REVISITING THE SYSTEMATICS OF THE GENERA *GRATELOUPIA*, *PHYLLYMENIA* AND *PRIONITIS* (HALYMENIACEAE, RHODOPHYTA) WITH A DESCRIPTION OF A NEW SPECIES - *PRIONITIS TAIWANI-BOREALIS*

Conxi Rodríguez-Prieto

Department of Environmental Sciences, Faculty of Sciences, University of Girona, M. Aurèlia Capmany 69, 17003 Girona, Spain

Olivier De Clerck

Phycology Research Group and Centre for Molecular Phylogenetics and Evolution, Ghent University, Ghent, Belgium

Michael D. Guiry

AlgaeBase, Ryan Institute, National University of Ireland, University Road, Galway, H91 TK33, Ireland

Showe-Mei Lin

Institute of Marine Biology, National Taiwan Ocean University, Keelung 20224, Taiwan, R.O.C.

[1]Received _____. Accepted _____.

[2]Author for correspondence: e-mail linsm@ntou.edu.tw

Running title: **Systematics of *Grateloupia*, *Phyllymenia* and *Prionitis***

The taxonomy of the genera *Grateloupia*, *Phyllymenia* and *Prionitis* has been considerably revised but remains controversial. A combination of vegetative and female reproductive structure is used to define these genera. However, the architecture and behavior of the auxiliary cell ampullae before and after diploidization are not well documented for most species. We examined the female reproductive structures of a new species (*Prionitis taiwani-borealis* sp. nov.) from Taiwan and compared our observations to the species currently placed in the *Phyllymenia/Prionitis* complex. The female reproductive structures of the *Phyllymenia/Prionitis* complex are characterized by: 1) 2-celled carpogonial branches with the supporting cell being the basal cell of a third-order ampullar filament; 2) auxiliary cell ampullae composed of three orders of unbranched ampullar filaments before diploidization; 3) cells of auxiliary cell ampullar filaments forming a cellular cluster after diploidization and surrounding the developing gonimoblasts; 4) gonimoblast initials produced from the diploidized auxiliary cells before fusing with them; 5) branched auxiliary cell ampullar and secondary medullary filaments involved in early pericarp formation. A monophyletic relationship of species possessing female structures similar to those of *Pr. taiwani-borealis* and related species was highly supported based on combined *rbc*L and LSU rDNA sequence analyses. The female reproductive structures of other species of *Grateloupia sensu lato* phylogenetically closely related to the *Prionitis* and *Phyllymenia* assemblage, require reinvestigation as correct interpretations of pre- and post-fertilization events have proven to be highly informative for resolving the systematics of the Halymeniaceae.

***Key index words*: Auxiliary cell ampullae, *Phyllymenia gibbesii* comb. nov.; *Phyllymenia huangiae* comb. nov.; *Phyllymenia taiwanensis* comb. nov.; *Prionitis taiwani-borealis* sp. nov.; systematics**

Abbreviations: BI, Bayesian Inference; GTR, General Time Reversible model; HGI, Herbarium of University of Girona (Spain); NTOU, National Taiwan Ocean University

The genus *Prionitis* is widely distributed in both the Pacific and Atlantic Oceans and is currently considered to contain 17 species (Guiry and Guiry 2021). The generic name *Prionitis* [from the Greek πριόνι (prion), a saw, and the Greek suffix -ῖτις (-itis), pertaining to] was introduced by J.Agardh (1851: 185) for nine species (numbers 1-3, 3a, 4-8): *Pr. microcarpa* (C.Agardh) J.Agardh [currently *Polyopes constrictus* (Turner) J.Agardh (Kawaguchi et al. 2002)], *Pr. chondrophylla* J.Agardh, *Pr. australis* (J.Agardh) J.Agardh, *Pr. decipiens* (Montagne) J.Agardh [currently *Neorubra decipiens* (Montagne) M.S.Calderon, G.H.Boo & S.M.Boo (Calderon et al. 2014a)], *Pr. pectinata* J.Agardh (a later heterotypic synonym of *Pr. decipiens*; Calderon et al. 2014a), *Pr. ligulata* J.Agardh, *Pr. sternbergii* (C.Agardh) J.Agardh, *Pr. jubata* J.Agardh (a later heterotypic synonym of *Pr. sternbergii*) (Gabrielson 2008) and *Pr. crinita* (S.G.Gmelin) J.Agardh [currently *Tichocarpus crinitus* (S.G.Gmelin) Ruprecht; Klochkova et al. 2009]. A generitype was not designated by J.Agardh as was often the practice at the time. Schmitz (1889: 452) selected *Prionitis lanceolata* (Harvey) Harvey [as "*Prionitis lanceolata* (Harvey) J.Agardh"] as the type [lectotype] but this typification was not valid as *Pr. lanceolata* was not amongst the nine species included in the genus by J.Agardh (1851). De Toni (1936: 6) recognised that *Prionitis* J.Agardh (1851) was a later homonym of *Prionitis* Adanson (1763), a genus of flowering plants [presently a synonym of *Falcaria* Fabricius, nom. cons. (Apiaceae)], and proposed the replacement name *Zanardinula* G.De Toni. Papenfuss (1950: 180), however, pointed out that *Prionitis* J.Agardh had been in use for a genus of algae for almost a century and proposed it for conservation, which was subsequently accepted. Papenfuss (1950: 180) also recognised Schmitz's (1889) typification and showed that *Pr. lanceolata* (Harvey) Harvey was the taxonomically correct name for *Pr. ligulata* J.Agardh. Silva (1952: 283) accepted *Prionitis* J.Agardh as proposed by Papenfuss but listed the "lectotype" as "*Prionitis ligulata* Grev[ille] ex J.Ag[ardh] = *Pr. lanceolata* (Harv.) Harv.". *Prionitis* J.Agardh is currently listed in the on-line *Index Nominum Genericorum* as a conserved name with *Pr. ligulata* as "type". As *Prionitis* J.Agardh is a conserved name, the type is also conserved and any lectotypifications are superfluous. The attribution by Silva (1952: 283) of the name to Greville is incorrect as the manuscript name referred to by J. Agardh (1851: 189-190) was an invalid designation “*Grateloupia ligulata*” Greville. Chiang (1970: 28) in his morphological study of the family Cryptonemiaceae [now the Halymeniaceae] listed *Pr. lanceolata* as the “type species” and treated *Pr. ligulata* J.Agardh as a synonym as Harvey (1833: 164) had earlier described this species as *Gelidium lanceolatum* Harvey based upon material collected by David Douglas (1799–1834) at Monterey, California, during the voyage of Captain Beechy (Chiang 1970: 28). *Prionitis ligulata* J.Agardh is thus a later heterotypic synonym of *Pr. lanceolata* (Harvey) Harvey (1853:197) and is the correct name for the type of *Prionitis* J.Agardh.

The genus *Phyllymenia* was established by J. Agardh (1848: 47) based on a single species, *Ph. hieroglyphica* J.Agardh, from the Cape of Good Hope, South Africa. J. Agardh (1851: 183) transferred *Ph. hieroglyphica* to *Grateloupia* C.Agardh *nom. cons.* (Agardh 1822: 211), but later, Schmitz and Hauptfleisch (1897: 511) placed *Grateloupia hieroglyphica* (J.Agardh) C.Agardh together with *Iridaea cornea* Kützing [currently known as *Pachymenia cornea* (Kützing) Chiang] in the genus *Cyrtymenia* F.Schmitz (Schmitz 1896: 16). Setchell and Gardner (1936) resurrected *Phyllymenia* and indicated that *Iridaea belangeri* Bory (in Bélanger & Bory 1834) from False Bay, Cape Province, South Africa was identical to *Ph. hieroglyphica,* and made a new combination, *Ph. belangeri* (Bory) Setchell & N.L.Gardner 1936, as the generitype of *Phyllymenia*.

Wang et al. (2001) proposed the merger of the genus *Prionitis* with *Grateloupia* mainly based on similarities in the structure of the auxiliary cell ampullae as described by Chiang (1970) and phylogenetic analyses of the *rbc*L gene. However, Wang et al. (2001) did not transfer all the species of *Prionitis* into *Grateloupia* as several species of *Prionitis* from both tropical and temperate regions needed to be re-investigated based upon their reproductive structure and DNA sequencing. Subsequently, De Clerck et al. (2005a) proposed the merger of the genus *Phyllymenia* into *Grateloupia* as both genera had almost identical auxiliary cell ampullae morphology. After observing the development of the auxiliary cell ampullae in two species of *Grateloupia* from Taiwan in northwestern Pacific Ocean, Lin et al. (2008) described two distinct types of auxiliary cell ampullae occurring in *Grateloupia sensu lato*: (1) ampullae consisting of three orders of unbranched filaments that branch after diploidization of the auxiliary cell, and form a pericarp together with modified medullary filaments ("*G. taiwanensis* type"), and (2) ampullae composed of only two orders of unbranched filaments in which only a few cells are incorporated into a basal fusion cell after diploidization of the auxiliary cell, and the pericarp is made up entirely of secondarily produced medullary filaments ("*G. orientalis* type"). Gargiulo et al. (2013) reinstated the genera *Prionitis* and *Phyllymenia* together with *Dermocorynus* P.Crouan & H.Crouan and *Pachymeniopsis* Yamada ex Kawabata based upon molecular analyses and a reinterpretation of the female reproductive structures. Subsequently, the new genera *Neorubra* M.S.Calderon, G.H.Boo & S.M.Boo (2014a) and *Mariaramirezia* M.S.Calderon, G.H.Boo, A.Mansilla & S.M.Boo (2016) [as *Ramirezia* M.S.Calderon et al., nom. illeg. in Calderon et al. (2014b)] were proposed based on some species previously placed in *Grateloupia sensu lato*. However, the female reproductive structures for some species of *Grateloupia sensu lato* that are closely related to this complex based on *rbc*L sequence analyses (see Lin and Liang 2011; Rodríguez-Prieto et al. 2021) were shown to have similar developmental patterns to the "*G. taiwanensis* type" (Lin et al. 2008).

