



# Species diversity and molecular phylogeny of non-geniculate coralline algae (Corallinophycidae, Rhodophyta) from Taoyuan algal reefs in northern Taiwan, including *Crustaphytum* gen. nov. and three new species

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## Abstract

In Taiwan the algal reefs in Taoyuan County are the largest, composed of recent and fossil non-geniculate coralline algae. However, their diversity and phylogenetics in the region have never been documented. In this study, we analyzed the phylogenetic relationships of the non-geniculate coralline algae species collected from Taoyuan algal reefs and related non-geniculate species from other places in Taiwan and around the world based on *psbA* and SSU sequences. The molecular analyses revealed that at least 12 non-geniculate coralline species belonging in six evolutionary clades (*Harveyolithon*, *Lithophyllum*, *Pneophyllum*, *Crustaphytum* gen. nov., and *Phymatolithon*, *Sporolithon*) occur in Taoyuan algal reefs. Eleven of these species do not match any described species and one (*Lithophyllum margaritae*) is a new record for the marine flora of Taiwan. We also describe a new genus (*Crustaphytum* gen. nov.) and three new non-geniculate coralline species (*Crustaphytum pacificum* sp. nov., *Harveyolithon rosea* sp. nov., and *Phymatolithon margoundulatus* sp. nov.) for the most dominant and commonly seen species revealed by the molecular analyses. Among the latter three species, *P. margoundulatus* is only found in Taoyuan County and is the most dominant species there, comprising over 30% of the total cover, whereas *C. pacificum* has the widest distribution in the western Pacific Ocean (Taiwan and New Caledonia). *Harveyolithon rosea* is a common species occurring in both algal reefs and rocky shores in the northern Taiwan. The other undescribed CCA species will be published when more specimens with reproductive structures are collected.

**Keywords** *Crustaphytum pacificum* gen. and sp. nov. · *Harveyolithon rosea* sp. nov. · *Phymatolithon margoundulatus* sp. nov. · *psbA* · Red algae · SSU · Taiwan

## Introduction

Non-geniculate coralline algae (Corallinophycidae, Rhodophyta) are important marine phototrophic organisms. These red algae occur abundantly on rocky shores or on hard substrate

environments in the euphotic zone (Steneck 1986; Adey 1998; Harvey et al. 2005). Morphological features of non-geniculate coralline algae may indicate adaptations to various environmental factors that led to their classification into different functional groups (Adey 1973; Littler and Littler 1980, 1984; Steneck 1986). They are characterized by calcified thalli, with calcite in their cell walls, and lack genicula (noncalcified vegetative segments). Along with geniculate coralline algae, they share similar reproductive structures that are confined in either conceptacles (i.e., the Corallinales and Sporolithales; see Harvey et al. 2003) or smaller compartments (i.e., the Sporolithales; Le Gall et al. 2010) and by tri-phasic life histories (Woelkerling 1988). Crustose coralline algae (CCA) are a group of non-geniculate coralline algae that have crustose thalli. In tropical waters, CCA play an important ecological role by contributing to the construction of biological or coral reefs (Bosence 1983a; Steneck 1986). They also provide food for herbivores and a

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nursery for the settlement of invertebrate larvae (Steneck and Dethier 1994; Adey 1998). In tropical waters CCA play an important ecological role by contributing to the construction of biological or coral reefs (Bosence 1983a; Steneck 1986). In temperate waters, non-geniculate coralline algae (NCA) often form extensive free-living rhodolith beds (often called by the Breton name, maerl), which constitute important habitats for a diverse assemblage of marine organisms (Cabioch 1970; Adey 1973, 1998; Steneck and Dethier 1994; Amado-Filho et al. 2007). CCA are an important link in the calcium cycle in the marine food web providing food for herbivores and a nursery for the settlement of invertebrate larvae (Steneck and Dethier 1994; Adey 1998). CCA are by far the best fossilized red algal orders (Aguirre et al. 2010), and coralline algal fossils have been useful in inferring paleo-climate and paleo-environments (Foster 2001; Cabioch et al. 2008).

Algal reefs are biological reefs, mainly made of crustose coralline algae and are important ecosystems with a wide distribution in both temperate and tropical areas from deep (Bosence 1983a, b) to shallow waters (Adey 1975; Freiwald and Henrich 1994). Coralline algal reef frameworks and composition have been largely reviewed by Bosence (1983a). He illustrated the formation of the biostrome made up of leafy crusts (e.g., *Mesophyllum* Me. Lemoine and *Lithophyllum* Philippi), rhodoliths (e.g., *Lithothamnion* Heydrich as *Lithothamnium* and *Phymatolithon* Foslie), and branches arising from crusts (e.g., *Neogoniolithon* Setchell & L.R.Mason) of different non-geniculate coralline species. Among the reef-forming coralline genera, *Mesophyllum* and *Lithophyllum* are commonly found in temperate (e.g., the Mediterranean and NE Atlantic) and warm waters (e.g., St. Croix and Florida) (reviewed by Bosence 1983a). On the other hand, *Phymatolithon* is mostly restricted to temperate or colder waters (Adey and McKibbin 1970; Wilks and Woelkerling 1994; Peña et al. 2015). Despite their ecological roles and their paleontological significance, CCA identification is hampered by their highly variable morphology, which depends on environmental conditions, as well as the need to decalcify them prior to the observation of anatomical features (Steneck and Adey 1976; Woelkerling et al. 1993; Maneveldt et al. 2008).

Dai et al. (2009) documented the distribution and formation history of the algal reefs along the coastlines of Taiwan. They reported that the algal reefs occurring along the seashore of Taoyuan County are the most extensive, about 27 km long and 100–450 m wide. Taoyuan algal reefs are mainly composed of non-geniculate coralline algae with crustose thalli that grow in the intertidal zone and in shallow waters 1–2 m deep. The oldest Taoyuan algal reefs were formed 7500 BP (years Before the Present) based on carbon-14 dating (Wang 2008; Dai et al. 2009). Few CCA names were briefly mentioned from the algal reefs in Taoyuan County in the northern Taiwan, e.g., *Lithophyllum* sp. (Wang 2008, 2010; Dai et al. 2009), *Mesophyllum*, *Phymatolithon*, and *Harveyolithon*

A.Rösler, Perfectti, V.Peña & J.C.Braga (Liou et al. 2017). It is important to document the CCA species in Taoyuan County as the algal reefs have been damaged by industrial pollution and habitat destruction as well as covered by drift sand (Lin et al. 2013; Liou et al. 2017). Although many algal reefs have been reported from Taiwan (Wang 2008; Dai et al. 2009), the CCA species diversity and composition in the algal reefs remain poorly known.

Recently, our research team has undertaken an ecological survey of the species diversity and composition of the Taoyuan algal reefs. We found that the CCA species in the region are difficult to identify based only on morphological features alone. In order to understand the species diversity and the CCA phylogenetic relationships occurring in Taoyuan algal reefs, we analyzed the SSU and *psbA* genes for Taoyuan CCA collections and from other localities with similar CCA species in the northern Taiwan. Both *psbA* and SSU sequence analyses detected many undescribed CCA species in Taoyuan algal reefs. We describe one new genus (*Crustaphyllum* L.-C.Liu & Showe M.Lin gen. nov.) and three new species (*Crustaphyllum pacificum* L.-C.Liu & Showe M.Lin sp. nov., *Harveyolithon rosea* L.-C.Liu & Showe M.Lin sp. nov., and *Phymatolithon margoundulatus* L.-C.Liu & Showe M.Lin sp. nov.) in the order Hapalidiales for the dominant or common CCA species found in Taoyuan algal reefs based on vegetative and reproductive morphologies.

## Materials and methods

**Sampling and preservation** CCA samples were collected by the hand or using a hammer and chisel from the algal reefs in the intertidal zone, or in the rocky subtidal by snorkeling or SCUBA diving. Each CCA sample was first photographed; then, a small part of the sample was preserved in silica gel for subsequent DNA extraction, whereas the remaining sample was first fixed in 10% formalin-seawater and then stored in 5% formalin in seawater or air-dried for morphological studies. Initially, more than 1300 crustose coralline algal specimens were collected from Taoyuan algal reefs and neighboring areas in the northern Taiwan. After a comparison of the external morphology of the CCA collections, ca. 120 individuals were used for further morphological and molecular analyses. Type collections and voucher specimens were deposited in the HAST herbarium located at Academia Sinica, Taipei, and the seaweed laboratory at the National Taiwan Ocean University ('NTOU'), Taiwan. For anatomical studies, hand-cut sections were stained with 1% aniline blue and mounted in 50% Karo light corn syrup or were treated with aceto-iron-hematoxylin-chloral hydrate and mounted in 50% Hoyer's mounting medium as described in Lin et al. (2004). Photomicrographs were taken on an Olympus BX51 microscope with a Q-imaging digital camera (Canada) or by SEM

(Hitachi SEM S-3400N, Japan). Habit images were taken with a Nikon D300 digital camera (Seiko Epson Corp., Japan).

