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A New Shallow-Water Species, *Polycyathus chaishanensis* sp. nov. (Scleractinia: Caryophylliidae), from Chaishan, Kaohsiung, Taiwan

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Mei-Fang Lin, Marcelo V. Kitahara, Hiroyuki Tachikawa, Shashank Keshavmurthy, and Chaolun Allen Chen (2012) A new shallow-water species, *Polycyathus chaishanensis* sp. nov. (Scleractinia: Caryophylliidae), from Chaishan, Kaohsiung, Taiwan. *Zoological Studies* **51**(2): 213-221. A small population of a new species of zooxanthellate scleractinian coral, *Polycyathus chaishanensis* sp. nov., is described from shallow water (< 3 m) off Chiashan, Kaohsiung, an uplifted Pleistocene reef located on the southwest coast of Taiwan. *Polycyathus chaishanensis* sp. nov. is a zooxanthellate coral associated with *Symbiodinium* C1 and forms small encrusting colonies. *Polycyathus chaishanensis* sp. nov. differs from other *Polycyathus* by having (1) the smallest corallites (2.0-3.7 mm in calicular diameter) reported in the genus *Polycyathus*; (2) septa hexamerally arranged in 4 incomplete cycles displaying dentate or laciniate axial edges; (3) crispate and well-developed pali before the secondary septa; and (4) light brown pigmented pali/columellar elements. When expanded, vivid-red to brown polyps rise considerably above the calice, and long and slender tentacles are covered with white nematocyst batteries. *Polycyathus chaishanensis* is the only species of *Polycyathus* known from Taiwanese waters and appears to be endemic to a small region at Chaishan. The small population of this new species raises concerns as to its vulnerability to natural and anthropogenic threats.
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Key words: Scleractinia, *Polycyathus chaishanensis*, Zooxanthellae, Chaishan, Shallow water.

Described from specimens collected near St. Helena, in the South Atlantic Ocean, the genus *Polycyathus* Duncan, 1876 (Anthozoa: Scleractinia: Caryophylliidae) is characterized by small reptoid to plocoid colonies that form through corallites that grow close to the base of their neighbors and become sparser with age. The corallites are cylindrical to slightly conical in shape, bud from a common coenosteum or from stolons (Cairns 1995), and are epithecated. There are 3-5 irregularly arranged septal cycles, of which the last is usually incomplete, and the 1st and 2nd are the most distinct and exsert. Two crowns of well-developed pali (P1 and P2) are present before the 2nd and 3rd septal cycles, of which P2 is usually more difficult to distinguish from columellar elements than P3 (Wijsman-Best 1970). The fossa is deep and contains a papillose columella. According to Duncan (1876), septa that are not incised and the absence of endotheca are diagnostic characters of this genus.

Ranging from shallow to waters deeper

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than 400 m (Cairns 1999), the vast majority of *Polycyathus* representatives are reported from the Pacific Ocean (Fig. 1), of which 5 are known to occur in southern Pacific waters (*P. verrilli* Duncan 1876, *P. octuplus* Cairns 1999, *P. fulvus* Wijsman-Best 1970, *P. norfolkensis* Cairns 1995, and *P. andamanensis* Alcock 1893). In the northwestern Pacific, 3 *Polycyathus* species are described from the Philippines, in waters deeper than 35 m (Verheij and Best 1987). Among them, *P. hodgsoni* Verheij & Best 1978 and *P. marigondoni* Verheij & Best 1978 have the lowest and highest number of septal cycles (3 and 5, respectively) compared to their congeners.

In the present study, a new species of *Polycyathus* is described. This new species inhabits a shallow-water area of Chaishan, an uplifted reef developed about 0.6 Mya (Fig. 2). Chaishan is about 6 km long and is home to about 15.62% of coastal habitats of Kaohsiung City, southwestern Taiwan (CPAMI 2008). The beach at Chaishan is composed of scattered hard substrates of carbonaceous rocks of various sizes which originated from nearby coastal hills. The water column contains a high concentration of particles which increases the turbidity of the water and might be one of the contributing factors to the low number of scleractinian corals reported in this area. Nonetheless, the new species of *Polycyathus* described herein appears to be endemic to this small Taiwanese region, as so far, it has not been found anywhere else in Taiwan.