In order to arrive at a generic classification of *Grateloupia sensu lato*, we re-examined the behavior of the auxiliary cell ampullae pre- and post-diploidization of two species that are molecularly closely related to *Phyllymenia* and *Prionitis* based on a preliminary *rbc*L sequence analysis. The generic delineation between the genera *Grateloupia*, *Phyllymenia* and *Prionitis* will also be discussed based on their gonimoblast development prior to and after diploidization as well as *rbc*L+LSU sequence analyses.

Comentat [crp1]: 2 spaces

MATERIALS AND METHODS

Specimens were collected in the shallow subtidal by snorkelling or SCUBA diving at depths of 2-10 m. For morphological studies, algal samples were preserved in 3-5% Formalin in seawater or preserved as herbarium sheets. A fragment of each specimen was preserved in silica gel or in 95% ethanol for subsequent DNA extraction. Hand sections were stained with 1% aniline blue acidified with 1% HCl or treated with Wittmann's aceto-iron-hematoxylin-chloral hydrate (Wittmann 1965) and mounted in 50% Hoyer's mounting medium modified by Lin et al. (2004) and Rodríguez-Prieto and Hommersand (2009). Habit images were taken with an Epson scanner (Tokyo, Japan), a Canon EOS 350D (Canon, Tokyo, Japan) or an Olympus underwater camera (Tough, Tokyo, Japan), and photomicrographs were made with an AxioCam MRc attached to an Axioskop 2 plus microscope (Carl Zeiss, Oberkochen, Germany) or an Olympus BX51 microscope with a Q-imaging digital camera (Burnaby, British Columbia, Canada). Voucher specimens were deposited in the Herbaria of the Institute of Marine Biology of National Taiwan Ocean University (NTOU) and the University of Girona, Spain (HGI). Herbarium abbreviations follow Thiers (2021).

DNA from silica-dried specimens was extracted using a DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. DNA amplification and sequencing procedures of the LSU rDNA (BB+V, BB+G, F449+G, X+28F, Z+J) and *rbc*L genes (F64+R1150, F645+RbcSstart) are as described in Freshwater et al. (1999), Lin et al. (2001) and Saunders and Moore (2013). Newly generated sequence data were integrated with a selection of sequences available from GenBank and aligned using MUSCLE (Edgar 2004). Regions of the LSU sequences that were ambiguously aligned were removed manually. The taxon sampling aimed to present the major lineages of the *Grateloupia sensu lato* clade. For the combined *rbc*L and LSU analyses, a subset of sequences was used and both sequences for each species were derived from the same individual (see Table S1). Only for *Prionitis sternbergii* were LSU and *rbc*L sequences generated from 2 different specimens. A selection of *Halymenia* C.Agardh and *Cryptonemia* J.Agardh sequences were used as outgroup. The *rbc*L and LSU datasets were analyzed separately as well as combined using maximum likelihood (ML) analyses with IQ-TREE (Nguyen et al. 2015). For the combined *rbc*L – LSU analyses separate partitions were created for the LSU and *rbc*L genes. Substitution models were automatically selected based on BIC values using ModelFinder (Kalyaanamoorthy et al. 2017). Both the LSU and *rbc*L separate partitions were assigned a TN model (code: 010020) with empirical base frequencies (+F), a proportion of invariant site (+I) and rate heterogeneity approximated by a gamma distribution and 4 rate categories (+G4). The robustness of the resulting phylogenies was tested using 1000 replicates of an ultrafast bootstrap heuristic (UFBoot; Minh et al. 2013) in combination with a nonparametric Shimodaira–Hasegawa approximate likelihood-ratio test (SH-aLRT test; Guindon et al. 2010). Additionally, a Bayesian tree was estimated for the *rbc*L – LSU dataset using MrBayes 3.2.7 (Ronquist et al. 2012), applying a GTR+GAMMA model applied to every partition separately (prset rate, pr=variable). Two runs consisting of 4 chains each were run for 5 million generations. Stationarity and convergence of the runs was assessed visually using Tracer v.1.6 (Rambaut et al. 2014) and a majority rule consensus tree was calculated after removal of a burnin fraction of 20 %.

RESULTS

Molecular analyses

The *rbc*L tree (Fig. S1) containing 92 terminal taxa, rooted with *Cryptonemia*/*Halymenia*, placed *Yonagunia* as the sister taxon of *Grateloupia sensu lato*. The latter consisted of several well supported clades, e.g. the *Dermocorynus* – *Grateloupia minima* clade, a *Neorubra* clade, a clade consisting of *Mariaramirezia osornoensis* with *G. doryphora* and *G. schizophylla*, a clade of predominantly tropical and warm temperate *Grateloupia* species (*G. filicina*-clade), a clade with South African *Prionitis* species, New Zealand *G. stipitata* and G. *prolifera*. Relationships among these clades however were generally unresolved. *Prionitis* and *Phyllymenia* species were resolved in a large clade together with numerous *Grateloupia* species as well as species formerly attributed to *Pachymeniopsis*. *Prionitis taiwani-borealis* from Taiwan positioned in the *Prionitis/Pachymeniopsis* clade including the generitypes *Pr. lanceolata* and *Pachymeniopsis lanceolata* (Okamura) Yamada ex Kawabata. The phylogenetic tree of the LSU dataset (Fig. S2) revealed the same major clades as the *rbc*L tree, but contrary to the *rbc*L tree, the LSU analysis positioned *Yonagunia* as the sister taxon of the *Grateloupia filicina* clade, rendering *Grateloupia sensu lato* paraphyletic. Support along the backbone of the phylogeny was very low in the LSU analyses. *Prionitis* and *Phyllymenia* species were resolved in a well-supported clade together with *Grateloupia* species as well as species formerly attributed to *Pachymeniopsis*.

The *rbc*L – LSU dataset for phylogenetic analyses consisted of 4501 characters (LSU = 2705 bp; *rbc*L = 1346bp) and 47 taxa. The combined analyses of *rbc*L and LSU genes resulted in trees that were overall very well-supported. Trees were characterized by 6 (= 7??), 12 and 8 branches out of a total 91 (=126??) with support values lower than 80% - SH-aLRT, 95% UFBOOT and 90% Bayesian posterior probabilities, respectively (Fig. 1). The topology of the ML and Bayesian analyses, however, differed with respect to the monophyly of *Grateloupia sensu lato*. The ML topology resolves *Yonagunia* sister to the *Dermocorynus*/*Grateloupia minima* clade, while *Yonagunia* is sister to *Grateloupia sensu lato* in the Bayesian topology (Fig. S3). It should be noted that neither relationship is well supported in the ML and Bayesian analyses. Further differences relate to the relative placement of the major clades along the backbone of the phylogeny. Again, support is lacking in both analyses. *Grateloupia sensu lato* was recovered in several clades. The generitype, *G. filicina* formed a highly supported clade together with *G. catenata*, *G. lithophila* and *G. ramosissima*. *Grateloupia carnosa* from Japan was resolved as the sister taxon of *Mariaramirezia osornoensis*, although it was only weakly supported. Two additional clades contained the species of *Prionitis* from South Africa as well as *Grateloupia stipitata* from New Zealand, and *Dermocorynus* (including *G. minima*) from the Western Atlantic, respectively. Lastly, species of *Phyllymenia* and *Prionitis* from Northeast Pacific and the remainder of the *Grateloupia* species were well-resolved with high bootstrapping values.

Comentat [u2]: Dear Olivier, I have recount the number of the support values. In total, **there are 126 support values** in *rbc*L-LUS tree (Fig. 1) based on the three methods. Could you please recheck again?--SML

Morphological observations

Prionitis taiwani-borealis* Showe M.Lin, Rodríguez-Prieto, De Clerck & Guiry, *sp. nov. (Figs 2-5)

DESCRIPTION: Thalli prostrate, cartilaginous, 3-5 cm in length, composed of 3-7 (-10) main dorsoventrally compressed axes arising from a discoid holdfast; main axes often arranged in a rosette-like array, bearing 3-4 orders of densely arranged, subdichotomous branches 9-12 mm long and branchlets 0.9-3.0 mm long; mature tetrasporangia 10-11 µm wide by 46-52 µm long; gametophytes dioecious or monoecious; spermatangia 0.7-1.0 µm wide by 2.1-3.1 µm long; gonimoblasts 90-145 µm in diameter.

TYPE LOCALITY: Chaojing, East Keelung City, Taiwan (25.1433° N, 121.8037° E).

HOLOTYPE: A preserved female gametophyte, deposited at the herbarium of the seaweed laboratory at the National Taiwan Ocean University, collected by L.C. Liu from a rocky shore, Chaojing, East Keelung City, Taiwan, 8 June 2009, 6 m in depth (NTOU 001524a).

ISOTYPES: NTOU 001525–001532, HGI-A 20835.

ETYMOLOGY: "*taiwani-borealis*" refers to locality of the type collection, northern Taiwan.

DISTRIBUTION: The species has been found in scattered localities along the coasts of northern Taiwan.

HABITAT AND SEASONALITY: Plants were found in shallow coral reefs or rocky shores, in water depths of 2–12 m. Plants can be found all year round, growing abundantly from May to June.

