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Deltocyathiidae, an early-diverging family of Robust corals (Anthozoa, Scleractinia)

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Over the last decade, molecular phylogenetics has called into question some fundamental aspects of coral systematics. Within the Scleractinia, most families composed exclusively by zooxanthellate species are polyphyletic on the basis of molecular data, and the second most speciose coral family, the Caryophylliidae (most members of which are azooxanthellate), is an unnatural grouping. As part of the process of resolving taxonomic affinities of 'caryophylliids', here a new 'Robust' scleractinian family (Deltocyathiidae fam. n.) is proposed on the basis of combined molecular (CO1 and 28S rDNA) and morphological data, accommodating the early-diverging clade of traditional caryophylliids (represented today by the genus *Deltocyathus*). Whereas this family captures the full morphological diversity of the genus *Deltocyathus*, one species, *Deltocyathus magnificus*, is an outlier in terms of molecular data, and groups with the 'Complex" coral family Turbinoliidae. Ultrastructural data, however, place *D. magnificus* within Deltocyathiidae fam. nov. Unfortunately, limited ultrastructural data are as yet available for turbinoliids, but *D. magnificus* may represent the first documented case of morphological convergence at the microstructural level among scleractinian corals. *Marcelo V. Kitahara, Centro de Biologia Marinha, Universidade de São Paulo, São Sebastião, S.P. 11600-000, Brazil. E-mail: kitahara@usp.br*
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Introduction

The Caryophylliidae is one of the most diverse and ecologically successful scleractinian families and comprises more than 300 extant species (Cairns 2009); caryophylliids are near ubiquitous, occurring from the Antarctic (Cairns 1982) to the Arctic (Roberts *et al.* 2003) and at depths ranging from the intertidal (e.g. *Paracyathus darwinensis*) to waters deeper than 5000 m (e.g. *Deltocyathus parvulus*). Although the vast majority of extant caryophyliid species are azooxanthellate and inhabit waters deeper than 200 m, some rely on symbiosis with dinoflagellates, and at least three species occur as both forms (Cairns *et al.* 1999). This family captures a wide spectrum of morphological diversity, accommodating small solitary forms < 5 mm in calicular diameter (e.g. *Caryophyllia aspera*) to colonial species that can attain heights of several metres (e.g. *Lophelia pertusa*).

Molecular phylogenetic analyses have challenged the validity of Caryophylliidae on the basis of both mitochondrial (Romano & Cairns 2000; Le Goff-Vitry *et al.* 2004; Kitahara *et al.* 2010a,b; Stolarski *et al.* 2011) and nuclear (Cuif *et al.* 2003; Barbeitos *et al.* 2010; Stolarski *et al.* 2011) markers (see also Huang 2012). Although to date relatively few representatives have been included in molecular analyses, the family 'Caryophylliidae' form at least eight different clusters that are scattered throughout both the 'Complex' and 'Robust' coral clades. Two of these clusters centre around the caryophyliid genera *Caryophyllia* (Kitahara *et al.* 2010a) and *Dactylotrochus* (Kitahara *et al.* 2012).

Deltocyathus is the third most speciose of 'caryophylliid' genera, comprising 25 extant species, and is particularly interesting due to its position in preliminary molecular phylogenetic analyses (Kitahara *et al.* 2010b). *Deltocyathus*

spp. are azooxanthellate (heterotrophic) and occur in all but the polar oceans between 44 and 5080 m. The macromorphology of the genus has been recently reviewed (Kitahara & Cairns 2009), and in molecular analyses, most *Deltocyathus* representatives studied clustered as a well-supported clade which, with *Anthemiphyllia patera costata* as sister group, formed the most basal lineage of the 'Robust' coral clade (Kitahara *et al.* 2010b). The sole exception to this pattern was *D. magnificus*, which unexpectedly clustered with the 'Complex' coral family Turbinoliidae (Kitahara *et al.* 2010b; Stolarski *et al.* 2011).

On the basis of additional molecular data, we propose elevating the genus *Deltocyathus* to family level (Deltocyathiidae fam. n.) within the 'Robust' coral clade and report the identification of micromorphological characters that support this re-ranking. The phylogenetic position of *D. magnificus*, however, remains enigmatic. On the basis of both classical morphological criteria and the new micromorphological characters identified here, *D. magnificus* is a typical deltocyathiid. However, despite adding more molecular data and the inclusion of additional specimens, *D. magnificus* consistently clustered as a sister group of turbinoliids irrespective of the molecular marker or the phylogenetic reconstruction method employed. *D. magnificus* may therefore be the first documented case of morphological convergence at the microstructural level among scleractinian corals.

Material and methods

Morphological

Specimens analysed in the present study were collected using Warren dredge or beam-trawl off New Caledonia (MNHN and IRD-Nouméa) and Australian (CSIRO) waters between 1994 and 2005, and preserved dry (only skeleton) or in high-grade ethanol. Specimens were identified based on macromorphological characters following Cairns (1995, 1998, 1999, 2004), Cairns & Zibrowius (1997) and Kitahara & Cairns (2009). Data on specimens sampling dates and locations are found in the study by Kitahara *et al.* (2010b) and Stolarski *et al.* (2011). Specimens preserved in ethanol and covered by the polyp had tissue from one half-system extracted using forceps, enabling the analysis of the skeleton. In an attempt to preserve the specimens illustrated in this study, only small pieces of dried skeleton (usually between 1/3 and 1/2) were subjected to destructive analyses. The remaining skeleton of each specimen is deposited at the Australian Commonwealth Scientific and Research Organization (CSIRO – Hobart), National Museum of Natural History (NMNH, Washington, DC) or the Muséum national d'Histoire naturelle (MNHN, Paris, France).

