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300 million years apart: the extreme case of macromorphological skeletal convergence between deltocyathids and a turbinoliid coral (Anthozoa, Scleractinia)

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ABSTRACT

The integration of morphological and molecular lines of evidence has enabled the family Deltocyathidae to be erected to accommodate *Deltocyathus* species that were previously ascribed to the family Caryophylliidae. However, although displaying the same morphological characteristics as other species of *Deltocyathus*, molecular data suggested that *D. magnificus* was phylogenetically distant from Deltocyathidae, falling within the family Turbinoliidae instead. To elucidate the enigmatic evolutionary history of this species and skeletal microstructural features, the phylogenetic relationships of Deltocyathidae and Turbinoliidae were investigated using nuclear ultraconserved and exon loci and complete mitochondrial genomes. Both nuclear and mitochondrial phylogenomic reconstructions confirmed the position of *D. magnificus* within turbinolids. Furthermore, a novel mitochondrial gene order was uncovered for Deltocyathidae species. This gene order was not present in Turbinoliidae or in *D. magnificus* that both have the scleractinian canonical gene order, further indicating the taxonomic utility of mitochondrial gene order. *D. magnificus* is therefore formally moved to the family Turbinoliidae and accommodated in a new genus (*Dennantotrochus* Kitahara, Vaga & Stolarski, gen. nov.). Surprisingly, turbinolids and deltocyathids do not differ in microstructural organisation of the skeleton that consists of densely packed, individualised rapid accretion deposits and thickening deposits composed of fibres perpendicular to the skeleton surface. Therefore, although both families are clearly evolutionarily divergent, macromorphological features indicate a case of skeletal convergence while these may still share conservative biomineralisation mechanisms.

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Introduction

Since the seminal molecular-based evolutionary reconstructions of the order Scleractinia (Chen *et al.* 1995; Romano and Palumbi 1996, 1997; Veron *et al.* 1996), the systematics of the order have been in revision. Although morphological data suggested that Scleractinia was subdivided into 5 (Wells 1956) or even 13 suborders (Veron 1995), molecular data have pointed to only 2 (Romano and Palumbi 1996; Fukami *et al.* 2008; Quattrini *et al.* 2020; Quek *et al.* 2023) or 3 main clades (Kitahara *et al.* 2010; Stolarski *et al.* 2011; Seiblitz *et al.* 2020). Apart from indicating that the supposed morphological synapomorphies of suborders were not consistent, molecular data have also revealed that several families are *para-* or even polyphyletic (e.g. Fukami *et al.* 2008; Huang *et al.* 2011; Arrigoni *et al.* 2014; Kitahara *et al.* 2016). As a result, several studies have been tackling these discrepancies using integrative approaches that combine molecular analyses and morphological observations (e.g. Arrigoni *et al.* 2021; Seiblitz *et al.* 2022).

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More recently, high-throughput sequencing has enabled the inclusion of vast amounts of data in large-scale phylogenomic studies (Goodwin *et al.* 2016; Kulkarni and Frommolt 2017). Among the most recent methods, the target enrichment of nuclear ultraconserved elements (UCEs) and exons has been used to successfully untangle the systematic and evolutionary history at different taxonomic levels, including in cnidarians (e.g. class Anthozoa Ehrenberg, 1834; Quattrini *et al.* 2018, 2020; McFadden *et al.* 2021; subclass Octocorallia Haeckel, 1866; Erickson *et al.* 2021; McFadden *et al.* 2022; and family Acroporidae Verrill, 1901; Cowman *et al.* 2020; Bridge *et al.* 2023). Moreover, target enrichment and low coverage sequencing methods can also be used to recover entire or nearly entire mitogenomes (e.g. Seiblitz *et al.* 2020; Quattrini *et al.* 2023). Although most scleractinian mitochondrial (mt) genomes show the same mt gene order (herein called canonical order), some rearrangements have been found across lineages (Chen *et al.* 2008; Emblem *et al.* 2011; Lin *et al.* 2012; Flot *et al.* 2013). Recently, Seiblitz *et al.* (2022) proposed that mt gene transpositions could be used as additional taxonomic characters and synapomorphies of specific lineages and could therefore help resolve the evolutionary history of some non-monophyletic taxa.

Among the scleractinian families revealed to be polyphyletic, the Caryophylliidae Dana, 1846 deserves particular attention. This family comprises 42 extant genera that harbour hundreds of species (B. Hoeksema and S. Cairns, World Register of Marine Species, see https://www.marinespecies.org, accessed 20 July 2023) that have been recovered in at least 9 clades (Kitahara *et al.* 2010, 2016) from the 2 main coral groups – 'Complex'–Refertina and 'Robust'–Vacatina. To reconcile molecular and morphological data within caryophylliids, Kitahara *et al.* (2012) elevated the genus *Deltocyathus* Milne Edwards & Haime, 1848 to family rank (i.e. Deltocyathidae Kitahara, Cairns, Stolarski & Miller, 2012) within vacatinian corals. The latter family currently comprises 27 extant species (World Register of Marine Species, see https://www.marinespecies.org) that are within the more commonly sampled deepwater scleractinians, with unique and congruent macro and micromorphological features. However, molecular results repeatedly recovered the Indo-Pacific species *Deltocyathus magnificus* Moseley, 1876 within the refertinian family Turbinoliidae Milne Edwards & Haime, 1848 (Kitahara *et al.* 2010, 2012; Stolarski *et al.* 2011; Campoy *et al.* 2020; Quek *et al.* 2023). This position was not supported by morphology (i.e. *D. magnificus* shares morphological characteristics with other species of *Deltocyathus*), thereby suggesting a possible case of morphological convergence at macro- and micromorphological levels (see Kitahara *et al.* 2012). In terms of divergence time, the most recent common ancestor between Deltocyathidae and Turbinoliidae is estimated to be *c*. 332–382 Ma (Campoy *et al.* 2020; Quattrini *et al.* 2020). Although *D. magnificus* was retained in the Deltocyathidae, the corallum completely encapsulated by tissue was suggested to be a character not shared with congeners but with turbinolids (Kitahara *et al.* 2010, 2012). While morphological convergence has already been proposed as a possible reason for obscuring taxonomy within species belonging to the same genus (e.g. *Acropora* Oken, 1815; van Oppen *et al.* 2001; *Stylophora* Schweigger, 1820; Flot *et al.* 2011), this has not been reported between scleractinian species belonging to such phylogenetically distant lineages.