Specimens examined: Keelung City: (1) Chaojing, coll. L.C Liu and H.J. Hsueh, 8 June 2009 (tetrasporic and females, NTOU001532, type collection), 27 August 2009 (tetrasporic, males and females, CJ-27Aug2009-H1~15; male and female, HGI-A 20835), 19 October 2009 (tetrasporic and females, CJ-19Oct2009-H1~5), 18 March 2010 (vegetative, CJ-18Mar2010-H1~2), 27 May 2010 (tetrasporic, males and females, CJ-27May2010-H1~2), coll. L.C Liu and Y.H. Chang, 16 October (tetrasporangial, CJ-16Oct2010-H1); (2) Dawulun, coll. Y.H. Chang, 20 October 2014 (male and female HGI-A 20836; tetrasporangial #DWL20x2014-H1~2), 30 March 2015 (sterile, #DWL30iii2015-H1~2); (3) Waimushan, coll. Y.H. Chang, 20 October 2014 (male and female HGI-A 20836; tetrasporangial #DWL20x2014-H1~2), 30 March 2015 (tetrasporangial, #DWL30iii2015-H1~2). New Taipei City: Lungdong, coll. S.-M. Lin, 7 June 2009 (female, #LDW7vi2009-H1), coll. L.C Liu, 7 June 2009 (female, tetrasporangial, #LDW13vi2010-H1, HGI-A 20840).

Habit and vegetative morphology: Thalli prostrate, composed of 3-7 (up to 10) dorsoventrally compressed main axes, arranged in rosettes and partially overlapping one another, up to 3-5 cm long, arising from discoid holdfasts, with stipes up to 12 mm long (Fig. 2, A-C). Thallus texture is cartilaginous and thalli become very tough when dried. Fresh thalli are orange to red in color and the distal ends of branches are yellowish (Fig. 2C), but became dark red when old or dried (Fig. 2A). Margins of axes are slightly to strongly curved inwards. Main axes are subdichotomously branched, with branches 9-12 mm long that bear 3-4 orders of alternately to subdichotomously arranged short branchlets, 0.9-3.0 mm long, with acute-blunt apices (Fig. 2, B-C). Thallus structure is multiaxial (Fig. 2, D-G) and growth is led by many obliquely dividing apical cells. Cortex is composed of anticlinal filaments, up to 3 cells long in upper parts of branches (Fig. 2D) and to 5-6 (-8) cells long in lower parts (Fig. 2, E-G). Subcortical cells are compactly arranged, rounded to oblong, 1.8-2.7 µm in diameter, outer cortical cells elongate abruptly (Fig. 2D white arrows) to 4.4-8.6 µm long. Innermost cortical cells (Fig. 2D, arrowheads) are slightly stellate, 4.5-10.3 µm in diameter, and secondarily pit-connected to form a network that is composed of 2-3 (up to 7 in old branches) cell layers, parallel to the thallus surface. The medulla is composed of densely and mostly longitudinally arranged tubular filaments, with oblong cells, 3.9-13.0 µm in diameter by 8.2-35.1 µm long. Medullary filaments (Fig. 2E, arrows) are secondarily pit-connected and become densely intermixed with numerous rhizoidal filaments in older branches (Fig. 2, F-G). In reproductive branches, some medullary cells are slightly to markedly enlarged and darkly stained (Fig. 2, H-I, arrowheads) among medullary and rhizoidal filaments (Fig. 2, H-I, arrows). These darkly stained medullary cells are rounded in cross section (Fig. 2I, arrowheads), but ovoid in longitudinal section, 10-25 µm wide by 12-68 µm long.

Reproductive structures: Gametophytes are dioecious or monoecious and are morphologically similar to tetrasporophytes. Tetrasporangial sori are scattered over the fertile branchlets or the swollen ends of fertile branches (Fig. 2, B-C). The branches or branchlets bearing tetrasporangia are darker red when dried in comparison to the vegetative branches, which are lighter red. Tetrasporangial initials are cut off laterally from subcortical cells in the mid cortex (Fig. 3 A), elongate (Fig. 3, A-B) and probably undergo meiosis to divide transversely first (Fig. 3C). At this stage, the fertile subcortical cell is still pit-connected (Fig. 3C, pc) to the lateral side of the base of the developing tetrasporangium, which, in turn, divides longitudinally to produce cruciately or decussately arranged tetraspores (Fig. 3D). Mature tetrasporangia are 10-11 µm wide by 46-52 µm long. Spermatangial sori are scattered over the fertile branchlets or distal ends of branches. Initially, surface cells elongate and differentiate into uninucleate spermatangial initials, 1.8-2.1 µm wide by 5.8-19.9 µm long. Spermatangia are formed singly and on maturity are 0.7-1.0 µm wide by 2.1-3.1

µm long (Fig. 3E, arrows), produced sequentially and arranged in terminal chains (Fig. 3F) from spermatangial parental cells. Fully developed spermatangial sori are raised at the thallus surface (Fig. 3G).

Cystocarps are scattered over the distal ends of fertile branches or more commonly on the swollen and rounded apices of branchlets (Fig. 3H-I, arrows). Gonimoblasts can be directly observed under a dissecting microscope (Fig. 3J, white arrows) without any sectioning, and mature carposporophytes are deeply embedded in the inner cortex (Fig. 3K, white arrows). The female reproductive system is non-procarpic; in other words, the auxiliary cells and carpogonial branches arise from separate cortical filaments, termed auxiliary cell ampullae (Fig. 4, A-D) and carpogonial branch ampullae (Fig. 4E). Functional auxiliary cell ampullae are abundant in the tips of young fertile branches, whereas functional carpogonial branches are rarely observed. Each auxiliary cell ampulla is composed of three orders of ampullar filaments, and the auxiliary cell is always the basal cell of a third-order filament (Fig. 4D). The architecture of carpogonial branch ampullae is similar to that of the auxiliary cell ampullae and is composed of three orders of filaments including two ampullar filaments and one carpogonial branch, which represents the replacement of a third-order ampullar filament (Fig. 4E). The carpogonial branch is borne on a supporting cell, and it is two-celled, including the hypogynous cell and carpogonium with a terminal, relatively extended trichogyne (Fig. 4E). Initially, the first order of ampullar filaments in auxiliary cell ampullae is produced laterally from a basal-inner cortical cell (Fig. 4A), and the cortical cells in the vicinity are slightly elongate and stain darkly (Fig. 4, A-B, white arrows). Soon afterwards, the initial of the second order ampullar filament is produced from the basal cell of the first order of ampullar filament (Fig. 4, B-C). The ampullar filaments in fully developed auxiliary cell ampullae are unbranched, 5-9 cells long, and the basal cell of the third ampullar filament enlarges and becomes darkly stained, functioning as the auxiliary cell (Fig. 4D). The cells of the ampullar filaments are initially rounded (Fig. 4, A-C), but the cells in the distal ends gradually elongate in fully developed ampullae (Fig. 4D). Direct diploidization was not seen, but presumably the auxiliary cell is diploidized via the terminal cell of the connecting filament. After diploidization, the cells of the ampullar filaments elongate and branch once or twice (Fig. 4 F, black arrows). At this stage, secondary medullary cells (Fig. 4F, white arrows) are produced from surrounding vegetative cells. In early development, a gonimoblast initial is cut off from the diploidized auxiliary cell and produces a primary gonimolobe. The branched ampullar filaments elongate further and branch a few more times to form a cellular cluster surrounding the developing gonimoblasts (Figs 4G, 5A, black arrows). The secondary medullary filaments (Fig. 4 G, white arrows) become pit-connected to the distal cells of the branched ampullar filaments to form a primary pericarp. As the development of gonimoblasts continues, a cavity is formed around the developing gonimoblasts between the inner cortex and the medulla (Fig. 5B) and a thickened, ostiolate pericarp is formed, composed of secondary medullary filaments issued from inner cortical cells and thickened cortex (Fig. 5 B). The auxiliary cell subsequently fuses with its neighboring ampullar cells to form a branched fusion cell (Fig. 5C), and the terminal cells of the branched ampullar filaments are pit-connected to the secondary medullary filaments (Fig. 5, D-E). The pit connection between the gonimoblast initial and fusion cell becomes widened through maturation of gonimoblasts, but the gonimoblast initial remains distinct (Fig. 5, D-E). At maturity, the branched ampullar filaments become inconspicuous and the fusion cell becomes highly branched (Fig. 5F). At least two gonimolobes are produced from a gonimoblast initial and mature gonimoblasts are up to 90-145 µm in diameter (Figs 3K, 5F). Most cells of gonimoblasts differentiate into spherical to ovoid carposporangia, 7.5-13.8 µm wide by 8.9-16.7 µm long (Fig. 5F).

Phyllymenia gibbesii* (Harvey) Showe M.Lin, Rodríguez-Prieto, De Clerck & Guiry, *comb. nov. (Fig. 6)

BASIONYM: *Grateloupia gibbesii* Harvey *Smithsonian Contributions to Knowledge* 5(5) [*Nereis boreali-americana* Pt. II, *Rhodospermeae*]: 199, pl. 26 (as "*Gibbesii*"), 1853.

TYPE LOCALITY: Sullivan's Island, Charleston, South Carolina, USA.

LECTOTYPE: TCD 0011818 (here designated), a preserved specimen deposited at Trinity College Dublin, Ireland (TCD). A lectotype for this species has not been formally designated in accordance with the ICN (Turland et al. 2018), although Reid Wiseman annotated TCD 0011818 as such, he did not publish the lectotypification as required by ICN Arts 9.19, 9.20, and 10.5. Furthermore, Rodríguez-Prieto et al. (2021: 85) did not use the words "here designated" in their attempted selection of a lectotype as required by ICN Art. 7.11.

DISTRIBUTION: Western Atlantic Ocean [South Carolina (USA), Colombia, Venezuela]; southern Mediterranean Sea (Egypt), where it is likely to be adventive.

