

## ***Grateloupia huangiae* (Halymeniaceae, Rhodophyta), a new species from Taiwan previously confused with *Polyopes lancifolius*, with emphasis on the development of the auxiliary-cell ampullae**

SHOWE-MEI LIN<sup>1\*</sup> AND HONG-YAN LIANG<sup>2</sup>

<sup>1</sup>*Institute of Marine Biology, National Taiwan Ocean University, Keelung 20224, Taiwan, Republic of China*

<sup>2</sup>*Marine Fisheries Division, Fishery Research Institute, Keelung 20246, Taiwan, Republic of China*

LIN S.-M., AND LIANG H.-Y. 2011. *Grateloupia huangiae* (Halymeniaceae, Rhodophyta), a new species from Taiwan previously confused with *Polyopes lancifolius*, with emphasis on the development of the auxiliary-cell ampullae. *Phycologia* 50: 232–240. DOI: 10.2216/10-29.1

*Grateloupia huangiae* S.-M. Lin & H.-Y. Liang *sp. nov.* was previously recorded as *G. okamurae* Yamada [currently recognized as *Polyopes lancifolius* (Harvey) Kawaguchi & H.W. Wang] in Taiwan. Molecular phylogenetic analysis shows that *G. huangiae* is unrelated to *P. lancifolius* but is closely related to *Grateloupia taiwanensis* S.-M. Lin & H.-Y. Liang. Although *G. huangiae* superficially resembles *P. lancifolius* in having a leafy thallus and short proliferations borne on the thallus surface, the structure of the auxiliary-cell ampulla is very different. The auxiliary-cell ampulla of *G. huangiae* is composed of three orders of unbranched filaments before diploidization, each of which is 11–13 cells long. The auxiliary cell is the first cell of the third-order ampullar filament, which is cut off from the first cell of the second-order ampullar filament. This type of auxiliary-cell ampulla is the same as that found in *G. taiwanensis*. On the other hand, *P. lancifolius* has a more complex auxiliary-cell ampulla in which the ampullar filaments may bear up to five to six orders of branches before diploidization. A detailed study of the development of auxiliary-cell ampullae before and after diploidization is needed in other species and genera within the Halymeniaceae to better delineate generic concepts in the family.

KEY WORDS: Auxiliary-cell ampullae, *Grateloupia huangiae* *sp. nov.*, Halymeniaceae, *Polyopes lancifolius*, Rhodophyta, Taiwan, taxonomy

### INTRODUCTION

The genus *Polyopes* J. Agardh is widely distributed in the Indo-western Pacific Oceans, in southern Australia and South Africa (Chiang 1970; Womersley & Lewis 1994; Kawaguchi *et al.* 2002; for a summary, see Guiry & Guiry 2010). Chiang (1970) examined the genotype *P. constrictus* (Turner) J. Agardh from Australia and South Africa and characterized *Polyopes* as possessing thick, turfy thalli and an *Aeodes*-type auxiliary-cell ampulla that is bushy with up to four to five orders of ampullar filaments. Kawaguchi *et al.* (2002) transferred several species from the genera *Carpopeltis* F. Schmitz and *Sinkoraena* H.-B. Lee, J.A. Lewis, Kraft & I.K. Lee to *Polyopes* after comparing them morphologically and molecularly with *P. constrictus*. They argued that the species placed in the genus *Sinkoraena* showed few morphological and anatomical characters that were different from those of *Polyopes*. Kawaguchi *et al.* (2002) also suggested that the presence of embedded tetrasporangia in thickened nemathecium, which had been used to characterize *Sinkoraena*, is a species, rather than a generic, character in the Halymeniaceae. Moreover, their molecular analysis did not support the separation of the two genera. Accordingly, *Sinkoraena* was merged with *Polyopes*, and the species previously placed in the former were transferred to the latter genus, including *P. lancifolius*

(Harvey) Kawaguchi & H.W. Wang and *P. tasmanicus* (Womersley & J.A. Lewis) Kawaguchi & J.A. Lewis.

*Grateloupia* C. Agardh is the largest genus in the family Halymeniaceae, containing more than 90 species, and has a wide distribution in temperate to warm waters (see Kawaguchi *et al.* 2001; Wang *et al.* 2001; Gavio & Fredericq 2002; De Clerck *et al.*, 2005a, b; Wilkes *et al.* 2005; Lin *et al.* 2008; for a summary, see Guiry & Guiry 2010). Chiang (1970) proposed that the *Grateloupia*-type auxiliary cell ampulla consists of a primary filament and two or three secondary filaments. On the other hand, Lin *et al.* (2008) examined the auxiliary-cell ampullae in three species of *Grateloupia* from Taiwan. They showed two different patterns of development of auxiliary-cell ampullae: one consisting of three orders of unbranched filaments that branch after diploidization of the auxiliary cell (*G. taiwanensis*-type) and the other consisting of two orders of unbranched filaments that do not branch after diploidization of the auxiliary cell (*G. orientalis* type).

Chiang (1973) first recorded *Polyopes lancifolius* (as *Grateloupia okamurae* Yamada) from Tali (Taipei Co.) and Patoutzu (Keelung City) in northeastern Taiwan. Our recent collections from the northeastern and southern coasts of Taiwan contain numerous female gametophytes of '*P. lancifolius*'. Our *rbcL* sequence analysis shows that '*P. lancifolius*' from Taiwan does not belong in *Polyopes* but, instead, is a species of *Grateloupia*. In this study, we describe *G. huangiae* S.-M. Lin & H.-Y. Liang *sp. nov.*, a red alga previously confused with *P. lancifolius* from Taiwan,

\* Corresponding author (linsm@ntou.edu.tw).

emphasizing the development of the auxiliary-cell ampullae. The phylogenetic relationships among *G. huangiae* and the other species of *Grateloupia* are discussed based on morphological observations and *rbcL* sequence analysis.

## MATERIAL AND METHODS

Collections were made intertidally or by snorkeling in depths of 0.5–2.0 m. Algal samples for morphological study were preserved in 3–5% formalin–seawater or pressed on herbarium sheets, whereas materials used in the molecular studies were desiccated in 95% alcohol. Voucher specimens are deposited in the Herbarium of the National Taiwan Ocean University, Taiwan (NTOU). Hand sections were stained with 1% aniline blue acidified with 1% HCl and mounted in 25–30% Karo® syrup (Englewood Cliffs, CA, USA) 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 (Tokyo, Japan) with a Q-imaging digital camera (Burnaby, BC, Canada) and habit views reproduced with an Epson scanner (Tokyo, Japan).