The recent recognition that the scleractinian skeleton is likely biologically controlled and not easily perturbed by environmental factors at the microstructural level – skeleton organic matrices composed of proteins and polysaccharides control nucleation, spatial delineation and organisation of microstructural units (see Cuif *et al.* 2003; Janiszewska *et al.* 2011, 2013) – has led to more detailed subcorallite observations (Budd *et al.* 2012; Kitahara *et al.* 2012, 2013; Huang *et al.* 2014; Janiszewska *et al.* 2015). Indeed, greater attention has been given to previously overlooked micromorphological and microstructural characters that have elucidated the taxonomy and evolutionary history of several scleractinian taxa (e.g. Benzoni *et al.* 2012; Stolarski *et al.* 2021; Juszkiewicz *et al.* 2022; Seiblitz *et al.* 2022; Arrigoni *et al.* 2023).

To further investigate the potential of such an extreme case of morphological convergence, both macro and micromorphological features were coupled with phylogenies based on mitogenomes, and nuclear UCEs and exons. Our results uncovered a novel mt gene order for representatives of the genus *Deltocyathus* and confirmed the early-diverging position within vacatinian corals. Based on the results of this integrative approach, we also propose that *D. magnificus* is a Turbinoliidae with unique morphological characteristics. Therefore, to accommodate this species within turbinolids, we propose a new genus named *Dennantotrochus* Kitahara, Vaga & Stolarski, gen. nov.

Material and methods

DNA extractions, library preparation and sequencing

Total genomic DNA extraction of specimens analysed in this study (details in Table 1) was performed using the DNeasy Blood and Tissue kit (Qiagen) following the manufacturer's animal tissue protocol. DNA purity and integrity were assessed on a spectrophotometer (Nanodrop, ThermoFisher Scientific) and in a 1% agarose gel electrophoresis respectively. Libraries were prepared using the TruSeq DNA Nano library preparation kit (Illumina) with modifications in index adapter concentration and number of PCR cycles as proposed by Seiblitz *et al.* (2022). DNA concentrations before and after library preparation were quantified on a Qubit fluorometer (ver. 2.0, ThermoFisher Scientific) and size distributions were assessed on a Bioanalyser (Agilent). For three turbinolids (i.e. *Cryptotrochus brevipalus* Cairns,

Table 1. Species name, museum catalog number, sequencing platform, and nuclear and mitochondrial data information included in this study.

Species	MNHN (and ZPAL) collection numbers	MyBaits capture	Illumina platform	Number of UCEs and exon loci recovered	Average assembly coverage	Mitogenome length (bp)	Mitogenome GC content (%)
Cryptotrochus brevipalus	IK-2016-2466 (ZPAL H.25/164)	Yes	MiSeq	1,162	858.7	>19,776	39.1
Cyathotrochus pileus	IK-2012-3825 (ZPAL H.25/162)	No	MiSeq	665	29.9	20,581	38.4
Deltocyathus cameratus	IK-2012-3824 (ZPAL H.25/159)	No	MiSeq	406	39.6	16,301	39.0
Deltocyathus heteroclitus	IK-2012-17830 (–)	No	NovaSeq	1,084	68.3	16,298	39.0
Dennantotrochus magnificus gen. nov.	IK-2012-3811 (ZPAL H.25/161)	No	MiSeq	626	16.3	19,736	37.8
Deltocyathus rotulus	IK-2012-3830 (ZPAL H.25/158)	No	MiSeq	131	23.3	16,294	39.0
Deltocyathus suluensis	IK-2016-2519 (ZPAL H.25/160)	No	NovaSeq	1,247	101.0	16,267	38.6
Notocyathus venustus	IK-2016-2126 (ZPAL H.25/163)	Yes	MiSeq	1,221	880.0	>20,500	38.8
Thrypticotrochus petterdi	IK-2012-17939 (–)	Yes	NovaSeq	1,320	3,292.4	>21,300	39.1
Tropidocyathus labidus	IK-2012-3828	No	MiSeq	90	814.0	20,240	38.9

MNHN represents the *Muséum National d'Histoire Naturelle* (Paris, France); sample fragments used for microstructural analyses are housed in the Institute of Paleobiology, Polish Academy of Sciences (coll. abbreviated ZPAL). Average assembly coverage refers only to the mitochondrial genome. Length is approximate when the mitogenome was not retrieved as complete.

1999, *Notocyathus venustus* (Alcock, 1902) and *Thrypticotrochus petterdi* (Dennant, 1906)), the MyBaits protocol (ver. IV, Arbor BioSciences) was used to target and enrich UCEs and exons with the hexacoral–scleractinian combined baits set developed by Quattrini *et al.* (2018), Cowman *et al.* (2020) and Quek *et al.* (2020). The remaining species were sequenced using a genome skimming method. Illumina sequencing was performed either on a MiSeq (ver. 3, 300-bp PE reads) at the Genome Investigation and Analysis Laboratory of the Centro de Facilidades para a Pesquisa (GENIAL-CEFAP, USP) or a NovaSeq. 6000 (150-bp PE reads) at the Human Genome and Stem Cell Research Center (CEGH-CEL, USP) (details in Table 1).

Mitochondrial genome analyses

Quality control of sequencing data was performed on Trimmomatic (ver. 0.39, see http://www.usadellab.org/cms/index.php?page=trimmomatic; Bolger *et al.* 2014). Trimmed sequences were assembled either with MITObim (ver. 0.39, see https://github.com/chrishah/MITObim; Hahn *et al.* 2013) or into contigs using SPAdes (ver. 3.14.0, see https://github.com/ablab/spades; Bankevich *et al.* 2012; with the --careful parameter). Genes were annotated using the MITOS2 online tool (ver. 2.1.8, see http://mitos.bioinf.uni-leipzig.de/; Bernt *et al.* 2013) with the parameters genetic code 4 (mold) and RefSeq. 89 Metazoa. Annotation was manually verified using Geneious Prime (ver. 2022.2.1, Biomatters Ltd, Auckland, New Zealand). For *Deltocyathus cameratus* Cairns, 1999, *Deltocyathus rotulus* (Alcock, 1898) and *Deltocyathus magnificus*, mitogenome fragments previously determined using Sanger sequencing on the same specimens (primer sequences and PCR settings from Lin *et al.* 2011) were compared to assemblies obtained with Illumina sequencing data using BLAST (Altschul *et al.* 1990). Boundaries of all genes were confirmed using BLAST against either the NCBI nucleotide database or non-redundant protein sequences database. Once mitogenomes were fully annotated, these were included in a phylogenetic reconstruction together with 60 published mitogenomes (see Supplementary Table S1). Sequence alignments of protein coding, transfer RNA and ribosomal RNA genes were performed with MUSCLE (ver. 3.8.425, see http://www.drive5.com/muscle/; Edgar 2004). Alignments were visually inspected for ambiguous sites and successively concatenated resulting in a final alignment of 14,976 bp.