HABITAT & SEASONALITY: Plants grow on rocky substrata in shallow water, in an eutrophic embayment. Reproductive thalli were found from late March to mid-June.

SPECIMENS EXAMINED: Egypt: Alexandria, Eastern Harbour, coll. S. Shabaka, 9 May 2019 (20 May 2019 (–2 m, HGI-A 20420 male and female).

Description. Habit, vegetative, tetrasporangial and spermatangial morphology are as the recent description by Rodríguez-Prieto et al. (2021: 85, figs 2-20).

The development of female reproductive structures in *Ph. gibbesii* (Fig. 6, A-H) is similar to that of *Pr. taiwani-borealis*. The female reproductive system is non-procarpic as the auxiliary cell (Fig. 6C) and carpogonial branches (Fig. 6D) ampullae are produced from different cortical filaments. The auxiliary cell ampulla is initiated from a subcortical cell (Fig. 6A), composed of three orders of ampullar filaments (Fig. 6B), and the auxiliary cell is always the enlarged basal cell of the third-order filament (Fig. 6C). The carpogonial branch is two-celled, including the hypogynous cell and carpogonium, borne on a supporting cell (= the basal cell of the second order ampullar filament) (Fig. 6D). Fertilization was not observed. In later stages, cells of the fertilized carpogonial branch fuse with the supporting cell, which produces a primary connecting filament (Fig. 6E, cf). Later, a network formed by secondarily produced connecting filaments (Fig. 6E, cf'), seems to function as the center of diploidization. The auxiliary cell then becomes diploidized through the terminal end of a secondary connecting filament (Fig. 6E, cf') fusing with a basal cell (Fig. 6E, bc) of an ampullar filament in the vicinity of the auxiliary cell. Noticeably, some cells of the ampullar filaments in the diploidized auxiliary cell ampulla enlarge or divide once laterally (Fig. 6E, white arrows), whereas those cells (Fig. 6E, black arrowheads) in the fertilized carpogonial branch ampulla remain the same size and do not divide. The early development of gonimoblasts is similar to that found in *Pr. taiwani-borealis*. After diploidization, the cells of the ampullar filaments elongate and branch once or twice (Fig. 6F, white arrows), and a gonimoblast initial is cut off from the diploidized auxiliary cell (Fig. 6F, gi). As gonimoblast development continues, the gonimoblast initial produces a primary gonimolobe (Fig. 6 G) and cells of the ampullar filaments divide a few more times to form a cellular cluster surrounding the developing gonimoblasts (Fig. 6H, white arrows). At this time, secondary medullary cells (Fig. 6H, black arrows) are produced from surrounding vegetative cells to form an early pericarp. The maturation stages are as described in Rodríguez-Prieto et al. (2021: 86, figs 30-35). The auxiliary cell eventually fuses with its neighboring ampullar cells to form a branched fusion cell and the branched ampullar filaments surrounding the basal part of the gonimoblasts are gradually fading out. Two to three gonimolobes are produced from a gonimoblast initial and most cells of gonimoblasts differentiate into spherical to ovoid carposporangia.

DISCUSSION

The Halymeniaceae is one of the largest families in the Florideophyceae, currently including 38 genera (two of which are hemiparasites) and more than 348 species (Guiry and Guiry 2021). The main center of distribution of the Halymeniaceae is situated in warm-temperate regions (Chiang 1970, Womersley and Lewis 1994, Yoshida 1998, Saunders and Kraft 2002, Wang et al. 2001, see Lin and Guiry 2017 for a summary). The generic diagnostic features in the Halymeniaceae include a combination of auxiliary cell ampullae features, cystocarp morphology and vegetative structure (Chiang 1970, Womersley and Lewis 1994, Lin et al. 2008, 2020) as well as gene phylogeny (Wang et al. 2001, Kawaguchi et al. 2004, Hommersand et al. 2010, Calderon et al. 2014a, 2014b, D’Archino et al. 2015). However, the development of the auxiliary cell ampullar complex and carposporophytes is obscure and controversial in many genera, especially in the *Grateloupia/Phyllymenia/Prionitis* complex (Lin et al. 2008, Gargiulo et al. 2013, Rodríguez-Prieto et al. 2021). The LSU+*rbc*L phylogenetic analyses in this study (see Fig. 1) supported a separation

Comentat [crp3]:

of the *Phyllymenia*/*Prionitis* complex from *Grateloupia sensu stricto*. The species currently placed in the genera *Prionitis* and *Phyllymenia*, which were shown to have a cellular cluster formed by divided and branched ampullar filaments after diploidization. This result concurs with Lin et al. (2008) and Lin & Liang (2011), who showed that some species of *Grateloupia sensu lato*, specifically species formerly placed in the genera *Phyllymenia*, *Prionitis* and *Pachymeniopsis*, possessed ampullar filaments developing into branched filamentous clusters after diploidization and formed a distinct clade based on *rbc*L gene sequence analyses. The carposporophyte development of many species in *Grateloupia sensu lato*, which lack critical stages of postfertilization, need to be reexamined before making further taxonomic changes.

Chiang (1970) proposed five types of secondary ampullar filaments in the auxiliary cell ampullae in the genera of the Halymeniaceae: the *Grateloupia*-type, the *Thamnoclonium*-type, the *Aeodes*-type, the *Cryptonemia*-type, and the *Halymenia*-type. In Chiang's scheme (1970: 71), the *Grateloupia*-type, as seen in the genera *Grateloupia*, *Prionitis*, *Phyllymenia*, and *Pachymeniopsis,* was composed of a single primary ampullar filament and two or three 7- to 13-celled, unbranched secondary ampullar filaments. Chiang also pointed out that the auxiliary cell is usually the basal cell of the secondary ampullar filament, which is produced by the third, fourth or fifth cell of the primary ampullar filament. Chiang's interpretation was largely followed in later taxonomic studies of the Halymeniaceae (Wang et al. 2001, De Clerck et al. 2005a, 2005b, Kawaguchi et al. 2004, Wilkes et al. 2005, Calderon et al. 2014a, 2014b). Lin et al. (2008) were the first to document the pre- and post-fertilization of the auxiliary cell ampullae using the labeling and the sequence of first-, second-, and third-order ampullar filaments based on the ontogeny of the auxiliary cell ampullae based on *Grateloupia taiwanensis* positioned in the *Phyllymenia/Prionitis* complex and *Grateloupia orientalis* Showe M.Lin & H.-Y. Liang positioned in the *Grateloupia* clade. Here, we follow the labeling system of Lin et al. (2008) for numbering the auxiliary cell ampullar filaments. In *Phyllymenia* and *Prionitis*, the auxiliary cell ampullae are composed of three orders of unbranched ampullar filaments before diploidization and the auxiliary cell is the basal cell of a third-order ampullar filament, and the cells of ampullar filaments in diploidized auxiliary cell ampullae divide and branch many times to form a cellular cluster. On the other hand, the auxiliary cell ampullae in species of *Grateloupia sensu stricto*, as seen in *G. orientalis* and *G. ramosissima*, are composed of two orders of simple and unbranched ampullar filaments before diploidization and the auxiliary cell is the basal cell of a second-order ampullar filament, and the cells of ampullar filaments in diploidized auxiliary cell ampullae only divide few times but do not form a cellular cluster. A comparison of the ontogeny of the carpogonial and auxiliary ampullae, and pericarp formation as interpreted by Chiang (1970), Lin et al. (2008, this study) and Gargiulo et al. (2013) is shown in Table 1. Significant differences between Lin et al. (2008, this study) and Gargiulo et al. (2013) include: 1) the origin of the auxiliary cells which are always the basal cells of the second-order (*G. orientalis* type in *Grateloupia sensu stricto*) or the third-order (*"G." taiwanensis* type in the *Prionitis/Phyllymenia* complex) of ampullar filaments according to Lin et al. (2008, this study). Our observations agree with Chiang's observations (Chiang 1970: 32), in which the auxiliary cells can be the innermost (= basal) cells of the secondary filaments in *Prionitis*. According to Gargiulo et al. (2013), however, the auxiliary cell is the terminal cell of a 3-5 celled auxiliary cell branch in a fully developed auxiliary cell ampulla. In some cases, the diploidized auxiliary cells can produce nutritive cells. - 2) the origin of the supporting cell: Lin et al. (2008, this study) followed Chiang's (1970) interpretation that the supporting cell is a subcortical cell positioned immediately beneath a 2-celled carpogonial branch. In contrast, Gargiulo et al. (2013) considered the supporting cell to be an innermost subcortical cell bearing the whole carpogonial branch ampulla. - 3) The origin of involucral filaments (= nutritive filaments) and fate of auxiliary cell ampullae: according to Lin et al. (2008, this study), the involucral filaments are produced from the subcortical cells in the vicinity of the diploidized auxiliary cell ampullae or from cells of the auxiliary cell ampullar filaments after diploidization, and the diploidized auxiliary cells never produce any nutritive cells or involucral filaments. In Gargiulo et al. (2013), the nutritive filaments can be produced from the diploidized auxiliary cells or the fusion cell resulting from a diploidized auxiliary cell branch.