DNA samples were prepared using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) following the instructions of the manufacturer. DNA sequencing procedures were as described in Lin *et al.* (2001). New sequence data and those available from GenBank were compiled and aligned with Sequencher (Gene Codes Corp., Ann Arbor, MI, USA) and exported for phylogenetic analysis. Three *rbcL* sequences for *G. huangiae* from Linping (type locality), Longdong and Makang, Taiwan, generated in this study are listed in Table 1 together with the sequences obtained from GenBank. Phylogenetic analyses were performed using parsimony heuristic searches [the maximum parsimony (MP)], Bayesian analysis (BA), and calculation of bootstrap proportion values were conducted as described in Lin *et al.* (2008). MP analysis and bootstrapping methods are available in the computer programs PAUP\* v4.0 (Swofford 2003), with 500 bootstrap replicates completed for the MP analysis. Support for nodes for parsimony trees was assessed by calculating 500 bootstrap resamplings of the heuristic searches based on random stepwise additions, MULPARS and the tree bisection–reconnection (TBR) algorithm. The model used in the BA available in the computer program MrBayes v 3.1.2 (Huelsenbeck and Ronquist 2001) was the general-time-reversible model of nucleotide substitution with invariable sites and gamma-distributed rates for the variable sites (GTR + I + G). For the BA, we ran four chains of the Markov chain Monte Carlo (one hot and three cold), sampling one tree every 100 generations for 500,000 generations starting with a random tree. Stationarity was reached at generation 5000. Thus, trees saved until generation 5000 were the ‘burn-in’ of the chain, and inferences about the phylogeny were based on those trees sampled after generation 5000. A 50% consensus tree (majority rule as implemented by PAUP\*) was computed from the 4950 + 1 trees saved after the burn-in point.

## RESULTS

### Molecular analyses

A set of 40 *rbcL* gene sequences from representative taxa within the genera *Grateloupia*, *Polyopes* and related species in the Halymeniaceae were selected for a phylogenetic analysis with two species of *Halymenia* C. Agardh serving as the out-group (see Table 1). The *rbcL* alignment excluded the first 106 sites from the 5' end and last 102 sites from the 3' end from the analyses. The analyzed matrix included 1259 total and 324 parsimony informative sites (25.73%). Parsimony analysis of the *rbcL* data set obtained from heuristic searches resulted in 10 most parsimonious trees (MPT) (tree length = 1331 confidence interval = 0.4147, retention index (RI) = 0.6003). The tree topology of MPT and Bayesian trees of *rbcL* were largely congruent, so that only the MPT was presented (Fig. 1). The bootstrap proportion values derived from parsimony and the confidence values obtained from the consensus of the 4950 + 1 Bayesian trees were shown above and below the nodes of the phylogenetic tree, respectively.

With two species of *Halymenia* from the family Halymeniaceae used as the out-group, *G. huangiae* was placed in a single clade with all other species of *Grateloupia* included in the analysis, including the generitype of *Grateloupia*, *G. filicina* (J.V. Lamouroux) C. Agardh from Italy, and the other species placed in *Grateloupia* with 74% bootstrap support. The *Grateloupia* clade was clearly separated from the *Polyopes* clade containing the generitype *P. constrictus* from Australia and *P. lancifolius* from Japan in both the MPT (Fig. 1) and the Bayesian tree (not shown). The interspecific genetic distance of the *rbcL* gene among the species of *Grateloupia* analyzed here ranged from 1.360% to 13.65%.

*Grateloupia huangiae* is sister to *G. taiwanensis* S.-M. Lin & H.-Y. Liang with 40–41 out of 1359 base-pair differences (= 2.94–3.02% genetic divergence). The populations of *G. huangiae* from northeastern Taiwan diverged by one base pair from the population occurring in Linping, southwestern Taiwan. The *G. huangiae*/*G. taiwanensis* clade from Taiwan showed a close relationship to the terete to flattened or blade-like species: *G. subpectinata* Holmes, *G. sparsa* (Okamura) Chiang, *G. turuturu* Yamada from Japan and *G. phuquocensis* Tanaka & H.H. Pham from Hawaii with strong bootstrap support (100%). Their interspecific *rbcL* gene sequence divergence (Kimura two-parameter distance) ranged from 2.25% to 4.55%.

### MORPHOLOGICAL OBSERVATIONS

#### *Grateloupia huangiae* S.-M. Lin & H.-Y. Liang sp. nov.

Figs 2–24

*Thalli usque ad 15 cm alti, leviter plani et foliosi, ruber vel atrobrunnei, gelatinosi vel cartilaginei, constans ex laminis 3–5, 10–25 cm longis 0.5–3 cm latis, ex haptero parvo discoideo oriens, 2–4 mm diametro, laminis ferre laminulis numerosis marginales vel superficies lanceolatas 4–50 mm longas 2–6 mm latas, cortex stratorum 4–6, cellulis moniliformibus vel*

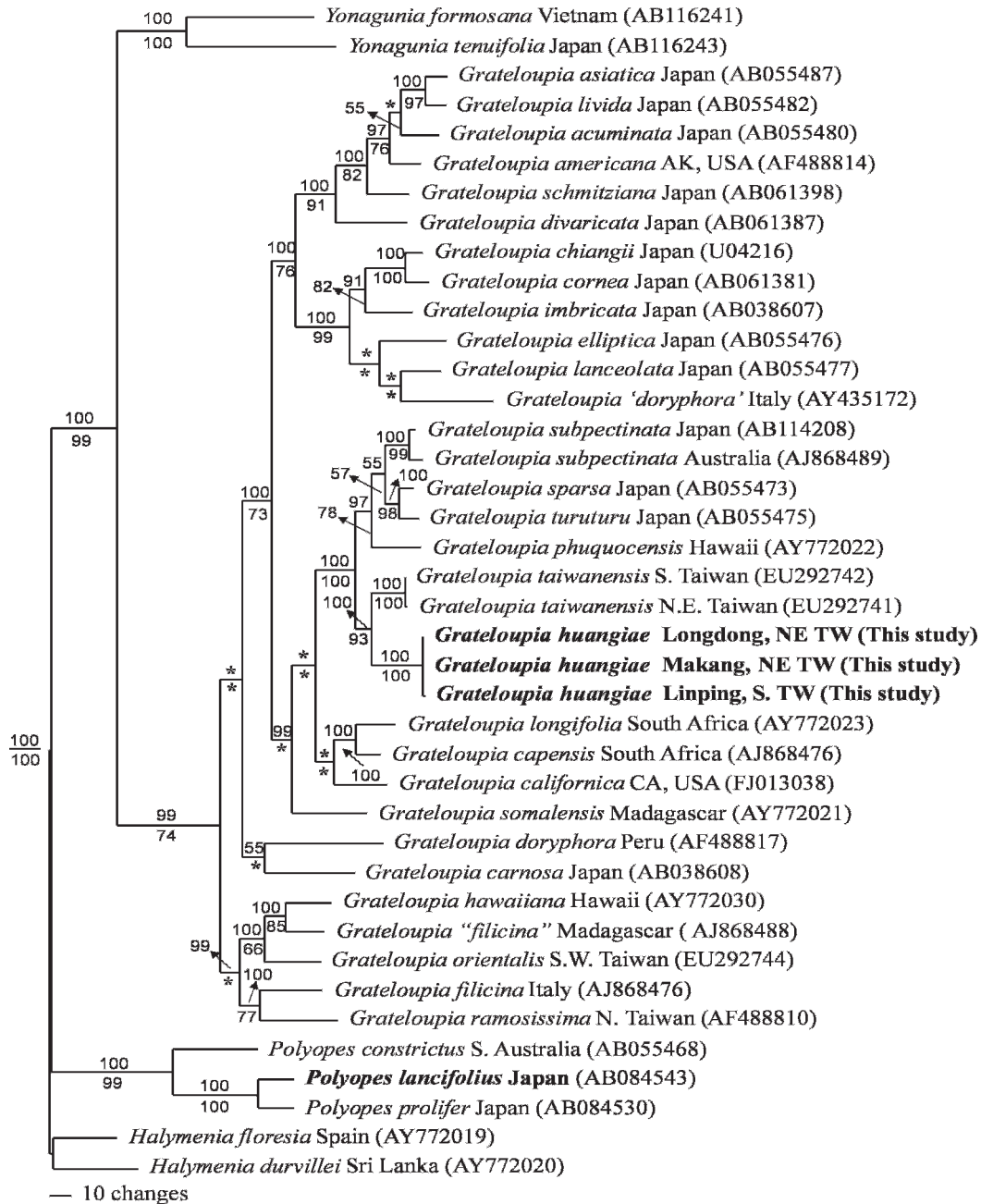
**Table 1.** List of species used in *rbcL* analysis and accession numbers in GenBank. \*<sup>1</sup> = Kawaguchi *et al.* (2001); \*<sup>2</sup> = Gavio & Fredericq (2002); \*<sup>3</sup> = Miller *et al.* (2009); \*<sup>4</sup> = Wang *et al.* (2001); \*<sup>5</sup> = De Clerck *et al.* (2005a); \*<sup>6</sup> = De Clerck *et al.* (2005b); \*<sup>7</sup> = Lin *et al.* (2008); \*<sup>8</sup> = Faye *et al.* (2004); \*<sup>9</sup> = Kawaguchi *et al.* (2002); \*<sup>10</sup> = Kawaguchi *et al.* (2004); \*<sup>11</sup> = Wang *et al.* (2000); \*<sup>12</sup> = Fredericq *et al.* (1996); \*<sup>13</sup> = this study.

