i>Pavona clavus</i>	Agariciidae	DQ643836	C18,315 bp	ND5+ (11,129 bp)	COI–
<i>Pavona decussata</i>	Agariciidae	KP231535	C18,378 bp	ND5+ (11,129 bp)	COI–
<i>Dendrophyllia arbuscula</i>	Dendrophyllidae	KR824937	C19,069 bp	ND5+ (11,299 bp)	884_COI+ (964 bp)
<i>Dendrophyllia cribrosa</i>	Dendrophyllidae	JQ290080	C19,072 bp	ND5+ (11,282 bp)	884_COI+ (964 bp)
<i>Tubastraea coccinea</i>	Dendrophyllidae	KX024566	C19,094 bp	ND5+ (11,322 bp)	884_COI+ (964 bp)
<i>Tubastraea coccinea</i>	Dendrophyllidae	JQ290078	C19,070 bp	ND5+ (11,300 bp)	884_COI+ (964 bp)

Species	Family	Accession no	Mt size <sup>1</sup>	ND5 intron (size) <sup>2</sup>	COI intron (size) <sup>3</sup>
<i>Tubastraea tagusensis</i>	Dendrophyllidae	KX024567	C19,094 bp	ND5+ (11,324 bp)	884_COI+ (964 bp)
<i>Turbinaria peltata</i>	Dendrophyllidae	KJ725201	C18,966 bp	ND5+ (11,332 bp)	884_COI+ (964 bp)
<i>Euphyllia ancora</i>	Euphylliidae	JF825139	C18,875 bp	ND5+ (11,866 bp)	COI–
<i>Galaxea fascicularis</i>	Euphylliidae	KU159433	C18,751 bp	ND5+ (12,022 bp)	COI–
<i>Fungiacyathus stephanus</i>	Fungiacyathidae	JF825138	C19,381 bp	ND5+ (10,932 bp)	COI+ (961 bp)
<i>Alveopora japonica</i>	Poritidae	MG851913	C18,144 bp	ND5+ (11,621 bp)	COI–
<i>Alveopora</i> sp.	Poritidae	KJ634271	C18,146 bp	ND5+ (11,621 bp)	COI–
<i>Goniopora columna</i>	Poritidae	JF825141	C18,766 bp	ND5+ (11,175 bp)	884_COI+ (964 bp)
<i>Porites fontanesii</i>	Poritidae	NC_037434	C18,658 bp	ND5+ (11,131 bp)	884_COI+ (965 bp)
<i>Porites harrisoni</i>	Poritidae	NC_037435	C18,630 bp	ND5+ (11,133 bp)	884_COI+ (965 bp)
<i>Porites lobata</i>	Poritidae	KU572435	C18,647 bp	ND5+ (11,133 bp)	884_COI+ (965 bp)
<i>Porites lutea</i>	Poritidae	KU159432	C18,646 bp	ND5+ (11,130 bp)	884_COI+ (971 bp)
<i>Porites okinawensis</i>	Poritidae	JF825142	C18,647 bp	ND5+ (11,133 bp)	884_COI+ (965 bp)
<i>Porites panamensis</i>	Poritidae	KJ546638	C18,628 bp	ND5+ (11,117 bp)	884_COI+ (965 bp)
<i>Porites poritis</i>	Poritidae	DQ643837	C18,648 bp	ND5+ (11,135 bp)	884_COI+ (965 bp)
<i>Porites rus</i>	Poritidae	LN864762	C18,647 bp	ND5+ (11,133 bp)	884_COI+ (971 bp)
<i>Pseudosiderastrea formosa</i>	Siderastreidae	KP260632	C19,475 bp	ND5+ (11,524 bp)	884_COI+ (970 bp)
<i>Pseudosiderastrea tayami</i>	Siderastreidae	KP260633	C19,475 bp	ND5+ (11,524 bp)	884_COI+ (970 bp)
<i>Siderastrea radians</i>	Siderastreidae	DQ643838	C19,387 bp	ND5+ (11,463 bp)	884_COI+ (988 bp)
<i>Robust clade</i>					
<i>Madracis decactis</i>	Astrocoeniidae	KX982259	C16,970 bp	ND5+ (10,435 bp)	COI–
<i>Madracis mirabilis</i>	Astrocoeniidae	EU400212	C16,951 bp	ND5+ (10,415 bp)	COI–