Bioinformatic processing, assembly and alignment of UCEs

Resulting contig files from the SPAdes assembler were used for processing nuclear data. Assembled reads were processed using the Phyluce pipeline (Faircloth 2016). At this stage, previously published genomic and transcriptomic scleractinian data and corallimorpharian species (as outgroup taxa) were included in the analyses (see Supplementary Table S1).

'Phyluce_assembly_match_contigs_to_probes' was used to match the bait set (combined hexacoral and scleractinian bait set developed by Quattrini *et al.* 2018, Cowman *et al.* 2020 and Quek *et al.* 2020) to the contigs to identify loci with a minimum coverage of 70% and a minimum identity of 70%. Loci were subsequently extracted into separate FASTA files using 'phyluce_assembly_get_fastas_from_match_counts' and aligned with default parameters using 'phyluce_align_seqcap_align' in MAFFT (ver. 7, see https://mafft.cbrc.jp/alignment/software/; Katoh *et al.* 2002). Loci were internally trimmed with 'phyluce_align_get_gblocks_trimmed_alignments_from_untrimmed' that uses GBlocks (ver. 0.91, see http://phylogeny.lirmm.fr/phylo_cgi/one_task.cgi?task_type=gblocks; Castresana 2000). Multiple data matrices of locus alignments were created using 'phyluce_align_get_only_loci_with_min_taxa', in which each locus had either 60 or 75% species occupancy and 'phyluce_align_get_informative_sites' was applied to calculate the number of parsimony informative sites.

Phylogenomic analyses

Prior to the phylogenetic analyses, a saturation test was run on the nuclear loci (PhyloMad; Duchêne *et al.* 2021), using entropy models on all sites. All loci displaying substitution saturation were removed from further analyses. For both the mitochondrial and nuclear datasets a partitioned phylogenomic analysis was conducted using maximum likelihood (ML) in IQ-TREE (ver. 2.1, see http://www.iqtree.org/; Nguyen *et al.* 2015). The best-fit models and best partition scheme were selected by ModelFinder (Kalyaanamoorthy *et al.* 2017) implemented in the IQTree (ver. 2.1). Ultrafast bootstrap approximation (UFBoot) (-B 1000; Hoang *et al.* 2018) was conducted as was the Sh-like approximate likelihood ratio test (-alrt 1000; Anisimova *et al.* 2011). Both reconstructions utilised Corallimorpharia as outgroup.

Morphological analyses

The structural analyses of skeletons of some of the coral species we sequenced were performed to provide additional information relevant for nesting *D. magnificus* within the turbinolid clade. Kitahara *et al.* (2013) provided ultrastructural skeletal data of diverse deltocyathid species (*D. cameratus, D. corrugatus, D. crassiseptum, D. heteroclitus, D. inusitatus, D. ornatus, D. rotulus* and *D. suluensis*) that were also compared with those of *D. magnificus*. Focusing on skeletal characters potentially informative in a phylogenetic context (such as distribution pattern of Rapid Accretion Deposits, pattern of fibre distribution in Thickening Deposits; Stolarski 2003), the growing edges of septa, the etched transverse sections, and septal granulation patterns from deltocyathids (*D. rotulus*, *D. cameratus* and *D. suluensis*), turbinolids (*Cyathotrochus pileus*, *Notocyathus venustus* and *Cryptotrochus brevipalus*) and *D. magnificus* were compared qualitatively. All structural components were visualised with Philips/FEI XL20 Scanning Electron Microscopy; the samples were sputter coated with platinum and photographed; and polished sections were lightly etched in Mutvei's solution following the protocol of Schöne *et al.* (2005). Skeletal fragments that were analysed are housed in the Institute of Paleobiology, Polish Academy of Sciences (ZPAL abbreviation, Table 1).

Results

Nuclear data

For the nuclear dataset, a total of 2479 loci (out of 2490) were recovered from the assembled contigs. The final alignment included 58 scleractinian species (one representative per species), 10 of which were sequenced for this study (sequences deposited as a Targeted Locus Study (TLS) at DDBJ/EMBL/GenBank under the BioProject PRJNA1071668, BioSamples #SAMN39709812-21, Accession numbers KIFA00000000–KIFJ00000000) and five corallimorpharians that were used as outgroup. The number of loci recovered from each species ranged from 90 to 1649 per sample (mean ± s.d. 780 ± 365 loci), with a range between 90 and 1320 (795 ± 473 loci) for the species newly sequenced, and from 195 to 1649 (778 ± 347 loci) for the previously published data. As expected, the three species sequenced for this study that had UCEs and exon loci captured *in vitro* prior to sequencing held a higher average number of recovered loci irrespective of the Illumina platform used (see Table 1), as compared to species sequenced through the genome skimming approach. On the other hand, species that were sequenced through the MiSeq platform and not previously target-enriched had fewer loci recovered. This result is due to the lower quantities of raw data per sample retrieved with the MiSeq platform (compared to NovaSeq). Only 3% of the loci had a risk of substitution saturation and were therefore removed from further analyses. The 60 and 75% taxon occupancy matrices resulted in similar, well-supported ML topologies. The final 60% matrix (resulting ML tree shown in Fig. 1) included a concatenated alignment of 298 UCE and exon loci with an alignment length of 54,969 bp of which 42.5% were phylogenetically informative.

In the final ML phylogeny, 94% of the nodes had support equal to or higher than 90% (SH-aLRT and UFBoot values; Fig. 1). *D. cameratus*, *D. heteroclitus*, *D. rotulus* and *D. suluensis* were recovered in a clade with maximum support (SH-aLRT = 100, UFBoot = 100) together with *Paraconotrochus antarcticus* (SH-aLRT = 100, UFBoot = 100), and as sister to the remaining species of the 'Robust'–Vacatina group (SH-aLRT = 100, UFBoot = 100). *D. magnificus* and the sequenced turbinolids were recovered as a clade (SH-aLRT = 100, UFBoot = 100) within the 'Complex'–Refertina group. Specifically, *D. magnificus* was sister to *Cyathotrochus pileus* (SH-aLRT = 98, UFBoot = 97).