Species	Collection location	GenBank accession number
<i>Grateloupia acuminata</i> Holmes	Katase, Fujisawa, Kanagawa Prefecture, Japan	AB055480* <sup>1</sup>
<i>Grateloupia americana</i> Kawaguchi & H.-W. Wang	Whan Park, near Sirka, Alaska, USA	AF488814* <sup>2</sup>
<i>Grateloupia asiatica</i> Kawaguchi & H.-W. Wang	Tsuyazaki, Fukuoka Prefecture, Japan	AB055487* <sup>1</sup>
<i>Grateloupia californica</i> Kylin	Monterey, California, USA	FJ013038* <sup>3</sup>
<i>Grateloupia carnosus</i> Yamada & Segawa	Oryuzako, Miyazaki Prefecture, Japan	AB038608* <sup>11</sup>
<i>Grateloupia chiangii</i> Kawaguchi & H.-W. Wang	Pigeon Pt, San Mateo Co., California, USA	U04216* <sup>12</sup>
<i>Grateloupia cornea</i> Okamura	Ohara, Chiba Prefecture, Japan	AB061381* <sup>4</sup>
<i>Grateloupia capensis</i> De Clerck	Yzerfonteyn, western Cape Province, South Africa	AJ868467* <sup>6</sup>
<i>Grateloupia divaricata</i> Okamura	Izu-misaki, Miyake Island, Tokyo, Japan	AB061387* <sup>4</sup>
<i>Grateloupia doryphora</i> (Montagne) M. Howe	Sicily, Italy (Wilkes <i>et al.</i> unpublished data in GenBank)	AY435172
<i>Grateloupia doryphora</i> (Montagne) M. Howe	Playa de San Francisco, Bahía de Ancón, Ancón, Lima, Peru	AF488817* <sup>2</sup>
<i>Grateloupia elliptica</i> Holmes	Goshikinohama, Usa, Tosa, Kochi Prefecture, Japan	AB055476* <sup>1</sup>
<i>Grateloupia filicina</i> (J.V. Lamouroux) C. Agardh	Tulér, Madagascar	AJ868488* <sup>6</sup>
<i>Grateloupia filicina</i> (J.V. Lamouroux) C. Agardh	Ponteleria, Sicily, Italy	AJ868476* <sup>6</sup>
<i>Grateloupia hawaiiiana</i> E.Y. Dawson	Maui, Hawaii	AY772030* <sup>5</sup>
<i>Grateloupia huangiae</i> S.-M. Lin & H.-Y. Liang <i>sp. nov.</i>	Longdong, Taipei Co., N.E. Taiwan, coll. A. Liu, 20 Apr. 2003.	HM590408* <sup>13</sup>
<i>Grateloupia huangiae</i> S.-M. Lin & H.-Y. Liang <i>sp. nov.</i>	Linping, Pintung Co., S. Taiwan, coll. S.-M. Lin & H.-Y. Liang, 17 Jan. 2005.	HM590409* <sup>13</sup>
<i>Grateloupia huangiae</i> S.-M. Lin & H.-Y. Liang <i>sp. nov.</i>	Makang, Taipei Co., N.E. Taiwan, coll. S.-M. Lin, 30 Apr. 2002.	HM590410* <sup>13</sup>
<i>Grateloupia imbricata</i> Holmes	Tsuyazaki, Fukuoka Prefecture, Japan	AB038607* <sup>4</sup>
<i>Grateloupia lanceolata</i> (Okamura) Kawaguchi	Shikanoshima, Fukuoka Prefecture, Japan	AB055477* <sup>1</sup>
<i>Grateloupia livida</i> (Harvey) Yamada	Muroto-misaki, Kochi Prefecture, Japan	AB055482* <sup>1</sup>
<i>Grateloupia longifolia</i> Kylin	Yzerfonteyn, Western Cape Province, South Africa	AY772023* <sup>5</sup>
<i>Grateloupia orientalis</i> S.-M. Lin & H.-Y. Liang	Linyuan, Pintung Co., Taiwan	EU292744* <sup>7</sup>
<i>Grateloupia phuquocensis</i> Tanaka & H.H. Pham	Kaalawai, Oahu, Hawaii	AY772022* <sup>5</sup>
<i>Grateloupia ramosissima</i> Okamura	Ho Ping Island, Keelung City, Taiwan	AF488810* <sup>2</sup>
<i>Grateloupia schmitziana</i> (Okamura) Kawaguchi & H.W. Wang	Shichirigahama, Kamakura, Kanagawa Prefecture, Japan	AB061398* <sup>4</sup>
<i>Grateloupia somalensis</i> Hauck	Plage de Monseigneur, Fort Dauphin, Madagascar	AY772021* <sup>5</sup>
<i>Grateloupia sparsa</i> (Okamura) Chiang	Ohara, Chiba Prefecture, Japan	AB055473* <sup>1</sup>
<i>Grateloupia subpectinata</i> Holmes	Williamstown, Victoria, Australia (as <i>Grateloupia luxurians</i> )	AJ868489* <sup>6</sup>
<i>Grateloupia subpectinata</i> Holmes	Seto, Shirahama, Wakayama Prefecture, Japan	AB114208* <sup>8</sup>
<i>Grateloupia taiwanensis</i> S.-M. Lin & H.-Y. Liang	Sail Rock, Pintung Co., S. Taiwan	EU292742* <sup>7</sup>
<i>Grateloupia taiwanensis</i> S.-M. Lin & H.-Y. Liang	Makang, Taipei Co., N.E. Taiwan	EU292741* <sup>7</sup>
<i>Grateloupia turuturu</i> Yamada	Onahama, Iwaki, Fukushima Prefecture, Japan	AB055475* <sup>1</sup>
<i>Halymenia durvillei</i> Bory	Beruwela, Sri Lanka	AY772020* <sup>5</sup>
<i>Halymenia floesia</i> (Clemente) C. Agardh	Illes Formigues, Palamos, Girona, Spain	AY772019* <sup>5</sup>
<i>Polyopes constrictus</i> (Turner) J. Agardh	Point Lonsdale, Victoria, Australia	AB055468* <sup>1</sup>
<i>Polyopes lancifolius</i> (Harvey) Kawaguchi & H.-W. Wang	Inoshiri, Usa, Tosa, Kochi Prefecture, Japan	AB084543* <sup>9</sup>
<i>Polyopes prolifer</i> (Hariot) Kawaguchi & H.-W. Wang	Kannonzaki, Yokosuka, Kanagawa Prefecture, Japan	AB084530* <sup>9</sup>
<i>Yonagunia formosana</i> (Okamura) Kawaguchi & Masuda	Hon Miew, Nha Trang, Khanh Hoa Province, Vietnam	AB116241* <sup>10</sup>
<i>Yonagunia tenuifolia</i> Kawaguchi & Masuda	Agarizaki, Yonaguni Island, Japan	AB116243* <sup>10</sup>

