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Molecular identification of *Grateloupia* and *Phyllymenia* (Grateloupiaceae) in Vietnam reveals a new record of *G. yangjiangensis* based on *rbc*L gene sequences analysis

Nhu-Thuy Nguyen-Nhat[1], Manh Linh Nguyen[2,3], Duc Tien Dam[2], Xuan Thuy Nguyen[1],
Trung Hieu Nguyen[1], Viet Ha Dao[1,3], Xuan Vy Nguyen[1,3,*]

[1]*Institute of Oceanography, VAST, Vietnam*
[2]*Institute of Marine Environment and Resources, VAST, Vietnam*
[3]*Graduate University of Science and Technology, VAST, Vietnam*

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ABSTRACT

Worldwide, the marine macroalgae '*Grateloupia filicina*' showed several lineages. Among them, *Grateloupia filicina* is limited to the Mediterranean basin. Previously, several cryptic species were identified as '*Grateloupia filicina*'. In this study, numerous *Grateloupia* spp. collected from coastal Vietnam and voucher specimens were used for morphological observation and phylogenetic analyses. Both analyses for samples collected at Nha Trang reveal a new record of *G. yangjiangensis*. The phylogenetic trees indicated that the herbarium vouchers labeled '*G filicina*' and '*G. livida*' from other locations of Vietnam group to *G. yinggehaiensis* clade. In addition, the phylogenetic trees also showed that the unknown *Grateloupia* sp. recently collected from Cat Ba Island is *Phyllymenia taiwanensis*. Therefore, a new record, *G. yangjiangensis*, is added to the Vietnamese flora.

Keywords: Cystocarp, *Grateloupia*, new records, *rbc*L, Viet Nam.

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[*] Corresponding author at: Institute of Oceanography, 01 Cau Da St., Nha Trang city 650000, Khanh Hoa, Vietnam. *E-mail addresses:* nguyenxuanvi@gmail.com

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INTRODUCTION

Previously, the family name Grateloupiaceae was proposed by Schmitz [1] as a part of the suborder Cryptoneminae, which included 11 genera: *Grateloupia, Cryptonemia, Halymenia, Aeodes*, *Corynomorpha*, *Pachymenia, Polyopes, Prionitis* J. Agardh, *Carpopeltis* F. Schmitz, J. Agardh, *Dermocorynus* P. Crouan and H. Crouan, and *Thamnoclonium* Kützing. The latter study synonymized the Grateloupiaceae with the Cryptonemiaceae [2]. However, Guiry [3] reinstated the Halymeniaceae Bory based on nomenclatural priority. Therefore, the Grateloupiaceae and Cryptonemiaceae have been considered synonyms of the Halymeniaceae. Recently, Kim et al. [4] proposed that Grateloupiaceae was a distinct family, which is distinguished from the Halymeniaceae by the following three main characteristics: (i) simple unbranched and unilateral types of auxiliary cell ampullae, (ii) pericarp formed densely by the fusion of secondary medullary filaments from subcortical cells and lateral ampullary filaments from a fusion cell complex, and (iii) tetrasporangia originating laterally from the outer cortex. The result of phylogenetic analyses based on multi-genes (*rbc*L, *psa*A, *psb*A, *cox*1, and LSU) also supported that Grateloupiaceae, Halymeniaceae and Tsengiaceae are sister families.

Grateloupia C. Agardh comprises 94 species in the world [5]. Members of *Grateloupia sensu lato* show highly diverse morphological traits, and it is one of the genera presenting challenging species classification [6]. Based on the species *Grateloupia orientalis* S.-M. Lin *et* H.-Y. Liang. Lin et al., [7] indicated that auxiliary cell ampullae abundant in *Grateloupia* are composed of two unbranched filaments, the auxiliary cell being the basal cell of the second order ampullar filament, which is cut off from the first cell of the first order ampullar filament. Those morphological characteristics were the main differences of *Grateloupia* from the remaining genera of Grateloupiaceae. Based on the carpogonial branch ampullae and auxiliary cell branch, a new species, *Grateloupia oligoclora* H. W. Wang *et* Y. Bian was proposed as typical Grateloupia -type in China [8]. *Grateloupia orientalis* was also found in India [9]. *Phyllymenia* was distinguished from *Grateloupia* (two orders) by comprising three orders of ampullar filaments [7]. Worldwide, phylogenetic analyses were unequivocal in that *G. filicina* (J. V. Lamouroux) C. Agardh samples were placed in several separate lineages [6]. Based on morphological observations and genetic markers, De Clerck et al., [6] proposed "*G. filicina*" contributes from the Atlantic Ocean (Portugal to southern England) as a new species, namely *G. minima* P. Crouan *et* H. Crouan; transferring "*G. Filicina*" in South Africa to new species, namely *G. capensis* O. De Clerck (latterly identified as *Phyllymenia capensis* (O.De Clerck) Gargiulo, M. Morabito *et* Manghisi) [10]. In China, the new species *Grateloupia yangjiangensis* W.-X. Li *et* Z.-F. Ding and *G. yinggehaiensis* H. W. Wang *et* R. X. Luan was misidentified as *G. filicina* previously [11, 12]. *G. yangjiangensis* was distinguished from *G. filicina* by several morphological features such as slightly compressed branches; thickness 0.25–0.38 mm. For the applied genetic maker, the phylogeny based on a single *rbc*L can resolved the boundaries among members of *Phyllymenia* and *Grateloupia*. Therefore, *rbc*L was considered as a DNA barcode for this group [6, 10, 12].

