

The mitochondrial genomes of Crispatotrochus rubescens and Crispatotrochus rugosus (Hexacorallia; Scleractinia): new insights on the phylogeny of the family Caryophylliidae.

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Short Report

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Abstract

Background Caryophylliidae is one of the most diverse scleractinian families, however it was recovered as polyphyletic in multiple molecular studies. Recently, the mitochondrial gene order was proposed as a character for a taxonomic revision of the family. Here we describe the first mitogenome of the caryophylliid genus *Crispatotrochus*, whose phylogenetic position remains uncertain.

Methods and results The complete mitochondrial genomes of *Crispatotrochus rubescens* and *Crispatotrochus rugosus* were sequenced, assembled, and annotated. The two mitogenomes are identical and circular, have a length of 16,536 bp, a GC content of 35.9%, and contain 13 protein-coding genes, 2 ribosomal RNAs and 2 transfer RNAs. Both species have a transposition of a three-genes block – *cob*, *nad2*, and *nad6* – similarly to a group of caryophylliid genera that were recovered as monophyletic and include the type species (*Caryophyllia*) of the family. The phylogenetic analyses recovered *Crispatotrochus* within the clade that presents the gene rearrangement and specifically as sister taxa of the genus *Caryophyllia*, a result consistent with previous studies and the similar gross morphology of the two genera.

Conclusions We determined the mitochondrial genomes of the genus *Crispatotrochus* to investigate their relations within Scleractinia. Results from this study provide insights on the phylogenetic position of the genus and corroborate that the mitochondrial gene order could be used as taxonomic character for the family Caryophylliidae.

Introduction

Corals belonging to the order Scleractinia are distributed worldwide and are the engineers of complex shallow and deep-water reef ecosystems. Despite their importance, a well-resolved phylogeny of the order has not yet been achieved (e.g., [1]), hampering the study of longstanding evolutionary questions. Since the advent of the first molecular studies, scleractinian corals have been divided into two (e.g., [2-4]) or three (e.g., [5-6]) main clades at the suborder level, and several families and genera have been recovered as para- or polyphyletic (see [1]).

Within the Vacatina/Robust clade, the family Caryophylliidae Dana, 1846 is currently one of the most diverse, comprising solitary and colonial species, of which the latter includes important components of deep-water reefs [7]. However, recent phylogenetic reconstructions recovered the family as polyphyletic with its members divided into several clades spread across the scleractinian phylogeny (e.g., [1, 5]). Moreover, many taxa belonging to this family still lack molecular information. Therefore, the evolutionary history of this family is still obscure, and its taxonomic revision is in progress. In a recent study, Seiblitz and colleagues [6] showed that all components recovered in a monophyletic clade that harbors the caryophylliid genera *Caryophyllia* (type taxon of the family), *Desmophyllum, Premocyathus*, and *Solenosmilia* have a transposition of a three-genes block (Fig. 1) from the canonical scleractinian mitochondrial gene (mtgene) order (rearrangement previously known only for the genera *Desmophyllum*)

and *Solenosmilia* [8–11]). However, this rearrangement was not observed in any other analyzed mitogenome from genera formally belonging to the family, such as *Heterocyathus*, *Polycyathus*, and *Trochocyathus*. Therefore, such a mtgene order was proposed as a taxonomic character/synapomorphy of the "true" Caryophylliidae, leading to the hypothesis that this family is much smaller than previously thought – but data from several genera are still needed in order to come to a definitive conclusion.

The caryophylliid genus *Crispatotrochus* Tenison-Woods, 1878 is distributed worldwide and comprehends 14 extant azooxanthellate and solitary species. Despite being recovered as closely related to *Caryophyllia* in three studies [1, 12–13], only *C. rugosus* has nucleotide sequences available to date (two nuclear markers – i.e., 28S rDNA, 12S rDNA, and one mitochondrial marker – 16S rDNA). Hence the phylogenetic position of the genus is still under debate. In this study, we obtained the complete mitochondrial genomes of *C. rubescens* and *C. rugosus*, compared them with other caryophylliid mitogenomes, and investigated the phylogenetic position of the genus. The data herein represent a step forward for untangling relationships between azooxanthellate species and will be fundamental for a future revision of the family.