*polygonis et medulla filamentosa filis laxis, tetrasporophyta atque gametophyta isomorphica, gametophyta dioecia, cellulae matriciales spermatangiarum e cellulis superficialibus transformatae, spermatangias 3–5 µm diametro, carposporophyta matura in lamina fertilibus medulla immersa, ampullae cellulae auxiliaris constantes ex gradibus 3 filorum ampullarium e cellula subbasali corticis interioris natorum; cellula auxiliara cellula basala fila grada terta abscissa e cellula prima fila ampullara grada secunda, fila ampullae 11–13 cellae longa, post diploidizationem, fila ampullaria 3- vel 5-plo ramificant ut faciant fascicula filamentosa cingentem*

*carposporophyta cystocarpium tempore subolesco, carposporangia pyriformibus vel globosis, 10–12 µm latis 12–15 µm longis, tetrasporangia e cellula cortica interiora nata, 16–20 µm latis 40–45 µm longis, in maturitate cruciatim divisa.*

Thalli up to 25 cm in length, slightly flattened and foliose, red to dark brown, gelatinous to cartilaginous in texture and composed of three to five main blades, 10–25 cm long and 0.5–3.0 cm wide, arising from a small discoid holdfast 2–4 mm in diameter; blades bearing numerous marginal or superficial, lanceolate proliferations with tapering tips, 4–50 mm long by 2–6 mm wide; cortex four

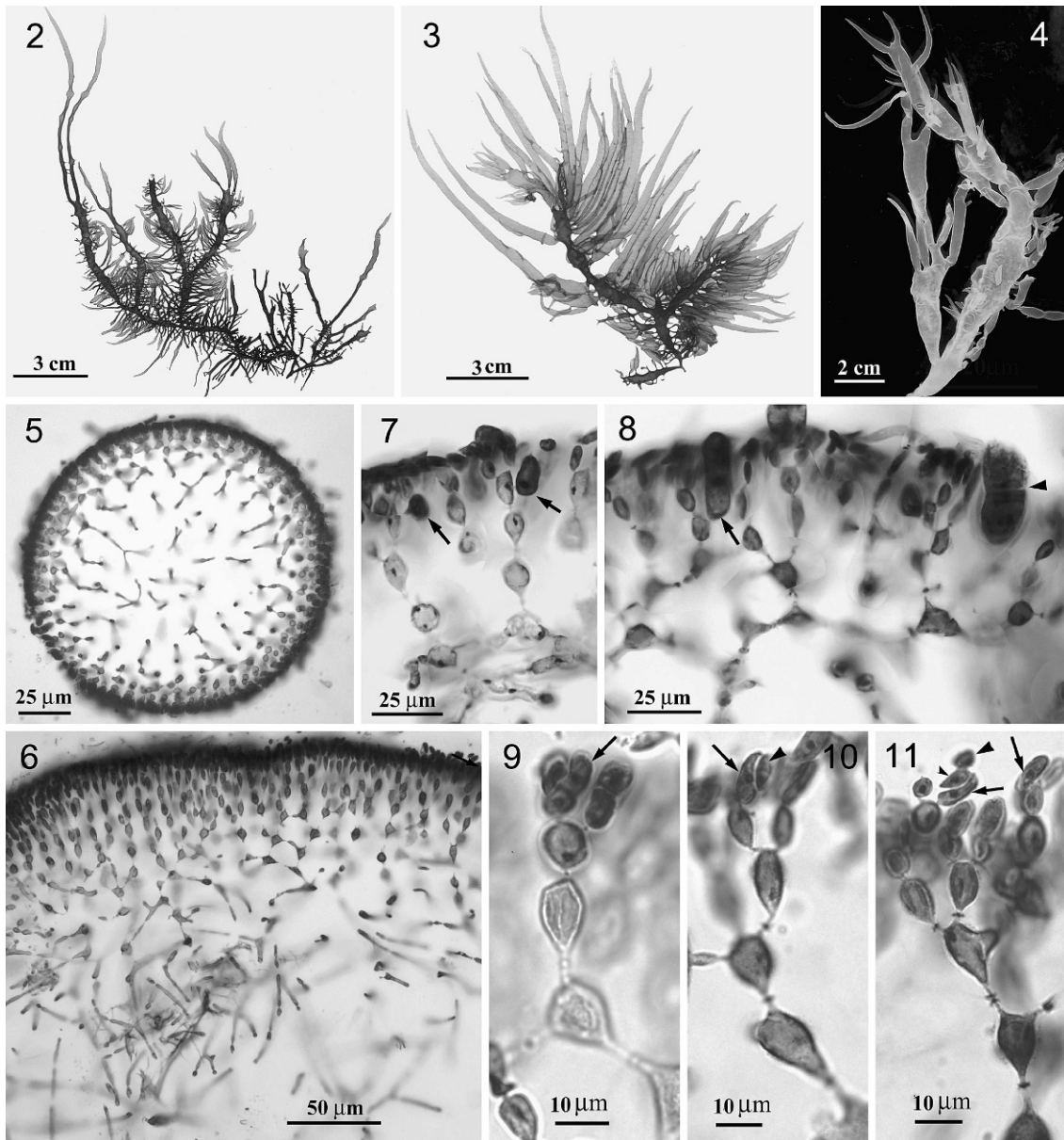


**Fig. 1.** One of 10 *rbcL* MPTs showing the inter- and intrageneric relationships among species of *Grateloupia*, *Polyopes* and *Yonagunia*. The numbers above the branches are the posterior probabilities (PPs) obtained from the consensus of 4950 + 1 Bayesian trees; whereas, the numbers below the branches are the maximum-parsimony bootstrapping proportion (BP). Asterisks (\*) refer to the BP or PPs < 50%. Branch lengths are proportional to the amount of sequence change.

to six layers thick, inner cortical cells polygonal in shape and the medullary filaments laxly arranged; gametophytic and tetrasporophytic plants isomorphic with the gametophytes dioecious; spermatangial parent cells transformed from surface cells and bearing spermatangia 3–5 µm in diameter; carposporophytes deeply embedded in the medulla of fertile blades; auxiliary-cell ampullae consisting of three orders of unbranched filaments with the auxiliary cell being the basal cell of the third-order ampullar filament cut off from the first cell of the second-order filament, with each ampullar filament 11–13 cells in length

at maturity; ampullar filaments branching laterally three to five times after diploidization to form a dense filamentous cluster surrounding the developing carposporophyte, carposporangia pyriform or rounded, 10–12 µm wide by 12–15 µm long; tetrasporocytes initiated from inner cortical cells, the tetrasporangia 16–20 µm wide by 40–45 µm long and bearing cruciately arranged tetraspores when mature.