Calderon et al. (2014a) established *Neorubra* based on J. Agardh's *"Prionitis" decipiens* and characterized the genus as possessing a 5-celled carpogonial branch (fig. 29) and 4-celled auxiliary cell branch (fig. 32). Calderon et al. (2014a) treated the basal cells (= innermost subcortical cells) bearing the whole carpogonial branch ampulla as the supporting cells based on the homologous ontogeny of the auxiliary cell ampullae. In other words, the first cells (= the basal cells) of the carpogonial branch ampullae are the supporting cells of the ampullae as initially proposed by Gargiulo et al. (2013). The interpretation of the structure of the carpogonial branches in Gargiulo et al. (2013) and Calderon et al. (2014a) is very different from that in Chiang (1970) and later authors (e.g., Womersley and Lewis 1994, Kawaguchi 1989, 1997, Wang et al. 2001, Faye et al. 2004, Kawaguchi et al. 2004, Lin et al. 2008, Hommersand et al. 2010, Lin and Liang 2011). In this more widely accepted interpretation, a carpogonial branch in the Halymeniaceae consists of a 2-celled unbranched filament composed of a carpogonium and a hypogynous cell borne on a modified/fertile subcortical cell (= the supporting cell) (see Chiang 1970, fig. 13d for *Prionitis lanceolata* and fig.16d for *Phyllymenia belangeri*). Moreover, the way the cells of the auxiliary cell filaments are numbered in Calderon et al. (2014a) differs from the numbering used here, in which the auxiliary cell is the basal cell of a third-order of ampullar filament, as first proposed by Lin et al. (2008). Lin et al. (2012, 2020) reinforced the taxonomic value of using hematoxylin staining to study critical stages of postfertilization, as the stain enables distinguishing modified vegetative cells from reproductive cells, which is very important in the correct interpretation of pre- and post-fertilization/diploidization events.

Prionitis taiwani-borealis was previously misidentified by Lin et al. (2018) as *Carpopeltis maillardii* Montagne & Millardet [≡ *Yonagunia maillardii* (Montagne & Millardet) Showe M.Lin, Y.-C.Chuang & De Clerck] as they share a similar thallus morphology and vegetative structure. However, *Ph. taiwani-borealis* can be separated from *Y. maillardii* by the female reproductive structures, in which the ampullar filaments in the auxiliary cell ampullae in the latter do not branch to form a cellular cluster as seen in the former (see Lin et al. 2020, Fig. 3). *Prionitis taiwani-borealis* is only found in the subtidal zones in northern Taiwan and its thallus morphology (prostrate thallus with densely arranged flattened branches) is very different from the described species in the *Prionitis*/Phyllymenia complex (having erect bladed, cylindrical or terete thalli) from China (Xia

Comentat [crp4]: This is not in italics

2004:81-140) and Japan (Yoshida 1998: 725-734). Gargiulo et al. (2013) showed that *Pr. taiwani-borealis* (as *Carpopeltis maillardii*) clustered in the *“Grateloupia lanceolata”* [≡ *Pachymeniopsis lanceolata*]/*Grateloupia americana* (= *Prionitis lanceolata*) clade based on *rbc*L sequence analyses. Kawabata (1954, fig. 3, d-f; 1962, pl. 3, e-g) clearly showed that the ampullar filaments in *Pachymeniopsis lanceolata* cuts off a number of branchlets (= branched ampullar filaments in this study), which were then inter-connected with medullary filaments and inner cortical cells. Both studies suggested that the development of the auxiliary cell ampullae after diploidization in *Pachymeniopsis lanceolata* is largely similar to that of *Phyllymenia*. The other species of “*Grateloupia*” and “*Prionitis/Pachymeniopsis*”, which share similar female reproductive development or cluster with *Phyllymenia* in molecular analyses, require re-investigation.

Taxonomic conclusions

Grateloupia taiwanensis and *Grateloupia huangiae* have been shown to have a similar female reproductive developmental pattern to that seen in *Phyllymenia gibbesii*. Accordingly, we formerly transfer *“G.” taiwanensis and “G.” huangiae* into *Phyllymenia*. The other species of *“Prionitis”* and *“Grateloupia”* clustered in the *Prionitis*/*Phyllymenia* clade will be transferred when their female reproductive structures are better documented. The emended generic description and new combinations of *Phyllymenia* are as follows:

***Phyllymenia* J.Agardh (1848: 47)**

Type: *Phyllymenia hieroglyphica* J.Agardh, = *Phyllymenia belangeri* (Bory) Setchell & N.L.Gardner, 1936 [≡ *Iridaea belangeri* Bory, 1834, ≡*Grateloupia belangeri* (Bory) De Clerck, Gavio, Fredericq, Cocquyt & Coppejans, 2005a]

Revised description: thalli terete or bladed, stipes arising from discoid holdfasts, blades or branches composed of compact or loosely arranged cortical cells and relatively larger or filamentous medullary cells, secondary pit-connections abundant among inner cortical cells,; before diploidization, auxiliary cell ampullae composed of three orders of unbranched ampullar filaments, in which the first-order of ampullar filaments are cut off laterally from an innermost cortical cell, initials of the second- and third-order of ampullar filaments are cut off from the first cell of the first- and second-order of ampullar filaments, respectively, auxiliary cells being always the first cells of a third-order ampullar filament in fully developed auxiliary cell ampullae; in early gonimoblast development, the auxiliary cell ampullar filaments divide and branch several times to form a cellular cluster, which is intermixed with secondarily produced medullary filaments in the early formation of the pericarp. Development of spermatangia and tetrasporangia is similar to that found in *Grateloupia*.

Phyllymenia taiwanensis **(Showe M.Lin & H.-Y.Liang) Showe M.Lin, Rodríguez-Prieto, De Clerck & Guiry, *comb. nov*.**

BASIONYM: *Grateloupia taiwanensis* Showe M.Lin & H.Y.Liang, *Journal of Phycology* 44: 198, figs. 1-3, 7, 2008.

HOLOTYPE: Female, NTOU Jan-15-06-SR-taiwanensis-1, collected on 15 January 2006 by S.-L. Liu and H.-Y. Liang, deposited at the seaweed herbarium of Institute of Marine Biology, NTOU.

TYPE LOCALITY: Sail Rock, Kenting National Park, Pingtung County, southern Taiwan. Subtidal zone (1–2 m deep).

DISTRIBUTION: northern Pacific Ocean (Taiwan, China – Yi et al. 2018), South China Sea (Vietnam – Nguyen et al. 2019), Gulf of Mexico (Alabama – DePriest and Lopez-Bautista 2012), probably adventive.

Phyllymenia huangiae **(Showe M.Lin & H.-Y.Liang) Showe M.Lin, Rodríguez-Prieto, De Clerck & Guiry, *comb. nov*.**

BASIONYM: *Grateloupia huangiae* Showe M.Lin & H.-Y.Liang, *Phycologia* 50(3): 233, figs 2-24, 2011.

HOLOTYPE: 172005-1-Holo-Gh, Female plant, 17 January 2005, S.-M. Lin and H.-Y. Liang (NTOU).

TYPE LOCALITY: Linping, Pingtung County, southwestern Taiwan. Intertidal and subtidal zones (0–2 m deep).

DISTRIBUTION: northern Pacific Ocean (Taiwan).

ACKNOWLEDGMENTS

This work was partially supported by the Haiken Algal Foundation grants (107G28902) and from the Ministry of Science and Technology of Taiwan (MOST 104-2621-B-019-001 & 107-2621-B-019 -002 -MY3) to SML. We thank Soha Shobaka, Li-Chia Liu and Yu-Shan Qiu for helping with some specimen collecting and sorting. This work makes use of resources and facilities provided by UGent as part of the Belgian contribution to EMBRC-ERIC (FWO GOH3817N).

REFERENCES

Adanson, M. 1763. *Familles des plantes*. II. Partie [Vol. 2]. Chez Vincent, Imprimeur-Librarie de Mgr le Comte de Provence, rue S. Servin, Paris.

Agardh, C. A. 1822. *Species algarum rite cognitae, cum synonymis, differentiis specificis et descriptionibus succinctis. Volumen primum pars posterior.* Ex officina Berlingiana, Lundae [Lund].

Agardh, J. G. 1848. *Anadema*, ett nytt slägte bland Algerne. *Öfvers. Kongl. Vetensk.-Akad. Förh. Stockholm* 1846:1-16.

Agardh, J. G. 1851. *Species genera et ordines algarum*, seu descriptiones succinctae specierum, generum et ordinum, quibus algarum regnum constituitur. Volumen secundum: algas florideas complectens. Part 1. C.W.K. Gleerup, Lundae [Lund].

Bélanger, C. & Bory de Saint-Vincent, J. B. G. M. 1834. *Voyage aux Indes-Orientales. Botanique IIe Partie. Cryptogamie*. A. Bertrand, Paris, France.

Calderon, M.S., Boo, G.H. & Boo, S.M. 2014a. *Neorubra decipiens* gen. & comb. nov. and

Phyllymenia lancifolia comb. nov. (Halymeniales, Rhodophyta) from South America. *Phycologia* 53:409-22.

Calderon, M. S., Boo, G. H. & Boo, S. M. 2014b. Morphology and phylogeny of *Ramirezia osornoensis* gen. & sp. nov. and *Phyllymenia acletoi* sp. nov. (Halymeniales, Rhodophyta) from South America. *Phycologia* 53:23-36.

Calderón, M. S., Boo, G. H. & Boo, S. M. 2016. Correction to the paper "Morphology and phylogeny of *Ramirezia osornoensis gen. & sp. nov.* and *Phyllymenia acletoi sp. nov.* (Halymeniales, Rhodophyta) from South America". *Phycologia* 55(5): 610.

Chiang, Y.-M. 1970. Morphological studies of red algae of the family Cryptonemiaceae. *Univ. Calif. Publ. Bot.* 58:1-95.

D'Archino, R., Nelson, W. A. & Zuccarello, G. C. 2015. *Amalthea* and *Galene*, two new genera of Halymeniaceae (Rhodophyta) from New Zealand. *Bot. Mar.* 57:185-201.

De Clerck, O., Gavio, B., Fredericq, S., Cocquyt, E. & Coppejans, E. 2005a. Systematic reassessment of the red algal genus *Phyllymenia* (Halymeniaceae, Rhodophyta). *Eur. J. Phycol.* 40:169-78.