**DNA extraction, PCR amplification, and sequencing** DNA from silica gel dried specimens was extracted using the DNeasy Plant Mini Kit (Qiagen, USA) following the manufacturer's instructions. The DNA sequencing procedures were as described in Lin et al. (2001). Two molecular markers, the small subunit of the ribosomal rDNA (SSU, ca. 1700 base pairs) and the Photosystem II protein D1 coding gene in the chloroplast genome (*psbA*, ca. 850 base pairs), were amplified in order to infer the phylogenetic relationships of the target CCA species. The SSU primers used were as described in Saunders and Kraft (1996; G01 + G04) and Nakayama et al. (1998; SR4 + SR9) in addition to two newly designed primers F1072 (5'-ACGATCAGATACCGTCGTA-3') and R1953 (5'-AACTAAGAACGGCCATGCAC-3') specifically for the species of *Crustaphytum* and *Phymatolithon*. The newly generated sequences and those available from GenBank were compiled and aligned with Sequencher (Gene Codes Corp., USA) and exported for phylogenetic analysis.

**Molecular analyses** Phylogenetic analyses were performed using maximum likelihood (ML), maximum parsimony (MP), and Bayesian analysis. MP and nonparametric bootstrapping followed Lin et al. (2001). The genera *Ahnfeltia* (Ahnfeltiales) and *Nemalion* (Nemaliales) were selected as outgroups following Le Gall et al. (2010) and Nelson et al. (2015). We used PAUP\* v4.0 (Swofford 2003) for MP analyses. Initial searches consisted of 100 random sequence additions, MULPARS (but holding five trees at each step), STEEPEST DESCENT, and nearest neighbor interchange branch swapping trees leading to most parsimonious solution. Trees found in these initial searches were then used as starting points for further searches with MULPARS, STEEPEST DESCENT, and tree bisection reconnection algorithm until final swapping was complete. GARLI Gari 0.96 beta (Zwickl 2006) was used for ML analyses. The substitution model for ML was general-time reversible with gamma-distributed rate heterogeneity (GTR + I +  $\Gamma$ ) as suggested by running "Find best DNA model" implemented in MEGA 6 (Tamura et al. 2013). Bootstrap proportion (BP) values involved 1000 and 500 replicates for MP and ML analyses, respectively. The substitution model for ML was the general-time-reversible with gamma-distributed rate heterogeneity (GTR + I +  $\Gamma$ ) as defaults. A Bayesian analysis (BA) was performed in MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) using a GTR + I +  $\Gamma$  model, which allowed for rate variation among different codon positions. The analysis consisted of four chains (one hot and three cold), which were run for  $10^6$  generations with sampling every 100 generations. Stationarity of SSU and *psbA* genes was reached at generation 20,000/60,000, respectively, which were the "burn-in" of the

chain. Inferences about the phylogeny were based on those trees sampled after generation 20,000/60,000, respectively. A 50% consensus tree (majority rule as implemented by PAUP\* v4.0) was computed from the 9800 + 1/9400 + 1 trees saved after the burn-in point.

## Results

### Molecular analyses

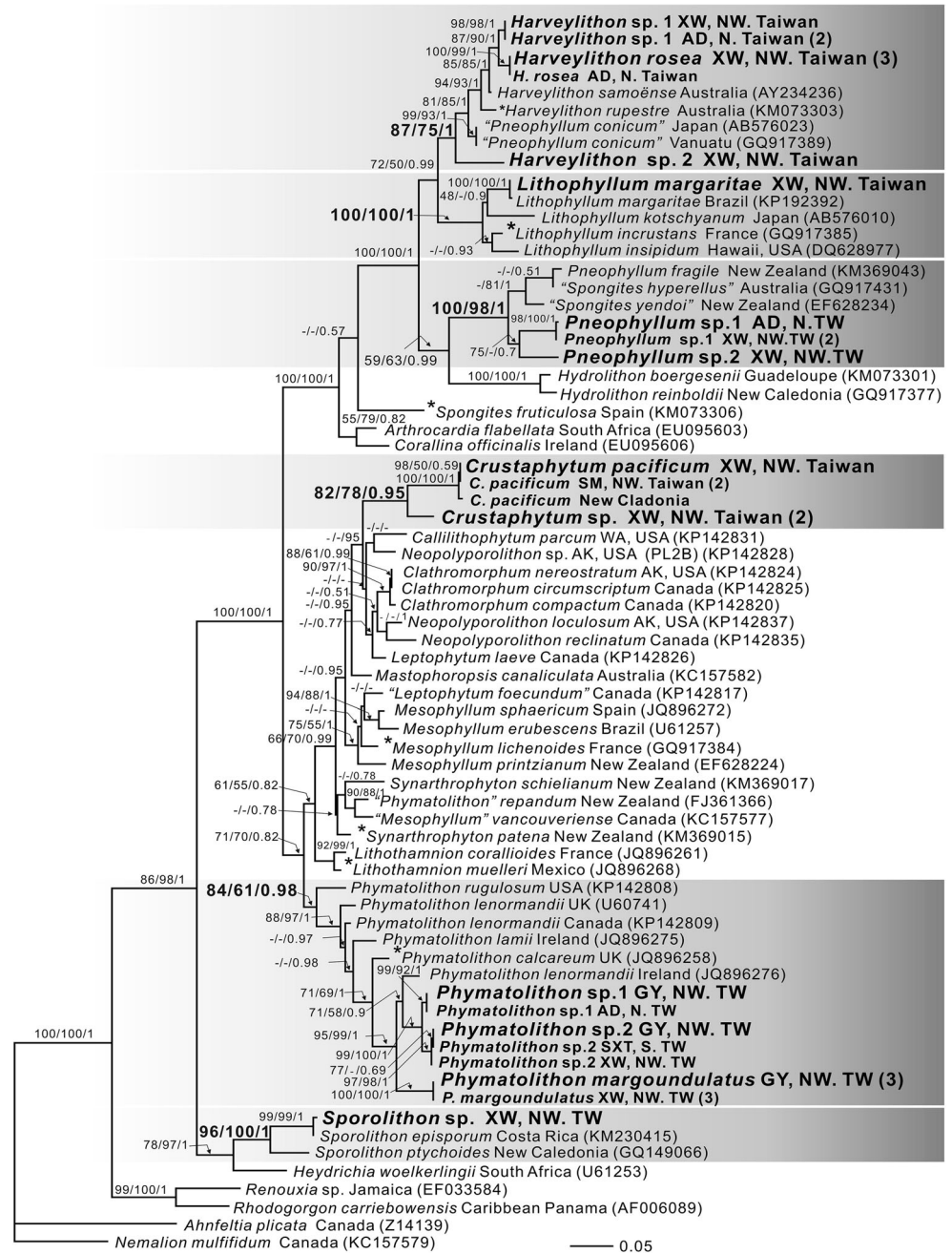
A total of 70 SSU and 84 *psbA* sequences of the crustose coralline algae from Taiwan and related CCA sequences available from GenBank (see Table S1, supplementary information), including 20 SSU and 31 *psbA* sequences from Taoyuan algal reefs and other regions in Taiwan, were used for the final phylogenetic analyses. The analyzed data matrix included 834 base pairs (bp) for *psbA* with 260 parsimony-informative sites and 1687 bp for SSU with 314 parsimony-informative sites. No insertion or deletion mutations were found in the dataset of *psbA*, whereas the indels (gaps) in the SSU alignments were treated as the fifth character. The topologies of the MP, ML, and BA trees were largely congruent, and only the ML trees were shown for the SSU (Fig. 1) and *psbA* (Fig. 2) datasets. The MP and Bayesian trees for the SSU (Figs. S1, S2) and *psbA* (Figs. S3, S4) datasets are in supplement materials.

In the SSU tree the CCA from Taoyuan algal reefs along with the taxa from other localities in Taiwan were grouped into six genera, *Harveyolithon* A.Rösler, Perfectti, V.Peña & J.C.Braga, *Lithophyllum* Philippi, and *Pneophyllum* Kützing in the Corallinales; *Crustaphytum* L.-C. Liu & Showe M.Lin gen. nov. and *Phymatolithon* Foslie in the Hapalidiales; and *Sporolithon* Heydrich in the Sporolithales with moderate to strong supports (Fig. 1) with 12 species (Fig. 2, clades I–XII). Among the six genera detected from Taoyuan algal reefs (see Fig. 2), *Harveyolithon* and *Phymatolithon* were the most dominant genera, and both contained three species, *Harveyolithon* sp. 1 (clade I), *Harveyolithon rosea* L.-C.Liu & Showe M.Lin sp. nov. (clade II), *Harveyolithon* sp. 2 (clade III), *Phymatolithon* sp. 1 (clade VII), *Phymatolithon* sp. 2 (clade VIII), and *Phymatolithon margoundulatus* L.-C.Liu & Showe M.Lin sp. nov. (clade IX). *Pneophyllum* and *Crustaphytum* gen. nov. were the second most speciose genera in the algal reefs, each with two species, treated as *Pneophyllum* sp. 1 (clade V), *Pneophyllum* sp. 2 (clade VI), *Crustaphytum pacificum* sp. nov. (clade X), and *Crustaphytum* sp. (clade XI). *Lithophyllum* (clade IV, *L. margaritae* (Hariot) Heydrich) and *Sporolithon* [clade XII, *Sporolithon* sp.] were the least diverse genera in the region having only one species in each genus.