Mitochondrial (mt) 16S ribosomal (r)RNA gene sequences were amplified and aligned with previously published sequences from 8 representatives of morphologically related caryophylliid genera (including *P. muellerae* Abel 1959) and 13 representatives of non-caryophylliid families to investigate the validity of this genus. Following Kitahara et al. (2010a b), the phylogenetic analysis did not indicate that the Caryophylliidae is a monophyletic family, and also raises concerns about the validity of *Polycyathus*, which is one of the less-understood scleractinian genera.

MATERIALS AND METHODS

Specimens examined in the present study were collected by snorkeling in 2000, 2005, and 2008 from a tidal pool (< 3 m in depth) at Chaishan, Kaohsiung, Taiwan (22°38'18"N; 120°15'19"E) (Fig. 2). Colonies were photographed *in situ* using an Olympus SP350 camera (Center Valley, PA, USA) with an underwater housing. Collected specimens were bleached to remove soft tissues, rinsed with fresh water, thoroughly dried, and photographed using a Nikon D200 (Tokyo, Japan) camera. Morphological observations were carried out using an Olympus SZ-ST stereomicroscope equipped with an ocular graticule. Scanning electron microscopy (SEM) was performed on a FEI Quanta 200/Quorum PP2000TR FEI, 2007 (Hillsboro, OR,

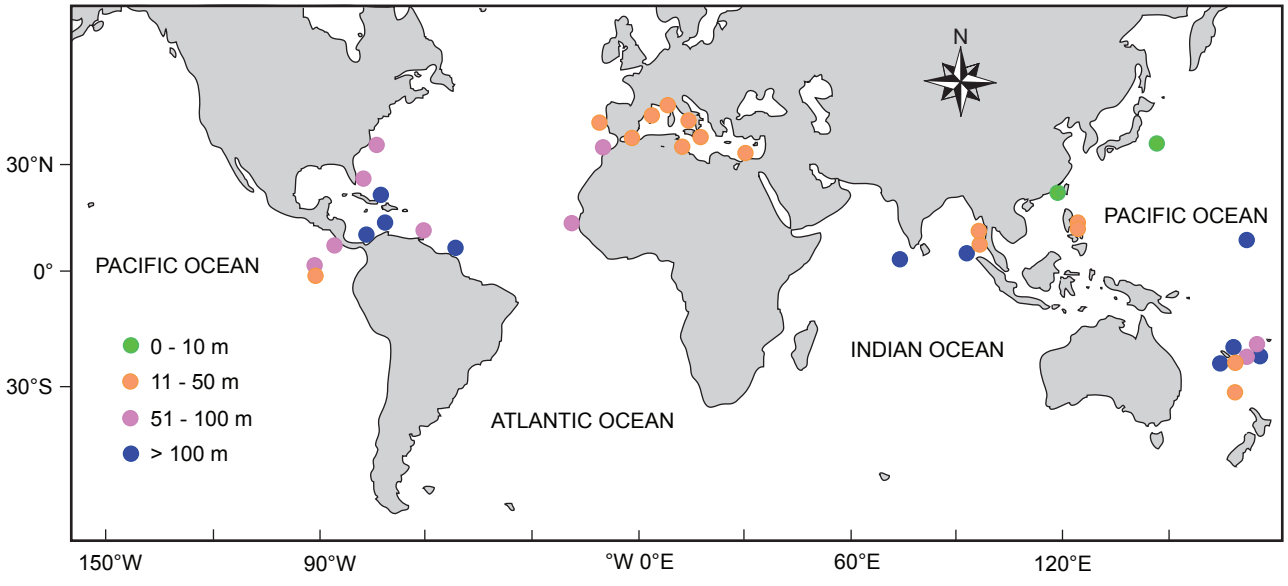

Fig. 1. Worldwide distribution and depths of *Polycyathus* spp.