**ETYMOLOGY:** ‘*huangiae*’ named in honor of Dr. Su-Fang Huang at the Taiwan Museum in Taipei City, who has



**Figs 2–11.** *Grateloupia huangiae* S.-M. Lin & H.-Y. Liang *sp. nov.* Habit, vegetative, tetrasporangial and spermatangial structures (Figs 1, 2, 4, 5, 8–10: Lingping, Pingtung Co.; Figs 3, 6, 7: Makang, Taipei Co.).

**Fig. 2.** Holotype, a female plant (NTOU17i2005-1-Holo-Gh).

**Fig. 3.** Isotype, a male plant (NTOU17i2005-2-iso-Gh).

**Fig. 4.** A tetrasporic plant (MK30iv2002T-F).

**Fig. 5.** Cross section through the tip of a young bladelet showing cortex and medulla (LP17i2005TX-yV).

**Fig. 6.** Cross section through a main blade showing the cellular cortex and filamentous medulla (LP17i2005TX-mV).

**Fig. 7.** Cross section through the middle part of a tetrasporic blade showing tetrasporocyst initials (arrows) cut off laterally from intercalary cortical cells (MK30iv2002T-TX).

**Fig. 8.** Cross section of a tetrasporangia-bearing blade showing an elongated tetrasporocyst (arrow) and a cruciately divided tetrasporangium (arrowhead) (MK30iv2002T-TX).

**Fig. 9.** Cross section of a spermatangia-bearing bladelet showing a developing spermatangial parent cell (arrow) (LP17i2005L-yM).

**Fig. 10.** Cross section of a spermatangia-bearing bladelet showing a spermatangial parent cell (arrow) cutting off a spermatangium (arrowhead) obliquely (LP17i2005L-yM).

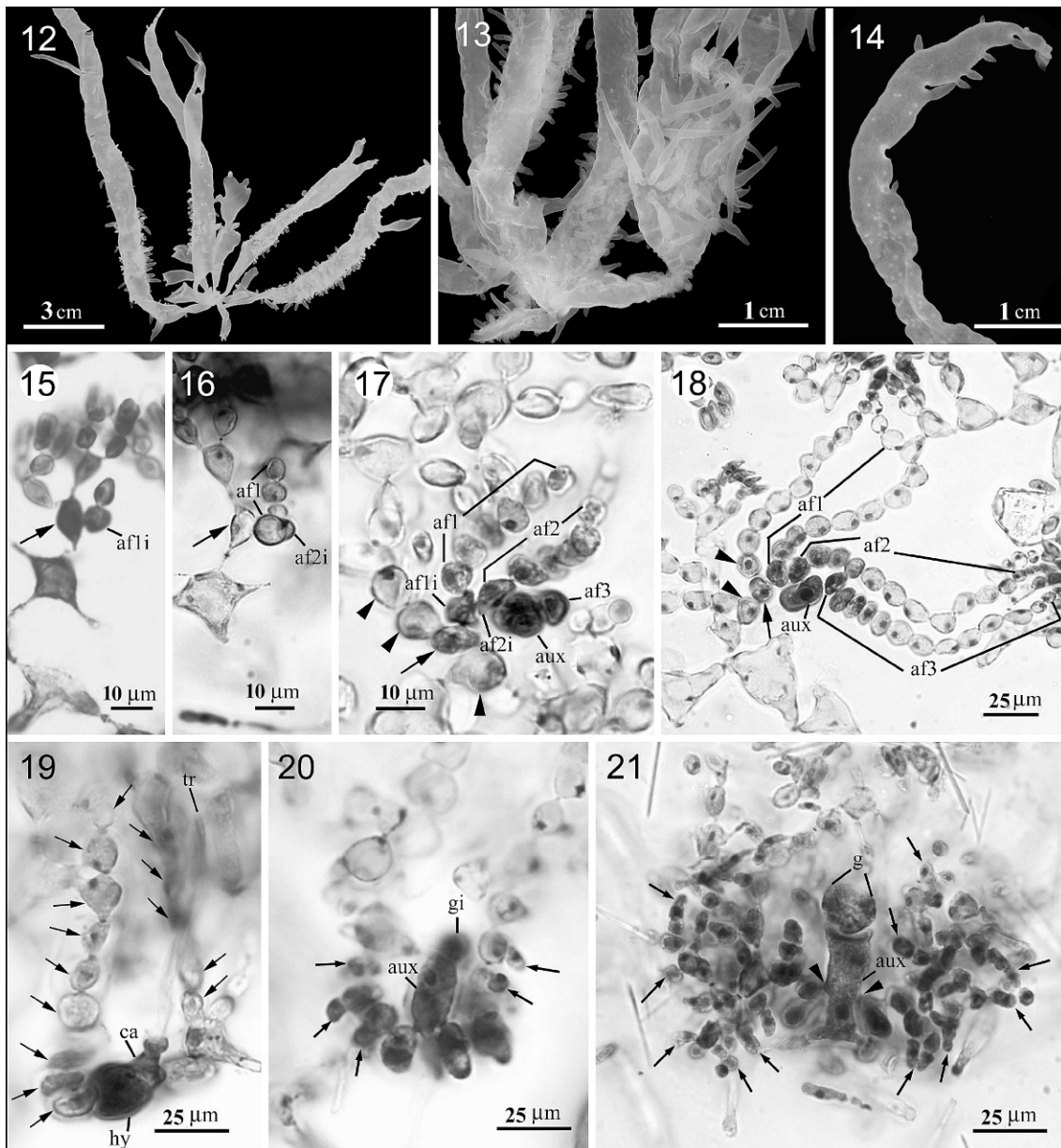
**Fig. 11.** Cross section through a spermatangia-bearing, main blade with spermatangial parent cells (arrows) and spermatangia (arrowheads) (LP17i2005L-mM).

made significant contributions to the marine flora of Taiwan over the past two decades.

**HOLOTYPE:** In NTOU: NTOU17i2005-1-Holo-Gh, ♀ (Fig. 1), coll. S.-M. Lin & H.-Y. Liang, 17 January 2005.

**Isotypes:** NTOU17i2005-2-iso-Gh to no. NTOU17i2005-18-iso-Gh.

**TYPE LOCALITY:** Lingping, Pingtung Co., southwestern Taiwan (120°30'E, 22°25'N).



**Figs 12–21.** *Grateloupia huangiae* S.-M. Lin & H.-Y. Liang sp. nov. Female plants, auxiliary cell ampullar formation and postfertilization stages (Figs 11–17: Makang, Taipei Co.; Figs 18–20: Lingping, Pingtung Co.).

**Fig. 12.** A female plant after bleaching showing numerous, short proliferations borne on the surface of the middle to basal part of thallus (MK30iv2002F-F).