Based on *psbA* sequences, *Phymatolithon* was the most dominant genus in Taoyuan algal reefs in terms of coverage



**Fig. 1** SSU phylogeny of the non-geniculate coralline algae from Taoyuan algal reefs with related species from the northern Taiwan and around the world (maximum likelihood method,  $-\ln L = -9228.5568$ ). Bootstrap values of ML and MP and Bayesian posterior probabilities of BI, respectively, are shown above branches. Hyphen (-) indicates low support (< 50%). Boldface denotes the newly generated sequences in this study. Asterisk denotes generic types. The Arabic numbers in brackets after the species names represent the number of specimens with identical sequences



and species number (Fig. 2, clades IX~XI). *Crustaphyllum* gen. nov. was the second most dominant genus on the reefs, represented by *C. pacificum* sp. nov. with 0.48% (4 bp difference within the 834 bp of the *psbA* sequences) and 0.12% (2 bp difference within the 1687 bp of the SSU sequences) genetic divergence between the populations from Taiwan and New Caledonia, and by *Crustaphyllum* sp., which is restricted to Taoyuan algal reefs. *Harveyolithon rosea* sp. nov. was found in both the algal reefs and rocky shores in the northern Taiwan. In the *psbA* phylogenetic tree (Fig. 2), three “*Pneoephyllum*” from the western Pacific Ocean (Vanuatu, Fiji, and Japan) as well as “*Porolithon onkodes*” from

Indonesia were positioned within the *Harveyolithon* assemblage. In *Pneoephyllum*, one species (clade V, *P. sp. 1*) appeared to occur in both algal reefs and rocky shores in the northern Taiwan, whereas *Pneoephyllum sp. 2* was restricted to Taoyuan algal reefs.

### Morphological observations

Both SSU (Fig. 1) and *psbA* (Fig. 2) sequence analyses revealed at least twelve CCA species in Taoyuan algal reefs. Accordingly, we describe a new genus (*Crustaphyllum* L.-C.Liu & Showe M.Lin gen. nov.) and three new species





(*Crustaphytum pacificum*, *Harveyolithon rosea*, *Phymatolithon margoundulatus*) for the dominant species found in Taoyuan reefs. Descriptions of the other CCA species detected by DNA sequence analysis must await new material, as there are not enough specimens at present with well-developed reproductive structures for a detailed morphological study.

### *Crustaphytum* L.-C.Liu & Showe M.Lin *gen. nov.*

**Description** Thalli non-geniculate, epilithic, encrusting, rugose, protuberances branches absent; thallus organization dorsiventral, monomerous composed of a coaxial core growing more or less parallel to thallus surface; peripheral region composed of cellular filaments initiated from the core growing and curving outwards; outermost epithallial cells with rounded or flattened walls but not flared; subepithallial initials either the same length or shorter than derivative cells positioned below; calcified crystals in the cell wall radially arranged; cells in contiguous filaments joined by cell fusions, secondary pit-connections absent; tetrasporangial conceptacle chambers multiporate, tetrasporangia zonately divided only; mature spermatangia produced in terminal, branched clusters from distal ends of spermatangial parental filaments.

**Etymology** The genus epithet “*Crusta*” refers to its crustose thallus, whereas “*phytum*” means plant.

**Generitype** *Crustaphytum pacificum* L.-C.Liu & Showe M.Lin sp. nov.

**Distribution** Currently known only in the western Pacific Ocean (Taiwan and New Caledonia).

### *Crustaphytum pacificum* L.-C.Liu & Showe M.Lin sp. nov. (Figs. 3 and 4)

**Description** Crustose thalli elliptical, with rough surfaces and wavy margins or irregular in outline, 4–10 cm long by 3–5 cm wide, epilithic not firmly attached by ventral thallus surface, thallus thickness 100–300  $\mu\text{m}$  and 35–45 celled layers, thallus composed of one or two layers of roundish to flattened epithallial cells 1.0–1.8  $\mu\text{m}$  long by 3.5–2.5  $\mu\text{m}$  wide, subepithallial initials 2.5–5.0  $\mu\text{m}$  long by 3.3–4.7  $\mu\text{m}$  wide; trichocytes borne on subepithallial cells and usually form a group of 4–5 in the epithallial layer; tetrasporophytes with densely grouped multiporate conceptacles with elliptical chambers, 130–155  $\mu\text{m}$  high by 115–200  $\mu\text{m}$  wide, pore canals of tetrasporangial conceptacle filled with plugs, 15–22  $\mu\text{m}$  in length, basal parts of plugs 8–10  $\mu\text{m}$  in wide; spermatangial conceptacle uniporate, chambers, 40–50  $\mu\text{m}$  high by 90–135  $\mu\text{m}$  wide.

**Holotype** HAST143457 (male gametophyte), collected by S.-M. Lin & L.-C. Liu on May 6, 2016, deposited in the herbarium of the Academia Sinica, Taipei (HAST) (see Fig. 3a).

**Isotypes** HAST143458 (tetrasporophyte), NTOU 001369–NTOU001371, male gametophyte and tetrasporophytes, deposited in the herbaria of the Academia Sinica, Taipei (HAST) and the Institute of Marine Biology, National Taiwan Ocean University (“NTOU”).

**Type locality** Xinwu Township, Taoyuan County, Taiwan. (25° 00' 32.6" N, 121° 01' 14.2" E).

**Etymology** The species name “*pacificum*” refers to the Pacific Ocean, the geographic location in which this new species is found.

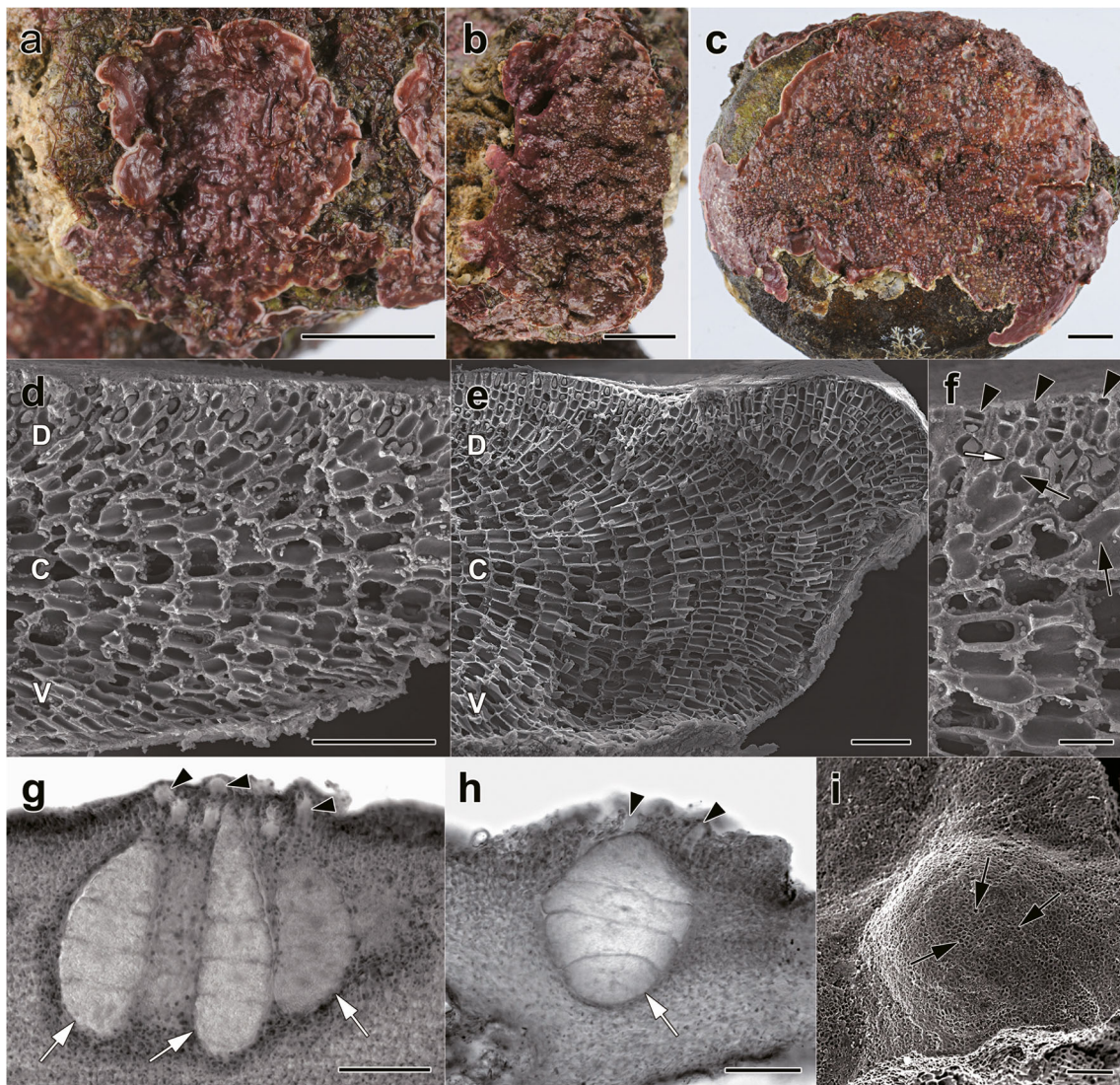
**Distribution** Currently known from the western Pacific Ocean (Taiwan and New Caledonia). In Taiwan, this species is distributed along the coastlines of Taoyuan County to New Taipei City in northern Taiwan.