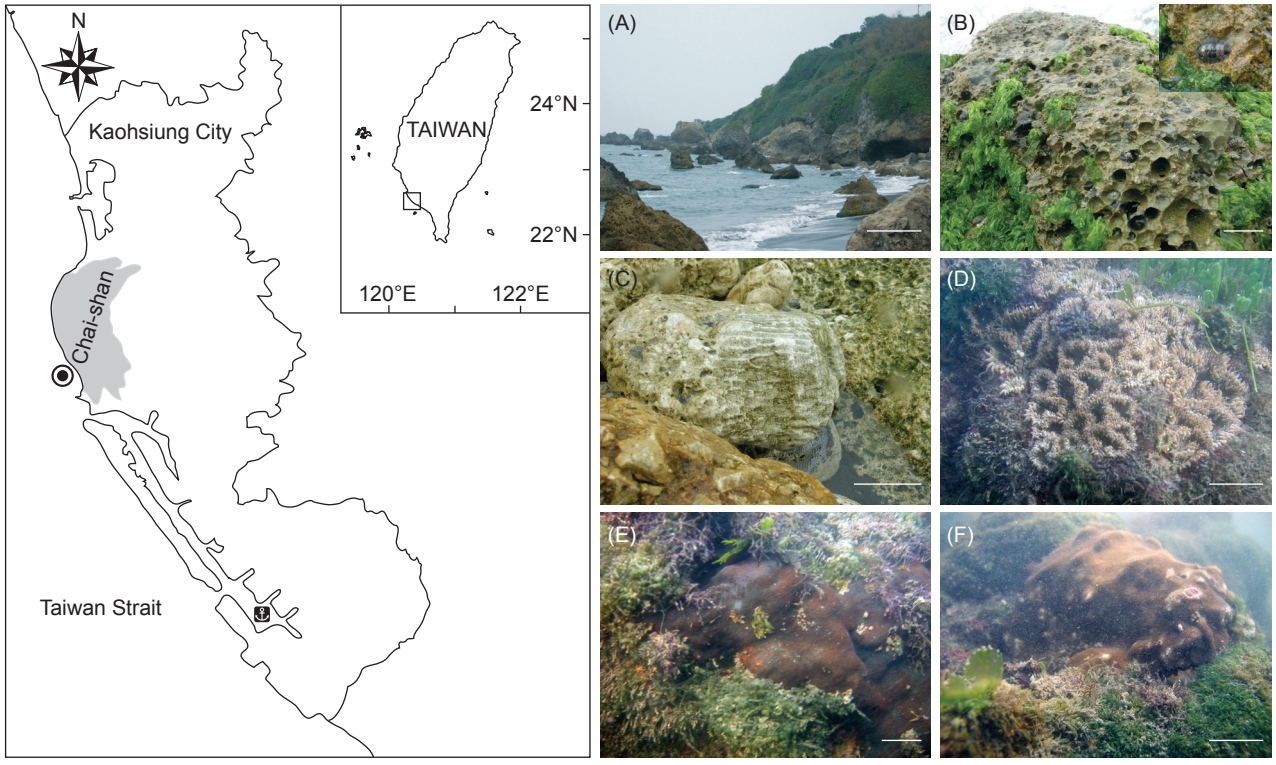

Fig. 2. Map of sampling localities of *Polycyathus chaishanensis* sp. nov. (A) Landscape of an uplifted coral reef; (B) patches of limestone dominated by *Ulva* sp., chiton, and barnacles; (C) ancient coral; (D) *Anthopleura* sp.; (E) *Psammocora* sp.; (F) *Porites okinawanensis*.

