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Three New Species of the Sun Coral Genus *Tubastraea* (Scleractinia: Dendrophylliidae) from Hong Kong, China

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Tubastraea is a genus of azooxanthellate scleractinian corals belonging to the family Dendrophylliidae, which are commonly called sun corals. This genus currently has only seven recognized species. In this paper, we report three new species of *Tubastraea*, including *T. dendroida* sp. nov., which has a tree-like colony, *T. violacea* sp. nov., which has violet polyps, and *T. chloromura* sp. nov., which has olive green polyps. These species are distinct in their septal structures, as well as their *rDNA* sequences including the entire *ITS1*, *5.8S* and *ITS2*, and a segment of the *18S* and *28S* genes.

Key words: Scleractinian coral, Azooxanthellate, Ahermatypic coral, Dendrophylliid, South China Sea.

BACKGROUND

Ahermatypic corals are scleractinian corals formed by a solitary individual or a small colony of individuals that do not develop into a reef structure (Scott 1984; Schumacher and Zibrowius 1985; Veron 1993). They are typically small, azooxanthellate and live in deeper waters, and therefore are often overlooked in coral community surveys (Lam et al. 2008). This is the case for *Tubastraea* Lesson, 1830, a genus of azooxanthellate ahermatypic corals in the family Dendrophylliidae. This genus consisting of the species commonly known as sun corals, is mainly distributed in the Indo-Pacific region, but also well-known in the Atlantic, where they were introduced (Creed et al. 2017). *Tubastraea* currently comprises only seven extant species (Hoeksema and Cairns 2021), including *T. coccinea* Lesson, 1830, *T. diaphana* (Dana, 1846), *T. faulkneri* Wells, 1982, *T. floreana* Wells, 1982, *T. megacorallita* Yiu, Chung & Qiu 2021, *T. micranthus* (Ehernberg, 1834) and *T. tagusensis* Wells, 1982. Recently, *T. aurea* (Quoy & Gaimard, 1833) was classified as *Australopsammia aurea* (Quoy & Gaimard, 1833) (Rowlett 2020). However, in this paper we consider *T. aurea* (Quoy & Gaimard, 1833) as the valid name, since the decision to classify it otherwise was based only on the fact that the type locality of this species is in the southern hemisphere, rather than on morphological and phylogenetic analysis. The change in classification contradicted the result of a phylogenetic study showing that it is nested within a clade of *Turbastraea* (Arrigoni et al. 2014).

Species of *Tubastraea* share several characteristics: 1) their colonies develop from a common basal coenosteum by budding with clear connection among polyps, and their columella are small to moderate size and lack an epitheca; 2) their colonial coralla are firmly attached to substrate; 3) their septal cycles are hexamerally arranged and typically inserted with spongy columella; and 4), their coralla exhibit a rough texture (Cairns 2001; Cairns and Kitahara 2012). Although in most *Tubastraea* species the septa are not arranged in a

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Pourtalès plan, those of *T. megacorallita* are. Because of this fact, Yiu et al. (2021a) removed the lack of this structure from the diagnostic features of this genus. Molecular phylogenetic analysis based on multigene markers (*i.e.*, *COI*, intergenic spacer between *COI* and *16S*, and a *rDNA* marker including *ITS1*, *5.8S*, *ITS2*, and a segment of *18S* and *28S*) have clarified the classification of Dendrophylliidae (Arrigoni et al. 2014), revealing that, while most other genera of the family represented by at least two species are non-monophyletic, *Tubastraea* is monophyletic with a strong bootstrap support.

In Hong Kong, five species of *Tubastraea* have been recorded: *T. coccinea*, *T. diaphana*, *T. faulkneri*, *T. megacorallita* and *T. micranthus* (Scott 1984; Clark 1997; Lam et al. 2008; Yiu et al. 2021a). Compared to the zooxanthellate corals that mostly inhabit the shallow waters (< 10 m) (Yeung et al. 2021), azooxanthellate corals including these *Tubatraea* spp. are mainly distributed in the deeper waters (≥ 10 m), with the exception of *T. aurea* which was reported from "snorkelling depths" (Scott 1984). While implementing a project studying corallivorous nudibranchs in Hong Kong waters (Hu et al. 2020a b), we found four undescribed species of *Tubastraea.* One of them, *T. megacorallita*, has been described recently (Yiu et al. 2021a). In this paper we describe the other three species based on morphological and molecular analyses.

MATERIALS AND METHODS

Sample collection

All samples were collected by SCUBA from Sung Kong and Waglan Island in eastern Hong Kong waters (Fig. 1). They were preserved in 95% ethanol and deposited in the Tropical Marine Biodiversity Collections of the South China Sea (TMBC), Chinese Academy of Sciences, Guangzhou.