Species	Family	Accession no	Mt size <sup>1</sup>	ND5 intron (size) <sup>2</sup>	COI intron (size) <sup>3</sup>
<i>Lopheila pertusa</i> <sup>7</sup>	Caryophyllidae	FR821799	C16,150 bp	ND5+ (6460 bp)	COI–
<i>Lophelia pertusa</i> <sup>7</sup>	Caryophyllidae	KC875348	C16,149 bp	ND5+ (6460 bp)	COI–
<i>Lophelia pertusa</i> <sup>7</sup>	Caryophyllidae	KC875349	C16,149 bp	ND5+ (6460 bp)	COI–
<i>Solenosmilia variabilis</i>	Caryophyllidae	KM609293	C15,968 bp	ND5+ (6459 bp)	COI–
<i>Solenosmilia variabilis</i>	Caryophyllidae	KM609294	C15,968 bp	ND5+ (6459 bp)	COI–
<i>Colpopyllia natans</i>	Flaviidae	DQ643833	C16,906 bp	ND5+ (10,445 bp)	COI–
<i>Plesiastrea versipora</i>	Flaviidae	MH025639	C15,320 bp	ND5+ (9398 bp)	COI–
<i>Echinophyllia aspera</i>	Lobophylliidae	MG792550	C17,697 bp	ND5+ (10,136 bp)	720_COI+ (1077 bp)
<i>Sclerophyllia maxima</i> <sup>8</sup>	Lobophylliidae	FO904931	C18,168 bp	ND5+ (10,760 bp)	720_COI+ (1074 bp)
<i>Dipsastraea rotumana</i>	Merulinidae	MH119077	C16,466 bp	ND5+ (10,149 bp)	COI–
<i>Flavites halicora</i>	Merulinidae	MH794283	C17,033 bp	ND5+ (11,150 bp)	COI–
<i>Hydnopora exesa</i>	Merulinidae	MH086217	C17,790 bp	ND5+ (10,243 bp)	COI–
<i>Orbicella annularis</i>	Merulinidae	AP008973	C16,138 bp	ND5+ (9540 bp)	COI–
<i>Orbicella annularis</i>	Merulinidae	AP008974	C16,138 bp	ND5+ (9540 bp)	COI–
<i>Orbicella faveolata</i>	Merulinidae	AP008977	C16,138 bp	ND5+ (9540 bp)	COI–
<i>Orbicella faveolata</i>	Merulinidae	AP008978	C16,138 bp	ND5+ (9540 bp)	COI–
<i>Orbicella franksi</i>	Merulinidae	AP008975	C16,138 bp	ND5+ (9540 bp)	COI–
<i>Orbicella franksi</i>	Merulinidae	AP008976	C16,137 bp	ND5+ (9539 bp)	COI–
<i>Polycyathus</i> sp.	Merulinidae	JF825140	C15,357 bp	ND5+ (9438 bp)	COI–
<i>Platygyra carnosa</i>	Merulinidae	JX911333	C16,463 bp	ND5+ (10,164 bp)	COI–
<i>Mussa angulosa</i>	Mussidae	DQ643834	C17,245 bp	ND5+ (10,636 bp)	COI–
<i>Madrepora oculata</i>	Oculinidae	JX236041	C15,841 bp	ND5+ (10,140 bp)	COI–



Species	Family	Accession no	Mt size <sup>1</sup>	ND5 intron (size) <sup>2</sup>	COI intron (size) <sup>3</sup>
<i>Pocillopora damicornis</i>	Pocilloporidae	EU400213	C17,425 bp	ND5+ (10,864 bp)	COI–
<i>Pocillopora damicornis</i>	Pocilloporidae	EF526302	C17,415 bp	ND5+ (10,863 bp)	COI–
<i>Pocillopora eydouxi</i>	Pocilloporidae	EF526303	C17,422 bp	ND5+ (10,863 bp)	COI–
<i>Seriatopora caliendrum</i>	Pocilloporidae	EF633601	C17,010 bp	ND5+ (10,467 bp)	COI–
<i>Seriatopora hystrix</i>	Pocilloporidae	EF633600	C17,059 bp	ND5+ (10,465 bp)	COI–
<i>Stylophora pistillata</i>	Pocilloporidae	EU400214	C17,177 bp	ND5+ (10,583 bp)	COI–
<i>Astrangia</i> sp.	Rhizangiidae	DQ643832	C14,853 bp	ND5+ (9258 bp)	COI–
<sup>1</sup> Size of mitochondrial genome. C, completely sequenced; P, partial/almost completely sequenced.					
<sup>2</sup> Size of ND5-717 group I intron.					
<sup>3</sup> Size of COI group I intron. COI–, no COI intron present; 720, 867, or 884 introns indicated.					
<sup>4</sup> The sea anemone <i>Aiptasia pulcella</i> may also be annotated as <i>Exaiptasia pallida</i> .					
<sup>5</sup> Information from our unpublished complete mitochondrial genome sequence of <i>Stichodactyla helianthus</i> .					
<sup>6</sup> The black coral <i>Cirrihipathes lutkeni</i> may also be annotated as <i>Strichpates lutkeni</i> .					
<sup>7</sup> The stony coral <i>Lophelia pertusa</i> may also be annotated as <i>Desmophyllum pertusum</i> .					
<sup>8</sup> The stony coral <i>Sclerophyllia maxima</i> may also be annotated as <i>Acanthastrea maxima</i> .					

**Appendix Table 1.**  
Key features of group I introns in hexacoral mitogenomes.

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