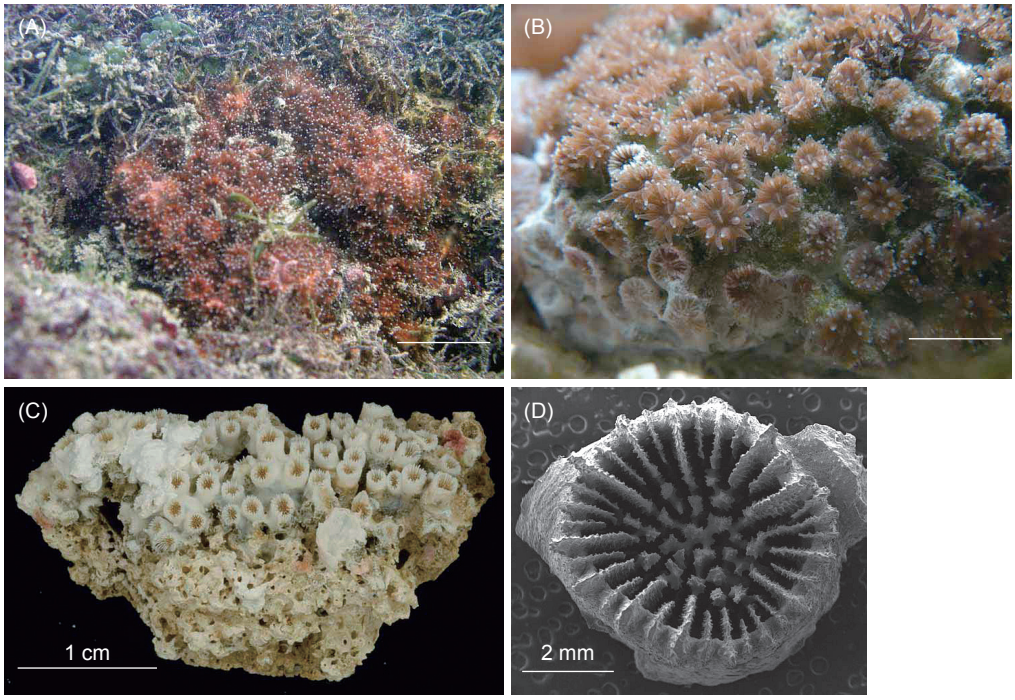

Fig. 3. *Polycyathus chaishanensis* sp. nov. (A) *Caulerpa racemosa* and calcified red algae; (B) a specimen with brown tissues indicating the presence of zooxanthellae; (C) colony view of the holotype (NMNS-6309-001) consisting of 73 corallites in different stages of development; (D) calicular view (SEM) of 1 corallite of the holotypic colony (NMNS-6309-001).

USA) instrument.

Skeleton vouchers were deposited at the National Museum of Natural Science (NMNS), Taichung, Taiwan and at the Museum of Tropical Queensland (MTQ), Townsville, Australia. In the morphological description, the following abbreviations were used: CD, calicular diameter; GCD, great CD; Sx, septa of the x order; Px, pali of the x order; and H, height. Tissue samples preserved in CHAOS solution (Fukami 2004) were used for DNA extraction.

Symbiodinium identification

Following LaJeunesse (2002), denaturing gradient gel electrophoresis (DGGE) of the internal transcribed spacer (ITS)-2 region was performed to identify the *Symbiodinium* clade present in *P. chaishanensis* sp. nov. The ITS-2 region was amplified using primers ITS2 clamp and ITSintfor 2 developed by LaJeunesse and Trench (2000). A polymerase chain reaction (PCR) was performed with a touch-down cycle according to LaJeunesse (2002). PCR products were subjected to electrophoresis for 15-16 h on denaturing gradient gels (45%-80%) using a CBS Scientific System (Del Mar, CA, USA). Gels were stained with SYBR green (Molecular Probes, Eugene, OR, USA) for 20 min, and photographed for further analysis. Bands were excised from the gel and sent for direct sequencing. Resulting sequences were deposited in the NCBI database (with accession nos.: 180016-180021)

Sequence analysis and phylogeny

Forty mt16S rDNA and the cytochrome *c* oxidase subunit I (COI) sequences, including these 2 regions from the complete mt genome of *P. chaishanensis* sp. nov (Lin et al. 2011), were retrieved from GenBank. This dataset contained 11 robust and 4 complex scleractinian families. Phylogenetic analyses were performed using MEGA 4.0 (Tamura et al. 2007) for Neighbor-joining (NJ) and MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001) for Bayesian inference (BI). The most appropriate model of nucleotides was determined to be HKY+I using MrModeltest vers. 2.3 (Nylander 2004). The NJ analyses were performed with 500 replicates, and for the BI, 2 runs each of 10^6 generations were calculated for each marker with topologies saved every 100 generations. The 1st quarter of the saved topologies were discarded as burn-in, and the remaining ones were used to calculate posterior probabilities.

RESULTS

Systematic description

Subclass Hexacorallia.
Order Scleractinia Bourne, 1900.
Suborder Caryophylliina Vaughan & Wells, 1943.
Family Caryophylliidae Dana, 1846.
Genus *Polycyathus* Duncan, 1876.
Polycyathus chaishanensis sp. nov.
Illustrations of the holotype are given in figures 3C, D, 4A-C; and illustrations of the paratype are given in figure 4D, E.

Materials examined: Holotype: NMNS-6309-001 (Taichung, Taiwan). Paratypes: NMNS-6309-002, NMNS-6309-003 (Taichung, Taiwan), and MTQ G64703 (Queensland, Australia, 1 specimen). Type locality: 22°38'18''N, 120°15'19''E (Taiwan), 3 m in depth.

Description: Small reptoid colonies formed by closely spaced cylindrical corallites arising from a common coenosteum or from stolons. Holotypic colony consisting of approximately 70 corallites. Extratentacular budding common; however, some corallites displaying intratentacular division. Calice circular to slightly elliptical. Largest corallite examined 3.65 × 3.73 mm in CD and 4.0 mm in H. Theca thick. Costae more prominent near calicular edge. All costae equal in width (about 0.21 mm wide), slightly convex, and bearing low, coarse granules. Intercostal striae deep and flat near calicular edge, becoming less distinct in direction of base. Coenosteum and theca white, but columellar elements usually light-brown pigmented. Vivid-red to dark brown sub-pellucid polyps considerably expanded above calicular edge; tentacles long, slender, with knobby end, and covered by small white verruca.