**Fig. 13.** Short proliferations borne in the middle to basal parts of the thallus (MK30iv2002F-F).

**Fig. 14.** Fertile proliferation showing carposporophytes (white spots) (MK30iv2002F-F).

**Fig. 15.** Cross section of a fertile blade showing the first-order ampullar filament (af1i) at the two-celled stage borne on a basal inner cortical cell (arrow) (MK30iv2002F-fb).

**Fig. 16.** Later stage of an auxiliary cell ampulla borne on a basal inner cortical cell (arrow) showing the first-order ampullar filament (af1) at the four-celled stage and the initial of the second-order filament (af2i) borne laterally on the first cell of the first-order ampullar filament (MK30iv2002F-eaf).

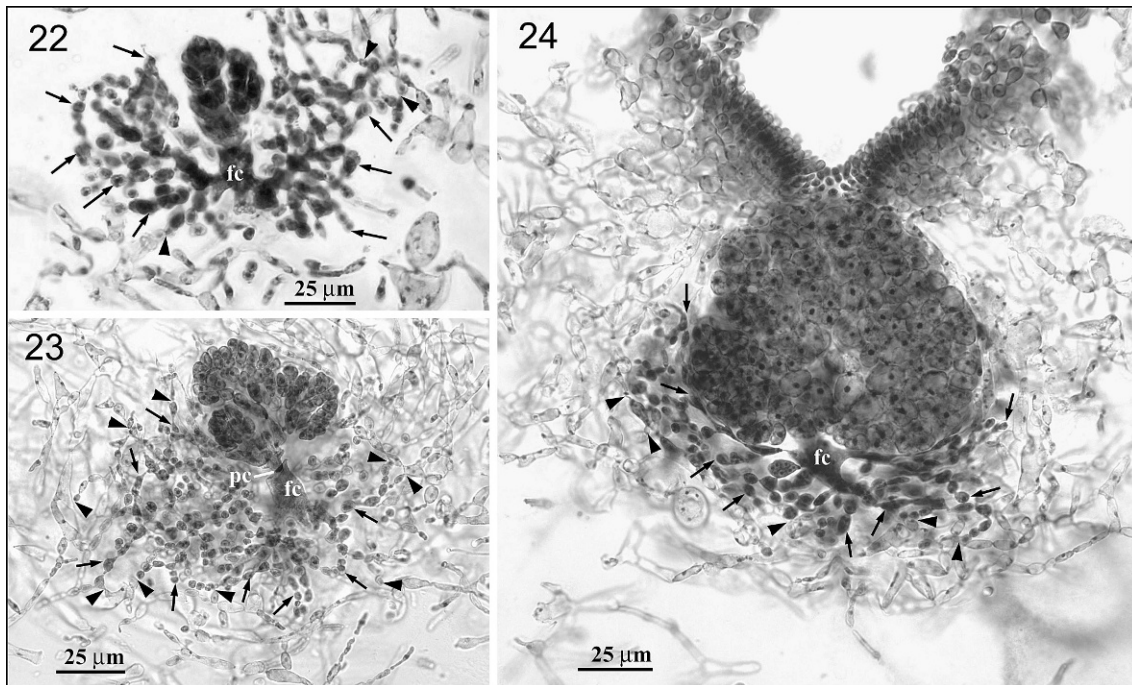
**Fig. 17.** A developing auxiliary-cell ampulla borne on a basal inner cortical cell (arrow) showing the first-order (af1), second-order (af2) and third-order (af3) ampullar filaments. Note that the inner cortical cells (arrowheads) near the auxiliary-cell ampulla stain as darkly as the ampullar filaments themselves (MK30iv2002F-daf).

**Fig. 18.** A fully developed auxiliary-cell ampulla borne on a basal inner cortical cell (arrow) showing the first-order (af1) and two unbranched second-order ampullar filaments (af2 and af3). Note that the nucleus of the auxiliary cell (aux) is distinct and is surrounded by a clear hyaline region. The inner cortical cells (arrowheads) near the auxiliary-cell ampulla stain as darkly as the ampullar filaments (MK30iv2002F-faf).

**Fig. 19.** Carpegonial ampulla showing a fertilized carpegonium (ca), the hypogenous cell (hy), the remaining trichogyne (tr) and ampullar filaments (arrows) (LP17i2005L-cb).

**Fig. 20.** Early fertilization stage showing a gonimoblast initial (gi) cut off from the auxiliary cell (aux) and the first, lateral cell divisions (arrows) of the ampullar filaments (LP17i2005L-gi).

**Fig. 21.** Developing gonimolobe (g) surrounded by the developing, branched ampullar filaments (arrows) and the auxiliary cell (aux), which is initiating fusion with its neighboring ampullar cells (arrowheads) (LP17i2005L-dg).



**Figs 22–24.** *Grateloupia huangiae* S.-M. Lin & H.-Y. Liang *sp. nov.* Cystocarp development. (Lingping, Pingtung Co.).

**Fig. 22.** A young gonimolobe showing the newly formed fusion cell (fc) and branched auxiliary cell ampullar filaments (arrows). Note that some the distal cells of the ampullar filaments have pit connections (arrowheads) to cells of filaments derived from cortical cells in their vicinity (LP17i2005L-egb).

**Fig. 23.** Immature cystocarp showing the broadened pit connection between the fusion cell (fc) and the primary gonimoblast cell and the continued branching of the ampullar filaments (arrows). Note that pericarp formation has involved the branched ampullar filaments and secondary medullary filaments (arrowheads) derived from the cortical cells in the vicinity of the gonimoblasts (LP17i2005L-ime).

**Fig. 24.** A nearly mature cystocarp showing the carposporophyte with a basal, branched fusion cell (fc). Note that the pericarp consists of the remaining ampullar filaments (arrows) mixed with the secondary medullary filaments (arrowheads) (LP17i2005L-mc).

**DISTRIBUTION:** Widely distributed in Taipei Co. and I-Lan Co. (northeast) and Pingtung Co. (south and southwest), Taiwan.

**HABITAT AND SEASONALITY:** Plants were frequently found from January through May in Pingtung Co. including Kenting National Park but occur from March through June in northeastern Taiwan. Plants were attached on rocky reefs at 0–2-m depth.

**SPECIMENS EXAMINED:** Northeastern Taiwan: (1) Taipei County: Lungdong, coll. *S.-L. Liu*, 20 April 2003 (NTOU-LD-20iv20003Gh, ♀ ♂), coll. *S.-M. Lin*, 8 June 2009 (NTOU-KL-8vi2009-t1, ⊕); Makang, coll. *S.-M. Lin*, 30 April 2002 (NTOU-MK-20iv2002Gh, ♀ ♂ ⊕); (2) I-Lan County: Toucheng, coll. *S.-M. Lin*, 19 May 2007 (NTOU-TC-19v2007Gh); Wushih Harbor, coll. *S.-M. Lin*, 29 August 2007 (NTOU-WSH-29viii2007Gh, ⊕); (3) Keelung City: Patoutzu, coll. *H.-J. Hsueh*, 18 March 2010 (NTOU-PTZ-18iii2010Gh, ⊕). Pingtung Co., southern Taiwan: (1) Kenting National Park: Sail Rock, coll. *H.-Y. Liang* & *S.-M. Lin*, 17 April 2005 (NTOU-SR-17iv2005Gh, ♀ ♂ ⊕); Wan Lee Dong, coll. *S.-M. Lin*, 3 May 2003 (NTOU-WLD-3v2003Gh, ⊕); (2) Lingping, coll. *S.-M. Lin* & *H.-Y. Liang*, 17 January 2005 (NTOU-PT-17i2005, ♀ ♂), coll. *S.-L. Liu* & *H.-Y. Liang*, 16 March 2006 (NTOU-LP-16iii2006Gh, ♀ ♂).