In Vietnam, the review studies showed nine species of *Grateloupia* and two species of *Phyllymenia* in the checklist of marine algae of Vietnam [13, 14]. The Herbarium of the Institute of Oceanography (VMO) comprises about 100 voucher specimens labeled *Grateloupia* spp. Almost all specimens are too old (collected about 40–70 years ago), and both specimens of *G. dichotoma* (Kützing) Ardissone (= *G. proteus*) and *G. lithophila* Børgesen are missing. Recently, two new records, including *P. taiwanensis* and *P. huangiae* (previously treated as members of *Grateloupia*), were found in Da Nang and Quy Nhon, respectively [15, 16]. Among members of *Grateloupia*, *'G. filicina'* are common species found on hard substrata with wave exposure, intertidal to 1 m depth [17, 18]. This species grows well from January to March annually at Xom Co, Nha Trang [19]. Therefore, it leads to the research question that which lineages *"G. filicina"* from Vietnam belongs. Is the unidentified *Grateloupia* sp. collected Cat Ba Island, a member of *Grateloupia* or *Phyllymenia*? Can the DNA barcoding approach identify the old voucher specimens?

MATERIALS AND METHODS

Sample collection

The new collection of ‘*Grateloupia filicina*’ and *G. ramosissima* were collected in the shallow waters of 1–2 m in depth along the coast of Vietnam, including Cat Ba Island (1), Quy Nhon (6) and Nha Trang (7). In addition, the old materials, namely ‘*G. livida*’ and ‘*G. filicina*’ collected at Hon Ne (2) Sam Son (3), Thanh Hoa province; Vinh Linh (4), Quang Tri province; Tu Hien estuary (5) Hue city (Figure 1, Appendix 1) were used in phylogenetic analyses. Voucher specimens are deposited at VMO, Institute of Oceanography.

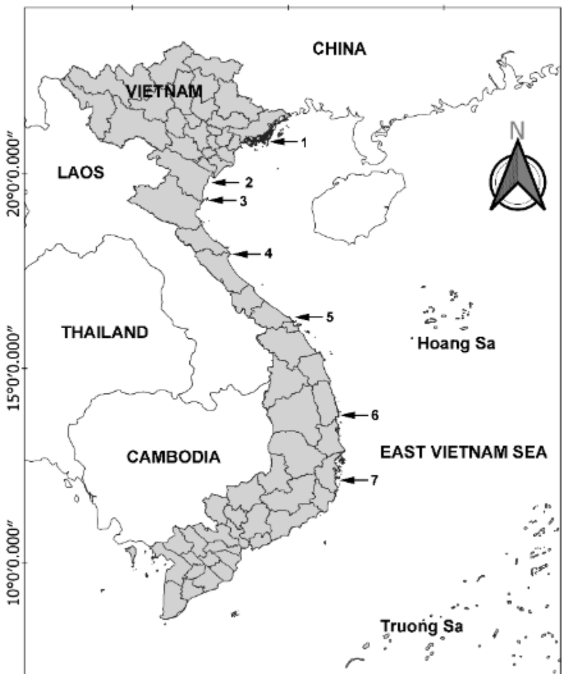

Figure 1. Map of Vienam and sampling sites (1–7). 1- Cat Ba island; 2- Hon Ne island; 3- Sam Son; 4- Vinh Linh; 5- Tu Hien; 6- Quy Nhon; 7- Nha Trang

Morphological observation

Only fresh materials of ‘G. filicina’ collected at Xom Con, Nha Trang (the same site as Dawson [19]) were used for morphological observation. Sections were made by hand, treated with acetoin-hematoxylin-chloral hydrate and mounted in 50% Hoyer’s mounting medium. Photomicrographs were taken using an Olympus BX51 microscope (Tokyo, Japan) with a DP27 digital camera (Olympus, Tokyo, Canada), and habit views were reproduced with an Epson scanner (Tokyo, Japan) at the Institute of Oceanography, Vietnam.