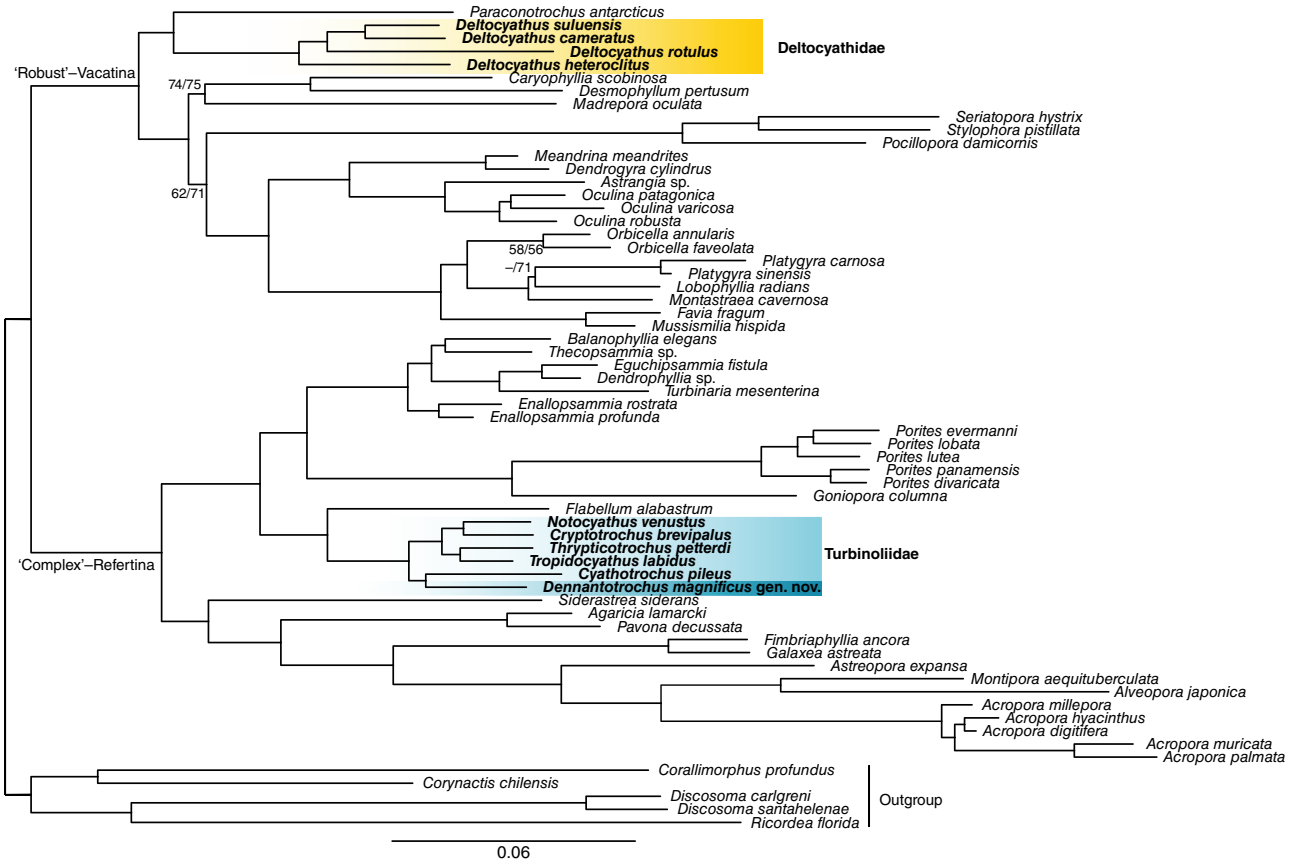

Fig. 1. Maximum likelihood phylogeny based on the nuclear dataset (concatenated alignment of 298 UCE and exon loci with an alignment length of 54,969 bp). Numbers at nodes indicate SH-aLRT and UFBoot values 50–95. Nodes without numbers indicate that SH-aLRT and UFBoot are >95. Light blue box indicates the family Turbinoliidae while darker blue indicates the new genus. Yellow box indicates the family Deltocyathidae. Specimens sequenced for this study are in bold.

Mitochondrial data

The average assembly coverages ranged from 16.3 to 3292.4× (Table 1). The mitogenomes of *D. cameratus*, *D. heteroclitus*, *D. rotulus* and *D. suluensis* (GenBank Accession numbers OR625182–OR625185) are circular and very similar to each other, ranging between 16,267 and 16,301 bp in length and having the GC content varying from 38.6 to 39.0%. The mitogenomes of the Turbinoliidae and *D. magnificus* were more diverse among these (complete mt genomes: GenBank Accession numbers OR625186–OR625188; protein coding genes from the incomplete mt genomes: GenBank Accession numbers PP376102–PP376128). These mitogenomes ranged in length between 19,736 and >21,300 bp with a GC content from 37.8 to 39.1% (Table 1). Circularising the mitogenomes of *Cryptotrochus brevipalus*, *Notocyathus venustus* and *Thrypticotrochus petterdi* was not possible as some parts were missing. Therefore these are considered to be incomplete with mt genomes longer than the observed lengths. Distinct from the sequenced *Deltocyathus, D. magnificus* and the turbinolids have an intron in the *cox1* gene that has been previously observed in other scleractinians (Celis *et al.* 2017; Chuang *et al.* 2017). Moreover, all *Deltocyathus* have the genes *cox3* and *cox2* positioned between the *nad3* and *nad5-3′*, whereas in the scleractinian canonical order (including those found in turbinolids and *D. magnificus*), these genes are found between the *12S* and *nad4l* (Fig. 2). This particular mt gene transposition and order has not been detected in scleractinians.

In the final ML phylogeny, 92% of the nodes had support equal to or higher than 95% in both SH-aLRT and UFBoot values (Fig. 3). The species with the mt gene rearrangement mentioned were recovered as a group within the 'Robust'–Vacatina clade (SH-aLRT = 100, UFBoot = 100) as sister to all remaining robust corals, mirroring the nuclear based results. On the other hand, *D. magnificus* and the turbinolids were recovered as a group (SH-aLRT = 100, UFBoot = 100) in the 'Complex'–Refertina clade but differently from the topology based on nuclear data. *D. magnificus* was sister to the turbinolids species.