Morphological analysis

Photographs of the specimens were taken using an

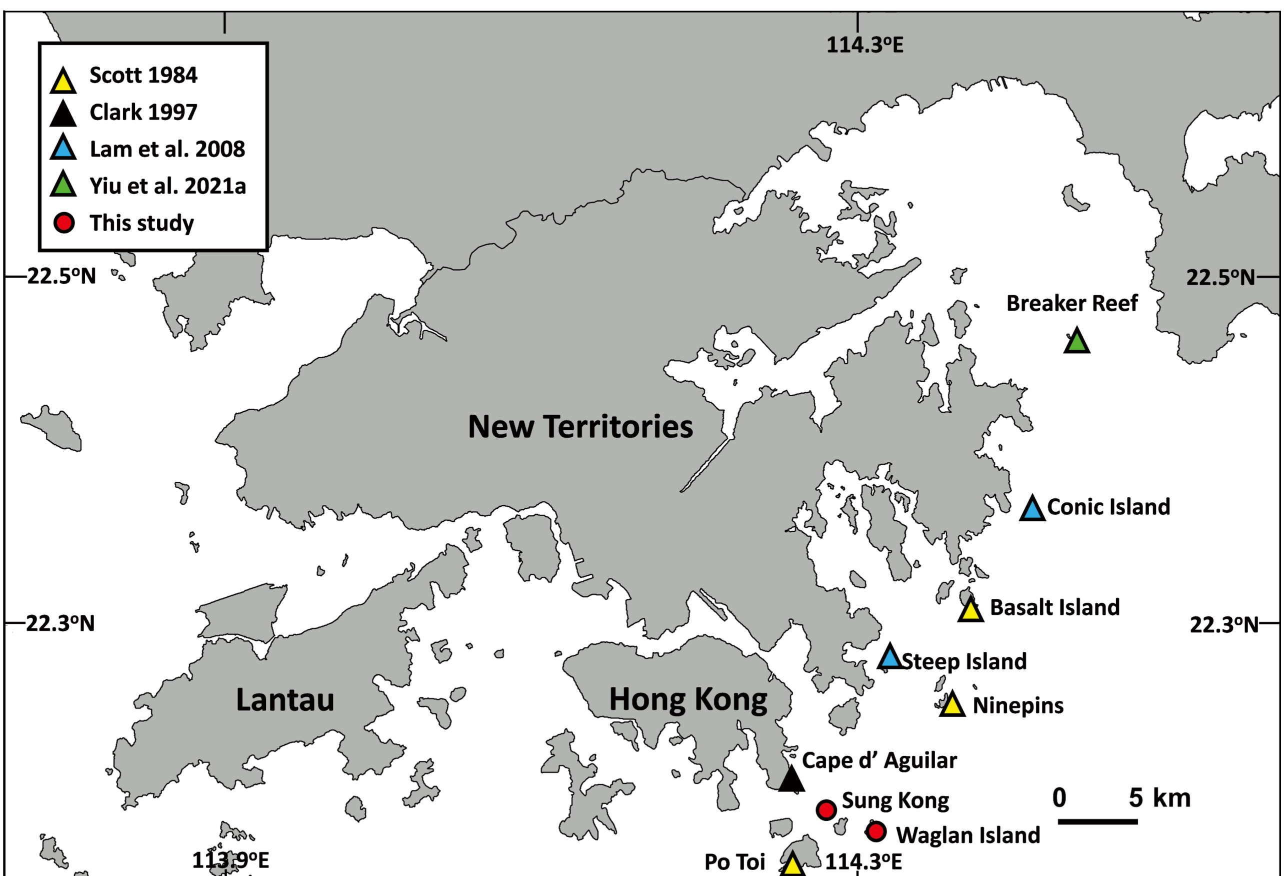

Fig. 1. A map of Hong Kong showing the two sampling sites in this study (red dots), as well as the sampling sites (triangles) of ahermatypic corals in previous studies (Scott 1984; Clark 1997; Lam et al. 2008; Yiu et al. 2021a).

Olympus OM-D EM1markII camera with a M. Zuiko Digital ED 60mm f2.8 macro lens. Morphological characters defined by Cairns (2001) and Cairns and Kitahara (2012) were used for species description. They included whole colony size, corallite size, fossa depth, intercorallite distance and septa arrangement. The size measurements were performed using a ruler. The following abbreviations were used: GCD, greater calicular diameter; LCD, lesser calicular diameter; S, septa.

DNA extraction, PCR, sequencing and analysis

Genomic DNA was extracted from two specimens in each species using the CTAB method (Stewart and Via 1993). DNA quantity was measured, and purity determined using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, USA). DNA quality was checked by 1% agarose gel electrophoresis. Sequences from two mitochondrial regions and one nuclear region were targeted. The first mitochondrial region covers a portion of cytochrome oxidase 1 (*COI*) gene, while the second mitochondrial region covers the 3' end of *COI*, intergenic spacer (*IGR*) between *COI* and trnM, trnM and the 5'-end of large ribosomal subunit (*16S*), namely *IGR* in the rest of the text. The nuclear region covered a portion of *rDNA* including the entire sequences of *ITS1*, *5.8S* and *ITS2*, and a portion of *18S* and *28S*.

Polymerase chain reaction (PCR) was conducted using the extracted DNA as templates to amplify the *COI*, *IGR* and *rDNA*. The partial *COI* (~750 bp) was amplified using primers designed by Arrigoni et al. (2014): COIDENL (5'- CGCTGGGCGTTTTCTACTAA -3') and COIDENR (5'-GAAATCATTCCAAAGCCAGGT -3'). The amplification program consisted of an initial denaturation step of 94°C for 2 min, followed by 35 cycles of 94°C for 30 sec, 53°C for 1 min, 72°C for 1 min and finally a 7 min extension step at 72°C. The *IGR* (~500 bp) was amplified using primers designed by Arrigoni et al. (2014): AGAL (5'-CGCATTGAAACACGAGCTTA -3') and DENF (5'-TTTGCTGGTTGGAATTTGGT -3'). Amplification reactions were carried out using the following program: 94°C for 4 min, 35 cycles of 94°C for 1 min, 51°C for 1 min, 72°C for 1 min and a final phase at 72°C for 5 min. The *rDNA* (~750 bp) was amplified using primers A18S (5'- GATCGAACGGTTTAGTGAGG -3') (Takabayashi et al. 1998) and ITS4 (5'-TCCTCCGCTTATTGATATGC -3') (White et al. 1990), amplifications were performed with the following program; 94°C for 4 min, 40 cycles of 15 sec at 94°C,1 min at 55°C, 30 sec at 72°C and a final phase at 72°C for 5 min. PCR products were sent to BGI Hong Kong for sequencing on an ABI 310 Genetic Analyzer. All new sequences were deposted into GenBank (Table S1).

Alignments of the three genes were conducted separately and trimmed manually to 601 bp for *COI*, 448 bp for *IGR* and 684 bp for *rDNA* using MEGA 7. Sequences were concatenated using SequenceMatrix v.1.7.8 (Vaidya et al. 2011) and then imported to the website version of IQ-Tree (http://iqtree.cibiv.univie.ac.at/; Nguyen et al. 2015) for Maximum Likelihood tree reconstruction with 1,000 ultrafast bootstrap pseudoreplicates (Hoang et al. 2017). ModelTest (Kalyaanamoorthy et al. 2017) incorporated in IQ-Tree was applied for each partition of the concatenated sequences, which detected TPM3u+F+I as the best model for *COI*, HKY+F+G4 for *IGR* and K2P+I+G4 for *rDNA* based on Bayesian Information Criterion. MrBayes v.3.2.7a (Ronquist and Huelsenbeck 2003) was used to perform the Bayesian Inference analysis with four Metropolis-coupled Markov Chain Monte Carlo applied to 10 million generations, sampled at every 1,000 generations with a 25% burn-in. Since the best models detected for the concatenated dataset by ModelTest were not available in MrBayes, they were substituted by the closest overparameterized models (Huelsenbeck and Rannala 2004): GTR+I+G for *COI*, HKY+I+G for *IGR* and K2P+I+G for *rDNA*. The phylogenetic trees were visualized and edited using FigTree v1.4.4. Pairwise genetic distances for the respective *COI*, *IGR* and *rDNA* genes were estimated using MEGA 7 separately based on the p-distance method using the bootstrap method with 10,000 pseudoreplicates for variance estimation. Rates among sites were gamma distributed with invariant sites (G+I) and the gamma parameter was set to four.

RESULTS

SYSTEMATICS

Class ANTHOZOA Ehrenberg, 1834
Order SCLERACTINIA Bourne, 1900
Family DENDROPHYLLIIDAE Gray, 1847
Genus *Tubastraea* Lesson, 1830

Tubastraea dendroida sp. nov.