Complete tissue removing was achieved by overnight immersion in 3% sodium hypochlorite (NaOCl) solution. Skeletons were rinsed in Milli-Q water and washed in an ultrasonic cleaner for ca. 2 min. All species were studied with transmitted light microscope (TLM) and scanning electron microscope (SEM). Thin (ca. 30-μm-thick) sections of various skeletal elements were observed and photographed with a Nikon (Tokyo, Japan) Eclipse 80i TLM. Skeletal microarchitectural and microstructural features were visualized with Philips (Amsterdam, Netherlands) XL 20 or Jeol (Tokyo, Japan) JSM5410LV SEM microscopes. Specimens were observed intact (septal surfaces), as broken but not etched skeletal samples or as broken/polished and etched samples. Transverse or longitudinal polished or broken sections of septa were exposed for ca. 20 s of etching in 0.1% formic acid solution, following Stolarski (2003). The etched samples were rinsed with distilled water and air-dried. Once dried, the samples were mounted on stubs and sputter-coated with gold or conductive platinum film. Resulting thin sections and skeletal fragments attached to microscope stubs are housed at the Institute of Paleobiology (ZPAL, Warsaw) or at the Muséum national d'Hisotire naturelle (MNHN, Paris).

A fossil (Middle Jurassic, Bajocian) specimen of *Deltocyathus* sp. is deposited at the Museo di Paleontologia Università di Roma (MPUR).

Molecular

Tissue was collected from whole mesenteries dissected out using forceps and washed in absolute ethanol. Genomic DNA was extracted using the DNeasy Tissue Kit (QIAGEN, California, USA) following the manufacturer's instructions. For each species, the concentration of genomic DNA extracted was measured using a Nanodrop 1000 (Thermo Scientific, Massachusetts, USA), and an aliquot of the genomic DNA was diluted or concentrated to achieve the final concentration of 25 ng/μL. Polymerase chain reaction (PCR) amplification of the mitochondrial (16S rDNA and CO1) and nuclear (28S rDNA) followed Stolarski *et al.* (2011), using the primers listed in supplementary information 1.

Sequences were initially aligned using ClustalW version 2 (Larkin *et al.* 2007) and manually edited using JalView version 8.0 (Clamp *et al.* 2004). Poorly aligned positions in the 28S rDNA alignment were extracted using Gblocks (Castresana 2000). The appropriate model of DNA substitution for each gene was determined as by hierarchical likelihood ratio test implemented in MrModeltest (Nylander 2004).

Two different approaches were tested using sequences determined here and others retrieved from GenBank (Table 1). The first approach was based on concatenated CO1 and 28S rDNA sequences and included a broad range of scleractinian representatives that were selected based on previous studies that included azooxanthellated

Table 1 Taxonomic information, sampling locations, voucher deposition, GenBank accession numbers and references for mitochondrial cytochrome oxidase subunit 1 and nuclear 28S rDNA genes used in the present study. 16S rDNA GenBank accession numbers are provided by Romano & Cairns (2000), Le Goff-Vitry *et al.* (2004) and Stolarski *et al.* (2011). Asterisk denotes new sequences

Family	Genus	Species	GenBank accession number (reference)		Sampling location (expedition and station)/ voucher deposition (Only for new sequenced material)
			CO1	28S	
Anthemiphyllidae	*Anthemiphyllia*	*patera costata*	HM018604 (1)	HQ439609 (6)	–
'Caryophylliidae'	*Caryophyllia*	*lamellifera*	HM018616 (1)	HQ439616 (6)	–
		rugosa	HM018618 (1)	HQ439620 (6)	–
	Cladocora	*arbuscula*	AB117292 (2)	AF549226 (7)	–
	Conotrochus	*funicolumna*	HM018621 (1)	HQ439629 (6)	–
	Stenocyathus	*vermiformis*	HM018619 (1)	HQ439681 (6)	–
	Stephanocyathus	*spiniger*	HM018665 (1)	HQ439638 (6)	–
Deltocyathiidae fam.n.	*Deltocyathus*	*corrugatus*	–	JX486105 (*)	New Caledonia (Bathus 4, CP899)/NMNH
		inusitatus	HM018626 (1)	JX486108 (*)	New Caledonia (Norfolk 2, DW2157)/NMNH
		magnificus	HM018627 (1)	HQ439634 (6)	–
		ornatus	HM018628 (1)	–	–
		rotulus	HM018629 (1)	–	–
		sarsi	HM018630 (1)	JX486106 (*)	Australia (SS1005, 85-14)/CSIRO Hobart
		suluensis	HM018631 (1)	JX486109 (*)	New Caledonia (Norfolk 2, CP2143)/NMNH
		vaughani	–	JX486107 (*)	New Caledonia (Norfolk 2, DW2049)/NMNH
Dendrophylliidae	*Balanophyllia*	*cornu*	HM018605 (1)	HQ439647 (6)	–
	Enallopsammia	*rostrata*	HM018632 (1)	HQ439654 (6)	–
Faviidae	*Caulastraea*	*furcata*	AB117274 (2)	AF549224 (7)	–
	Diploastrea	*heliopora*	AB117290 (2)	AF549227 (7)	–
	Favia	*fragum*	AY451351 (3)	EU262856 (8)	–
		stelligera	AB117264 (2)	AF549223 (7)	–
	Leptoria	*phrygia*	AB117273 (2)	AF549228 (7)	–
	Manicina	*areolata*	AB117227 (2)	AF549255 (7)	–
	Montastraea	*annularis*	AB117260 (2)	AF549229 (7)	–
		curta	AB117278 (2)	AF549230 (7)	–
		franksi	AP008976 (4)	EU262849 (8)	–
Flabellidae	*Flabellum*	*lamellulosum*	HM018640 (1)	HQ439661 (6)	–
	Javania	*fusca*	HM018652 (1)	HQ439666 (6)	–
Gardineriidae	*Gardineria*	*hawaiiensis*	GQ868678 (1)	GQ868673 (6)	–
		paradoxa	GQ868682 (1)	GQ868671 (6)	–
Meandrinidae	*Dendrogyra*	*cylindrus*	AB117299 (2)	AF549249 (7)	–
	Dischocoenia	*stokesi*	AB117298 (2)	EU262875 (8)	–
Micrabaciidae	*Letepsammia*	*formosissima*	GQ868685 (1)	GQ868668 (6)	–
	Rhombopsammia	*niphada*	GQ868683 (1)	GQ868674 (6)	–
Mussidae	*Lobophyllia*	*corymbosa*	AB117241 (2)	AF549237 (7)	–
	Mussa	*angulosa*	AB117239 (2)	EU262869 (8)	–
Oculinidae	*Galaxea*	*fascicularis*	AB441201 (5)	AF263360 (7)	–
	Oculina	*diffusa*	AB117293 (2)	AF549240 (7)	–
Pectiniidae	*Echinophyllia*	*aspera*	AB117252 (2)	AF549241 (7)	–
Pocilloporidae	*Pocillopora*	*verrucosa*	AB441230 (5)	AF549252 (7)	–
	Stylophora	*pistillata*	AB441231 (5)	AF549253 (7)	–
Poritidae	*Porites*	*porites*	DQ643837 (5)	EU262878 (8)	–
Siderastreidae	*Siderastrea*	*radians*	AB441212 (5)	EU262861 (8)	–
Turbinoliidae	*Cyathotrochus*	*pileus*	HM018623 (1)	HQ439682 (6)	–
	Tropidocyathus	*lessoni*	HM018669 (1)	EU262782 (6)	–