Septa hexamerally arranged in 4 incomplete cycles, according to formula: $S_1 \geq S_2 > S_3 > S_4$. Corallites < 2 mm in GCD with 12 or fewer septa, but larger corallites (up to 3.7 mm in GCD) with several pairs of S4 totaling up to 34 septa. S1 exsert (0.5-0.7 mm), with straight and almost-vertical axial edges sometimes bearing small, cylindrical (0.24 mm in diameter) palus. S2 only slightly less exsert and equal or narrower than S1. S3 less exsert, thinner, and about 2/3 width of S2. Axial edges of S1-S2 dentate, those of S3 laciniated. S4 1/2-2/3 width of S3. Well-developed P3 (sometimes bilobated) present before S3. If

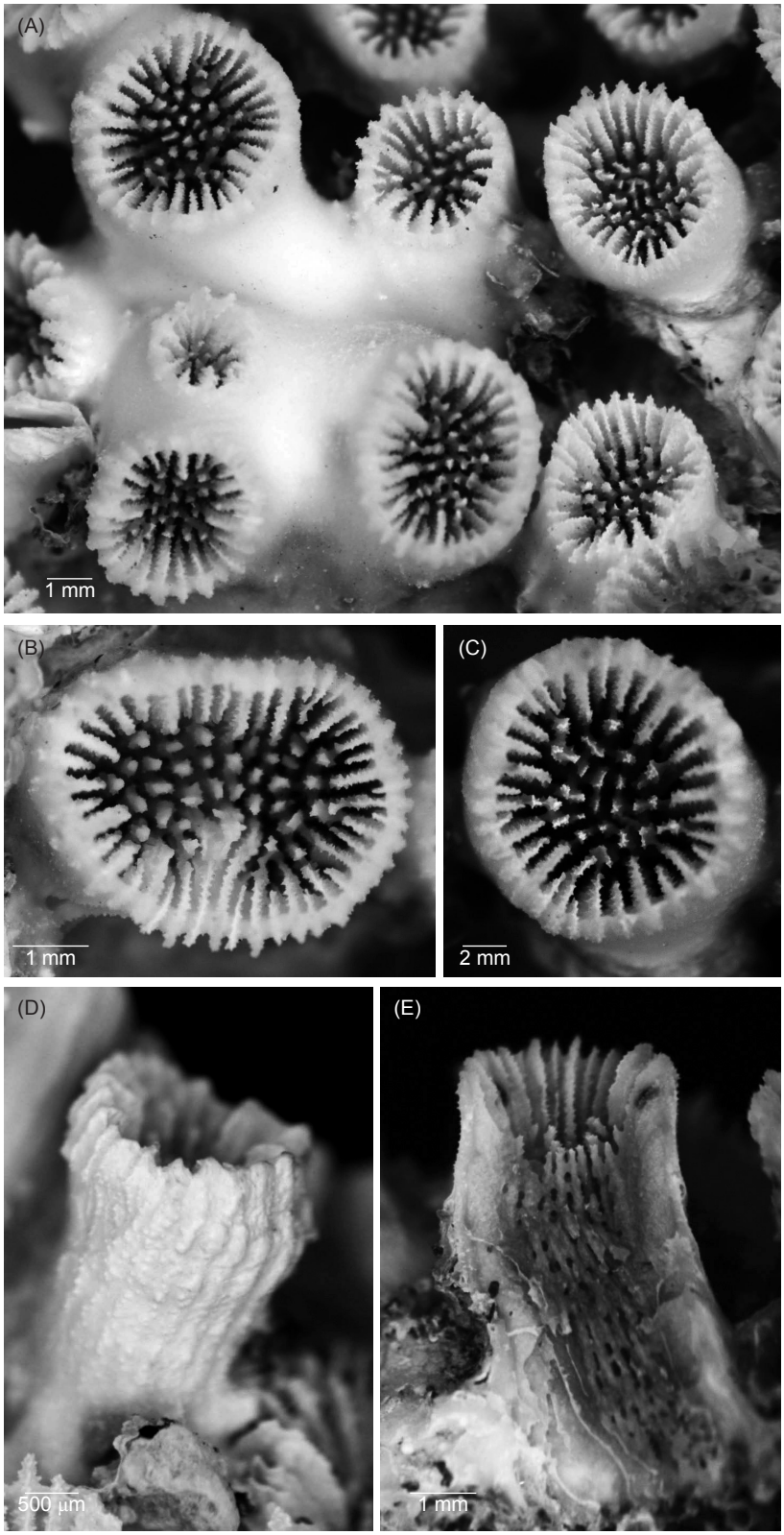

Fig. 4. (A) Calicular view of 1 corallite of the holotypic colony (NMNS-6309-001) undergoing extratentacular budding; (B) calicular view of 1 corallite of the holotypic colony (NMNS-6309-001) undergoing intratentacular budding; (C) calicular view of 1 corallite of the holotypic colony (NMNS-6309-001); (D) lateral view of a corallite from the paratype colony (NMNS-6309-002); (E) detail of columellar elements MTQ G64703.

present, P2 difficult to distinguish from columellar elements. Septal and palar faces bearing several pointed granules aligned perpendicular to septal/palar edges. Fossa moderately deep, containing elongate papillose columella. Columella composed of 5-7 slender, irregularly shaped rods.

Remarks: *Polycyathus chaishanensis* sp. nov. differs from all other known species of this genus by having a much smaller corallite. Twenty-one corallites examined from the holotype colony had a mean CD of 3.05 ± 0.26 mm (Fig. 5), whereas corallites among the other 18 valid *Polycyathus* species are significantly larger (mean CD of 4.38 ± 1.10 mm). In addition, *P. chaishanensis* sp. nov. has one of the shallowest bathymetric ranges known from representatives of