Skeletal data

Macromorphologically, all 26 extant species that belong to the genus *Deltocyathus* are characterised by solitary,

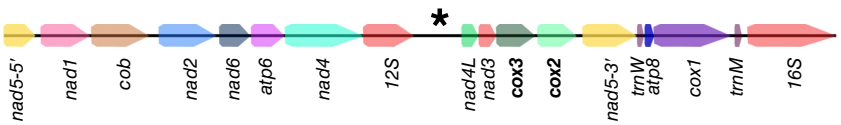

Fig. 2. Mitochondrial gene map of *Deltocyathus* species. Scaling is approximate. Protein-coding, tRNA and rRNA genes are abbreviated as in the text. Blank regions between genes represent intergenic spacers. The transposed genes (*cox3*, *cox2*) are marked in bold and the asterisk (*) indicates the canonical position of this gene for Scleractinia.

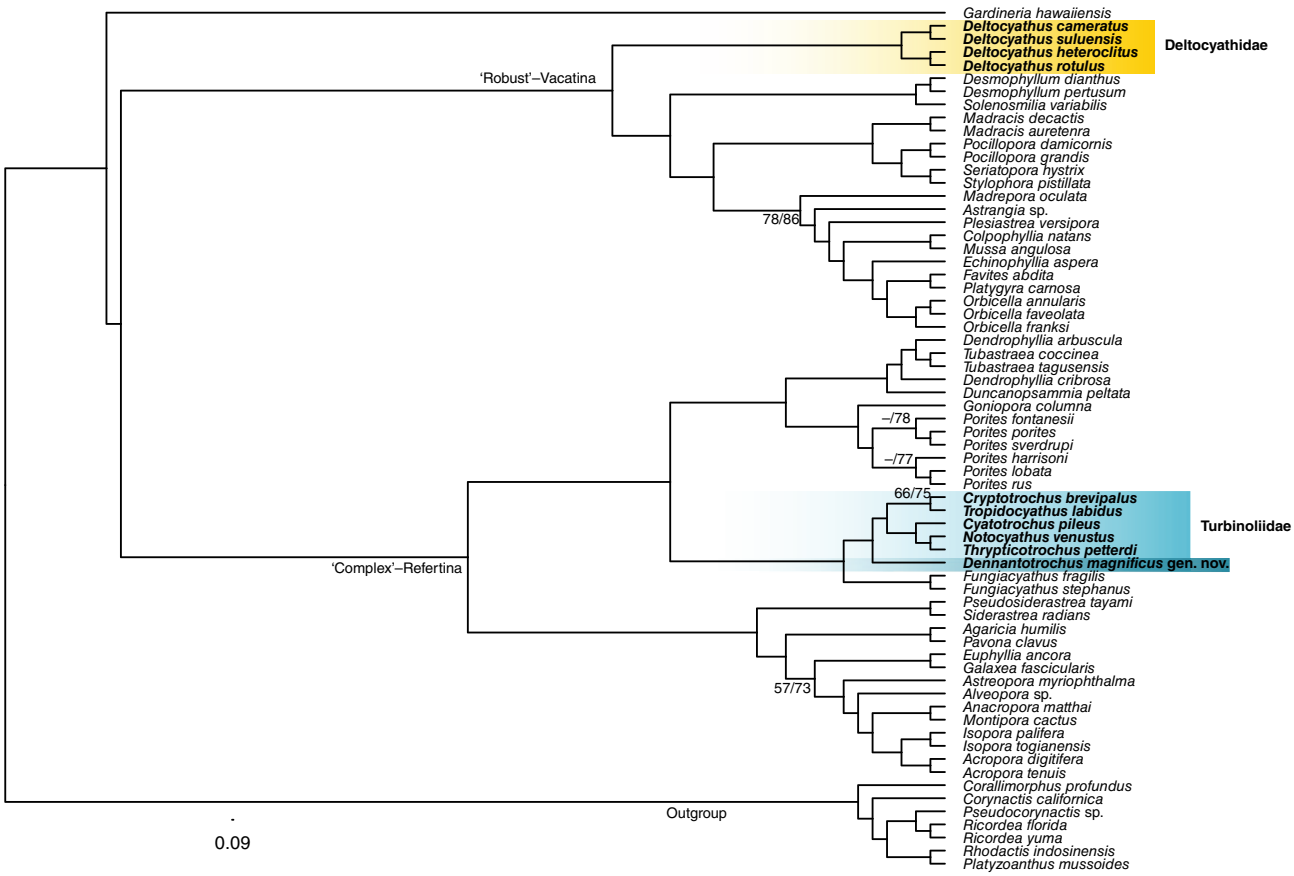

Fig. 3. Maximum likelihood phylogeny based on the mitochondrial dataset. Numbers at nodes indicate SH-aLRT and UFBoot values 50–95. Nodes without numbers indicate that SH-aLRT and UFBoot values are >95. Light blue box indicates the family Turbinoliidae whereas darker blue indicates the new genus. Yellow box indicates the family Deltocyathidae. Specimens sequenced for this study are highlighted in bold.

discoidal to patellate coralla (examples in Fig. $4a_{1,2}$–$c_{1,2}$). By contrast, all traditional turbinolid species have cylindrical (often the result of transverse division), bowl-shaped or conical coralla (examples in Fig. $4e_{1,2}$–$f_{1,2}$, $5f_{1,2}$). Distal septal edges of deltocyathid and turbinolid taxa are straight–undulated and rather smooth but consist of densely packed, individualised rapid accretion deposits (RADs) that are visible as densely packed hollowed-out regions (Fig. $4a_{3,4}$–$c_{3,4}$, e_4, f_4, $5b_{2,3}$) in transverse etched sections. Septal faces of deltocyathids and turbinolids are covered with numerous granulations of variable shapes i.e. sharp or with slightly rounded tips, narrow or slightly broader spines (Fig. $4a_3$–c_3, e_3, f_3, $5b_4$). Organisation pattern of thickening deposits (TDs) is also similar in deltocyathids and turbinolids: bundles of fibres are arranged approximately perpendicularly to the septal surface. Although *Dennantotrochus*, gen. nov. differs macromorphologically in corallum shape from typical turbinolids, ultrastructurally this is not distinguishable from either turbinolids or deltocyathids.