Molecular data acquisition

For each specimen, about 100 mg of dried materials (fresh materials of *‘G. filicina’* and old materials) was homogenized with a bead mill (MM 400, Retsch, Germany) at 22 Hz for 1 min. The finely powdered algal material was used for DNA extraction using the Quick-DNA^{TM} Miniprep Plus Kit (Zymo Research, CA, USA) with the manufacturer’s instructions. The regions selected for PCR amplification were portions of the plastid-encoded gene for the large ribulose-1,5-bisphosphate-carboxylase-oxygenase (*rbc*L) subunit. Two primer pairs [20] were combined to amplify the length of about 1,400 bp of *rbc*L [20]. The PCR conditions were followed Yang and Kim [21]. PCR products were cleaned using a GenEluteTM PCR Clean-Up kit (Sigma-Aldrich, St. Louis, MI, USA) following the manufacturer’s instructions. Direct Sanger sequencing of PCR products in both directions was done by 1ST BASE (Selangor, Malaysia). The consensus sequences were achieved by Clone Manager 9 (Sci-Ed, Cary, NC, USA).

Phylogenetic analysis

The dataset of 49 *rbc*L sequences included seven newly generated sequences obtained in this study, 29 sequences of known *Grateloupia*, and 11 sequences from members of *Phyllymenia* retrieved from GenBank (https://www.ncbi.nlm.nih.gov). Members of other genera, including *Dermocorynus, Mariaramirezia, Neorubra, Pachymeniopsis/Prionitis,* and *Yonagunia* within Grateloupiaceae were included for comparison (Appendix 1). *Halymenia durvillei* Bory (Halymeniaceae) used as out-group. Sequences were aligned by the MAFFT algorithm with the selection of the q-ins-i option and the alignment was further modified by eye. jModelTest version 2.1.6 and the corrected AIC (Akaike Information Criterion) were used to find the best model for the analysis. Phylogenetic analyses were performed using Maximum Likelihood (ML) in RAxML version 8.1 with the General Time Reversible (GTR) model and Bayesian Inference (BI) (Metropolis-coupled Markov chain Monte Carlo method, GTR model) performed in MrBayes v.3.2.2. In ML analysis, support for relationships was determined from 1,000 rapid bootstrap replicates. In the BI analysis, two parallel runs with four chains each (three heated and one cold) were performed for three million generations, sampling the tree every 100 generations. The consensus tree based on two different trees (achieved from the two methods) was constructed by Dendro Scope software, version 3.2.10.

RESULTS

Phylogenetic analysis of *rbc*L

The *rbc*L dataset for phylogenetic analyses consisted of 1,321 characters and 49 taxa. The alignment showed 824 bp (63.1%) of conserved sites, 397 bp (30.1%) of variable sites, 305 bp (23%) of parsimony informative characters, and 92 bp (7.0%) of singletons. Both phylogenetic analyses (ML and BI) grouped a new sequence of samples recently collected in Nha Trang bay (‘*G. filicina*’ - VMO-G42) into the clade with *Grateloupia yangjiensis* (previously identified as ‘*G. filicina’* in China and Hawaii); the new sequences of “*G. livida*” - VMO-G3 (Sam Son, Thanh Hoa), ‘*G. filicina*’ - VMO-G71 (Tu Hien, Hue), ‘*G. filicina*’ - VMO-G65 (Hon Ne, Thanh Hoa), ‘*G. filicina*’ - VMO-G4 (Vinh Linh, Quang Tri) into the clade with *Grateloupia yinggehaiensis*; the samples recently collected in Cat Ba, Hai Phong labeled as *Grateloupia* sp. (CB22a) grouped into the clade with *Phyllymenia taiwanensis* (Fig. 2). The intraspecific pairwise distance (*p*-distance) between all sequences of ‘*G. filicina*’/*G. yangjiangensis* (Clade I) from Hawaii, China, and Vietnam based on *rbc*L sequence analyses ranged from 0% (0 bp) to 0.24% (3 bp). The intraspecific pairwise distance (*p*-distance) among *‘G. filicina*’/*G. yinggehaiensis/"G. livida’* (Clade II) from Sri Lanka, Madagascar, and Vietnam also ranged from 0.1% (1 bp) to 0.24% (3 bp). The pairwise distances between *G. filicina* (clade III) and clade I and between clade III and Clade II are 5.3% (65 bp) and 5.8% (71 bp), respectively (Table 1). No nucleotide differences exist among *Phyllymenia tawainensis* collected from Vietnam, Taiwan and “*Grateloupia* sp.” From Cat Ba. Therefore, taxa in Clade I, including “*G. filicina*” collected in Nha Trang, should be treated as *G. yangjiangensis.* In contrast, taxa in Clade II including ‘*G. livida*’ and “*G. filicina*” from Vietnam, should be treated as *G. yinggehaiensis*. The ‘*Grateloupia* sp.’ sample from Cat Ba is *Phyllymenia taiwanensis*.