(1) - Kitahara *et al.* (2010b); (2) - Fukami *et al.* (2004); (3) - Shearer & Coffroth (2008); (4) – Fukami & Knowlton (2005); (5) - Fukami *et al.* (2008); (6) - Stolarski *et al.* (2011); (7) - Cuif *et al.* (2003); (8) - Barbeitos *et al.* (2010); (*) - Present study; NMNH – National Museum of Natural History, Washington D.C.; CSIRO – Commonwealth Scientific and Industrial Research Organization, Hobart.

scleractinians in molecular phylogenetic analysis (i.e. Kitahara *et al.* 2010b; Stolarski *et al.* 2011). This phylogenetic inference aimed to validate the position of the *Deltocyathus* clade within the order. The second approach included two phylogenetic reconstructions based on 16S rDNA (data not shown) and 28S rDNA and aimed to check the

intriguing position of *D. magnificus*. Phylogenetic analyses were performed using PhyML (Guindon & Gascuel 2003) for maximum likelihood (ML) and MrBayes version 3.1.2 (Huelsenbeck & Ronquist 2001) for Bayesian inference (BI). ML analyses were performed under the GTR model with a non-parametric Shimodaira–Hasegawa-like (SH) procedure, as well as with 104 bootstrap replicates. For the BI, two independent runs each containing four Markov Monte Carlo (MCMC) chains proceeded for 10 million generations, with trees being sampled every 1000 generations. The first quarter of the 10 000 saved topologies was discarded as burn-in for each run, ensuring that –l nL had plateaued. In addition, average standard deviation of split frequencies between BI runs was <0.001. Remaining topologies were used to calculate the posterior probability.

Results

Molecular

For *Deltocyathus inusitatus*, *D. magnificus*, *D. sarsi* and *D. suluensis*, sequences were obtained for both CO1 and 28S rDNA. However, for some other species, data for one or other molecular marker could not be obtained despite the use of a number of different DNA polymerases and PCR protocols. These failures are most probably a consequence of sample preservation conditions. For *D. corrugatus* and *D. vaughani*, only 28S rDNA was obtained, whereas for *D. ornatus* and *D. rotulus*, only CO1 data were acquired. In addition, attempts to amplify and sequence the (mitochondrial) 16S rDNA gene were made, but these failed in all cases except that of *D. magnificus* (data not shown).

For each gene analysed, the sequences from *Deltocyathus* species were aligned with published data for representatives of most scleractinian families (Table 1). After manual editing, the 28S rDNA alignment consisted of 620 bp, 247 of which were variable and 184 (~ 29.6%) were phylogenetically informative. The CO1 data were unambiguously alignable, and in this case, the alignment consisted of 595 bp in total, of which 263 positions were variable and 224 (~ 37.6%) phylogenetically informative. In terms of base composition at both CO1 and 28S rDNA loci, *D. magnificus* differed significantly to all other *Deltocyathus* representatives (Table S2). Pairwise analysis of the CO1 and 28S rDNA sequences conducted using the maximum composite likelihood method in MEGA ver. 4 (Tamura *et al.* 2007) confirmed that *D. magnificus* is genetically divergent to all of its congeners (Table S2). Average distance comparison (Kimura 2 under gamma distribution) of the CO1 data indicates that all of the *Deltocyathus* species studied, except *D. magnificus*, are more closely related to 'Robust'' Scleractinia than to representatives of the ''Complex'' or ''Basal'' corals (Table S2). However, analysis of the 28S rDNA sequences implied a closer affinity of most *Deltocyathus* species with ''Basal'' scleractinians. The latter result is attributed to the basal position of the *Deltocyathus* clade within the ''Robust'' coral clade (see Fig. 1). Uniquely among *Deltocyathus* species studied, the sequences from *D. magnificus* were always closer to those of turbinoliids in the ''Complex'' coral clade.

Phylogenetic analyses based on single genes recovered all of the *Deltocyathus* species except *D. magnificus* as a monophyletic group, and always placed the latter among ''Complex'' corals; this counterintuitive position was also seen in phylogenetic reconstructions based on 16S rDNA data (Fig. S1). However, the CO1 and 28S rDNA analyses differed with respect to the position of the *Deltocyathus* cluster; on the basis of CO1 data, *Deltocyathus* is basal within the ''Robust', whereas in 28S rDNA analyses, *Deltocyathus* clustered with 'Basal' Scleractinia. This latter position is most likely a long-branch attraction artefact due to nucleotide compositional biases; 28S rDNA analyses that exclude 'Basal' scleractinians place the *Deltocyathus* clade within 'Robust' corals (data not shown). Further investigation is needed to resolve this long-branch attraction problem, but more extensive taxon sampling ('one of the most important determinants of accurate phylogenetic estimation'; Heath *et al.* 2008: 239) may clarify the matter.