(Fig. 2)
urn:lsid:zoobank.org:act:774c42bb-bd84-4f0f-b00f-256ba8d1c90d

Materials examined: Holotype: Colony with 74 corallites, 60 mm in length and 94 mm in height (TMBC030974).

Paratype: Colony with 74 corallites, 13.5 mm in

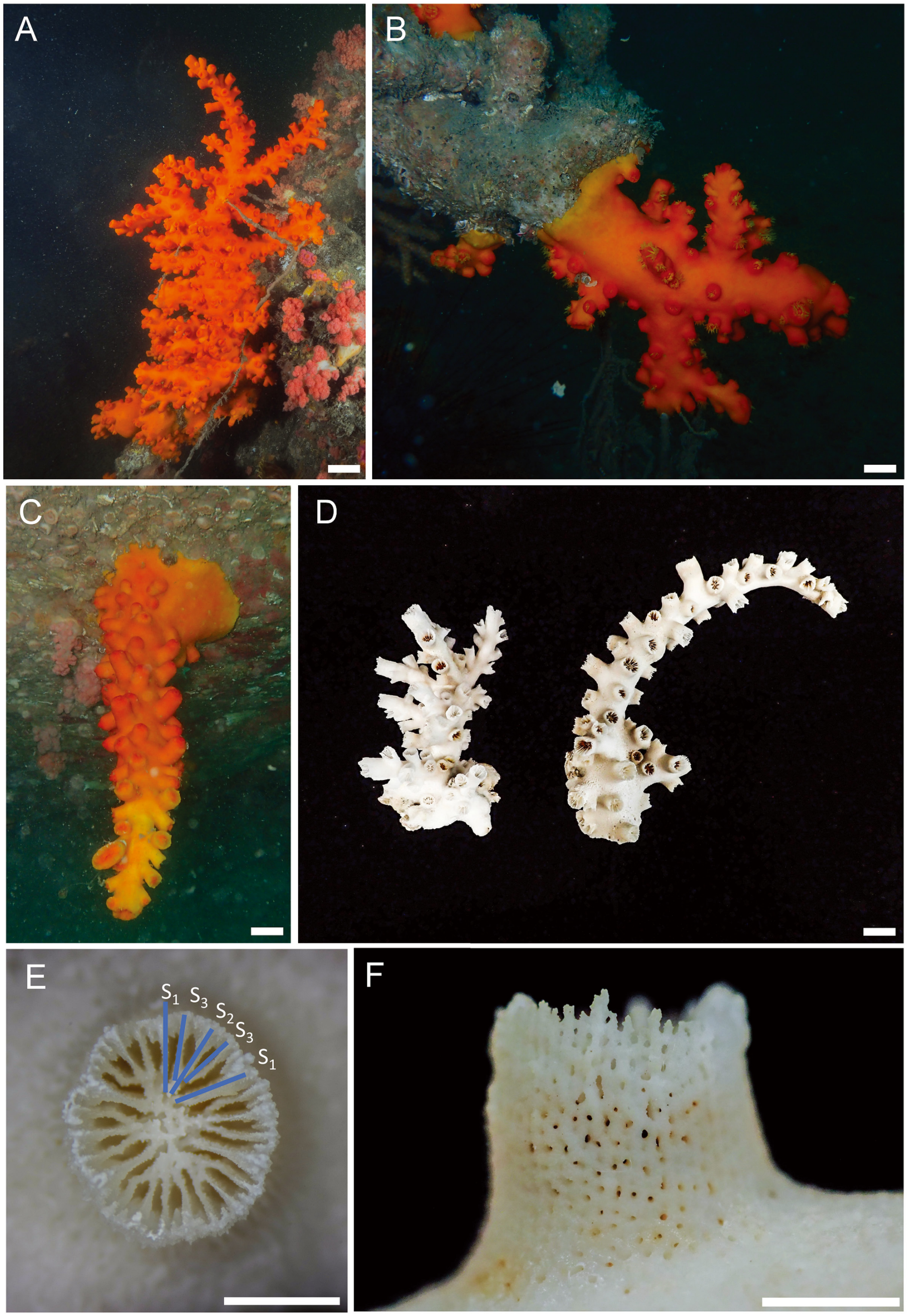

Fig. 2. *Tubastraea dendroida* sp. nov. A–C, photographs of colonies taken in the field. D, skeleton of holotype (left, TMBC030974) and paratype (right, TMBC030975). E, Cross-sectional view of a corallite showing three septal cycles. F, lateral view of a corallite. Scale bars: A–C = 3 cm; D = 1 cm; E–F = 2.5 mm.

length and 202 mm in height (TMBC030975).

Type locality: Both specimens were collected from Sung Kong (22°11'26.5"N, 114°16'48.4"E) on 28/09/2021 at 17 m depth (Fig. 1).

Etymology: *Tubastraea dendroida* sp. nov. looks like a tree branch. The species epithet reflects this morphological character.

Geographic distribution: Currently only known in Hong Kong.

Habitat: Exposed sites with moderate current, rocky substrate, 10–25 m water depth.

Description: Living specimen (Fig. 2A–C) with bright orange tissue covering epithecal wall and corallite. Tentacles yellow, usually withdrawn. Colony dendroid, branching uniplanar, with one elongate, straight axial corallite, and several side branches formed by extra-tentacular budding (Fig 2D). Each specimen with up to 74 corallites varying between 13.5–60 mm in length and 94–202 mm in height. Colony height up to 50 cm. Corallites circular or slightly elliptical (6–8 mm in GCD and 5–7 mm in LCD) with a thin wall. Axial corallites usually biggest among all corallites in a colony. A total of 24 septa present, with 10 to 16 of the septa fused with columella (Table 1). Septa hexamerously arranged (Table 2), containing 3 cycles, with size increasing from inner to outer as $S_1 = S_2 > S_3$. Septa usually arranged in a Pourtalès plan (Fig. 2E), curving from edge to columella. Columella (Fig. 2E)