this genus (≤ 3 m) (Fig. 1), and all colonies were found to inhabit tidal pools. Of the 18 extant *Polycyathus* species, 3 were described from the Atlantic Ocean (*P. atlanticus* Duncan, 1876 [depth unknown], *P. senegalensis* Chevalier 1966 [12-143 m], and *P. mayae* Cairns 2000 [110-309 m]; 5 from the Indian Ocean (*P. persicus* Duncan 1876 [depth unknown], *P. fuscomarginatus* Klunzinger 1879 [depth unknown], *P. verrilli* [depth unknown], *P. difficilis* Duncan 1889 [depth unknown], and *P. andamanensis* [depth unknown]); 1 species from the Mediterranean Sea (*P. muellerae* Abel 1959 [10-32 m]); and according to Cairns (1999), 9 species are known from Pacific waters (*P. palifera* Verrill 1869 [reef depth], *P. hondaensis* (Durham & Barnard 1952) [55-64 m], *P. fulvus* [30-50 m], *P. isabela* Wells, 1982 [14-23 m], *P. hodgsoni* [> 35 m]; *P. marigondoni* [35 m]; *P. furanaensis* Verheij & Best 1987 [6-52 m], *P. norfolkensis* [10-20 m], and *P. octuplus* [90-441 m]).

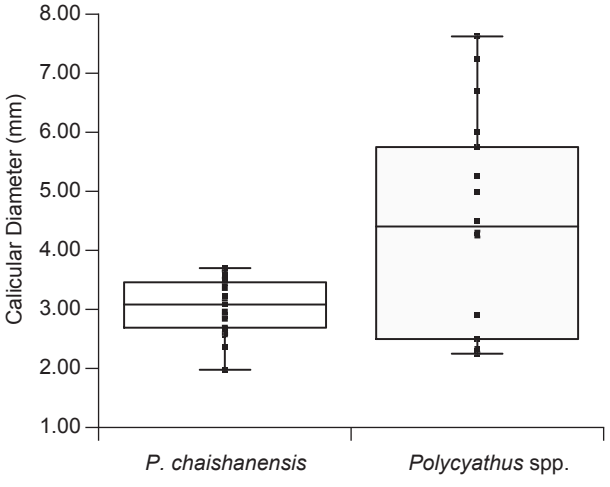

Fig. 5. Measurement of the calicular diameter (CD) of *P. chaishanensis* sp. nov. (21 corallites) and extant *Polycyathus* species (18 species). The CD of each *P. chaishanensis* corallite and its congeners are indicated by black circles in the box plot. The non-parametric Wilcoxon-Mann-Whitney rank sum test showed no significant difference (p = 0.1135) in calicular diameters between *P. chaishanensis* sp. nov. and extant *Polycyathus* species.

Among Pacific and Indian congeners that have small corallites, *P. chaishanensis* sp. nov. is most similar to *P. difficilis* (Mergui Archipelago). Both species have an exserted S1, indistinct P1, and S2 and S3 with dentate/laciniate axial edges. However, *P. chaishanensis* sp. nov. differs in having 4 incomplete cycles of septa, while *P. difficilis* has 3 cycles of septa.