Material And Methods

DNA extraction and genome sequencing

Total genomic DNA from *C. rubescens* and *C. rugosus* (MNHN-IK-2016-2379 and MNHN-IK-2012-17869, respectively) was extracted using the DNeasy Blood and Tissue kit (Qiagen) following the manufacturer's animal tissue protocol. DNA quality and integrity were assessed on a microvolume spectrophotometer (Nanodrop, Thermo Fisher Scientific) and in a 1% agarose gel electrophoresis, respectively. DNA concentration before and after library preparation was quantified with Qubit fluorometer (Thermo Fisher Scientific). Libraries were prepared using the TruSeq DNA Nano Library Preparation kit (Illumina) with modifications in index adapter concentration and the number of PCR cycles (see [6]). Libraries were then sequenced on an Illumina NovaSeq 6000 (150 bp PE reads, two lanes combined with 71 samples from other studies) at the Human Genome and Stem Cell Research Center (CEGH-CEL, USP).

Mitochondrial Genome Assembly And Annotation

Quality control of sequencing data was performed with Trimmomatic [14] and trimmed sequences were assembled into contigs using SPAdes v 3.1 [15] (–careful parameter). For both species, the mitogenome was recovered as a single and circular contig. Genes were annotated using MITOS2 online tool [16] with the parameters genetic code 4 (mold) and RefSeq 89 Metazoa. Annotation was manually verified using Geneious Prime 2022.2.1 (Biomatters Ltd. Auckland, New Zealand) with four published Caryophylliidae mitogenomes used as reference sequences (*Caryophyllia scobinosa*, OL584334; *Desmophyllum pertusum*, KC875348; *Desmophyllum dianthus*, KX000893; *Solenosmilia variabilis*, KM609293). Boundaries of all genes were then confirmed using BLAST [17] against either the NCBI nucleotide database or non-redundant protein sequences database.

Phylogenetic Analysis

Once mitogenomes were fully annotated, they were included in a phylogenetic reconstruction together with 25 published mitogenomes of species belonging to the Vacatina/Robust clade and one outgroup (*Porites lobata* belonging to the Refertina/Complex clade) for a total of 28 mitogenomes. Sequence alignments of protein coding, transfer RNA, and ribosomal RNA genes were performed with MUSCLE 3.8.425 [18]. Alignments were visually inspected for ambiguous sites and successively concatenated resulting in a final alignment of 14,741 bp. For the phylogenetic reconstruction a Maximum Likelihood analysis was performed with a gene partition set on RAxML v8.2.12 [19] using the GRT + GAMMA model, 1000 rapid bootstrap replicates and 20 random starting trees.

Results And Discussion

Mitochondrial genome features

The average assembly coverages for *C. rubescens* and *C. rugosus* were 232.2 and 245.1 X, respectively. The two determined mitochondrial genomes (Genbank accession numbers: XXXX; XXXX) are identical and circular, with a total length of 16,536 bp and a GC content of 35.9% (Fig. 1 and Table 1). With few exceptions, members of the two scleractinian groups Vacatina/Robust and Refertina/Complex exhibit divergent characteristics of their mitogenomes. In general, Refertina/Complex species show longer mitogenomes with higher GC content (Refertina/Complex: length from ~ 17.0 kbp to ~ 19.5 kbp, and from ~ 36.2 to ~ 40.5% GC; Vacatina/Robust: length from ~ 14.9 to ~ 17.8 kbp, and from ~ 29.1 to ~ 35.1% GC [but *Caryophyllia scobinosa* 36.6%]; [6, 20]). Hence, *Crispatotrochus* mitogenomes within "true" caryophyllids (clade comprised by all species with the mitochondrial transposition; Fig. 2) and have a GC content most similar to *C. scobinosa* (Table 1).

Table 1

Species, GenBank accession number, and information of the mitochondrial genomes available for the components of the clade that present the mtgene rearrangement.

Species	Genbank	Length (bp)	GC content %	References
Caryophyllia scobinosa	OL584334	16,105	36.63	Seiblitz et al. 2022
Crispatotrochus rubescens		16,536	35.90	This study
Crispatotrochus rugosus		16,536	35.90	This study
Desmophyllum dianthus	KX000893	16,310	35.10	Addamo et al. 2016
Desmophyllum dianthus	KX000894	16,229	35.10	Addamo et al. 2016
Desmophyllum pertusum	FR821799	16,150	34.90	Emblem et al. 2011
Desmophyllum pertusum	OL584333	16,150	34.87	Seiblitz et al. 2022
Desmophyllum pertusum	KC875349	16149	34.90	Flot et al. 2013
Desmophyllum pertusum	KC875348	16149	34.90	Flot et al. 2013
Solenosmilia variabilis	KM609293	15,968	34.70	Zeng et al. 2016
Solenosmilia variabilis	KM609294	15,968	34.70	Zeng et al. 2016
Solenosmilia variabilis	OL584335	15,969	34.67	Seiblitz et al. 2022
Premocyathus sp.	OL584331	15,816	34.97	Seiblitz et al. 2022