Table 1. Comparison of gross morphological characters among *Tubastraea* species

Species	Growth form	Intercorallite distance	Corallites		Columella	Fossa
			Shape	LCD × GCD (mm)	LCD × GCD (mm)/Size	Depth (mm)
T. coccinea[a,b,c,d]	Plocoid	Closely spaced	Circular	10.0–13.0	Large	Moderately deep
T. chloromura sp. nov.	Phaceloid	Closely spaced	Circular	6.0–10.0 × 6.0–11.0	1.5–4.0 × 4.0–6.0	4.0–8.0
T. dendroida sp. nov.	Dendroid/Branching uniplanar	Closely spaced/ widely spaced	Circular	5.0–7.0 × 6.0–8.0	1.0–2.0 × 2.0–3.0	3.0–11.0
T. diaphana[c,e]	Dendroid/Phaceloid		Circular	6.0–11.0		Deep
T. faulkner[b,e,f]	Plocoid	Closely spaced/ widely spaced	Circular	5.2–7.8/8.0–13.0	Large	Deep and spongy/Shallow
T. floreana[e,f]	Phaceloid	Closely spaced/ widely spaced	Cylindrical	4.0–6.0	Rudimentary	4.0–5.0; moderately deep
T. megacorallita[g]	Phaceloid	Closely spaced/ widely spaced	Elliptical	6.3–19.1 × 8.0–24.6	0.9–3.7 × 1.9–10.1	2.5–18.1
T. micranthus[c,h,i]	Dendroid/Branching uniplanar		Circular	4.5–6.5 × 5.0–7.5	Rudimentary	4.0–9.0
T. tagusensis[b,f]	Phaceloid	Closely spaced	Cylindrical	< 10.0	Rudimentary	Deep
T. violacea sp. nov.	Phaceloid	Closely spaced/ widely spaced	Cylindrical	8.0–14.0 × 10.0–18.0	2.5–4.0 × 6.0–8.0	9.0–11.0

[a]Wells (1983), [b]Cairns (1991), [c]Cairns & Zibrowius (1997), [d]Cairns (1994), [e]Lam et al. (2008), [f]Wells (1982), [g]Yiu et al. (2021a), [h]Nemenzo (1960), [i]Ogawa & Takahashi (1993).

Table 2. Comparison of septal arrangement among *Tubastraea* species

Species	Arrangement	Total number	No. of septa fused with columella	No. of cycles	Size	Fusion
T. coccinea[a,b,c,d]	Normal	Up to 48	12	4	$S_1 = S_2 > S_4 > S_3$	S_4 united with S_3
T. chloromura sp. nov.	Normal	34–40	10–12	4	$S_1 > S_2 > S_3 = S_4$	S_3 fused with S_4
T. dendroida sp. nov.	Pourtalès plan	24	11–12	3	$S_1 > S_2 = S_3$	No
T. diaphana[c,e]	Normal			4	$S_1 > S_2 >> S_3 > S_4$; $S_1 = S_2 > S_4 = S_3$	
T. faulkner[b,e,f]	Normal	48+		4	$S_1 > S_2 > S_4 > S_3$	
T. floreana[e,f]	Normal	24		3	$S_1 = S_2 > S_3$	No
T. megacorallita[g]	Pourtalès plan	34–92	10–26	5	$S_1 = S_2 > S_3 > S_4 = S_5$	S_5 fused with S_4 and S_3
T. micranthus[c,h,i]	Normal				$S_1 > S_2 >> S_3$	
T. tagusensis[b,f]	Normal	24/48		3 or 4	$S_1 = S_2 > S_3 > S_4$; $S_1 = S_2 = S_3 > S_4$	No
T. violacea sp. nov.	Pourtalès plan	75–87	23–26	5	$S_1 = S_2 > S_3 > S_5 > S_4$	S_5 fused with S_4 and S_3

[a]Wells (1983), [b]Cairns (1991), [c]Cairns & Zibrowius (1997), [d]Cairns (1994), [e]Lam et al. (2008), [f]Wells (1982), [g]Yiu et al. (2021a), [h]Nemenzo (1960), [i]Ogawa & Takahashi (1993).

spongy (2–3 mm in GCD and 1–2 mm in LCD). Fossa 3–11 mm deep. Costae granular. Intercostal striae porous (Fig. 2F).

Taxonomic remarks: *Tubastraea dendroida* sp. nov. resembles *T. micranthus* and *Dendrophyllia ijimai* in that their colonies are uniplanar (Filander et al. 2021). It can be distinguished from *T. micranthus* in that the latter has a slimmer main "stem", its three septal cycles are not arranged a Pourtalès plan, and it is usually in dark green or brown colour (Schuhmacher 1984; Cairns and Zibrowius 1997; Sammarco et al. 2010; Filander et al. 2021). However, Tachikawa (2005) reported that *T. micranthus* had both dark green and orange colour morphs in Japan, with the dark green morph more dominant. Whether this is true, or the orange morph is *T. dendroida* sp. nov. needs to be determined when specimens are available for examination. The septa of *Dendrophyllia ijimai* are also arranged in a Pourtalès plan, but this species differs from the new species in having four septal cycles instead of three septal cycles. It is the second species of *Tubastraea* known to have a Pourtalès plan.

***Tubastraea chloromura* sp. nov.**

(Fig. 3)

urn:lsid:zoobank.org:act:1d107473-b405-468f-9212-8a2fe77db739

Materials examined: *Holotype*: Colony with 26 corallites, 45 mm in length and 32 mm in height (TMBC030976).

Paratype: Colony with 16 corallites, 57 mm in length and 30 mm in height (TMBC030977).

Type locality: Both specimens were collected from Waglan Island (22°10'51.4"N, 114°18'11.8"E) on 14/03/2021 at 10 m depth (Fig. 1).

Etymology: *Tubastraea chloromura* sp. nov. has a distinct olive green epithecal wall. The species epithet *chloromura* (Latin chloro = green, murus = wall) reflects this morphological character.

Geographic distribution: Currently only known in Hong Kong.