Interestingly, DGGE from the ITS-2 confirmed the presence of *Symbiodinium* subclade C1 associated with *P. chaishanensis* sp. nov. Although Wijsman-Best (1970) described the association of zooxanthellae with *P. fulvus*, to date, all other representatives of this genus are considered azooxanthellate (Cairns et al. 1999). However, to reinvestigate this important ecological aspect of shallow-water *Polycyathus*, new samples enabling the examination of their tissue must be collected.

Etymology: This species is named for the uplifted reef in southern Taiwan (Chaishan) from which it was collected and to which it is possibly endemic.

Distribution: Known only from the sublittoral zone (< 3 m deep) near Chaishan, Kaohsiung, Taiwan (22°37'13"N, 120°15'56"E to 22°38'18"N, 120°15'19"E).

DISCUSSION

Phylogeny of *Polycyathus*

To test the hypothesis that *Polycyathus* is a natural genus, a 16S rRNA sequence was extracted from the *P. chaishanensis* sp. nov. mt genome (accession no.: NC 015642; Lin et al. 2011) and aligned with previously published sequences from 8 representatives of morphologically related caryophylliid genera and 13 representatives of non-caryophylliid families. Results of the phylogenetic analysis are summarized in figure 6, and following Romano and Cairns (2000), Le-Goff Vitry et al. (2004), and Fukami et al. (2008), sequences from 4 scleractinian species in the “complex” coral clade were used as an outgroup. Despite the fact that only 2 *Polycyathus* species were represented in