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. *J. Phycol.* 41: 391-410.

De Toni, G. 1936. *Noterelle di nomenclatura algologica. VII. Primo elenco di Floridee omonime*. Privately published, Brescia.

DePriest, M.S. & Lopez-Bautista, J.M. 2012. Sequencing of the *rbc*L Marker reveals the nonnative red Alga *Grateloupia taiwanensis* (Halymeniaceae, Rhodophyta) in Alabama. *Gulf Mex. Sci.* 30(1-2):7-13.

Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32:1792-7.

Faye, E. T., Wang, H. W., Kawaguchi, S., Shimada, S. & Masuda, M. 2004. Reinstatement of *Grateloupia subpectinata* (Rhodophyta, Halymeniaceae) based on morphology and *rbc*L sequences. *Phycol. Res.* 52:59-68.

Freshwater, D.W., Fredericq, S. & Bailey, J.C. 1999. Characteristics and utility of nuclear-encoded large-subunit ribosomal gene sequences in phylogenetic studies of red algae. *Phycol. Res.* 47:33-8.

Gabrielson, P.W. 2008. 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.

Gargiulo, G., Morabito, M. & Manghisi, A. 2013. A re-assessment of reproductive anatomy and postfertilization development in the systematics of *Grateloupia* (Halymeniales, Rhodophyta). *Crypt. Algol.* 34:3-35.

Guindon, S., Dufayard, J. F., Lefort, V., Anisimova, M., Hordijk, W. & Gascuel, O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst. Biol. 59:307-21.

Guiry, M. D. & Guiry, G. M. 2021. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. Available at: https://www.algaebase.org (last accessed 28 May 2021).

Harvey, W. H. 1833. [California] Ordo LVI. Algae. *In* Hooker, W. J. & Arnott, G. A. [Eds.] *The botany of Captain Beechey's voyage; …*, No. 4 York Street, Covent Garden, London, UK.

Harvey, W. H. 1853. *Nereis boreali-americana*; or, contributions towards a history of the marine algae of the atlantic and pacific coasts of North America. Part II. Rhodospermeae. *Smith. Contrib. Knowl.* 5(5):1-258.

Hommersand, M. H., Leister, G. L., Ramírez, M. E., Gabrielson, P. W., & Nelson, W. A. 2010. A morphological and phylogenetic study of *Glaphyrosiphon* gen. nov. (Halymeniaceae, Rhodophyta) based on *Grateloupia intestinalis* with descriptions of two new species: *Glaphyrosiphon lindaueri* from New Zealand and *Glaphyrosiphon chilensis* from Chile. *Phycologia* 49:554-73.

Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K. F., von Haeseler, A. & Jermiin, L. S. 2017.

ModelFinder: fast model selection for accurate phylogenetic estimates. Nat. Meth. 14:587-89.

Kawabata, S. 1954. On the structure of the frond, and the reproductive organ of *Pachymeniopsis lanceolata* Yamada (*Aeodes lanceolata* Okam.). *Jpn. J. Phycol. (Sôrui)* 2(3): 11-5, 67-71.

Kawaguchi, S. 1989. The genus *Prionitis* (Halymeniaceae, Rhodophyta) in Japan. *J. Fac. Sci., Hokkaido Univ., Ser. V (Bot.)* 16:193-257.

Kawaguchi, S. 1997. Taxonomic notes on the Halymeniaceae (Gigartinales, Rhodophyta) from Japan. III. Synonymization of *Pachymeniopsis* Yamada in Kawabata with *Grateloupia* C. Agardh. *Phycol. Res.* 45:9-21.

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* 41:619-35.

Kawaguchi, S., Shimada, S., Wang, H. W. & Masuda, M. 2004. The new genus *Yonagunia* Kawaguchi & Masuda (Halymeniaceae, Rhodophyta), based on *Y. tenuifolia* Kawaguchi & Masuda sp. nov. from southern Japan and including *Y. formosana* (Okamura) Kawaguchi & Masuda comb. nov. from southeast Asia. *J. Phycol.* 40:180-92.

Klochkova, N. G., Koroleva, T. N., Kusidi, A. E. 2009. *Atlas vodorosley-makrofitov prikamchatskikh vod [Atlas of algae-macrophytes of Kamchatka waters. Red algae]*. Izdaniya KamchatNIRO, Petropavlovsk-Kamchatski.

Lin S.-M., Hommersand, M. H. & Fredericq, S. 2004. Two new species of *Martensia* (Delesseriaceae, Rhodophyta) from Kenting National Park, southern Taiwan. *Phycologi*a 43:13-25.

Lin, S.-M. & 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* 50:232-40.

Lin, S.-M., D’Archino, R. & Hommersand, M. H. 2012. A new method of cystocarp development in the red algal genus *Callophyllis* (Kallymeniaceae) from Chile. *J. Phycol.* 48:784-972.

Lin, S.-M., Fredericq, S. & Hommersand, M. H. 2001. Systematics of the Delesseriaceae (Ceramiales, Rhodophyta) based on LSU rDNA and *rbc*L sequences, including the Phycodryoideae, subfam. nov. *J. Phycol.* 37:881-99.

Lin, S.-M., Liang, H.-Y. & Hommersand, M. H. 2008. Two types of auxiliary cell ampullae in *Grateloupia* (Halymeniaceae, Rhodophyta), including *G. taiwanensis* Lin et Liang sp. nov. and *G. orientalis* Lin et Liang sp. nov. from Taiwan based on *rbc*L sequence analysis and cystocarp development. *J. Phycol.* 44:196-214.

Lin, S.-M. & Guiry, M. 2017. Halymeniales. In Frey, W. [Eds.] Syllabus of Plant Families-A. Engler's Syllabus der Pflanzenfamilien Part 2/2: Photoautotrophic eukaryotic Algae-Rhodophyta. Borntraeger Science Publishers, Berlin, Germany.

Lin, S.-M., Tseng, L.-C., Put, A. Jr., Bolton, J., Liu L.-C. 2018. Long-term spatial and temporal variabilities in marine macroalgal biota along the coast of Northern Taiwan, southern East China Sea. *Mar. Biol.* 165:83.

Lin, S.-M., De Clerck, O., Leliaert, F. & Chuang, Y.-C. 2020. Systematics and biogeography of the red algal genus *Yonagunia* (Halymeniaceae, Rhodophyta) from the Indo-Pacific including the description of two new species from Taiwan. *J. Phycol.* 56:1542-56.

Minh, B. Q., Nguyen, M. A. T., Haeseler, A. N. 2013. Ultrafast Approximation for Phylogenetic Bootstrap. *Mol. Biol. Evol.* 30:1188-95.

Nguyen, X.-V., Nguyen, T.-H., Dao, V.-H & Liao, L. 2019. New record of *Grateloupia taiwanensis* S.-M. Lin et H.-Y. Liang in Vietnam: Evidence of morphological observation and *rbc*L sequence analysis. *Biodiversitas* 20:688-95.

Nguyen, L. T., Schmidt, H. A., von Haeseler, A. & Minh, B. Q. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol. Biol. Evol.* 32:268-74.

Papenfuss, G.F. 1950. Generic names of algae proposed for conservation. II. *Madroño*10(4):179-84.

Rambaut, A., Suchard, M. A., Xie, D. & Drummond, A. J. 2014. Tracer v1.6. Available at: http://beast.bio.ed.ac.uk/Tracer (last accessed 12 April 2021).

Rodríguez-Prieto, C. & Hommersand, M. H. 2009. Behaviour of the nuclei in pre- and post-fertilization stages in *Kallymenia* (Kallymeniaceae, Rhodophyta). *Phycologia* 48:138–55.

Rodríguez-Prieto, C., Shabaka, S. H., El-Din, N. S. & De Clerck, O. 2021. Morphological and molecular assessment of *Grateloupia* (Halymeniales, Rhodophyta) from Egypt revealed a new introduced species in the Mediterranean Sea, *Grateloupia gibbesii*. *Phycologia* 60:83-95.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Hohna, S., Larget, B., Liu, L., Suchard, M. A. & Huelsenbeck, J. P. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* 61:539-42.

Saunders, G. W. & Kraft, G. T. 2002: Two new Australian species of *Predaea* (Nemastomataceae, Rhodophyta) with taxonomic recommendations for an emended Nemastomatales and expanded Halymeniales. *J. Phycol.* 38:1245-60.

Saunders, G. W. & Moore, T. E. 2013. Refinements for the amplification and sequencing of red algal DNA barcode and RedToL phylogenetic markers: a summary of current primers, profiles and strategies. *Algae* 28:31-43.

Setchell, W. A. & Gardner, N. L. 1936. *Iridophycus* gen. nov. and its representation in South America. *Proc. Natl. Acad. Sci. U. S. A.* 22:469-73.

Schmitz, F. 1889. Systematische Übersicht der bisher bekannten Gattungen der Florideen. *Flora Allg. Bot. Ztg.* 72:435-56.

Schmitz, F. 1896. Kleinere Beiträge zur Kenntniss der Florideen. VI. *Nuova Notarisia* 7:1-22.

Schmitz, F. & Hauptfleisch, P. 1897. Grateloupiaceae. *In* Engler, A. & Prantl, K. [Eds] *Die natürlichen Pflanzenfamilien nebst ihren Gattungen und wichtigeren Arten insbesondere den Nutzpflanzen unter Mitwirkung zahlreicher hervorragender Fachgelehrten, Teil 1, Abteilung 2.*, pp. 508-14, Leipzig: verlag von Wilhelm Engelmann.

Silva, P. C. 1952. A review of nomenclatural conservation in the algae from the point of view of the type method. *Univ. Calif. Publ. Bot.* 25:241-323.