To better understand the evolutionary position of *Deltocyathus* species, the CO1 and 28S rDNA sequences were concatenated and aligned with data for representatives of other families, resulting in a data matrix of 1215 bp that included 43 scleractinians (21 exclusively azooxanthellate) representing 35 genera belonging to 15 families (Table 1). ML (SH-like and bootstrap) analyses of this matrix returned log-likelihood values of -10132.21. The Bayesian convergence diagnostic returned a potential scale reduction factor between 1.000 and 1.003, and -10157.52 as the arithmetic mean of the best state likelihood values between both cold chains (two runs).

Following Kitahara *et al.* (2010b) and Stolarski *et al.* (2011), representatives from the 'Basal' scleractinian clade (Gardineriidae and Micrabaciidae) were used to root the phylogeny. Both ML and BI methods gave strong support for the two major clades (i.e. 'Complex' and 'Robust') of Scleractinia implied by previous studies (Romano & Palumbi 1996, 1997; Romano & Cairns 2000; Chen *et al.* 2002; Cuif *et al.* 2003; Le Goff-Vitry *et al.* 2004; Barbeitos *et al.* 2010). However, the modest statistical support for the early split between these two clades seen here is probably due to the tendency of CO1-based phylogenetic reconstructions to place the 'Robust' corals as radiating from the 'Complex' clade (see Fukami *et al.* 2008; Kitahara *et al.* 2010b). In the phylogenetic tree resulting from the analyses of the concatenated CO1/28S rDNA data, the 'Complex'

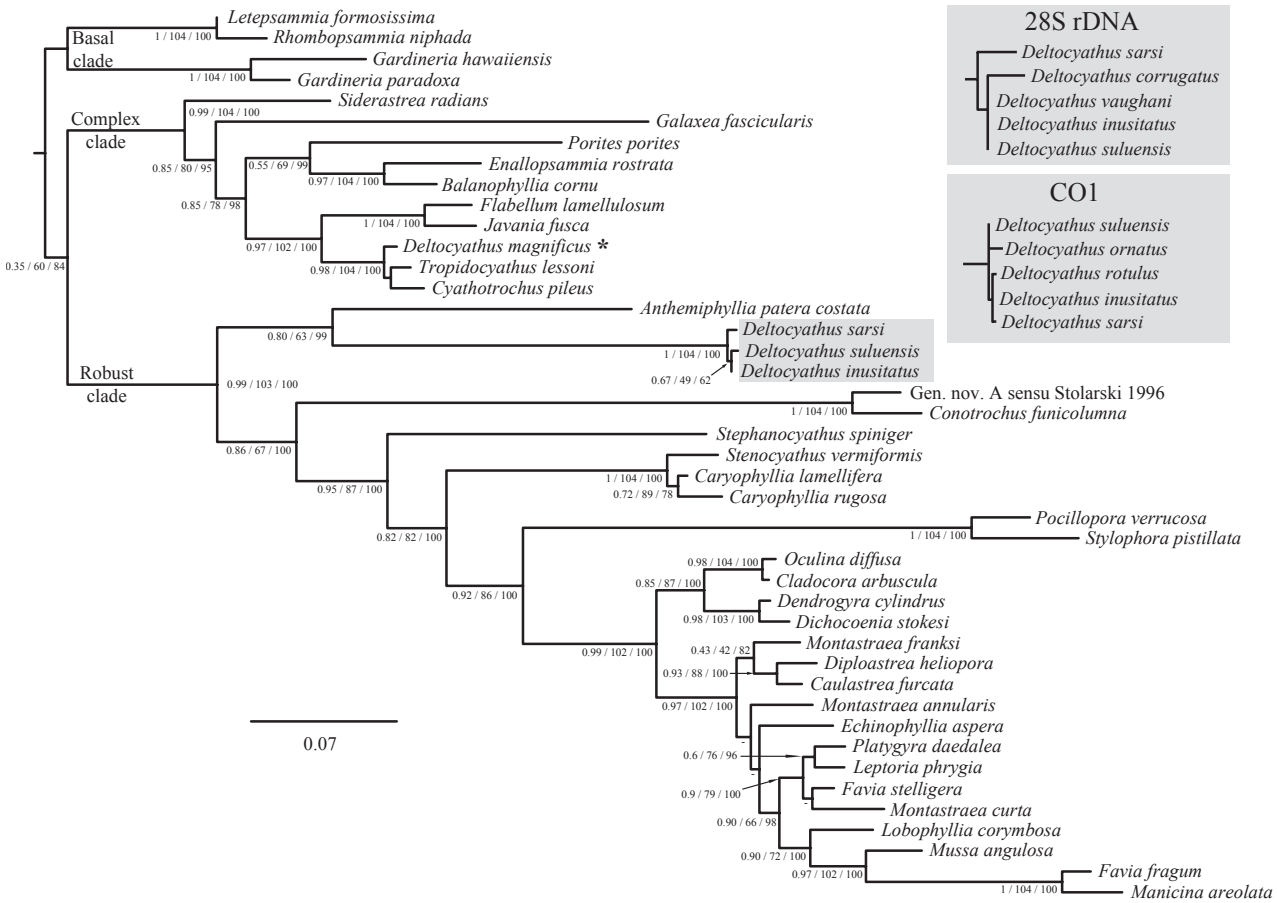

Fig. 1 —A. Phylogenetic tree of Scleractinia using the 5'end of the cytochrome oxidase subunit 1 (CO1) gene and the 1st and 2nd domains of the 28S rDNA with gardineriids and micrabaciids as outgroups. Numbers near nodes are ML SH-like values, ML bootstrap values (104 replicates) and the Bayesian posterior probabilities (percentages), respectively. Asterisks highlight the position of *D. magnificus* within 'Complex' corals. —B. The *Deltocyathus* clade recovered using partial 28S rDNA. —C. The *Deltocyathus* clade recovered using partial CO1. In an effort to rule out contamination issues during DNA extraction and/or PCR, 3 additional specimens of *D. magnificus* from different sampling locations had genomic DNA extracted and CO1 and 16S rDNA sequenced, resulting in identical sequences (both genes) to the ones previously used to infer the phylogenies.