**Habitat and seasonality** Plants were abundant in algal reef areas, growing abundantly from January through July and growing mostly in the lower intertidal zone attached to old algal reefs or pebbles, but sometimes found in the subtidal zones attached to rocks at 2–3 m depth. Tetrasporic thalli bearing mature conceptacles were often found in winter and spring (January through March), whereas male gametophytes were only observed in late spring (May).

**Additional specimens examined** Xinwu, Taoyuan County, Taiwan, January 25, 2015 (NTOU001359–NTOU001363, NTOU001479–NTOU001486), January 15, 2016 (NTOU001364–NTOU001365), May 6, 2016 (NTOU001366–NTOU001371); Guanyin, Taoyuan County, Taiwan, March 18, 2013 (NTOU001478), January 19, 2017 (NTOU001487–NTOU001490); Linshanbi, New Taipei City, Taiwan, July 5, 2016 (NTOU001372); Shimen, New Taipei City, Taiwan, June 25, 2013 (NTOU001357).

**Habit and vegetative morphology** Thalli are non-geniculate, crustose, 4–10 cm long by 3–5 cm wide, without bearing any protuberances or branches on surfaces, and dark purple or dark red in color (Fig. 3a–c). Thallus margins are slightly wavy or partially unattached to the substrate and the ventral surfaces are not firmly attached to old algal reefs. Crustose thalli are 100–300  $\mu\text{m}$  thick and are composed of 30–45 celled layers (Fig. 3d, e). Thallus growth is dorsiventral and monomerous (Fig. 3d, e). In a longitudinal view of thallus crust, the core area comprises approximately 70% of the thallus thickness and is composed of 25 layers of filaments, of the cells which are oblong or oval or slightly elongated, mostly 12–21  $\mu\text{m}$  long by 6–9  $\mu\text{m}$  wide (Fig. 3e). Perithallial filaments in the dorsal region are composed of 7–10 celled layers, including one or two layers of epithallial cells, 1.0–1.8  $\mu\text{m}$  long by 3.5–2.5  $\mu\text{m}$  wide, and one layer of subepithallial cells, 2.5–5.0  $\mu\text{m}$  long by 3.3–4.7  $\mu\text{m}$  wide in a vertical view (Fig. 3e, f). The calcified crystal filaments in the meristem region are radially arranged (Fig.





**Fig. 3** *Crustaphytum pacificum* L.-C.Liu & Showe M.Lin gen. and sp. nov. Habit, vegetative structure, and male and tetrasporangial reproductive structure [scale bars: a–c=1 cm; d–e, g–i=50 μm; f=10 μm]. **a** Holotype, male gametophyte (HAST143457). **b** Isotype, male gametophyte (HAST143458). **c** Isotype, tetrasporic plant (NTOU001370). **d** Longitudinal section through young thallus showing monomerous construction and ventral (V) and dorsal (D) parts of coaxial hypothallus (NTOU001361). **e** Longitudinal section through mature thallus showing thickened, coaxial hypothallus (NTOU001360). **f** Close up of **d** showing flattened epithallial cells (black arrowheads), radially

arranged calcified crystals (white arrow) in the meristem, and cell fusion (black arrows) between adjacent cells (NTOU001361). **g** Longitudinal section through a multiporate tetrasporangial conceptacle showing pore plugs (arrowheads) and developing tetrasporangia (arrows) (NTOU001361). **h** Longitudinal section through a mature tetrasporangial conceptacle showing pore plugs (arrowheads) and inflated tetrasporangium (arrows) and zonately arranged spores (NTOU001362). **i** Surface view of a multiporate tetrasporangial conceptacle showing pores (arrows) (NTOU001364)

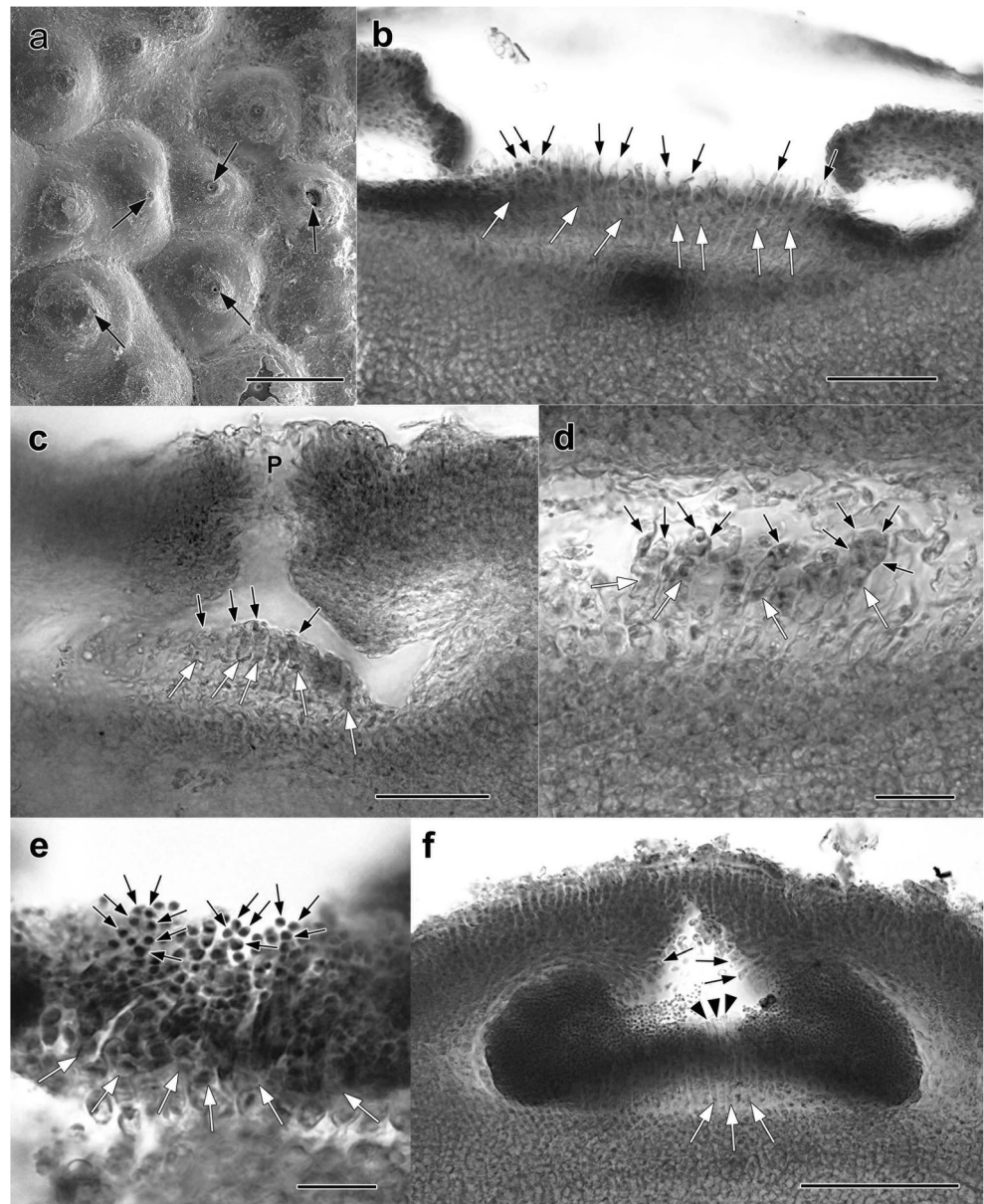
3f). Generally, subepithallial cells are either the same length or shorter than their derivative cells positioned below and cell fusion is commonly found between cells of neighboring filaments (Fig. 3f).