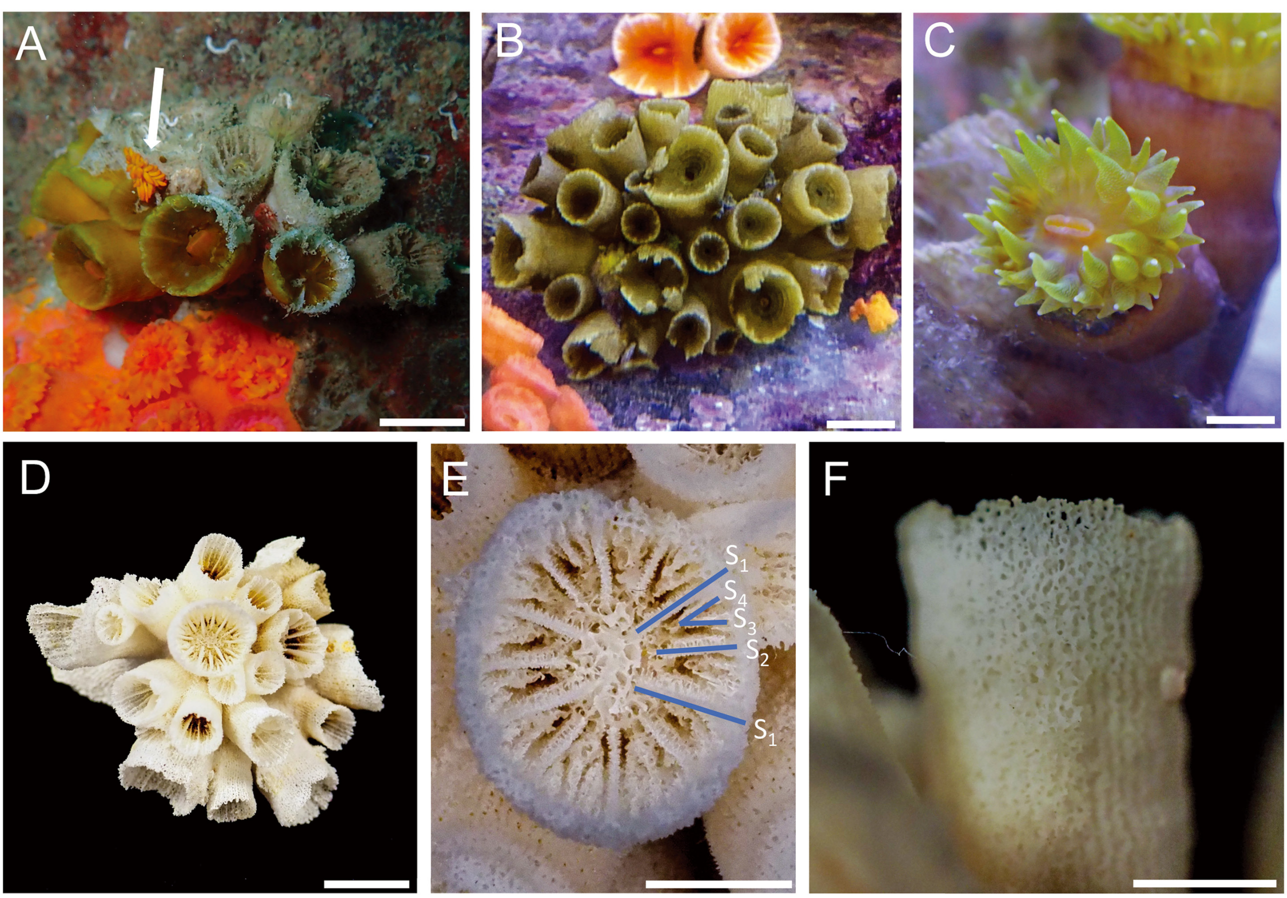

Fig. 3. *Tubastraea chloromura* sp. nov. A, a colony of the new species (olive green) next to a colony of *T. coccinea* (red) in the field, a nudibranch *Phestilla melanobranchia* (pointed by a white arrowhead). B, a colony of the new species (olive green) next to a colony of *T. coccinea* (red) in laboratory aquarium. C, open polyp. D, skeleton of holotype. E, cross-sectional view of a corallite showing four septal cycles. F, lateral view of a corallite. Scale bars: A–B = 1 cm; C = 5 mm; D = 1 cm; E–F = 5 mm.

Habitat: Exposed sites with moderate current, rocky substrate.

Description: Living specimen (Fig. 3A–B) with olive green tissue covering epithecal wall of corallite, and light green tentacles. Colony phaceloid. Corallites formed by extratentacular budding (Fig 3B), consisting of 16–26 corallites. Colonies between 45–57 mm in length and 30–32 mm in height. Corallites circular (6–10 mm in GCD and 6–11 mm in LCD) with a thin wall. A total of 34–40 septa present, with 10 to 12 of the septa fused with columella (Table 1). Septa hexamerously arranged (Table 2), containing 4 cycles, with increasing size from inner to outer as $S_1 > S_2 > S_3$ = S4. Septa normally inserted. Septa spongy, highly porous (Fig. 3D). Columella (Fig. 3D) spongy (1.5–4 mm in GCD and 4–6 mm in LCD). Fossa 4–8 mm deep. Costae granular. Intercostal striae porous (Fig. 3E).

Taxonomic remarks: *Tubastraea chloromura* sp. nov. resembles *T. coccinea* in gross colony morphology, but its tissues are olive green. It was thought by the first author as a colour variant of *T. coccinea* when he collected the samples. This new species exhibits sympatric distribution with *T. coccinea* (Fig 3A). In addition to having a unique tissue colour, *T. chloromura* sp. nov. has a thinner and more porous septal texture than other *Tubastraea* species.

Tubastraea violacea sp. nov.

(Fig. 4)
urn:lsid:zoobank.org:act:972E5F06-D434-45CB-8961-8CD65BC6DE38

Materials examined: *Holotype*: Colony with 130 corallites, 160 mm in length and 83 mm in height (TMBC030978).

Paratype: Colony with 16 corallites, 35 mm in length and 40 mm in height (TMBC030979).