**HABIT AND VEGETATIVE STRUCTURE:** Thalli (Figs 2–4, 12) are slightly flattened and foliose, composed of three to five

main blades, 10–25 cm long and 0.5–3.0 cm wide, arising from a small disc-shaped holdfast 2–4 mm in diameter. Blades are red to dark brown and bear many marginal or superficial, lanceolate proliferations with tapering tips, 4–50 mm long by 2–6 mm wide, variable in length and width (Figs 12, 13). Thalli are gelatinous to cartilaginous and richly mucilaginous internally. Internal anatomy varies slightly being different between young and old blades and different parts of the thallus. When young, the blades are composed of a compact, two- to three-layered moniliform cortex and laxly arranged medullary filaments (Fig. 5). With blade thickening, the outer cortical cells undergo both longitudinal and transverse divisions, becoming four to six layers thick, and the medullary filaments become stretched and laxly arranged leaving a little space, which is occupied with mucilage matrix in the center (Fig. 6). Inner cortical cells become polygonal in shape and pit-connected to their neighboring cells (Figs 7, 8).

**REPRODUCTIVE STRUCTURES:** Gametophytic and tetrasporophytic plants are isomorphic, with the gametophytes dioecious (Figs 2–4, 12). Tetrasporocytes are cut off laterally from inner cortical cells (Fig. 7) and later elongate and divide transversely and longitudinally maturing into tetrasporangia 16–20 μm wide by 40–45 μm long containing four cruciately, rarely decussately, arranged tetraspores (Fig. 8). Spermatangial parent cells are transformed from surface cells, which first divide longitudinally (Fig. 9, arrow) and then elongate and divide obliquely (Fig. 10).

Finally, they divide transversely to form terminal spermatangia, 3–5 µm in diameter (Fig. 11).

Carposporophytes are scattered over the thallus surface (Figs 12–14) and are deeply embedded in the medulla of the fertile blades (Figs 19–24). Early stage in the development of the carpogonium and carpogonial ampullae were not observed, but a mature carpogonial ampulla consists of a carpogonium with a long trichogyne, a hypogenous cell and two ampullar filaments (Fig. 19). Auxiliary-cell ampullae were abundant in all the female plants examined. Initials of auxiliary-cell ampullae are cut off from basal or subbasal inner cortical cells (Figs 15–18). The first-order ampullar filaments grow apically by transverse divisions. An initial of the second-order ampullar filament is cut off laterally from the first cell of the first-order ampullar filament when it reaches three to four cells long (Fig. 16). A third-order ampullar filament is produced laterally from the first cell of the second-order ampullar filament at the time when the second-order ampullar filament has reached five to seven cells in length. The basal cell of the third-order ampullar filament eventually enlarges and functions as the auxiliary cell (Fig. 17). In fully developed auxiliary-cell ampullae, each ampullar filament can reach 11–13 cells in length, and the enlarged auxiliary cell is distinct (Fig. 18).

Following presumed diploidization via a connecting filament from the fertilized carpogonium to the auxiliary cell, the auxiliary cell cuts off a gonimoblast initial transversely and the cells of the ampullar filaments divide longitudinally, initiating lateral branches (Fig. 20). The pit connection between the auxiliary cell and the gonimoblast initial broadens, and the gonimoblast initial undergoes multiple concavo-convex cell divisions to produce the cells of the primary gonimolobe (Fig. 21). Ampullar filaments continue to grow apically and branch laterally three to five times to form a dense filamentous cluster surrounding the developing carposporophyte (Figs 21, 22). As development of the carposporophyte continues, cell fusions takes place between the auxiliary cell and its neighboring ampullar cells to form a fusion cell (Figs 22, 23). During differentiation of the gonimolobes, the distal cells of branches of the ampullar filaments become secondarily pit-connected with numerous medullary filaments, which are derived from the inner cortical cells in the vicinity of the developing gonimoblasts and ramify around the developing carposporophyte (Figs 23, 24). The branched ampullar filaments and secondary medullary filaments together form the pericarp that surrounds the gonimolobes (Fig. 24). Fully developed, mature carposporangia-bearing gonimoblasts are conical, 150–250 µm in diameter, with a branched fusion cell at the base. Most cells of the gonimoblast filaments are transformed into pyriform or rounded carposporangia, 10–12 µm wide by 12–15 µm long.

## DISCUSSION

Recent molecular phylogenetic analyses of species assigned to *Grateloupia* and *Prionitis* call for a re-evaluation of species diversity and in-depth morphological studies (Wang *et al.* 2001; De Clerck *et al.* 2005a, b; Wilkes *et al.* 2005;

Gabrielson 2008a, b; Lin *et al.* 2008), and in this article we examined *G. huangiae* from Taiwan. The development of the auxiliary cell ampulla in *G. huangiae* is essentially the same as that of *G. taiwanensis*. In contrast to the *Grateloupia*-type auxiliary-cell ampulla described by Chiang (1970), the auxiliary-cell ampullae possess three orders of simple ampullar filaments before diploidization in both species. The cells of the ampullar filaments branch laterally and link to secondary medullary filaments derived from the inner cortical cells and form a pericarp of mixed gametophytic and ampullar filaments (Figs 23, 24). This study confirms the details in the development of the auxiliary-cell ampullae before and after presumed diploidization described earlier by Lin *et al.* (2008) in which they found two distinct growth patterns in three species of *Grateloupia* from Taiwan, one of which was referred to as the *G. taiwanensis* type and the other as the *G. orientalis* type. The ampulla-bearing, inner cortical cells can be confused as part of the auxiliary-cell ampulla, if the vegetative cells in their vicinity stain as darkly as the cells of the ampullar filaments themselves (see Figs 17, 18; see also Lin *et al.* 2008, fig. 5c).

Our *rbcL* sequence analysis (Fig. 1) showed that *G. huangiae* is genetically distinct from *P. lancifolius* from Japan, despite their superficial similarity in the presence of numerous short proliferations from the surface of the thallus. The structure of their auxiliary-cell ampullae is also very different in the two species. The mature auxiliary-cell ampulla of *P. lancifolius* is bushy with more than 10 branched ampullar filaments before diploidization (see Lee *et al.* 1997, figs 4, 14 16).

*Grateloupia huangiae* is also superficially similar to *G. capensis* De Clerck in De Clerck *et al.* (2005b) from South Africa in that both bear superficial proliferations. However, the latter species has a thicker thallus than the former, with up to 12 layers of cells in the cortex compared to three to five layers in *G. huangiae*. The two species are also genetically distinct; 74 base-pair distances separate their *rbcL* sequences (5.8%). In addition, stellate cells are abundant in the inner cortex of *G. capensis* with five to eight pit connections between cortical cells; whereas, stellate cells were rarely observed in *G. huangiae*. Furthermore, the texture of *G. capensis* is firm, differing to the soft, gelatinous thallus of *G. huangiae*.