clade contained representatives of the (morphologically defined) families Siderastreidae, Oculinidae, Poritidae, Dendrophylliidae, Flabellidae and Turbinoliidae, as well as *D. magnificus*, whereas the 'Robust' clade comprised all of the other *Deltocyathus* species, as well as members of families Anthemiphyllidae, Caryophylliidae, Stenocyathidae, Pocilloporidae, Oculinidae, Faviidae, Meandrinidae, Mussidae and Pectiniidae.

Morphological

Macromorphological characters – Macromorphologically, all 25 extant species that belong to the genus *Deltocyathus* are characterized in having a solitary, discoidal to patellate corallum. With the exception of *D. halianthus*, adult representatives of this genus are unattached, probably living on soft (sand/mud) substrate. Costae are well developed at least near the calicular edge (Fig. 2, A5-I5 [light-blue arrows]) and are separated by intercostal spaces that bear a row of small desmocyte attachment scars (Fig. 2, A5-I5 [yellow arrows]). In some species (e.g. *D. corrugatus*), 6–12 costal spines extend beyond the calicular edge (Fig. 2, B1, D1, F1) that together with basal shape (flat to conical, rarely slightly concave) are purported to be strategies to improve corallum stability with respect to substrate and currents. The vast majority of the species that do not have costal spines display a lanceted calicular edge, in which triangular to rectangular lancets (or apexes) correspond to costal projections beyond calicular edge (e.g. *D. rotulus* – Fig. 2, G1-2). Adult specimens usually have four to five septal cycles (48–96 septa), of which the primary and sometimes the secondary are

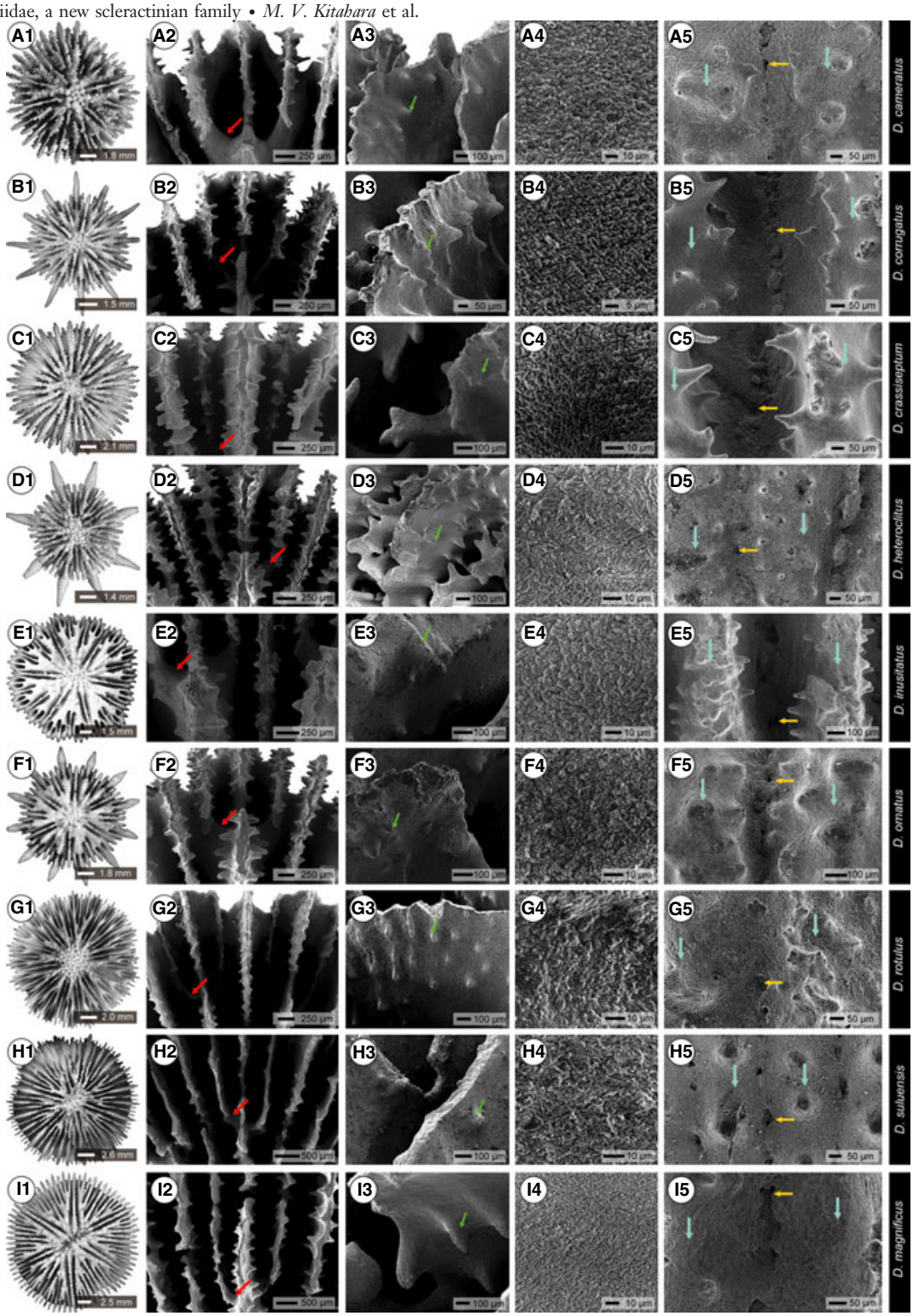

Fig. 2 Calicular views of 9 adult *Deltocyathus* species (subscripted with '1'); close-up of last cycle septa fusing to its adjacent septa or pali (red arrows) and some showing lancets formed beyond calicular edge (subscripted with '2'); septal faces bearing well-developed aligned granules (green arrows) (subscripted with '3'); enlarged view of septal face composed of crystal clusters (subscripted with '4'); close-up of base near calicular edge showing well-developed granular costae (blue arrows) and porous intercostal spaces (yellow arrows). — A1-5. *D. cameratus*; — B1-5. *D. corrugatus*; - C1-5. *D. crassiseptum*; —D1-5. *D. heteroclitus*; —E1-5. *D. inusitatus*; —F1-5. *D. ornatus*; —G1-5. *D. rotulus*; —H1-5. *D. suluensis*; —I1-5. *D. magnificus*.