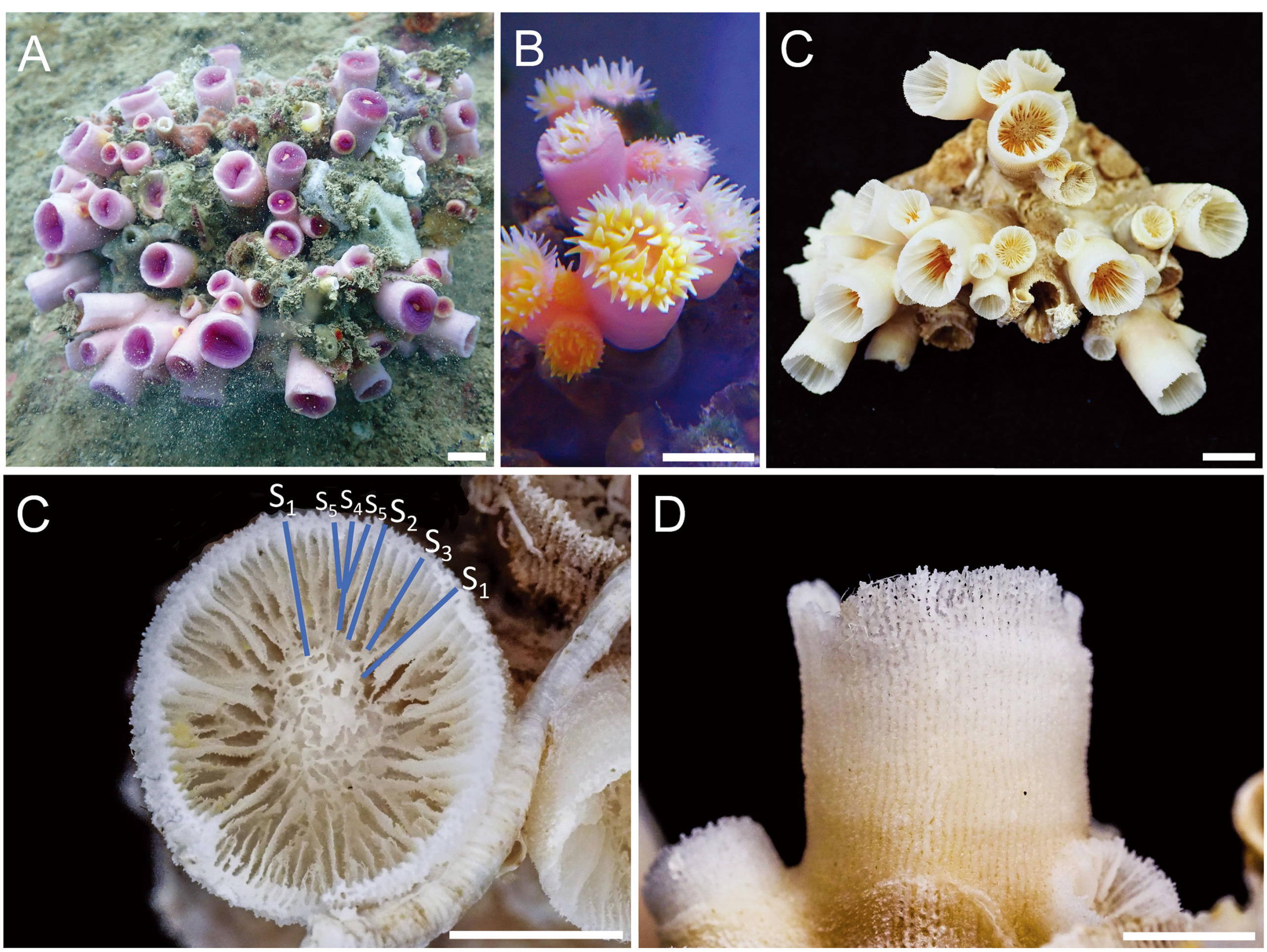

Fig. 4. *Tubastraea violacea* sp. nov. A, a colony of in the field and opened polyp at right top corner. B, open polyps. C, skeleton of part of the holotype (TMBC030978). C, cross-sectional view of corallite showing the five septal cycles. D, lateral view of a corallite. Scale bars: A–C = 1 cm; D–E = 5 mm.

Type locality: The holotype was collected from Sung Kung (22°11'26.5"N, 114°16'48.4"E) (Fig. 1) on 14/03/2021 at 10 m depth. The paratype was collected from Waglan Island (22°10'51.4"N, 114°18'11.8"E) on 28/09/2021 at 10 m depth.

Etymology: *Tubastraea violacea* sp. nov. has violet tissue covering the corallites. The species epithet reflects this morphological character.

Geographic distribution: Hong Kong (this study) and Canal Woodin, New Caledonia (based on the sequences of *Tubastraea* sp 2. in Arrigoni et al. 2014)

Habitat: Exposed sites with moderate current, rocky substrate.

Description: Living specimen (Fig. 4A) with pale purple tissue covering epithecal wall, violet tissue covering corallite and translucent yellow tentacles. Colony phaceloid with each long corallite having its own wall. Corallites formed by extratentacular budding (Fig. 4B), consisting of 16–130 corallites. Colonies 35–160 mm in length and 40–83 mm in height. Corallites cylindrical (10–18 mm in GCD and 8–14 mm in LCD) with a thick wall. A total number of 75–87 septa present, with 23–26 of the septa fused with columella (Table 1). Septa hexamerously arranged (Table 2), containing 5 cycles, with the size order of $S1 = S_2 > S_3 > S_5 > S_4$. Septa arranged in a Pourtalès plan. Columella (Fig. 4C) spongy (6.0–8.0 mm in GCD and 2.5–4.0 mm in LCD). Fossa 9–11 mm deep. Costae granular. Intercostal striae porous (Fig. 4D).

Taxonomic remarks: *Tubastraea violacea* sp. nov. is unique among the congeneric species in having violet tissue covering the corallites. It is also remarkable in having a Pourtalès plan of septal arrangement, and that is the third species of *Tubastraea* has this character. Yiu et al. (2021a) observed that the Pourtalès plan is absent in small corallites of *T. megacorallita* but it is usually present in corallites at least 17 mm × 15 mm (GCD × LCD) in size. However, all corallites of the type specimens of *T. violacea* sp. nov. were observed to exhibit a Pourtalès plan. Besides, the same as *T. megacorallita*, *T. violacea* sp. nov. has five septal cycles. All other *Tubatraea* species have three to four septal cycles. Moreover, *T. violacea* sp. nov. can be distinguished from *T. megacorallita* in having cylindrical corallites rather than elliptical corallites. Also, the two species are different in their septal size orders.

Molecular analyses

The alignment and concatenation resulted in a dataset of 1.733 bp (*COI*: 601 bp, *IGR*: 448 bp, *rDNA*: 684 bp). Pair-wise sequence comparisons were conducted to determine the inter- and intra-specific *p*-distances (Table 3). Intraspecific *p*-distances in the three species are in general very small, with 0% for *COI*, 0% for *IGR*, and 0.15% for *rDNA* in *T. violacea* sp. nov., 0% for *COI*, 0.22% for *IGR*, and 0% for *rDNA* in *T. chloromura* sp. nov., and 0% for *COI*, 0.22% for *IGR*, and 1.79% for *rDNA* in *T. dendroida* sp. nov. The relatively large *rDNA p*-distances between the two specimens of *T. dendroida* sp. nov. and between the two species of *T. micrnthus* indicate the potential of cryptic speciation in this species. *Tubastraea violacea* sp. nov. is most closely related to *Tubastraea* sp. 2. HS2883, an undescribed species collected from Caledonia (Arrigoni et al. 2014); the sequences between them had a *p*-distance of 0% for *COI*, 0% for *IGR*, and 0.32–0.48% for *rDNA*, indicating they are conspecific, despite their differences in tissue colour. The species closest to *T. chloromura* sp. nov. and *Tubastraea dendroida* sp. nov. is *T. micranthus*. Between *T. chloromura* sp. nov. and *T. micranthus*, the *p*-distance was 0.17% for *COI*, 0–0.22% for *IGR*, and 0.96% for *rDNA*. Between *T. dendroida* sp. nov. and *T. micranthus*, the *p*-distance was 0% for *COI*, 0–0.22% for *IGR* and 1.29–1.45% for *rDNA*. In

Table 3. *Tubastraea* intra- and inter-specific uncorrected *p*-distances (%) for *COI/IGR/rDNA*. Data obtained in this study are in bold