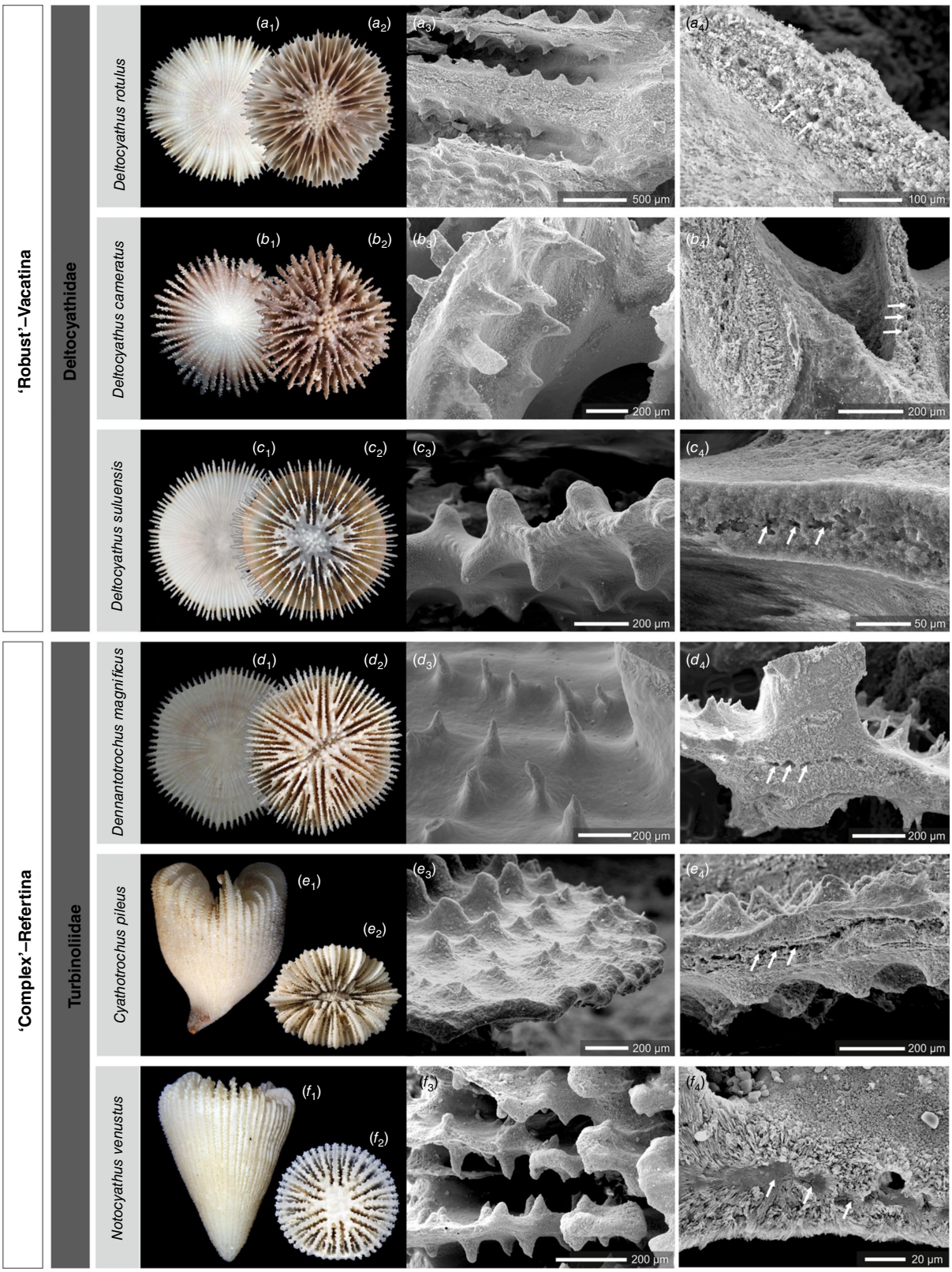

Fig. 4. (*Caption on next page*)

Fig. 4. Skeletal features of selected representatives of deltocyathid ('Robust'–Vacatina) and turbinoliid ('Complex'–Refertina) scleractinian coral clades. Deltocyathids: (*a*) *Deltocyathus rotulus*. (*b*) *Deltocyathus cameratus*. (*c*) *Deltocyathus suluensis*. Turbinoliids (*d*) *Dennantotrochus magnificus*. (*e*) *Cyathotrochus pileus*. (*f*) *Notocyathus venustus*.Overall basal–lateral (subscript 1) and distal (subscript 2) views of coralla. Distal edges of septa are straight–undulating and rather smooth but consisting of densely packed, individualised rapid accretion deposits (RAD) that are visible as densely packed hollowed-out regions in transverse etched sections (subscript 4, arrows); lateral sides of septa are covered with numerous granulations (subscript 3, variable shapes – sharp or slightly rounded tips, narrow or slightly broader spines). Microstructural organisation of thickening deposits is similar in deltocyathids and turbinolids: bundles of fibres are arranged perpendicularly to the septal surface. Microstructural details of the following specimens: (*a*) ZPAL H.25/158 (*b*) ZPAL H.25/159 (*c*) ZPAL H.25/160 (*d*) ZPAL H.25/161 (*e*) ZPAL H.25/162 (*f*) ZPAL H.25/163.