independent, usually extending from the columella to or beyond the calicular edge. The upper septal edge is straight to slightly sinuous. The axial septal edges are usually entire; however, those of the last cycle (S4 or S5) are often lacerated. The lower axial edge from septa of the last cycle (S4 or S5) fuses to its flanking septa (S3 or S4) or respective pali (P3 or P4). The septal faces bear rows of granules aligned perpendicular to the upper septal edge, giving to some species a robust appearance. Well-developed pali are present before all but last cycle of septa (*D. vaughani* is the only congener to have pali before all septal cycles).

Pali are usually well separated from their respective septa by a deep and narrow notch and display axial edges that fuse to adjacent lower cycle septal faces/pali forming the characteristic delta-shaped chevrons (e.g. Fig. 2 [red arrows], this distinguishes *Deltocyathus* spp. from all other 'caryophylliids'). In all septal systems, each pair of P3 always fuses to the P2. The columella is papillose and composed of a few to several regular shaped rods usually arranged in an elliptical field.

Micromorphological and Microstructural characters – At low/moderate magnifications (e.g. Fig. 2, subscripted with '2' and '3'), *Deltocyathus* skeletal surfaces are relatively smooth, but septal faces are covered with pointed granules aligned in rows, which occasionally fuse, forming short irregular ridges perpendicular to the septal edge (e.g. Fig. 2, B3). At higher magnification (Fig. 2, subscripted with '4') however, most of the skeletal surfaces are covered with crystal clusters irregularly arranged, which usually are smaller than 2 μm in length. The crystal clusters are less prominent at the tips of septal granules. Overall, two main components are typically recognized in the skeleton of the sectioned *Deltocyathus* coralla: rapid accretion deposits (RAD) and thickening deposits (TD) (Fig. 3, B, F and Fig. 4, B, F, J). According to Stolarski (2003) and Brahmi *et al.* (2010), RAD are skeletal deposits enriched in organic components formed within well-differentiated regions of skeletal rapid accretion. On the other hand, TD are skeletal structures deposited outside the areas of rapid accretion (typically consisting of layers of fibres continuous with those of RAD) and are poorer in organic components. All *Deltocyathus* representatives examined herein have in adult stage a septothecate corallum wall composed of thickened outer parts of septa.

The septotheca is distinguished in transverse sections of distal parts of coralla with rapid accretion deposits visible only in the narrow mid-septal zone (Fig. 3, A, B, E, F and Fig. 4, A, B, E, F, I, J) flanked with thickening deposits formed by successive layers of bundles of fibres. Rapid accretion deposits of trabeculothecal segments, which are typical of ontogenetically earlier type of the wall, are not recognizable at the adult stage (see Stolarski 1995).

In longitudinal sections of septa, RAD and TD are recognizable as alternations of darker and brighter compo-

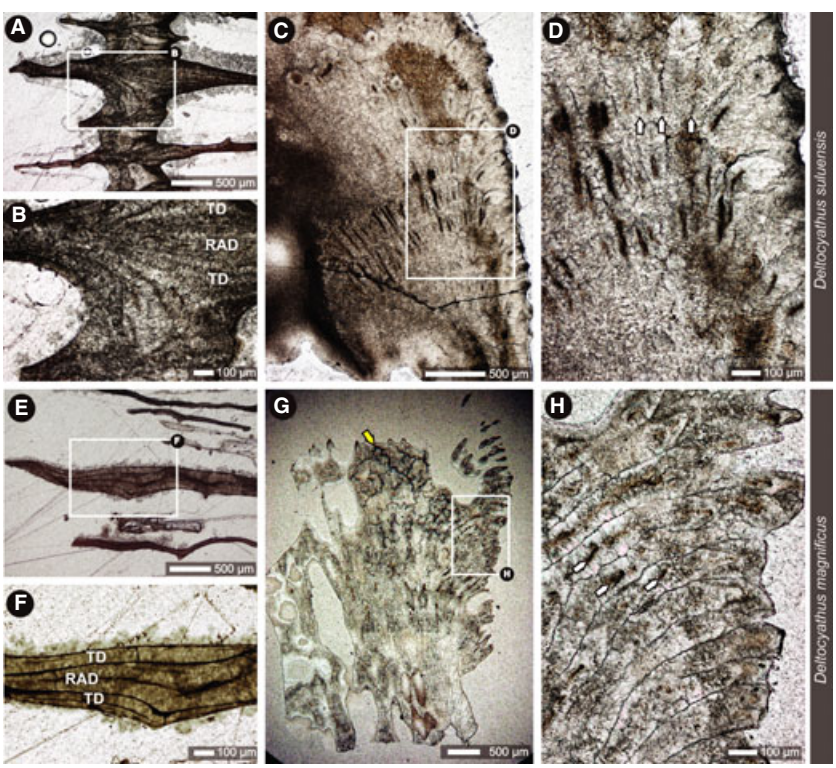

Fig. 3 Skeletal microstructure of *Deltocyathus suluensis* (R-SCL-588) (A–D) and *D. magnificus* (R-SCL-589) (E–H) that based on molecular markers group with 'Robust' and 'Complex' scleractinian clades, respectively (A, B, E, F - transverse sections; C, D, G, H - longitudinal sections across septa). In *D. magnificus* and other species of *Deltocyathus* (see also Fig. 4), rapid accretion deposits (RAD) are located in narrow mid-septal zone (A, B, E, F) and separated from each other ca. 50–100 μm (D, H); layers of septal thickening deposits (TD) flank each RAD and are formed by successive layers of bundles of fibres. Reticulated pattern in C (yellow arrow) is an artefact resulted from uneven adherence of the skeletal slice to the glass. White arrows indicate RAD.