**Reproductive morphology** Tetrasporophytes (Fig. 3c) and gametophytes (Fig. 3a, b) are similar in habit and size. Tetrasporangial conceptacles protrude slightly or are flattened and the chambers are elliptical, 130–155 μm high by 115–200 μm wide (Fig. 3g, h). Tetrasporangial conceptacle

roofs are flat-topped and multiporate. Each pore is surrounded by 6–7 cells in surface view and the pore canals are filled with plugs, 15–22 μm long by 8–10 μm wide at basal portion (Fig. 3i). The filaments around the pore canals are composed of 6–7 cells long, with the basal cells are smaller than the upper ones (Fig. 3g, h). Mature tetrasporangia are zonately divided (Fig. 3h), 80–100 μm long by 41–50 (up to 100) μm wide. Spermatangial conceptacles are hemispheric and uniporate (Fig. 4a, arrows) and the chambers are reniform (Fig. 4f), 40–50 μm high by



**Fig. 4** *Crustaphytum pacificum* L.-C.Liu & Showe M.Lin gen. & sp. nov. Development of spermatangial conceptacles [scale bars: **a**, **c** = 50  $\mu$ m; **b** = 25  $\mu$ m; **d**–**e** = 10  $\mu$ m; **f** = 50  $\mu$ m]. **a** Surface view of male conceptacles with unipores (arrows) (HAST143458). **b** Longitudinal section through young male conceptacles showing spermatangial parental cells (black arrows) and elongated subtending cells (white arrows) (HAST143458). **c** Longitudinal section through another young male conceptacle with thickened roof showing spermatangial parental cells (black arrows) and elongated subtending cells (white arrows) (HAST143457). **d** A later stage, showing spermatangial parental filaments (white arrows) bearing newly formed, short branches (black arrows) (HAST143457). **e** Details of fully developed spermatangia (black arrows) formed in densely packed clusters. Note that the subtending cells remain distinct (white arrows) (HAST143457). **f** Longitudinal section through a mature male conceptacle showing densely arranged spermatangia, remaining protective cells (arrowheads), and the innermost roof cells (black arrows) and vacuolated subtending cells (white arrows) (HAST143457)

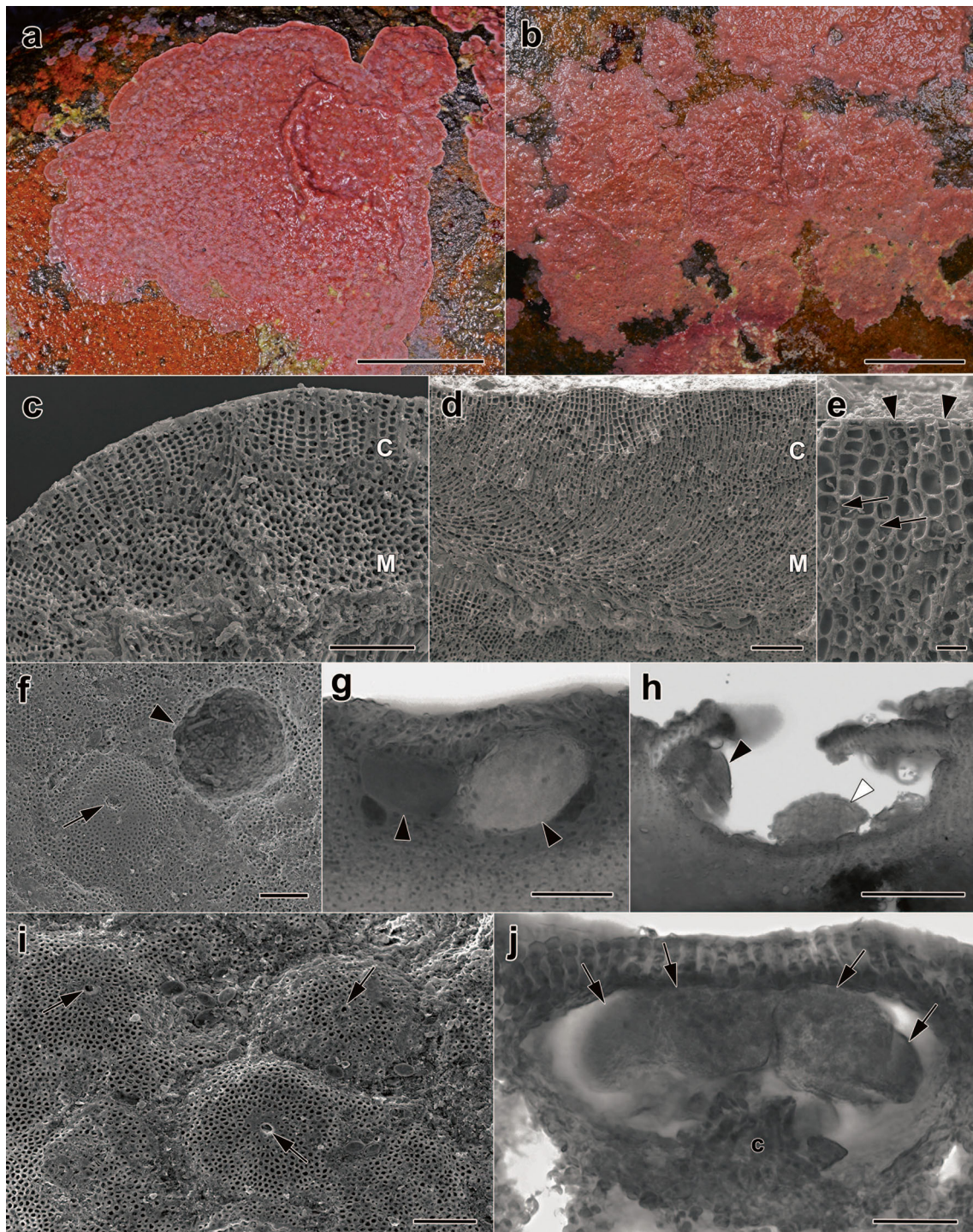


90–135  $\mu$ m wide. The very earliest stage of male conceptacle development is not found. At an early stage of spermatangial development, spermatangial parental filaments (Fig. 4b, c) divide two to three times transversely and then branched one to two times (Fig. 4d, black arrows), while their subtending cells become vacuolated and slightly elongated. Spermatangia are produced in terminal, branched clusters (Fig. 4e, f) from the distal ends of spermatangial parental filaments. In fully developed male conceptacles, a group of spermatangial parental filaments in the central portion do not divide further to produce spermatangia and the terminal cells become vacuolated (Fig. 4f, arrowheads). Spermatangial conceptacle roofs are 20–42  $\mu$ m thick and the innermost roof cells grow inward towards the pore canal (Fig. 4f, black arrows).

#### *Harveyolithon rosea* L.-C.Liu & Showe M.Lin sp. nov. (Fig. 5)

**Description** Thalli crustose, 2–5 cm long by 3–4 cm wide, elliptical or rounded with rough surfaces and slightly wavy or lobed margins, thallus 25–50 celled layers and 300–500  $\mu$ m thick, composed of a layer of roundish to flattened epithallial cells, 1–2  $\mu$ m in length and 3–5  $\mu$ m in diameter, a cortex of 15–30 celled layers, and a medulla of 10–20 celled layers; tetrasporangial conceptacles, 100–180  $\mu$ m in diameter in surface view, uniporate, roofs composed of 3–4 (up to 6) celled layers, slightly protruding above to flush, chambers elliptical or dome-shaped in longitudinal section view, 50–60  $\mu$ m high by 70–150  $\mu$ m in diameter, mature tetrasporangia 30–45  $\mu$ m long by 20–25  $\mu$ m wide; female conceptacles, 80–120  $\mu$ m in diameter in surface view, uniporate, chambers





**Fig. 5** *Harveyolithon rosea* L.-C.Liu & Showe M.Lin sp. nov. Habit, vegetative structure, and female and tetrasporangial structure [f, i, j SEM images; g–h light microscopic images; scale bars: a, b = 1 cm; c, d, f, h, i = 50  $\mu$ m; e = 10  $\mu$ m; g, j = 25  $\mu$ m]. a Holotype, female gametophyte (HAST143459). b Isotype, tetrasporophyte (NTOU001413). c Longitudinal section through thallus margin showing young monomerous construction, consisting of medullary (M) and cortical (C) cells (NTOU001399). d Longitudinal section through a thickened thallus showing monomerous construction composed of medulla (M) and cortex (C). Note that an older thallus is beneath of the thickened thallus (NTOU001409). e Close up of flattened epithallial cells (arrowheads) and cell fusion between neighboring cells (arrows)

(NTOU001409). f Surface view of tetrasporangial conceptacles with unipores (arrow), noting that one of the roofs being broken (arrowhead) (NTOU001409). g Longitudinal section of an immature tetrasporangial conceptacle showing developing tetrasporangia (arrowheads) (NTOU001409). h Longitudinal section of a fully developed tetrasporangial conceptacle showing dome-shaped chamber, zonately divided tetrasporangium (black arrowhead), and a remaining wall of discharged tetrasporangium (white arrowhead) (NTOU001409). i Surface view of uniporate (arrows) carposporangial conceptacles (NTOU001413). j Longitudinal section through a carposporophyte conceptacle showing carposporangia (arrows) surrounding the central columella (c) (HAST143459)



elliptical or dome-shaped in longitudinal section view, 40–55  $\mu\text{m}$  high by 80–100  $\mu\text{m}$  in diameter, mature carposporophyte bearing a small central columella on floor.