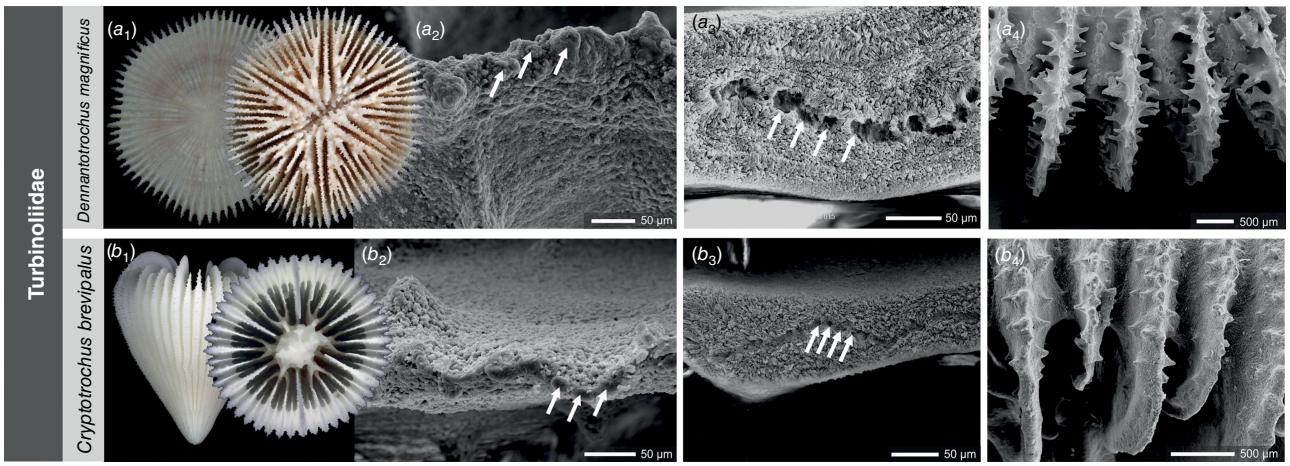

Fig. 5. Direct comparison between skeletal features of turbinolids with (*a*) flat and (*b*) conical coralla: *Dennantotrochus magnificus* and *Cryptotrochus brevipalus*. Overall basal–lateral (subscript 1) and distal (subscript 2) views of coralla. Close-ups of distal edges of septa with densely packed, individualised rapid accretion deposits (RAD; subscript 2, arrows) that sections are visible as densely packed hollowed-out regions in transverse etched sections (subscript 3, arrows); lateral sides of septa and costae are covered with numerous granulations (subscript 4). Thickening deposits composed of bundles of fibres arranged approximately perpendicularly to the septal surface (subscript 3). Microstructural details of the following specimens: (*a*) ZPAL H.25/161, (*b*) H.25/164.

Systematic account

Order **SCLERACTINIA** Bourne, 1900

'Complex'–**REFERTINA** scleractinian group

Family **TURBINOLIIDAE** Milne Edwards & Haime, 1848

Genus ***Dennantotrochus*** Kitahara, Vaga & Stolarski, gen. nov.

(Fig. 4, 5.)

ZooBank: urn:lsid:zoobank.org:act:0C22700D-5172-4DF2-AB89-0014FAE50C0

Type species

Deltocyathus magnificus Moseley, 1876, by monotypy.

Etymology

Named in honor of John Dennant for extensive work on extant and fossil turbinolids from Australia and New Zealand. The suffix is from the Greek *trochus* (round), a common suffix used in coral generic names.

Diagnosis

Corallum solitary, discoidal to hexagonal, free and encapsulated by tissue. Base flat to slightly concave. Corallum white. Septotheca costate. Costae slightly dentate and extend nearly equally beyond calicular margin. Septa arranged hexamerally, only S1 being independent. Axial edges of higher cycle pali join to faces of adjacent septa. Pali before all but last cycle. Columella papillose.

Remarks

Dennantotrochus, gen. nov. can be easily identified by unique characteristics not shared with any other turbinolid genera. The genus can be distinguished from *Cyathotrochus,* sister genus in the nuclear-based phylogeny, by the corallum shape, flat in *Dennantotrochus*, gen. nov. *v*. cuneiform in *Cyathotrochus* and the septa arrangement, always independent in *Cyathotrochus* whereas only S1 is independent in

Dennantotrochus, gen. nov. The genus is also easily distinguished from the other *Deltocyathus* species by being the only taxon to show a flat to slightly concave base. The distribution is restricted to the Western Pacific, from southern Australia to South Korea, and Hawaii (see Kitahara and Cairns 2021).

Discussion

Integrative approaches combining mitogenomes, nuclear UCEs and exon loci, and morphological data were used to elucidate the intriguing position of a previously named *Deltocyathus* species repeatedly recovered by molecular data within the family Turbinoliidae (Kitahara *et al.* 2010, 2012; Stolarski *et al.* 2011).

Molecularly, Turbinoliidae mitochondrial genomes (including that from *Dennantotrochus magnificus* – Table 1) match the GC content of those of the 'Complex'–Refertina taxa (~36.2 to ~40.5%; e.g. Kitahara *et al.* 2014). However, these are longer than those of other species of this group (~17.0 to ~19.5 kbp, e.g. Kitahara *et al.* 2014), especially for the species *Cyathotrochus pileus*, *Notocyathus venustus*, *Thrypticotrochus petterdi* and *Tropidocyathus labidus* that have mitogenomes as long as those from Corallimorpharia (Lin *et al.* 2014). By contrast, *Deltocyathus* species have mitochondrial genome lengths similar to those of 'Robust'–Vacatina taxa (from ~14.9 to ~17.8 kbp, e.g. Chen *et al.* 2008) but a GC content most similar to refertinian species. Seiblitz *et al.* (2022) already showed that some Caryophylliidae taxa have GC content more similar to 'Complex'–Refertina representatives than 'Robust'–Vacatina taxa. Therefore results from this study corroborate the hypothesis that the GC content in vacatinian mitogenomes is more variable than previously considered. Furthermore, both the scleractinian 'Basal' clade (*sensu* Stolarski *et al.* 2011 but see Quattrini *et al.* 2020 and Quek *et al.* 2023) and corallimorpharians (sister group to the order Scleractinia) show a higher GC content. Considering that the families Deltocyathidae and Caryophylliidae occupy early-diverging positions within vacatinian corals, a higher mitogenome GC content could be hypothesised to be an ancestral condition of the order Scleractinia but further analyses are needed to assess this conjecture.

Rearrangements of the mt gene order in scleractinian corals and hexacorals in general are purported to be rare compared to other cnidarian groups (e.g. octocorals; Quattrini *et al.* 2023). However, new transpositions have been recently uncovered in some vacatinian representatives such as the deep-water *Paraconotrochus antarcticus* (see Stolarski *et al.* 2021). More recently, the mt gene rearrangement has been proposed to be a synapomorphy of a specific clade (i.e. family Caryophylliidae; Seiblitz *et al.* 2022), thereby indicating the 'taxonomic' value. Indeed, our results show that all sequenced *Deltocyathus* species in the 'Robust'–Vacatina clade have the same mt gene rearrangement (Fig. 2). This specific mt gene transposition is not present in the mitogenome of *Dennantotrochus magnificus* and has never been observed in any other scleractinian taxa. We therefore propose this to be one of the potentially informative characters of the genus *Deltocyathus* and consequently of the family Deltocyathidae. Nonetheless, as *Deltocyathus* comprises 26 extant species of which 22 do not have the complete mitogenome sequenced to date and considering that different mt gene organisations have already been observed in species belonging to the same family (Chen *et al.* 2008), further analyses are necessary to assess the pervasiveness of the aforementioned Deltocyathidae mt gene transposition. The phylogenetic reconstructions resulting from both nuclear and mt data confirmed the monophyly and unique mt gene rearrangement of Deltocyathidae/*Deltocyathus* in the 'Robust'–Vacatina clade, and the inclusion of *Dennantotrochus magnificus* in the Turbinoliidae.