Thiers, B. 2021. *Index Herbariorum: A global directory of public herbaria and associated staff.* New York Botanical Garden's Virtual Herbarium. Available at: http://sweetgum.nybg.org/ih/

(last accessed 12 April 2021).

Turland, N. J., Wiersema, J. H., Barrie, F. R., Greuter, W., Hawksworth, D. L., Herendeen, P. S., Knapp, S., Kusber, W.-H., Li, D.-Z., Marhold, K., May, T. W., McNeill, J., Monro, A. M., Prado, J., Price, M. J. & Smith, G. F. [Eds]. 2018. *International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code)* adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. *Regnum Vegetabile*. Koeltz Botanical Books, Glashütten, Germany.

Wang, H. W., Kawaguchi, S., Horiguchi, T. & Masuda, M. 2001. A morphological and molecular assessment of the genus *Prionitis* J. Agardh (Halymeniaceae, Rhodophyta). *Phycol. Res.* 49:251-62.

Wilkes, R. J., McIvor, L. M. & Guiry, M. D. 2005. Using *rbc*L sequence data to reassess the taxonomic position of some *Grateloupia* and *Dermocorynus* species (Halymeniaceae, Rhodophyta) from the north-eastern Atlantic. *Eur. J. Phycol.* 40:53-60.

Wittmann, W. 1965. Aceto-iron-haematoxylin-chloral hydrate for chromosome staining. *Stain Tech.* 40:161-4.

Womersley, H. B. S. & Lewis, J. A. 1994. Family Halymeniaceae Bory 1828: 158. *In* Womersley, H.B.S. [Eds.] *The marine benthic flora of southern Australia. Part IIIA. Bangiophyceae and Florideophyceae (Acrochaetiales, Nemaliales, Gelidiales, Hildenbrandiales and Gigartinales sensu lato)*. Australian Biological Resources Study, Canberra, Australia.

Xia, B.-M. 2004. *Flora algarum marinarum sinicarum Tomus II Rhodophyta No. III Gelidiales Cryptonemiales Hildenbrandiales.* Science Press, Beijing, China [in Chinese].

Yi, P., Song, X.-W., Lou, Y., Wang, H.-W. 2018. *Grateloupia t*

aiwanensis (Rhodophyta), a new record of Hainan Island based on morphology, *rbc*L, and COI gene sequence analyses. *Oceanol. Limnol. Sin.* 49:604-13.

Yoshida, T. 1998. Marine Algae of Japan. Uchida Rokakuho Publishing, Tokyo, 1222 pp.

Table 1: A comparison on the structure of carpogonial branch and auxiliary cell ampullae and some critical stage of post-diploidization in *Grateloupia sensu lato* based on Chiang (1970), Lin et al. (2008, this study) and Gargiulo et al. (2013). Abbreviations: af = ampullar filament, auxb = auxiliary branches, auxc = auxiliary cell, bnf = branched nutritive filaments, cb = carpogonial branch, dauxc = diploidized auxiliary cell, down = downwardly directed, fc = fusion cell, gi = gonimoblast initial, *G.* = *Grateloupia*, inncc = intercalary inner cortical cell, nf = nutritive filaments, *Ph* = *Phyllymenia*, *Pr* = *Prionitis*, paf = primary ampullar filament, sb = sterile branches, up = upwardly directed. [*1:] referring to the species of *“Grateloupia” taiwanensis*, *“Grateloupia” huangiae*, *“Grateloupia” gibbesii*, *Prionitis taiwani-borealis* sp. nov. [*2:] referring to the species of *G. orientalis*, *G. ramosissima*

	Chiang (1970)	**Lin et al. (2008, this study)**	**Gargiulo et al. (2013)**
Carpogonial branches	2 celled & unbranched, composed of a carpogonium with a trichogyne and hypogynous cell borne on a supporting cell	2 celled & unbranched, composed of a carpogonium with a trichogyne and hypogynous cell borne on a supporting cell	4-6 celled & branched, including a carpogonium with a trichogyne, hypogynous, 1-3 sub-hypogynous cells, and basal cell
Origin of the supporting cell	Mostly the second, third or fourth cell (or sixth, rarely) of the primary ampullar filament	Two types: - The basal cell of the third-order of af as seen in the *Pr/Ph* complex[*1] - The basal cell of the second-order of af as seen in *G. sensu stricto*[*2]	An innermost subcortical cell bearing the whole carpogonial branch ampulla
Carpogonial branch ampullae	Two types: - composed of a cb borne on a paf, which produced one or two branches (i.e. *Prionitis*) - composed of a cb borne on a	Two types: - composed of three orders of unbranched af and the cb being a replacement of the third-order of af as seen in the *Pr/Ph* complex	At least three types: - composed a 4-celled cb and 3 sb (i.e. *G. filicina*) - composed a 5-celled cb and 4 or more sb (i.e. “*G. subpectinata*”)

	paf, which produced two or three branches (i.e. *Phyllymenia*)	- composed of two-orders of unbranched af and the cb being a replacement of the second-order of af in *Grateloupia sensu stricto*	- composed a 6-celled cb and 5 sterile branches (i.e. “*G. doryphora”*)
Origin of the Auxiliary cell	Two types: - an intercalary cell of the primary ampullar filament, usually the third, fourth or fifth cell of the filament - the lowermost/basal cell of a secondary ampullar branch, which originated from the second, third, fourth or fifth cell of the paf	Two types: (homologous to the supporting cells) - the basal cell of the third-order of af as seen in the *Pr/Ph* complex - the basal cell of the second-order ampullar filament as seen in *Grateloupia sensu stricto*	At least three types: - the terminal cell of a 3-celled branch borne on an inncc ((i.e. *G. filicina*) - the terminal cell of a 4-celled branch borne on an inncc (i.e. “*G. subpectinata”*) - the terminal cell of a 5-celled branch borne on an inncc (i.e. “*G. doryphora”*)
Auxiliary cell ampullae prior to diploidization	The *Grateloupia* type, composed a primary filament bearing two or three unbranched filaments (= secondary filaments) (i.e. *Grateloupia*, *Prionitis*, *Pachymeniopsis*, *Phyllymenia*)	Two types: - composed of three orders of unbranched af, aux being the basal cell of the third-order of af in the *Pr/Ph* complex - composed of two-orders of unbranched af, auxc being the basal cell of the second-order af in *Grateloupia sensu stricto*	At least three types: - composed of a 3-celled auxc branch and all cells of the branch produced simple lateral branch (i.e. *G. filicina*) - the terminal cell of a 4-celled auxc branch and 2-4 simple or sparingly branched laterals produced from some or all the cells of the branch (i.e. “*G. subpectinata*”)

			- the terminal cell of a 5-celled branch and all the cells of the branch produced simple or sparingly branched laterals (i.e. “*G. doryphora*”)
Behavior of diploidized auxiliary cell	Enlarged and cut off a gi at upper part before fused with neighboring ampullar cells to form a fc, but no nutritive filaments produced from dauxc (all genera of the Halymeniaceae)	Enlarged and cut off a gi at upper part before fused with neighboring ampullar cells to form a fc, but no nutritive filaments produced from dauxc (all genera of the Halymeniaceae)	Two types: - enlarged and cut off a gi at upper part and produced nf, no fc formed (i.e. *G. filicina*) - enlarged and cut off a gi at upper part fused with neighboring ampullar cells to form a fc, which produced branched nf (i.e. “*G. subpectinata*”)
Pericarp formation	Composed of branched auxc af (minority) and secondarily produced medullary filaments (majority) as seen in *Prionitis* & *Phyllymenia* (No information for *Grateloupia*)	Two types: - composed of branched auxc af and secondarily produced medullary filaments, aux af branched and elongated to form a filamentous/basal cellular cluster surrounding developing gonimoblasts in early formation (the *Pr/Ph* complex) - composed of secondarily produced medullary filaments only, auxc af degenerated in early stages	Four types: - composed of auxlb and up long bnf produced from dauxc (i.e. *G. filicina*) - composed of auxlb and up (long) & down (short) bnf produced from auxbfc (i.e. “*G. doryphora*”) - composed of auxlb and up & down short bnf produced from auxbfc (i.e. “*G. proteus*”) - composed of auxlb and up & down long bnf produced from auxbfc (i.e.

		(*Grateloupia sensu stricto*)	“*G. subpectinata*”)

FIGURE CAPTIONS

FIG. 1 Phylogenetic tree of the *rbc*L+LSU dataset of the *Grateloupia*/*Prionitis* complex from around the world with an emphasis on Taiwan’s species and with indication of posterior probabilities (first number) using Bayesian analyses and bootstrapping values using Maximum Likelihood (second number) and Maximum Parsimony (third number) methods in the tree branches.

FIG. 2. *Prionitis taiwani-borealis* Showe M.Lin, Rodríguez-Prieto, De Clerck & Guiry *sp. nov.* Habit and vegetative structure. Stained with aniline blue (D-F, H), hematoxylin (G, I).

(A) Holotype, female gametophyte (NTOU8vi2009CJ#3).

(B) Paratype, tetrasporphyte (NTOU27viii2009CJ-t).

(C) Underwater photo of tetrasporphyte (NTOU3vi2015DWL).

(D) Transverse section through a young part of a branch showing a 3 layered cellular cortex (white arrows), a thin layer of network-like sub-cortex (black arrowheads) and a thick layer of tubular medullary cells. Note that the medullary cells appear to be rounded in cross section (black arrows).

(E) Longitudinal section through an old part of a branch showing a thickened cortex composed of 5-6 layers of cells, a thick subcortex, and longitudinally orientated, tubular medullary cells (arrows) (HGI-A 20840).

(F) Transverse section through an old part of branch showing a thickened cortex and rounded sections of medullary filaments (arrowheads), intermixed with short filaments (arrowheads) (HGI-A 20840).