**Holotype** HAST14345 (female gametophyte), collected by S.-M. Lin & L.-C. Liu on April 15, 2014, deposited in the herbarium of the Academia Sinica, Taipei (HAST), Taiwan (see Fig. 5a).

**Isotypes** HAST14360–HAST14362 (sterile specimens), NTOU001411 (Fig. 5b)–NTOU001413, tetrasporophytes, collected by S.-M. Lin & L.-C. Liu on April 15, 2014, deposited in the herbaria of the Academia Sinica, Taipei (HAST) and the Institute of Marine Biology, National Taiwan Ocean University (“NTOU”).

**Type locality** Xinwu Township, Taoyuan County, Taiwan (25° 00′ 32.6″ N, 121° 01′ 14.2″ E).

**Etymology** The species name “*rosea*” refers to its rosy color.

**Distribution** Only found in the northern Taiwan, including Taoyuan County, New Taipei City, and Keelung City.

**Habitat and seasonality** Plants were very abundant on Taoyuan algal reefs and rocky shores in the northern coast, occurring almost year round and growing mostly in the lower intertidal or subtidal zones (1–2 m deep) attached to old algal reefs or rocks. Tetrasporic thalli bearing mature conceptacles were often found in late spring (April), whereas female gametophytes were only observed in autumn (November).

**Additional specimens examined** Xinwu, Taoyuan County, Taiwan, January 9, 2013 (NTOU001393–NTOU001396), April 15, 2014 (NTOU001410–NTOU001413, NTOU001491–NTOU001518), January 25, 2015 (NTOU001397, NTOU001414–NTOU001415, NTOU001519–NTOU001536), January 15, 2016 (NTOU001416, NTOU001537–NTOU001540), 6 May, 2016 (NTOU001541–NTOU001542); Aodi, New Taipei City, Taiwan, August 17, 2013 (NTOU001398–NTOU001399); Shimen, New Taipei City, Taiwan, June 25, 2013 (NTOU001397), August 27, 2013 (NTOU001400–NTOU001401), December 8, 2013 (NTOU001403), April 2, 2014 (NTOU001409); Longdongwan, New Taipei City, Taiwan, March 31, 2014 (NTOU001408); Chaojing, Keelung City, Taiwan, November 22, 2013 (NTOU001402).

**Habit and vegetative morphology** Thalli are crustose, elliptical or rounded, with slightly wavy or lobed margins (Fig. 5a, b), 2–5 cm long by 3–4 cm wide. Thallus color is pinkish to rosy when exposed to different lights. Thalli are monomerous and dorsiventrally organized, 25–50 celled layers (Fig. 5c, d), 300–500  $\mu\text{m}$  thick, including one layer of epithallial cells that are 1–2  $\mu\text{m}$  long by 3–5  $\mu\text{m}$  wide in a vertical view. In a longitudinal section of the thallus crust, the medullary area is composed of 15–20 layers of filaments composed of oblong to elongate cells (Fig. 5c, d, M), 5–7  $\mu\text{m}$  long by 2–5  $\mu\text{m}$  wide. Perithallial filaments in the cortical region are composed of

25–50 squat to oblong cells (Fig. 5c, d, C), 2–5  $\mu\text{m}$  long by 3–5  $\mu\text{m}$  wide. Cell fusion is commonly found between cells of neighboring filaments (Fig. 5e).

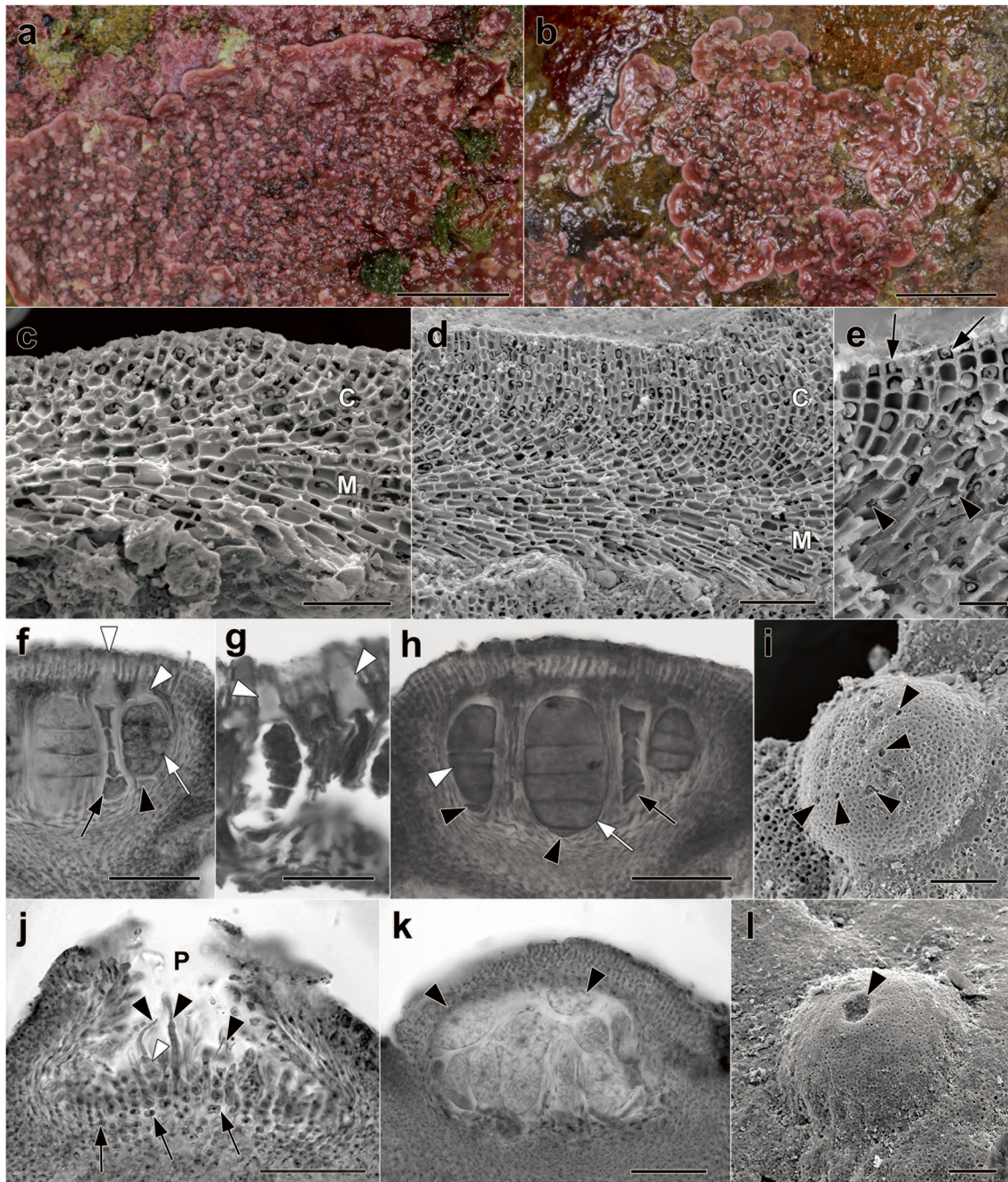
**Reproductive morphology** Gametophytes (Fig. 5a) and tetrasporophytes (Fig. 5b) are similar in habit and size. Tetrasporangial conceptacles do not protrude above the dorsal surfaces of thalli when young (Fig. 5g), but become slightly raised when old (Fig. 5f, h). Tetrasporangial chambers are elliptical or dome-shaped (Fig. 5h), 50–60  $\mu\text{m}$  high by 70–150  $\mu\text{m}$  in diameter. The roofs are flat-topped and uniporate and are composed of 3–4 (up to 6) celled layers, 18–20  $\mu\text{m}$  thick (Fig. 5h), including one or two layers of epithallial cells, a single layer of columnar meristematic cells that are two to three times as long as the length of the epithallial cells, and with one or two layers of small inner cells (Fig. 5g). Mature tetrasporangia are zonately divided (Fig. 5h), 30–45  $\mu\text{m}$  long by 20–25  $\mu\text{m}$  wide, and bisporangia are not observed. Carposporangial conceptacles are flat or slightly raised, uniporate (Fig. 5i), 80–120  $\mu\text{m}$  in diameter in surface view. Carposporangial chambers are elliptical or dome-shaped in longitudinal section view (Fig. 5j), 40–55  $\mu\text{m}$  high by 80–100  $\mu\text{m}$  in diameter. In mature conceptacles, gonimoblast filaments surround the central columella and carposporangia are produced terminally (Fig. 5j). The conceptacle roof cells are composed of oblong or cylindrical cells, 20–25  $\mu\text{m}$  thick, and carposporangia are pyriform, 25–33  $\mu\text{m}$  long by 30–35  $\mu\text{m}$  wide. Spermatangial plants were not found during the study.