## ACKNOWLEDGEMENTS

This project was partially supported by a National Science Council (Taiwan) grants (NSC 96-2628-B-019-006-MY3) and financial support from the Center for Marine Bio-environment and Biotechnology at NTOU (98529002C4) to S.-M. Lin.

## REFERENCES

- CHIANG Y.-M. 1970. Morphological studies of red algae of the family Cryptonemiaceae. *University of California Publication in Botany* 58: 95 pp.
- CHIANG Y.-M. 1973. Notes on marine algae of Taiwan. *Taiwania* 18: 13–17.



- DE CLERCK O., GAVIO B., FREDERICQ S., COCQUYT E. & COPPEJANS E. 2005a. Systematic reassessment of the red algal genus *Phyllymenia* (Halymeniaceae, Rhodophyta). *European Journal of Phycology* 40: 169–178.
- DE CLERCK O., GAVIO B., FREDERICQ S., BÁRBARA I. & COPPEJANS E. 2005b. Systematics of *Grateloupia filicina* (Halymeniaceae, Rhodophyta), based on *rbcL* sequence analyses and morphological evidence, including the reinstatement of *G. minima* and the description of *G. capensis* sp. nov. *Journal of Phycology* 41: 391–410.
- FAYE E.J., WANG H.W., KAWAGUCHI S., SHIMADA S. & MASUDA M. 2004. Reinstatement of *Grateloupia subpectinata* (Rhodophyta, Halymeniaceae) based on morphology and *rbcL* sequences. *Phycological Research* 52: 59–67.
- FREDERICQ S., HOMMERSAND M.H. & FRESHWATER D.W. 1996. The molecular systematics of some agar- and carrageenan-containing marine red algae based on *rbcL* sequence analysis. *Hydrobiologia* 326–327: 125–135.
- GABRIELSON P.W. 2008a. On the absence of previously reported Japanese and Peruvian species of *Prionitis* (Halymeniaceae, Rhodophyta) in the northeast Pacific. *Phycological Research* 56: 105–114.
- GABRIELSON P.W. 2008b. Molecular sequencing of northeast Pacific type material reveals two earlier names for *Prionitis lyallii*, *Prionitis jubata* and *Prionitis sternbergii*, with brief comments on *Grateloupia versicolor* (Halymeniaceae, Rhodophyta). *Phycologia* 47: 89–97.
- GAVIO B. & FREDERICQ S. 2002. *Grateloupia turuturu* (Halymeniaceae, Rhodophyta) is the correct name of the non-native species in the Atlantic known as *Grateloupia doryphora*. *European Journal of Phycology* 37: 349–359.
- GUIRY M.D. & GUIRY G.M. 2010. *AlgaeBase*. Worldwide electronic publication, National University of Ireland, Galway. Available at: <http://www.algaebase.org> (25 March 2010).
- HUELSENBECK J.P. & RONQUIST F. 2001. MrBayes: Bayesian inference of phylogeny. *Bioinformatics* 17: 754–755.
- KAWAGUCHI S., WANG H.W., HORIGUCHI T., SARTONI G. & MASUDA M. 2001. A comparative study of the red alga *Grateloupia filicina* (Halymeniaceae) from the northwestern Pacific and Mediterranean with the description of *Grateloupia asiatica*, sp. nov. *Journal of Phycology* 37: 433–442.
- KAWAGUCHI S., WANG H.-W., HORIGUCHI T., LEWIS J.A. & MASUDA M. 2002. Rejection of *Sinkoraena* and transfer of some species of *Carpopeltis* and *Sinkoraena* to *Polyopes* (Rhodophyta, Halymeniaceae). *Phycologia* 41: 619–635.
- KAWAGUCHI S., SHIMADA S., WANG H.-W. & MASUDA M. 2004. The new genus *Yonagunia* Kawaguchi et Masuda (Halymeniaceae, Rhodophyta), based on *Y. tenuifolia* Kawaguchi et Masuda sp. nov. from southern Japan and including *Y. formosana* (Okamura) Kawaguchi et Masuda comb. nov. from southeast Asia. *Journal of Phycology* 40: 180–192.
- LEE H.-B., LEWIS J.A., KRAFT G.T. & LEE I.K. 1997. *Sinkoraena* gen. nov. (Halymeniaceae, Rhodophyta) from Korea, Japan and southern Australia. *Phycologia* 36: 103–113.
- LIN S.-M., FREDERICQ S. & HOMMERSAND M.H. 2001. Systematics of the Delesseriaceae (Ceramiales, Rhodophyta) based on large subunit rDNA and *rbcL* sequences, including the Phycodryoidae, subfam. nov. *Journal of Phycology* 37: 881–899.
- LIN S.-M., HOMMERSAND M.H. & FREDERICQ S. 2004. Two new species of *Martensia* (Delesseriaceae, Rhodophyta) from Kenting National Park, southern Taiwan. *Phycologia* 43: 13–25.
- LIN S.-M., LIANG H.-Y. & HOMMERSAND M.H. 2008. Two types of auxiliary cell ampullae in *Grateloupia* (Halymeniaceae, Rhodophyta), including *G. taiwanensis* sp. nov. and *G. orientalis* sp. nov. from Taiwan based on *rbcL* gene sequence analysis and cystocarp development. *Journal of Phycology* 44: 196–214.
- MILLER K.A., HUGHEY J.R. & GABRIELSON P.W. 2009. Research note: First report of the Japanese species *Grateloupia lanceolata* (Halymeniaceae, Rhodophyta) from California, USA. *Phycological Research* 57: 238–241.
- SWOFFORD D.L. 2003. *PAUP\*: phylogenetic analysis using parsimony (\*and other methods)*. Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.
- WANG H.W., KAWAGUCHI S., HORIGUCHI T. & MASUDA M. 2000. Reinstatement of *Grateloupia catenata* (Rhodophyta, Halymeniaceae) on the basis of morphology and *rbcL* sequence. *Phycologia* 39: 228–237.
- WANG H.W., KAWAGUCHI S., HORIGUCHI T. & MASUDA M. 2001. A morphological and molecular assessment of the genus *Prionitis* J. Agardh (Halymeniaceae, Rhodophyta). *Phycological Research* 49: 251–261.
- WILKES R.J., MCVIVOR L.M. & GUIRY M.D. 2005. Using *rbcL* sequence data to reassess the taxonomic position of some *Grateloupia* and *Dermocorynus* species (Halymeniaceae, Rhodophyta) from the north-eastern Atlantic. *European Journal of Phycology* 40: 53–60.
- WOMERSLEY H.B.S., LEWIS J.A. 1994. Family Halymeniaceae Bory 1828: 158. In: *The marine benthic flora of southern Australia, Rhodophyta—Part IIIA—Bangiophyceae and Florideophyceae (Acrochaetiales, Nemaliales, Gelidiales, Hildenbrandiales and Gigartinales sensu lato)*, & H.B.S. WOMERSLEY 1994, pp. 167–218. Australian Biological Resources Study, Canberra, Australia.

Received: 15 April 2010; accepted: 14 September 2010  
Associate editor: Craig Schneider