Similar to other scleractinians, the studied mitogenomes contain 13 protein coding, 2 transfer RNA, and 2 ribosomal RNA genes (Fig. 1). A common feature found in several scleractinian mitogenomes is the presence of an intron in the gene *cox1* [21]. Nevertheless, the intron is absent in the mitogenomes from both *Crispatotrochus*, as in all caryophylliids sequenced to date [6]. Both *Crispatotrochus* mitogenomes have the transposition of three genes cob, nad2, and nad6, between the nad5 5' and the trn-Trp (Fig. 1), similarly to the other "true" caryophylliid species [6]. An interesting feature of the Crispatotrochus mitogenomes is the presence of a long intergenic region (IGR) between *atp8* and *cox1* (Fig. 1). Such a long IGR, not observed in any other caryophylliid mitogenome, results in the longest mitogenomes known for all representatives of the clade. Furthermore, Emblem and colleagues [8] reported the presence of repeated regions at the end of *nad1* and the beginning of *cob* in the mitogenome of *D. pertusum* and proposed they played a role in the mechanism of the genes transposition. Later, Seiblitz and colleagues [6] found the same repeated regions in *Caryophyllia* and *Solenosmilia*, but not in *Premocyathus*, possibly due to a secondary loss of this characteristic. The aforementioned repeated regions were also found in the Crispatotrochus mitogenomes but when their repeated regions are aligned, they have more base pair differences than Caryophyllia scobinosa, for example. This result supports the hypothesis that these repeated regions might have been independently lost more than once in the family Caryophylliidae.

Interestingly, although both species have marked morphological autapomorphies (see [22]), their mitogenomes are identical. This is somehow unusual since base pair variations have been recovered even within specimens belonging to the same species (e.g., *Desmophyllum pertusum* – see [6, 9]; and *Solenosmilia variabilis* – see [10]). Nevertheless, it is renowned that scleractinian mitochondrial genomes show slow rates of evolution [23], and their identical mitogenome sequences might indicate recent speciation.

Phylogenetic Position

In the phylogenetic reconstruction (Fig. 2), the genus *Crispatotrochus* was recovered inside the monophyletic clade composed by the caryophylliid genera that present the mtgene rearrangement, which is consistent with the mtgene order recovered for this genus. Specifically, *Crispatotrochus* was recovered as sister taxa of the genus *Caryophyllia*, a result that mirrors the similar gross morphological features of the two genera [24]. The phylogenetic position of *Crispatotrochus* is also consistent with the results from Romano and Cairns [12] and Barbeitos and colleagues [13] that recovered the genera *Caryophyllia* and *Crispatotrochus* as sister taxa using a combination of mitochondrial and nuclear markers (16S/28S and 12S/28S respectively).

Reliable phylogenetic reconstructions are the base for the understanding of diversification processes in different groups of animals, and evolutionary studies of the order Scleractinia have been long suffering an undersampling of azooxanthellate and deep-sea species [25]. In this scenario, results from this study expand our knowledge about molecular features of the azooxanthellate caryophylliid genus *Crispatotrochus*, provide the first molecular data available for the species *C. rubescens* and will be fundamental for a future revision of the taxonomic challenging family Caryophylliidae. Moreover, they add evidence to the hypothesis that the gene transposition could be a diagnostic feature and a synapomorphy of "true" caryophylliids and that, consequently, the family is in fact smaller than previously thought.

Declarations

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Competing Interests The authors have no relevant financial or non-financial interests to disclose.

Author Contributions CFV and MVK conceived and designed research. CFV conducted experiments. CFV, IGLS, and KCCC analyzed data. All authors wrote and approved the manuscript.

Ethical approval All applicable international, national, and/or institutional guidelines for animal testing and use of animals were followed by the authors.

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Figures



Figure 1

Mitochondrial gene map of the scleractinians Crispatotrochus rubescens and C. rugosus. Scaling is approximate. Protein-coding, tRNA, and rRNA genes were abbreviated as in the text. Blank regions between genes represent intergenic spacers. The ND5 intron is indicated by the inner gray line. Transposed genes are marked in bold and an asterisk (*) indicates the canonical position of this gene block for Scleractinia

Maximum Likelihood phylogenetic tree based on a concatenated alignment of all the protein-coding, ribosomal RNA, and transfer RNA genes. Number at nodes are bootstrap values. Sequences from this study are in bold. The green box indicates the monophyletic clade characterized by the gene block rearrangement