(G) Transverse section through an old part of a flattened branch showing a thickened cortex with elongated subcortical cells and dense medullary filaments (HGI-A 20835).

(H) Close up of a medulla in a cystocarp-bearing branch, showing secondarily produced, short rhizoidal filaments (arrows) intermixed with larger and darkly staining cells (arrowheads) (HGI-A 20835).

(I) A well-developed medulla in transverse section in a cystocarp-bearing branch, showing short medullary filaments (arrows) intermixed with larger and darkly staining cells (arrowheads) (HGI-A 20835).

FIG. 3. *Prionitis taiwani-borealis* Showe M.Lin, Rodríguez-Prieto, De Clerck & Guiry *sp. nov.* Reproductive structure. Stained with aniline blue (A-D), hematoxylin (E-G, H).

(A) Transverse section through a tetrasporangial sorus showing many tetrasporangial initials (arrows) produced from inner cortical cells (HGI-A 20840).

(B) Close up of (A) showing tetrasporangial initials (arrowheads) borne on inner cortical cells (arrowheads). Note that the tetrasporangial initials are produced from central parts of the fertile inner cortical cells. At the same time, a small cavity is formed near the tetrasporangial initial as the neighboring cortical cells are elongated and slightly incurved (HGI-A 20840).

(C) A later stage of (B), showing a developing tetrasporangium (it) which has undergone a transverse division. Note that the pit-connection (pc) between the tetrasporangium and the fertile inner cortical cell (arrow) persists is located at the lateral base of the tetrasporangium (HGI-A 20840).

(D) Close up of a mature tetrasporangium (t) which is cruciate-decussately cleaved (HGI-A 20840).

(E) Cross section through a developing spermatangial sorus showing spermatangial parental cells (arrows) differentiated from elongated surface cells. Note that the nuclei of spermatangial parental cells are enlarged, are positioned at the lower parts, and are darkly stained (HGI-A 20835).

(F) A later stage of (E) showing spermatangia (white arrows) cut off terminally from spermatangial initials (HGI-A 20835).

(G) Cross section through a fully developed spermatangial sorus showing spermatangia are produced sequentially. Note that the surface of the fertile branch has several swollen areas (arrows) causing by densely produced spermatangia (HGI-A 20835).

(H) Female gametophyte with cystocarp-bearing branchlets (arrows) (NTOU8vi2009CJ#4).

(I) Cystocarp-bearing branchlets showing swollen, rounded apices (arrows) (HGI-A 20836).

(J) Close-up of cystocarp-bearing branchlets showing several cystocarps (white arrows) produced in one swollen tip (NTOU8vi2009CJ#4)

(K) Cross section through a cystocarp-bearing branchlet showing many mature cystocarps (white arrows) embedded in the thickened inner cortex (HGI-A 20835).

FIG. 4. *Prionitis taiwani-borealis* Showe M.Lin, Rodríguez-Prieto, De Clerck & Guiry *sp. nov.* Formation of auxiliary cell and carpogonial branch ampullae, and early post-diploidization. Stained with aniline blue (A-C); hematoxylin (D-F, G).

(A) Cross-section through a fertile branchlet showing a darkly staining basal-inner cortical cell (black arrow), which initiated the first-order ampullar filament (af1). Note that the basal-inner cortical cell is stained as dark as the first cell of the first-order ampullar filament, and the cortical cells in the vicinity are also stained darkly and slightly elongated (white arrows) (HGI-A 20836).

(B) A later stage of (A) showing the initial of the second-order ampullar filament (af2i) cut off from the first cell (af1i) of the first-order ampullar filament (af1). Note that the fertile basal-inner cortical cell (black arrow) and the neighboring vegetative cells (white arrows) have elongated further (HGI-A 20836).

(C) Further development of the first-order (af1) and second-order (af2) ampullar filaments borne on the fertile basal-inner cortical cell (black arrow) (HGI-A 20836).

(D) Detail of a fully developed auxiliary cell ampulla showing the first-order (af1), second-order (af2) and third-order (af3) ampullar filaments. Note that the basal cell (af3i = auxiliary cell) of the third-order ampullar filament is largely inflated and darkly stained (HGI-A 20836).

(E) Detail of a fully developed carpogonial branch ampulla showing two-orders of ampullar filaments (af) flanking a carpogonial branch, which is composed of a carpogonium (cp) with a terminal, long trichogyne (tr), and a hypogynous cell (hy) borne on a supporting cell (sc) (HGI-A 20835).

(F) Subsurface view of early post-diploidization showing the auxiliary cell surrounded by branched ampullar filaments (black arrows) and secondary medullary cells produced from surrounding vegetative cells (white arrows) (HGI-A 20836).

(G) Cross section through a tip of fertile branchlet showing an auxiliary cell (aux) and young gonimoblast (g) borne on the gonimoblast initial (gi) and newly formed fusion cell (fc). Note the initiation of the lateral branches (black arrows) of the ampullar filaments and production of numerous secondary medullary filaments (white arrows) (HGI-A 20836).

FIG. 5. *Prionitis taiwani-borealis* Showe M.Lin, Rodríguez-Prieto, De Clerck & Guiry *sp. nov.* Cystocarp development. Stained with aniline blue (A, D, E), hematoxylin (B, C, F).

(A) Close up of young carposporophyte showing the primary gonimolobe (pg) and a newly formed fusion cell (fc) flanked by highly branched ampullar filaments (arrows) (HGI-A 20835).

(B) Cross section through a fertile branchlet showing developing gonimoblast (g) and connections (white arrows) between cells of ampullar filaments and secondary medullary cells (HGI-A 20835).

(C) Close up of an immature carposporophyte showing two gonimolobes (g1, g2) and branched fusion cell (fc). Note the remaining branched ampullar filaments (black arrows) and elongated secondarily produced medullary filaments (white arrows) (HGI-A 20835).

(D) Close up of another immature carposporophyte showing the two gonimolobes (g1, g2) borne on the gonimoblast initial (gi) and basal, small fusion cell (fc), and the connections (white arrows) between elongated ampullar filaments (black arrows) and neighboring secondary medullary filaments (HGI-A 20836).

(E) Transverse section through a nearly mature cystocarp showing the gonimolobes borne on the gonimoblast initial (gi) deeply embedded in the thickened cortex, weakly formed pericarp (white arrows), and fading fusion cell (fc) and ampullar filaments (black arrows) (HGI-A 20835).

(F) Transverse section through a fully developed cystocarp showing most cells of the gonimolobes (g1, g2) differentiating into carpsporangia and the weakly formed pericarp (white arrows). Note that the gonimoblast initial remains darkly stained and the fusion cell (fc) and ampullar filaments (black arrows) are still evident (HGI-A 20835).

FIG. 6. *Phyllymenia gibbesii* (Harvey) Showe M.Lin, Rodríguez-Prieto, De Clerck & Guiry *comb. nov.* Pre- and post-diploidization in early cystocarp development. Stained with aniline blue (A-D, F-H), hematoxylin (E). (HGI-A 20420).

(A) An early stage of an auxiliary cell ampulla showing the first- (af1) and second-order (af2) ampullar filaments. Note that the basal-inner cortical cell (arrow) is stained as dark as the cells of the first-order ampullar filament.

(B) Detail of a developing auxiliary cell ampulla borne on the basal-inner cortical cell (arrow), and the first-order (af1), second-order (af2) and third-order (af3) ampullar filaments. Note that the basal cell (af3i = auxiliary cell) of the third-order ampullar filament is slightly enlarged.

(C) Detail of a fully developed auxiliary cell ampulla and the first-order (af1), second-order (af2) and third-order (af3) ampullar filaments. Note that the basal cell (af3i = auxiliary cell) of the third-order ampullar filament is largely inflated.

(D) Detail of a fertilized carpogonial branch ampulla showing two-orders of ampullar filaments (af), the fertilized carpogonium (cp), and a fusion cell composed of the hypogynous cell and supporting cell. Note that at least two connecting filaments (cf) are cut off from the fusion cell.

(E) Detail of early post-diploidization showing the auxiliary cell (aux) diploidized through a basal ampullar cell (bc), a primary connecting filament (cf) produced by the fusion cell of the fertilized carpogonial branch ampulla, and a secondarily produced connecting filament (cf’) pit-connected with a basal cell next to the diploidized auxiliary cell

(F) An early post-fertilization stage showing a gonimoblast initial cut off from a swollen auxiliary cell. Note that the ampullar cells (black arrowheads) in the fertilized carpogonial branch ampulla do not divide, whereas some ampullar cells (white arrowheads) in the diploidized auxiliary cell ampulla either enlarge or divide laterally.

(G) A later stage of (F) showing the fusion cell (fc) connected with the remaining connecting filament (cf), and the gonimoblast initial cutting off some primary gonimoblast cells (pg). Note that the cells of the ampullar filaments (white arrows) are slightly elongated and branched a few more times.

(H) Detail of the fusion cell (fc) and gonimoblast initial (gi) bearing the primary gonimolobe (g) surrounded by highly branched ampullar filaments (white arrows). Note that secondarily produced medullary filaments (black arrows) are produced from the innermost cortical cells.

Table S1: Collection information for the species used in the *rbc*L+LSU sequence analyses.

Fig. S1. Phylogenetic tree of the *rbc*L dataset of *Grateloupia sensu lato*. The tree depicted is the maximum likelihood tree (log-likelihood: -12507.231) generated in IQ-TREE under a GTR+I+G model. Branch labels represent SH-aLRT support (%) / ultrafast bootstrap support (%).

Fig. S2. Topological comparison of phylogenetic trees of the combined LSU and *rbc*L dataset generated under a maximum likelihood framework (IQTree, left) and Bayesian analysis (MrBayes, right).