We found some discrepancies between our nuclear and mitochondrial phylogenies. Although in the nuclear reconstruction *D. magnificus* was recovered as sister to *Cyatotrochus pileus*, the mitochondrial reconstruction places this as sister to all the sequenced turbinolids. Additional data from other turbinolid genera and additional UCE loci may shed further light on the position of this newly erected turbinolid genus. This discrepancy could be driven by the fact that a comparatively low number of UCEs and exon loci were recovered for *Deltocyathus rotulus* and *Tropidoyathus labidus* (131 and 90 respectively). Nonetheless, studies (e.g. Derkarabetian *et al.* 2019) have shown that specimens with very few loci in the final matrices are successfully placed with congeners in phylogenetic reconstructions. Moreover, although species relationships may change within lineages – relationships within *Deltocyathus* species are also different between the two topologies –, noticing that family relationships and monophyly are congruent between the two phylogenies is important. Both the nuclear and mitochondrial phylogenomic reconstructions mirror those from Kitahara *et al.* (2010, 2013) and Stolarski *et al.* (2011) with representatives of the genus *Deltocyathus* forming an early-diverging vacatinian clade that is not closely related to the family Caryophylliidae.

Morphological convergence at the macromorphological level has already been proposed to explain similar morphotypes in several cnidarian taxa (e.g. corallites of species in the family Merulinidae; Huang *et al.* 2009; coiled growth form in octocorals and whip black corals; Bavestrello *et al.* 2012; and convergent functional morphology of the marginal musculature in zoantharians; Swain *et al.* 2015). Convergent morphologies and adaptations of a specific structure in phylogenetically distant lineages could be the result of a similar biological function or pressures from similar environmental conditions. Components of the family Turbinoliidae and Deltocyathidae have already been collected from the same location (see Kitahara and Cairns

2021) and identical substrate. However, as these are mainly restricted to deep waters, very little is known about the living habits of the representatives of these two families and the functions of different structures of the scleractinian corallum are still largely obscure. The only anatomical characteristic in common between *Dennantotrochus magnificus* and turbinolids is the complete investiture of the skeleton in the polyp tissue that has been proposed to be an adaptation to a semi-burrowing or interstitial habit (see Cairns 1997), a hypothesis later confirmed by Sentoku *et al.* (2016). Nevertheless, this characteristic is not exclusive to this clade, having also been observed in other scleractinian taxa, including some deep-water solitary species such as those of the families Micrabaciidae and Fungiacyathidae.

Many molecularly resolved clades of scleractinian corals are well supported by ultrastructural features (e.g. Stolarski 2003; Benzoni *et al.* 2012; Kitahara *et al.* 2012). Ultrastructural analysis of deltocyathid and turbinolid (including *Dennantotrochus*, gen. nov.) representatives does not indicate any major differences that would be expected for scleractinian clades (i.e. 'Complex'–Refertina, turbinolids, and 'Robust'–Vacatina, deltocyathids) separated by more than 300 Ma of divergence. Although molecular clock results vary depending on the molecular markers adopted and the set of species included in the analysed datasets, there is consensus that the onset of the order Scleractinia is placed between 415 and 383 Ma (Stolarski *et al.* 2011; Quattrini *et al.* 2020) and that the divergence of the two main clades occurred between 382 and 332 Ma (Campoy *et al.* 2020; Quattrini *et al.* 2020). Both clades are somewhat early-diverging scleractinian clades, therefore the shared ultrastructural characteristics may indicate the ancestral character state of earliest scleractinian corals, i.e. fairly smooth septal edges consisting of densely packed, individualised rapid accretion deposits (RAD). Indeed, among the earliest well-differentiated Triassic (*c*. 240 Ma) scleractinians, numerous representatives share such ultrastructural organisation (traditionally called 'minitrabecular organisation' e.g. Roniewicz 1984; Roniewicz and Morycowa 1993). These Triassic corals also had relatively simple organisation of the Thickening Deposits (i.e. arrangement of biomineral fibres more or less perpendicular to septal faces) that contrasts with the more recent families (e.g. acroporiid and flabellid mineral shingles whose fibres are parallel to skeletal surfaces) but is shared with deltocyathids and turbinolids. This suggests that some lineages of ancestral scleractinian corals, even if separated by 300 Ma of evolutionary history, could still share some conservative biomineralisation mechanisms.

Conclusions

Through an integrative approach that coupled morphological and molecular analysis, the phylogenetic position and traits of the enigmatic species *Deltocyathus magnificus* were investigated. The position with representatives of the family Turbinoliidae was confirmed by phylogenies built using both the complete mt genomes and hundreds of UCEs and exon loci. The species was therefore accommodated in the new turbinolid genus *Dennantotrochus*, gen. nov. Our results indicate that deltocyathid species are characterised by a specific mt gene rearrangement not observed in any other scleractinians, therefore possibly being a synapomorphy of the family. Interestingly, *D. magnificus* and deltocyathids have congruent macromorphological features (see Kitahara *et al.* 2012), and species from both families, belonging to phylogenetically distant scleractinian clades, do not differ in micromorphological characters, thereby indicating a possible ancestral character state of the earliest scleractinian corals. Our study represents an enhancement of our understanding of the systematics of two speciose azooxanthellate scleractinian families and fills some gaps in our understanding of the evolutionary history of Scleractinia.

Supplementary material

Supplementary material is available online.

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Data availability. Nuclear and mitochondrial data have been uploaded to GenBank. Complete mt genomes: GenBank Accession numbers OR625182–OR625185 and OR625186–OR625188; protein coding genes from the incomplete mt genomes: GenBank Accession numbers PP376102–PP376128. Nuclear UCEs and exon loci sequences were deposited as a Targeted Locus Study (TLS) at GenBank under the BioProject PRJNA1071668, BioSample #SAMN39709812-21, Accession numbers KIFA00000000-KIFJ00000000.

Conflicts of interest. The authors declare that they have no conflicts of interest.

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