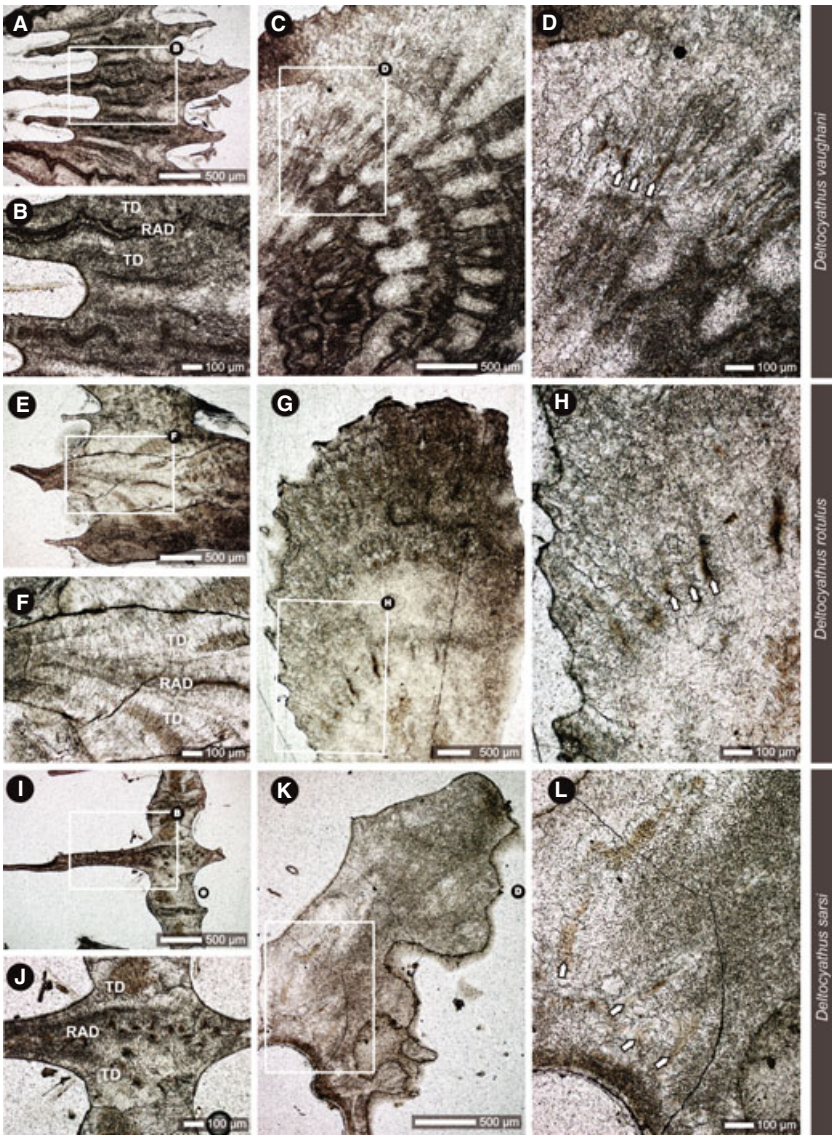

Fig. 4 Skeletal microstructure of *Deltocyathus vaughani* (R-SCL-592) (A–D), *D. rotulus* (R-SCL-594) (E–H) and *D. sarsi* (R-SCL-595) (I–L) that based on molecular markers (CO1 and 28S rDNA) group with *D. suluensis* (Fig. 1) within 'Robust' corals. All examined species show macromorphological and microstructural characteristics of *Deltocyathus* (see caption Fig. 3). White arrows mark RAD. In *D. sarsi*, rapid accretion centres are slightly dispersed in mid-septa region, but distances between them agree with those observed in other *Deltocyathus* species.

nents, and RAD zones are distributed every ca. 50–100 µm (Fig. 3, D, H). In these thin sections, boundaries between adjacent TD (developed around each RAD) are sharp, a feature traditionally described as trabeculae (see Wells 1956).

Discussion

In molecular phylogenetic analyses, most scleractinian families composed mainly of azooxanthellate representatives are monophyletic (Kitahara *et al.* 2010b; Stolarski *et al.* 2011). However, one exception to this is Caryophylliidae, the second most speciose scleractinian family, the validity of which has been challenged on the basis of analyses of 12S rDNA (Barbeitos *et al.* 2010), 16S rDNA (Romano & Cairns 2000; Le Goff-Vitry *et al.* 2004), CO1 (Kitahara *et al.* 2010b) and 28S rDNA (Stolarski *et al.* 2011) sequence data.

Of the 'caryophylliid' clades recovered in preliminary molecular analyses, which comprising most representatives of the genus *Deltocyathus*, is of particular interest as its position is consistent with the idea of a solitary and azooxanthellate ancestor for various scleractinian families that today are represented by zooxanthellate and colonial forms (e.g. Kitahara *et al.* 2012). The present study provides additional support for this idea; with the sole exception of *D. magnificus*, all of the *Deltocyathus* species examined formed a well-supported monophyletic group, which with *Anthemiphyllia patera costata* forms a basal lineage among 'Robust' scleractinians.

Whilst the 'oldest' *Deltocyathus* fossils reported so far are from the Upper Cretaceous (Squires 1958) and are more common from the Cenozoic (Palaeocene: Durham 1943; Eocene: Wells 1976), some fossil scleractinians from the Middle Jurassic (Bajocian) deposits of Monte Nerone, Pieia (Italy; for general stratigraphy of the site see Mariotti 2003), are morphologically indistinguishable from extant representatives of the genus (Fig. S4). These fossils (*Deltocyathus* sp.) have all of the diagnostic characters of the genus and are thus consistent with an earlier divergence of the *Deltocyathus* clade than has been assumed.