***Phymatolithon margoundulatus* L.-C.Liu & Showe M.Lin sp. nov. (Fig. 6)**

**Description** Thalli crustose, 2–4 cm long by 2–3 cm wide, fan-shaped or rosette-like with smooth or imbricate surfaces and strongly wavy or small lobed margins, with secondarily formed thallus lobes often growing over primary encrusts; thallus 35–45 celled layers and 100–250  $\mu\text{m}$  thick, composed of a layer of roundish to squat epithallial cells 2.5–3.5  $\mu\text{m}$  long by 2.5–3.0  $\mu\text{m}$  wide, cortex 10–15 celled layers when young, 20–35 celled layers when old, medulla 8–10 celled layers when young, 10–15 celled layers when old; tetrasporangial conceptacles hemispheric, 160–190  $\mu\text{m}$  in diameter in surface view, multiporate, roof, composed of 3–4 celled layers, smooth without uplifted rim, chambers round or oval-shaped in longitudinal section view, 80–100  $\mu\text{m}$  high by 110–210  $\mu\text{m}$  in diameter, mature tetrasporangia 75–80  $\mu\text{m}$  long by 35–42  $\mu\text{m}$  wide; female conceptacles, 100–150  $\mu\text{m}$  in diameter in surface view, uniporate, chambers rounded in longitudinal section view, 30–50  $\mu\text{m}$  high by 60–75  $\mu\text{m}$  in diameter, mature carposporangia 15–20  $\mu\text{m}$  long by 10–12  $\mu\text{m}$  wide.

**Holotype** HAST143463 (tetrasporophyte), collected by S.-M. Lin & L.-C. Liu on April 15, 2014, deposited in the





**Fig. 6** *Phymatolithon margoundulauts* L.-C.Liu & Showe M.Lin sp. nov. it, vegetative structure, and female and tetrasporangial structure [c–e, i, l SEM images; f–h, j–k light microscopic images; scale bars: a, b = 0.5 cm; c, g, j, k = 25  $\mu$ m; d, f, h, l = 50  $\mu$ m; e = 10  $\mu$ m]. **a** Holotype, tetrasporophyte (HAST143463). **b** Isotype, female gametophyte (NTOU001426). **c** Longitudinal section through thallus margin showing young monomerous construction, consisting of medullary (M) and cortical (C) cells (NTOU001427). **d** Longitudinal section through an old plant showing thickened monomerous construction composed of medullary (M) and cortical (C) cells (NTOU001419). **e** Close up of domed-shaped epithallial cells (arrowheads) and cell fusion between neighboring cells (arrows) (NTOU001419). **f** Longitudinal section through an immature multiporate tetrasporangial conceptacle stained with hematoxylin solution showing apical pore plugs (white arrowheads) in canals, an aborted tetrasporangium (black arrow), and a developing tetrasporangium (white arrow) borne on a stalk cell (black arrowhead)

(HAST143463). **g** Longitudinal section through a mature multiporate tetrasporangial conceptacle stained with aniline blue showing apical pore plugs (white arrowheads) in the canals in focus (HAST143463). **h** Longitudinal section through a mature multiporate tetrasporangial conceptacle with hematoxylin solution showing developing (white arrowhead) and fully developed (white arrow) tetrasporangia borne on stalk cells (black arrowheads), and aborted tetrasporangium (black arrow) (HAST143463). **i** Surface view of a tetrasporangia conceptacle with multiple pores (arrowheads) (NTOU001427). **j** Longitudinal section through a female conceptacle showing fully developed carpogonial branches, carpogonia (arrow) with terminal trichogynes (arrowhead) (NTOU001422). **k** Longitudinal section through a mature carposporophyte conceptacle showing dome-shaped chamber and carposporangia (arrowheads) (NTOU001422). **l** Surface view of a uniporate (arrowhead) female conceptacle (NTOU001422)



herbarium of the Academia Sinica, Taipei (HAST), Taiwan (see Fig. 6a).

**Isotype** NTOU001426 (tetrasporophyte), collected by S.-M. Lin & L.-C. Liu on April 15, 2014, deposited in the herbarium of the Institute of Marine Biology, National Taiwan Ocean University (“NTOU”), Taiwan.

**Syntypes** NTOU001420–NTOU001424, collected by S.-M. Lin on January 30, 2013; NTOU001543–NTOU001546, collected by S.-M. Lin & L.-C. Liu on April 15, 2013; NTOU001427, collected by S.-M. Lin & L.-C. Liu on January 25, 2015; NTOU001428–NTOU001429, NTOU001549–NTOU001550, collected by S.-M. Lin & L.-C. Liu on January 15, 2016; NTOU001551–NTOU001576, collected by S.-M. Lin & L.-C. Liu on May 6, 2016, deposited in the herbarium of the Institute of Marine Biology, National Taiwan Ocean University (“NTOU”), Taiwan.

**Type locality** Xinwu Township, Taoyuan County, Taiwan (25° 00′ 32.6″ N, 121° 01′ 14.2″ E).

**Etymology** The species name “*margoundulatus*” refers to the wavy margin of the thalli.

**Distribution** Only found in Taoyuan County, Northwest Taiwan.

**Habitat and seasonality** Plants were very abundantly found in Taoyuan algal reefs in the northeast coast, occurring from January to November and growing mostly in the lower intertidal attached to old algal reefs, but sometimes found in subtidal zones at 1–3 m depths. Tetrasporic thalli bearing mature conceptacles were often found in late spring (April), whereas female gametophytes were only observed in late winter (January).

**Additional specimens examined** Guanyin, Taoyuan County, Taiwan, November 12, 2012 (NTOU001417–NTOU001418, NTOU001547–NTOU001548), January 30, 2013 (NTOU001419).

**Habit and vegetative morphology** Thalli are non-geniculate, crustose, 2–4 cm long by 2–3 cm wide, fan-shaped or rosette-like with smooth or imbricate surfaces and strongly wavy or small lobed margins (Fig. 6a, b). The primary margins are slightly uplifted or not and secondarily formed thallus lobes often growing over primary encrusts, giving the thallus an imbricate appearance (Fig. 6a, b). Thallus color is variable, from purplish to dark red. Thalli are monomerous and dorsiventrally organized, but not coaxial (Fig. 6c), 100–200 µm thick with 30–50 celled layers (Fig. 6d), including one layer of rounded or flattened epithallial cells (Fig. 6e), 2.5–3.0 µm long by 2.5–3.5 µm wide in a vertical view. In a longitudinal view of thallus crust, the medullary region (M) is composed of 8–10 celled layers of filaments when young, 10–15 celled layers when old (Fig. 6d), 6–13 µm long by 4–5 µm wide. Cortical cells (C) are squat to rectangular, composed of 10–15 celled layers when young (Fig. 6c), 20–35 celled layers when old (Fig. 6d), 4.5–6.5 µm long by 3–4.5 µm wide. Generally,

subepithallial cells are either the same length or shorter than their derivative cells positioned below and cell fusion is commonly seen between neighboring cells in adjacent filaments (Fig. 6e).

**Reproductive morphology** Tetrasporophytes and gametophytes are similar in habit and size (Fig. 6a, b). Tetrasporangial conceptacles are hemispheric, multiporate (Fig. 6i), 160–190 µm in diameter in surface view and the chambers are round or oval-shaped in longitudinal section view (Fig. 6f–h), 80–100 µm high by 110–210 µm in diameter. Tetrasporangial conceptacle roofs are composed of 3–4 celled layers, 14–28 µm thick, and bear apical pore plugs (Fig. 6f–h, *white arrowheads*), 75–80 µm long by 35–42 µm wide. Mature tetrasporangia borne on stalk cells zonately divided, 75–80 µm long by 35–42 µm wide (Fig. 6h). Carposporangial conceptacles are hemispheric (Fig. 6j–l), uniporate (Fig. 6l), 100–150 µm in diameter in surface view, and the chambers are roundish in longitudinal section view, 30–50 µm high by 60–75 µm in diameter (Fig. 6k). Carpogonial branches developed from the floor cells, and each carpogonial branch is consisting of a carpogonium and a hypogynous cell borne on support cell (Fig. 6j). Mature carposporangia are 15–20 µm long by 10–12 µm wide (Fig. 6k). Spermatangial plants were not found during the study.