*Figure 2. rbc*L phylogeny of Grateloupiaceae inferred from Bayesian Inference and Maximum Likelihood. The posterior probability and bootstrap values are shown in each node, respectively. The species shown in boldface were sequenced in this study. See Fig. 1, Appendix 1 for more information on the data used

Table 1. Sequence divergence values (%) and nucleotide differences (bp) among clades

	Clade I (*G. yangjiangensis*)	Clade II (*G. yinggehaiensis*)	Clade III (*G. filicina*)
Clade I		3%	5.3%
Clade II	37		5.8%
Clade III	65	71	

Morphological observation

Grateloupia yangjiangensis W.-X. Li et Z.-F. Ding 2004

Order Halymeniales, Rhodophyta

References: Wang et al., (2014: 77–84, figs. 1–3)

Description: Thalli are fleshy-cartilaginous in texture and rusty red when living, consisting of multiple erect blades up to 10 cm high (Fig. 3A), arising from a discoid holdfast. The erect axes were terete below, becoming compressed above in branches bearing irregularly pinnate branchlets (Fig. 3B). The medulla is hollow in the center when young (Fig. 3C) but with the filaments densely interwoven in old branches (Fig. 3D). Cortex composed of five to six layers of outer moniliform cells and one to two layers of polygonal- to stellate-shaped inner cells (Fig. 3E). The gametophytes dioecious with the reproductive structures scattered over the entire thallus (Fig. 4A, arrows); carpogonial branches and auxiliary cells (Fig. 4B, arrowhead) were formed singly in separate ampulla produced in the inner cortex; The first-order auxiliary ampullar filament divides transversely to form a filament up to three cells long and cuts off the initial of the second order ampullar filament laterally from its first basal cell (Fig. 4C). Both ampullar filaments then continue dividing and extending toward the outer cortex (Figs. 4D, E). Early stage after diploidization shows undivided ampullar filaments (*af*1 and *af*2) and an auxiliary cell bearing the primary gonimolobe at a two-celled stage (arrows) borne on the gonimoblast initial (*gi*) (Fig. 4F). Gonimolobes (*g*) are flanked by the two ampullar filaments, whose cells may branch laterally (Figs. 5G, H). Fully developed, mature gonimoblasts comprise one to two gonimolobes and are 150–180 µm in diameter (Fig. 5I).

Type locality: Zhapo, Yangjiang, Guangdong Province, China

Habitat: Plants were attached on middle low tidal rock

Distribution: Xom Con, Nha Trang, Vietnam

Voucher: VMOG42-1, collected 10 February 2022 at Xom Con, Nha Trang city by Nguyen Nhat Nhu Thuy, VMOG42-3 collected 6 February 2018 at Xom Con, Nha Trang City by Nguyen Trung Hieu, N°11235 collected 02 February 1953 at Xom Con, Nha Trang by Yale Dawson, deposited at the VMO, Nha Trang, Vietnam.

Remarks: The herbarium voucher specimens deposited at VMO were misidentified as *Grateloupia filicina.*

DISCUSSION

This study describes a new record, *Grateloupia yangjiangensis*, from Vietnam. There has been no morphological observation of the herbarium voucher specimens of '*G. filicina*' and '*G. livida*' in the last 60-70 years. However, the genetic marker indicated that some were grouped into the *Grateloupia yinggehaiensis* clade. *Grateloupia yangjiangensis* and *yinggehaiensis* were first found in Hainan, China, near Vietnam.