		1	2	3	4	5	6	7	8	9	10	11
1	***T. violacea* sp. nov.**											
2	***T. violacea* sp. nov.**	0/0/0.15										
3	***T. chloromura* sp. nov.**	0.17/0/2.26	0.17/0/2.11									
4	***T. chloromura* sp. nov.**	0.17/0.22/2.26	0.17/0.22/2.11	0/0.22/0								
5	***T. dendroida* sp. nov.**	0/0/3.62	0/0/3.47	0.17/0/1.96	0.17/0.22/1.96							
6	***T. dendroida* sp. nov.**	0/0.22/3	0/0.22/2.85	0.17/0.22/1.35	0.17/0/1.35	0/0.22/1.79						
7	*T. coccinea* (AQ2)	0/0/1.43	0/0/1.27	0.17/0/2.06	0.17/0.22/2.06	0/0/3.01	0/0.22/2.69					
8	*T. diaphana* (AO101)	-/0.67/3.18	-/0.67/3.03	-/0.67/4.13	-/0.89/4.13	-/0.67/3.97	-/0.89/4.29	-/0.67/3.5				
9	*T. megacorallita*	0/0/4.6	0/0/4.44	0.17/0/4.28	0.17/0.22/4.28	0/0/4.43	0/0.22/4.59	0/0/3.96	-/0.67/4.77			
10	*T. micranthus* (HS3129)	0/0/1.61	0/0/1.45	0.17/0/0.96	0.17/0.22/0.96	0/0/1.45	0/0.22/1.29	0/0/1.29	-/0.67/3.55	0/0/3.54		
11	*T.* sp._1 (MY105)	0.83/0.45/5.14	0.83/0.45/4.98	1/0.45/4.65	1/0.67/4.65	0.83/0.45/4.82	0.83/0.67/4.82	0.83/0.45/4.65	-/0.67/5.15	0.83/0.45/6.1	0.83/0.45/4.22	
12	*T.* sp._2 (HS2883)	0/0/0.48	0/0/0.32	0.17/0/2.06	0.17/0.22/2.06	0/0/3.01	0/0.22/2.85	0/0/1.27	-/0.67/3.34	0/0/4.43	0/0/1.29	0.83/0.45/5.14

comparison, the *p*-distance between *T. micranthus* and *T. coccinea* was 0% for *COI*, 0% for *IGR* and 1.29% for *rDNA*. Overall, these genetic distances support our designation of the three new species.

The ML and BI trees constructed using the concatenated sequences showed the same topology (Fig. 5), with species of the genus *Tubastraea* forming a monophyletic clade within the family Dendrophylliidae. This result is in agreement with Arrigoni et al. (2014), and supports retention of the name *T. aurea*, rather than reclassifying the species as *Australopsammia aurea* (Rowlett 2020). Among the species of *Tubastraea* used in this analysis, *T. dendroida* sp. nov. is sister to *T. chloromura* sp. nov. The (*T. dendroida* sp. nov. + *T. chloromura* sp. nov.) clade is sister to *T. micranthus*. *Tubastraea violacea* sp. nov. along with *Tubastraea* sp. 2 HS2883 are sister to *Tubastraea* sp. 2 HS2884. The ((*T. violacea* sp. nov. + *Tubastraea* sp. 2 HS2883) +

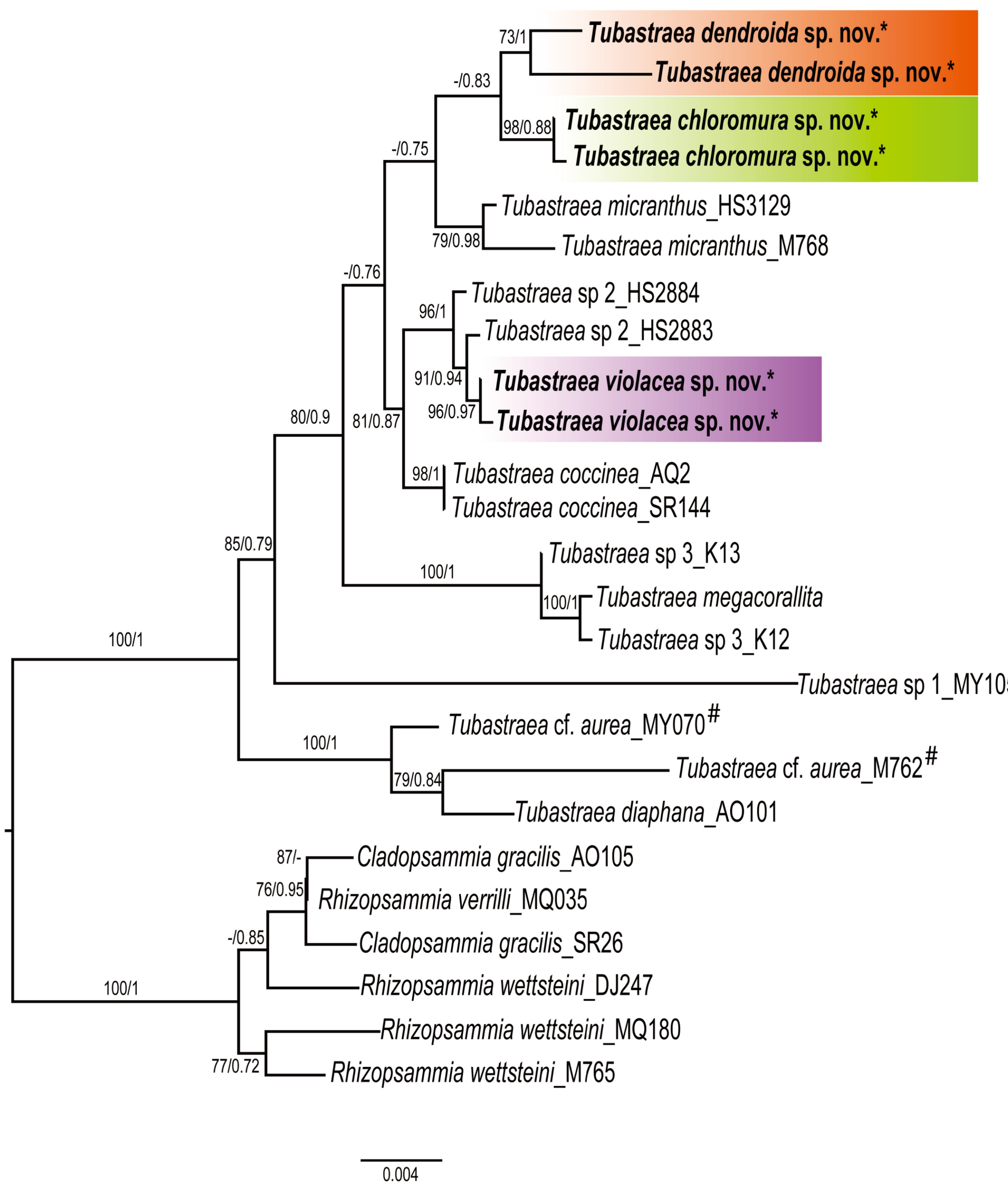

Fig. 5. Phylogenetic tree of the concatenated *COI/IGR/rDNA* dataset constructed using the Maximum Likelihood method and Bayesian Inference method. Bootstrap values > 70 and Bayesian Inference posterior probability values > 0.7 are shown in the nodes. Data obtained in this study are indicated by an asterisk. # indicates the species name in Rowlett (2020) was *Australopsammia aurea*.