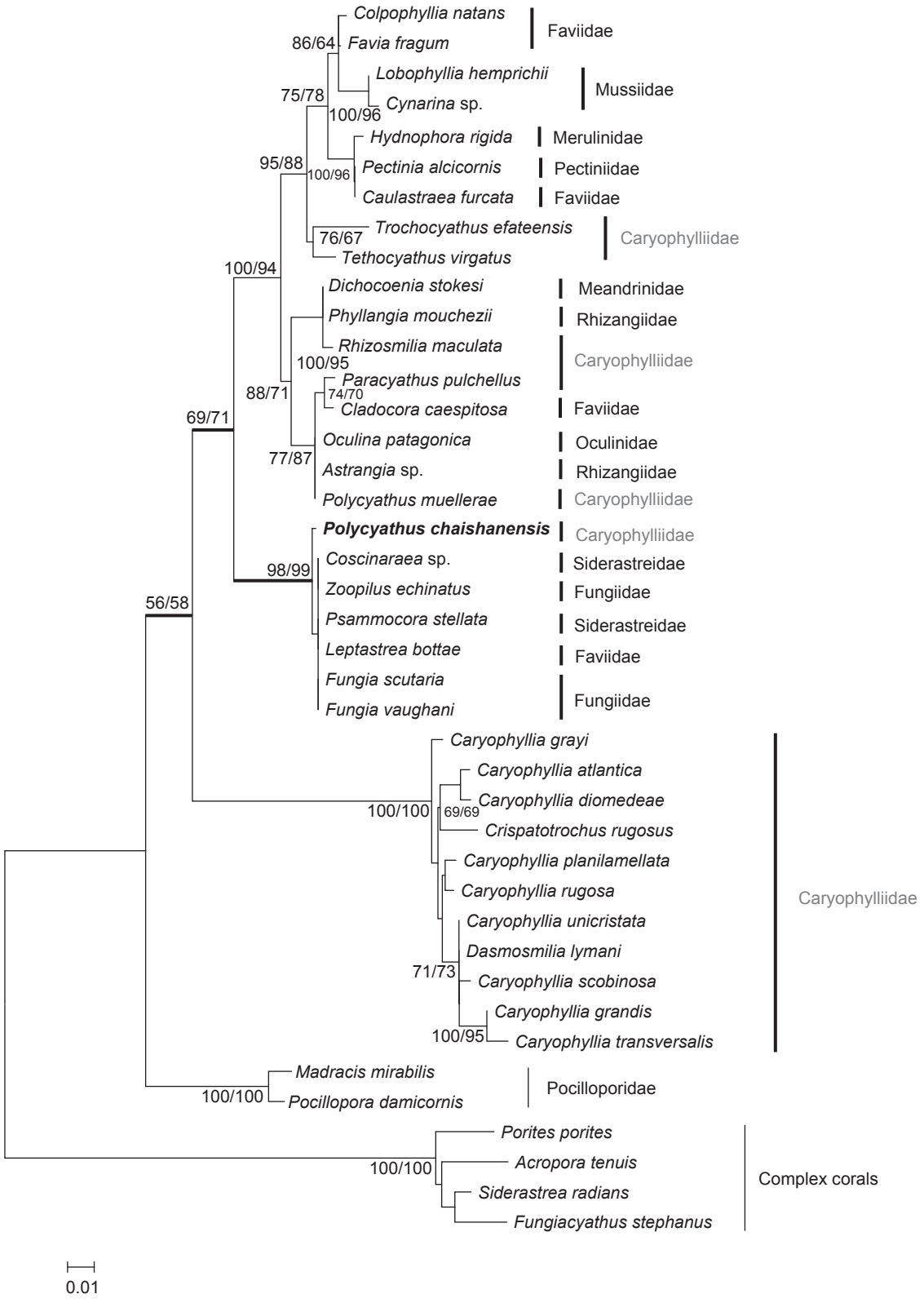

Fig. 6. Phylogenetic analyses based on Bayesian inference and Neighbor-joining analyses of the partial mitochondrial sequence of the 16S rRNA gene and the cytochrome oxidase subunit I gene from 41 scleractinian species. Numbers at the nodes correspond to Bayesian posterior probabilities and bootstrap support of the Neighbor-joining analysis, respectively. The scale unit is 0.01 substitutions per site.

the analysis, both the BI and NJ analyses indicated that this genus is not monophyletic. *Polycyathus chaishanensis* sp. nov. was not grouped with any other congener (*P. muellerae*) or any other caryophylliid representative. Instead, our results show that *P. chaishanensis* sp. nov. has a genetic immediacy to some representatives of the Siderastreidae (*Coscinaraea* and *Psammocora*), Fungiidae (*Zoopilus* and *Fungia*), and Faviidae (*Leptastrea*) (Fig. 6). In addition, our results support *P. muellerae* having a close relationship with *Paracyathus pulchellus* (Kitahara et al. 2010b) but not with *Rhizosmilia maculata*. These results were also supported by the COI sequence data (data not shown).

In previous molecular studies, many of the morphologically defined families, especially those composed of zooxanthellate species, showed extensive polyphyly (Romano and Cairns 2000, Le Goff-Vitry et al. 2004, Fukami et al. 2008, Kitahara et al. 2010a). In an attempt to clarify the validity of morphology-based taxonomy, additional taxon sampling, more-comprehensive morphological analyses, and additional molecular data are required (Fukami et al. 2008). Therefore, molecular data from other *Polycyathus* species are needed to clarify the phylogenetic status of this genus.

Ecology of *Polycyathus chaishanensis* sp. nov.

The rare distribution and the small-sized population of this new species raise several concerns as to its vulnerability to natural and anthropogenic threats, in a period of intense urban development at Chaishan.

Chaishan is an uplifted reef formed during the late Pleistocene (2.59-0.01 Mya; Gong et al. 1998). The Pleistocene reef limestone in southwestern Taiwan occurs in the Gutingkeng Formation near Kaohsiung (Gong et al. 1998). A debris avalanche and sandy substrate form the main characteristics of the Chaishan area and have contributed to the benthic communities of this region. Among hermatypic organisms reported from Chaishan's formation, the most important are scleractinian corals (such as *Acropora*, *Porites*, *Favia*, and *Favites*), mollusks, and encrusting calcareous red algae (Gong et al. 1998). Hard surfaces exposed to light in the Chaishan area were found to be heavily dominated by algae, primarily the green algae *Ulva fasciata* and *U. lactuca*, and some turf algae such as *Chaetomorpha antennina* (Huang 2003). Colonial zooxanthellate corals, *Psammocora* sp. and *Porites* sp., were found on limestone or among fleshy algae. However, most of these scleractinian species were found in tidal pools of < 5 m deep. Reefs in shallow water with less light are usually dominated by zoanthus and sea anemones, probably including *Anthopleura* sp. (Fig. 2). In addition, overhangs and overhanging surfaces with less light are primarily dominated by encrusting sponges. *Polycyathus chaishanensis* sp. nov. was only found on well-lit reefs dominated by green and encrusting calcareous red algae, and was generally rare (Fig. 2). However, this area is dominated by green algae and turbid waters caused by erosion, which may have inhibited the occurrence of most other scleractinians. The small population of this new species raises concerns as to its vulnerability to natural and anthropogenic threats.

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