On the other hand, irrespective of the locus or method of analysis used, *D. magnificus* always grouped with turbinoliids (i.e. *Tropidocyathus lessoni* and *Cyathotrochus pileus*) in the 'Complex' coral clade and were clearly resolved from other *Deltocyathus* spp. Doubled identification discarded the possibility of misidentification of the specimens sequenced. In order to exclude the possibility of contamination or mislabelling, data were obtained for three additional specimens of *D. magnificus* from three different sampling stations; in each case, the sequences (CO1 and 16S rDNA) were identical to those from the initial specimens. The molecular characteristics of CO1 and 28S rDNA sequences obtained for members of the *Deltocyathus* clade are similar to published data from typical 'Robust' corals. In contrast, sequences from *D. magnificus* are more like those of 'Complex' corals – the 16S rDNA is longer, and there are base composition biases in the CO1 and 28S rDNA sequences that resemble those seen in other 'Complex' coral species (higher cytosine and lower thymine content than in 'Robust' corals).

Although in the case of most zooxanthellate corals macromorphological characters are often inconsistent with molecular phylogenetics, in a number of recent studies fine-scale morphological characters have provided support for molecular-based clades (Cuif *et al.* 2003; Benzoni *et al.* 2007, 2010; Budd & Stolarski 2009, 2010; Gittenberger *et al.* 2011; Kitahara *et al.* 2012). In the present case, however, microstructural analysis failed to resolve *D. magnificus* from other *Deltocyathus* spp. Morphologically, *D. magnificus* is a typical member of the genus, not only in terms of macro- and micromorphology but also in all of the microstructural characteristics investigated. All *Deltocyathus* representatives have a similar rather simple microstructural organization of the skeleton (narrow rapid accretion zone; thickening deposits in a form of not differentiated bundles of fibres) that also occurs in many 'Robust' and 'Complex' corals (including turbinoliids). The only observation made that is unique to *D. magnificus* was the presence of soft tissue (edge zone) completely covering the skeleton, a trait not seen in any other congener examined (polyp terminate few millimetres beyond calicular edge), but characteristic of all turbinoliids. If the grouping with turbinoliids is confirmed, this would be the first case of morphological convergence at the macro- and microstructural levels among scleractinian corals. More in-depth microstructural and biogeochemical studies (as have been carried out by Janiszewska *et al.* 2011 for micrabaciids) are pending, and this may settle the position of *D. magnificus* among 'Robust' or 'Complex' corals.

The position of the main *Deltocyathus* clade as one of the most basal lineages within the 'Robust' corals and its genetic distance from other Caryophylliidae representatives support the proposal for its elevation to family rank. However, before additional detailed microstructural and biogeochemical analyses are undertaken for *Deltocyathus* representatives, in order to investigate whether there are skeleton-embedded features that support the position of *D. magnificus*, the latter will be tentatively classified together with other *Deltocyathus* in the Deltocyathiidae fam. n. Accordingly, below we describe the new family, based on the original description of the genus *Deltocyathus* and subsequent information acquired herein.

Order Scleractinia
'Robust' Scleractinian Group
Family Deltocyathiidae (fam. n.)

Diagnosis – Solitary, discoidal to patellate, usually free in adult stage. Well-developed costae bisected by desmocyte attachment scars. Septa composed of rapid accretion deposits located in narrow mid-septal zone flanked by successive layers of thickening deposits composed of bundles of fibres. Pali before all but last septal cycle; within each system, the axial edges of each pair of P3 fuse to its common P2 near columella, forming characteristic chevrons (delta shaped). Pali may also be present before fourth septal cycle. Columella papillose.

Genera included – *Deltocyathus* Milne Edwards & Haime, 1848

Type species: *Turbinolia italica* Michelotti 1838, by monotypy.

According to Chevalier (1961), the Michelotti type specimens of *T. italica* (Tortona, Italy – Miocene) are lost.

For a complete list of Recent valid *Deltocyathus* species, see Kitahara & Cairns (2009) and Cairns (2009).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Primers used in the present study.

Table S2. Mitochondrial CO1 and nuclear 28S rDNA nucleotide composition from all *Deltocyathus* representatives sequenced to date, estimates of evolutionary divergence between them and their comparison to scleractinian clades (average distance [calculated based on Kimura 2 evolutionary model under gamma distribution]). Evolutionary divergence results are based on the pairwise analysis using partial CO1 and first and second domains of the 28S rDNA. Analyses were conducted using the maximum composite likelihood method in Mega4 (Tamura *et al.* 2004; 2007). All positions containing gaps and/or missing data were eliminated from the data set.

Fig. S1. Phylogenetic tree of Scleractinia using the partial 16S rDNA gene with Corallimorpharia as outgroup. Numbers over or under branches are ML SH-like values and the Bayesian posterior probabilities (percentages), respectively. Grey box highlights the Turbinoliidae + *Trochocyathus rhombcolumna* and *Deltocyathus magnificus* clade, the latter also highlighted in red. Attempts to amplify the 16S rDNA segment from other *Deltocyathus* representatives failed notwithstanding different PCR protocols tested.

Fig. S2. Early representative of *Deltocyathus* (*Deltocyathus* sp. - MPUR NS 71/3) from the Middle Jurassic (Bajocian) deposits of Monte Nerone, Pieia (Italy). Specimens of these solitary, discoidal corals show diagnostic characters

of the genus: P_3 fused with P_2 (P in B) near papillose columella (septa S_3 and S_4 marked in B) to form deltas (A, B, D), and well-developed costae (C). Illustrated specimens show pentameral organization of septa according to the formula: $5S_1 > 5S_2 > 20S_4 > 10S_3$.