## Discussion

Although Taoyuan algal reefs are the most extensive in Taiwan (Dai et al. 2009), the CCA species diversity of these reefs had been largely ignored. This is the first attempt to document the species diversity and phylogenetic relationships based on DNA sequences of the CCA occurring in Taoyuan algal reefs. Phylogenetic analyses using SSU and *psbA* gene sequences identified 12 CCA species belonging to six genera (*Crustaphytum* gen. nov., *Harveyolithon*, *Lithophyllum*, *Pneophyllum*, *Phymatolithon*, *Sporolithon*; see Figs 1 and 2) from Taoyuan algal reefs, and all the CCA species found except one (*Lithophyllum margaritae*, a new record to Taiwan marine flora) are undescribed species and possibly new species. In general, the SSU tree is better for inferring the inter- and intra-generic relationships of the six genera we found from the algal reefs in Taoyuan, but their intra-specific divergence is relatively low (see Fig. 1 for species of *Harveyolithon* and *Crustaphytum pacificum*). Although the *psbA* tree did not resolve some of the inter- and intra-generic relationships well, the *psbA* sequences have higher intra-specific divergence (see Fig. 2).

In the past, only a few CCA genera were reported from Taiwan algal reefs. Liou et al. (2017) briefly mentioned three genera, *Mesophyllum*, *Harveyolithon*, and *Phymatolithon*, without any description, and Dai et al. (2009) listed an unnamed

species of *Lithophyllum* from the rocky shores and coral reefs in Taiwan. Although we have more than 1300 CCA collections from the algal reefs and rocky shores in the northern Taiwan, including Taoyuan County, New Taipei City, and Keelung City, most specimens are sterile. Thus, we describe only three new species (*Crustaphytum pacificum*, *Harveyolithon rosea*, and *Phymatolithon margoundulatus*) by focussing on the dominant CCA species occurring in Taoyuan algal reefs. *Mesophyllum*, a genus frequently reported from the coral reefs and rocky shores in Taiwan (Lewis and Norris 1987; Liou et al. 2017) is not found in Taoyuan algal reefs and the coral reefs and rocky shores around Taiwan (L.-C. Liu unpublished data), whereas *Phymatolithon* is first reported to the Taiwan marine flora. *Lithophyllum*, the only genus reported from Taoyuan algal reefs by Dai et al. (2009) without a species name, is represented by one species, *L. margaritae*. Interestingly, *Lithothamnion*, a genus commonly found in both coral reefs and rocky shores in Taiwan, is absent in Taoyuan algal reefs (L.-C. Liu & S.-M. Lin unpublished data).

*Crustaphytum* is only found in the algal reefs in the northern Taiwan (Shimen and Taoyuan) and New Caledonia in the south-western Pacific Ocean. *Crustaphytum* is a sister clade to several genera, e.g., *Clathromorphum*, *Callilithophytum*, *Leptophytum*, and *Neopolyporolithon*, based on both *psbA* and SSU sequence analyses (see Figs 1 and 2). *Crustaphytum* can be separated from the related genera mainly by possessing coaxial thallus and branched spermatangial filaments as well as tetrasporangial conceptacle chambers relatively smaller (130–155 µm high by 115–200 µm wide) (see Table 1). *Crustaphytum* differs from *Clathromorphum* (3–8 layers of epithallial cells, up to 14 layers in one species); *Callilithophytum* P.W. Gabrielson, W.H. Adey, G.P. Johnson & Hernández-Kantún (4–5 celled layers); and *Neopolyporolithon* W.H. Adey & H.W. Johansen (1–7 celled layers) in having a monomerous thallus, and coaxial core filaments coaxial and composed of one of two layers of epithallial cells and not bearing meristem split after cell division, whereas *Crustaphytum* differs from *Leptophytum* W.H. Adey (possessing unbranched spermatangia filaments) by having branched spermatangia filaments and spermatangia formed in terminal clusters. *Crustaphytum* is superficially similar to the genera *Mesophyllum*, *Melyvonnea* Athanasiadis & D.L. Ballantine and *Synarthrophyton* R.A. Townsend in thallus morphologies, but *Crustaphytum* can be separated from those genera by having different sizes of subepithallial cells and tetrasporangial conceptacles.

*Phymatolithon margoundulatus* is endemic to Taoyuan County and is the most dominant species occupying over 30% of the total coverage in Taoyuan algal reefs in spring (S.-M. Lin unpublished data), whereas *Harveyolithon rosea* is a common species occurring on both algal reefs and rocky shores in the northern Taiwan. Compared to most published species of *Harveyolithon* (tetrasporangial chambers more than 200 µm in diameter; see Rösler et al. 2016) except the

**Table 1** Generic features of *Crustaphytum* gen. nov. and related genera

Features	<i>Crustaphytum</i>	<i>Leptophytum</i>	<i>Neopolyporolithon</i>	<i>Clathromorphum</i>	<i>Callilithophytum</i>
Thallus morphology/thickness	Crustose with rugose surface/up to 300 µm	Crustose with smooth surface/1 mm	Leafy crustosing, or epiphytic on geniculate coralline algae/up to 2 cm	Crustose with smooth surface/up to 5 cm	Encrusting, parasitic on geniculate coralline algae/up to 3 mm
Growth pattern	Coaxial	Non-coaxial	Non-coaxial	Non-coaxial	Non-coaxial
Perithallial cells during thallus growth	Gradually elongated	Gradually elongated	Not all elongated, only the cells in the meristematic layer elongated	Not all elongated, only the cells in the meristematic layer elongated	Not all elongated, only the cells in the meristematic layer elongated
Orientation of calcified crystals in the meristem	Radially	Radially	Radially	Vertically and radially	Radially
Number of layers of epithallial cells	1–2	1–3	1–7	3–14	4–5*5
Sporangia formation	Tetrasporangia only	Tetra- and bisporangia	Tetra- and bisporangia	Tetra- and bisporangia	Tetrasporangia
Size of tetrasporangial conceptacles	130–155 µm high, 115–200 µm in diameter	180–330 µm high, 400–700 µm in diameter	216 µm high, 300 µm in diameter	185 µm high, 253 µm in diameter	253 µm high, 456 µm in diameter
Tetrasporangial conceptacle roof	Flat-topped, not sunken at maturity	Flat-topped, not sunken at maturity	Slightly protruding, sunken at maturity	Flat-topped, sunken at maturity	Flat-topped, not sunken at maturity
Spermatangial filaments	Branched	Unbranched	No data	Unbranched	Unbranched
References	This study	*1, *2, *3, *4	*5, *6	*6, *7, *8	*5, *6, *7

\*1 = Adey (1966), \*2 = Adey et al. (2001), \*3 = Athanasiadis (2016), \*4 = Athanasiadis and Adey (2006), \*5 = Adey and Johansen (1972), \*6 = Adey et al. (2015), \*7 = Lebednik (1977), \*8 = Adey (1965)



genotype *H. rupestre* (Foslie) A.Rössler, Perfectti, V.Peña & J.C.Braga (tetrasporangial chambers less than 100 µm in diameter), *H. rosea* is characterized by having relatively smaller tetrasporangial chambers (50–60 µm high by 70–150 µm in diameter) and more cells (3–4, up to 6 celled layers) of tetrasporangial conceptacle roofs. *Phymatolithon* is a large genus in terms of species number and has a worldwide distribution in both temperate and tropical waters (Woelkerling and Irvine 1986; Wilks and Woelkerling 1994; Peña et al. 2015). *Phymatolithon* currently contains ca. 17 species (see Guiry and Guiry 2018 for a review; this study) but the species identification is based largely on DNA sequence analyses and is difficult based only on morph-anatomical characters owing to character overlap or unreliability, e.g., the gametophytes and tetrasporophytes in some species have different forms (Wilks and Woelkerling 1994; Pardo et al. 2015; Wolf et al. 2016). Nevertheless, *Phymatolithon margoundulatus* can be separated from most species with crustose thalli (mostly up to 2–4 mm thick; see Kaleb et al. 2012; Peña et al. 2015) in the genus by having crustose and relatively thin thalli (100–200 µm thick). Moreover, the female conceptacles (30–50 µm high by 60–75 µm in diameter) in *P. margoundulatus* are relatively smaller compared to other species (up to 155–515 µm in diameter) (see Adey 1964; Chamberlain 1991; Van der Merwe and Maneveldt 2014).

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