Tubastraea sp. 2 HS2884) clade is sister to *T. coccinea*.

DISCUSSION

Our discovery of these three new species demonstrate Hong Kong's high diversity of sun corals. To date, eight of the ten recognized *Tubastraea* species have been recorded in Hong Kong waters (Scott 1984; Clark 1997; Lam et al. 2008; Yiu et al. 2021a; this study). The addition of these new species has changed our view on the use of several morphological characters in the taxonomy of *Tubastraea.* Among the previous described species, *T. micranthus* is the only species whose corallines form a tree-like structure, and the colonies are uniplanar. The discovery of *Tubastraea dendroida* sp. nov. having these properties yet the main stem of the colony attenuates from the base to the tip indicates the genus is more diverse in colony shape than previously thought. Moreover, a previous study suggested that *T. megacorallita* is unique among the congeneric species in having a Pourtalès plan of septal arrangement (Yiu et al. 2021a). Our finding of the Pourtalès plan in the corallite septa of *Tubastraea violacea* sp. nov., and *Tubastraea dendroida* sp. nov. supports our removal of this character as a diagnostic character of the genus (Yiu et al. 2021a).

We successfully used the three markers developed by Arrigoni et al. (2014) to classify our specimens. However, among them, only *rDNA* had adequate phylogenetic signal for species delimitation (Figs. S1–S3), although using the concatenated sequences resulted in improved support for some nodes of the trees. The result indicates the need for the adoption of more markers/molecular tools, such as transcriptome- or genome-wide target-enrichment baits (Quattrini et al. 2018; Quek et al. 2020), for more robust phylogenetic analysis of *Tubastraea*. Nevertheless, our integrated molecular and morphological analyses showed that the three species described in this study can be distinguished from their congeneric species reliably.

Among the three newly described species, *Tubastraea chloromura* sp. nov., is currently only known in Hong Kong. We found no other very similar gene sequences in public databases. Colonies very similar in morphology with *T. dendroida* sp. nov. have been reported in Japan as an orange form of *T. micranthus* (Tachikawa 2005), which indicates potential distribution of *T. dendroida* sp. nov. in Japan. Furthermore, the type specimens of *T. violacea* sp. nov. in Hong Kong are violet. However, Arrigoni et al. (2014) reported a yellow *Tubastraea* sp. 2 with sequences closely matching *T. violacea* sp. nov. in Canal Woodin, New Caledonia. Therefore, this species may show colour variation and have a wide distribution in tropical to subtropical western Pacific.

The distribution of the three new species is consistent with that of other azooxanthellate dendrophylliids in that they mainly live in exposed and deeper areas (Scott 1984). The nudibranch *Phestilla melanobrachia*, gastropods *Coralliophila costularis*, *Epidendrium* sp. (either *E. aureum* or *E. sordidum*) have been reported as common predators of *Tubastraea* spp. in Hong Kong (Yiu et al. 2021a b). During the field surveys, we also observed a juvenile *P. melanobrachia* feeding on a colony of *T. chloromura* sp. nov. (Fig 3A). Therefore, we suspect that it also preys on *T. dendroida* sp. nov. and *T. violacea* sp. nov.

Key to species of *Tubastraea*

1a. Colony branching uniplanar 2
1b. Colony not branching uniplanar 3
2a. Dark green, brown black coenosarc .... *T. micranthus*
2b. Orange coenosarc with yellow tentacles .... *T. dendroida* sp. nov.
3a. Colony plocoid 4
3b. Colony non-plocoid 5
4a. Corallite with moderately deep and up to 48 septa .... *T. coccinea*
4b. Corallite with deep and spongy/shallow fossa and more than 48 septa .... *T. faulkneri*
5a. Corallite elliptical .... *T. megacorallita*
5b. Corallite non-elliptical 6
6a. Corallite cylindrical 7
6b. Corallite circular 8
7a. Septal arranged in a Pourtalès plan .... *T. violacea* sp. nov.
7b. Septal arranged normally 9
8a. Septal size: $S_1 > S_2 >> S_3 > S_4$ or $S_1 = S_2 > S_4 = S_3$ .. *T. diaphana*
8b. Septal size: $S_1 > S_2 > S_3 = S_4$ .... *T. chloromura* sp. nov.
9a. Corallite with moderately deep fossa and 3 septal cycles .... *T. floreana*
9b. Corallite with deep fossa and 3 or 4 septal cycles .... *T. tagusensis*

CONCLUSIONS

We described *Tubastraea dendroida* sp. nov., *Tubastraea chloromura* sp. nov. and *Tubastraea violacea* sp. nov. from Hong Kong waters based on morphological and molecular analyses. Like other azooxanthellate corals, these new species occur at exposed areas and deeper waters. *Tubastraea dendroida* sp. nov. has a tree-like colony that is different in colour and shape from *T. micranthus*, the other species of this genus with a tree-like colony. *Tubastraea chloromura* sp. nov. and *Tubastraea violacea* sp. nov. have distinct colour in the tissues covering their corallites. The three species can be easily recognized by the shape and colour of the colonies. They are also distinct in their corallite structures as well as molecular marker sequences.

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Authors' contributions: JWQ initiated the study and revised the manuscript. SKFY conducted sampling, molecular and morphological analysis as well as drafted the manuscript.

Competing interests: SKFY and JWQ declares they have no conflict of interest.

Availability of data and materials: The accession numbers of specimens had been deposited on the GenBank.

Consent for publication: All of the authors agreed to publish the paper.

Ethics approval consent to participate: Not applicable.

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Supplementary materials

Fig. S1. Phylogenetic tree of the *COI* dataset constructed using Maximum Likelihood method. Bootstrap values > 70 are shown in the nodes. Data obtained from this study are indicated by an asterisk. # indicates the species name in Rowlett (2020) was *Australopsammia aurea*. (download)

Fig. S2. Phylogenetic tree of the *IGR* dataset constructed using Maximum Likelihood method. Bootstrap values > 70 are shown in the nodes. Data obtained from this study are indicated by an asterisk. # indicates the species name in Rowlett (2020) was *Australopsammia aurea*. (download)

Fig. S3. Phylogenetic tree of the concatenated *rDNA* dataset constructed using Maximum Likelihood method. Bootstrap values > 70 are shown in the nodes. Data obtained from this study are indicated by an asterisk. # indicates the species name in Rowlett (2020) was *Australopsammia aurea*. (download)

Table S1. GenBank accession numbers of the sequences used in phylogeny reconstruction and species delimitation retrieved from NCBI. Sequences of specimens from this study are in bold and sequences used in pair-wise distance calculations are indicated by an asterisk (*). (download)