So far, *Grateloupia yangjiangensis* was first mentioned in Flora Algarum Marinarum Sinicarum in China [22]. Based on external morphology, vegetative and reproductive structures, life history, and *rbc*L analysis, Wang et al. [11] re-identified and indicated that *G. yangjiangensis* was a single species within the genus *Grateloupia*. In this present study, the external morphology of '*Grateloupia filicina*' collected in Xom Con, Nha Trang, Vietnam also shows similar characteristics to *Grateloupia yangjiangensis* in China by the erect axes were terete below, becoming compressed above in branches bearing irregularly pinnate branchlets (Fig. 2A); blades were broad near the base and gradually broadening upward, then narrowing toward blunt to blade-like apices and sickle-like apices (Fig. 2B). This present study added more figures of cross-section of the branchlet that show the cellular cortex and hollow center which were not presented in Wang et al., [11]. The cellular cortex and hollow center of the branchlet of *G. yangjiangensis* were also found in other members of *Grateloupia*, such as *G. orientalis* [7]. For the reproductive anatomy, Chiang [23] indicated that the auxiliary cell is usually the basal cell of the secondary ampullar filament, produced by the third, fourth, or fifth cell of the primary ampullar filament. Our results on the reproductive anatomy indicated that the auxiliary cell ampullae are composed of two orders of unbranched ampullar filaments before fertilization (Figs. 3D, E). The trait of two filaments was also found *Grateloupia orientalis*, whereas three orders of filaments were found in the genus *Phyllymenia*, a sister genus of *Grateloupia*. The three orders of filaments were clearly illustrated in *P. taiwanensis* and *P. gibbesii* [24]. Comparing the vegetative anatomy of *Grateloupia yangjiangensis*, described by Wang et al. [11], our study added more figures on the formation of auxiliary cells and carpogonial branch ampullae and cystocarp development in detail. For example, the pit connection between the auxiliary cell and the gonimoblast initially showed better views.

In *rbc*L sequence analyses, '*Grateloupia filicina*' collected from Hawai'i, Nha Trang Bay, and *G. yangjiangensis* formed a distinct clade (Fig. 2, clade I). There are no or very low nucleotide differences (0-3 bp) among members of this clade. De Clerck et al. [6] indicated that *G. filicina* was originally described from the Mediterranean Sea. An *rbc*L-based molecular phylogeny by De Clerck et al. [6] showed that the encompassing samples covering the entire geographic distribution revealed many cryptic species. The *rbc*L sequences analysis of '*G. filicina*' collected from South Africa (GENT HEC 10860, GENT FL 110) revealed that it is a new species, namely *G. capensis* O. De Clerck. This species was transferred to *Phyllymenia* as *P. capensis* (O. De Clerck) Gargiulo, M. Morabito *et* Manghisi [10]. The tree based on *rbc*L sequence data by Wang et al., [11] did not include '*G. filicina*' from Hawai'i. Our analysis based on *rbc*L sequences showed that '*G. filicina*' from Hawai'I, Nha Trang, and *G. yangjiangensis* are one species. Combining external morphology, vegetative and reproductive structures, we propose that *Grateloupia yangjiangensis* is the correct name for '*G. filicina'* in Nha Trang bay, Vietnam.

The collection of '*G. filicina*' deposited at VMO was around 60–70 years ago, and the cross sections for those samples are impossible. Therefore, DNA barcodes (*rbc*L) were an advantage in determining which taxa the voucher specimens belong. In some cases, our PCRs for *rbc*L failed, and high-quality sequences were obtained from 30% of the herbarium samples. For higher plants, Korpelainen and Pietiläinen [25] successfully applied the *rbc*L from samples over more than 100 years old. However, another study indicated that 27.3% of samples (50–75 years) showed no DNA, and only 36.4% and 72.7% of those samples showed sequencing success for the lengths of 800 bp and 470 bp, respectively [26]. Therefore, our following study will design other primers for short sequencing (around 350–450 bp). It may be helpful for the old specimens. In our *rbc*L sequence analyses, some specimens are labeled as '*G. filicina'* and '*G. livida*' group to *G. yinggehaiensis* clade (Figure 2, clade II). However, we cannot observe the vegetative morphology and cystocarp development. The fresh materials of '*G. filicina*' and '*G. livida*' will be collected and observed in our subsequent studies.

Finally, this study revealed a new record of *Grateloupia yangjiangensis* from Vietnamese waters. The formation of auxiliary cells, carpogonial branch ampullae, and cystocarp development of *Grateloupia yangjiangensis* were observed in detail. *Phyllymenia taiwanensis* is also found in Cat Ba island. DNA barcoding can be applied to old herbarium samples.

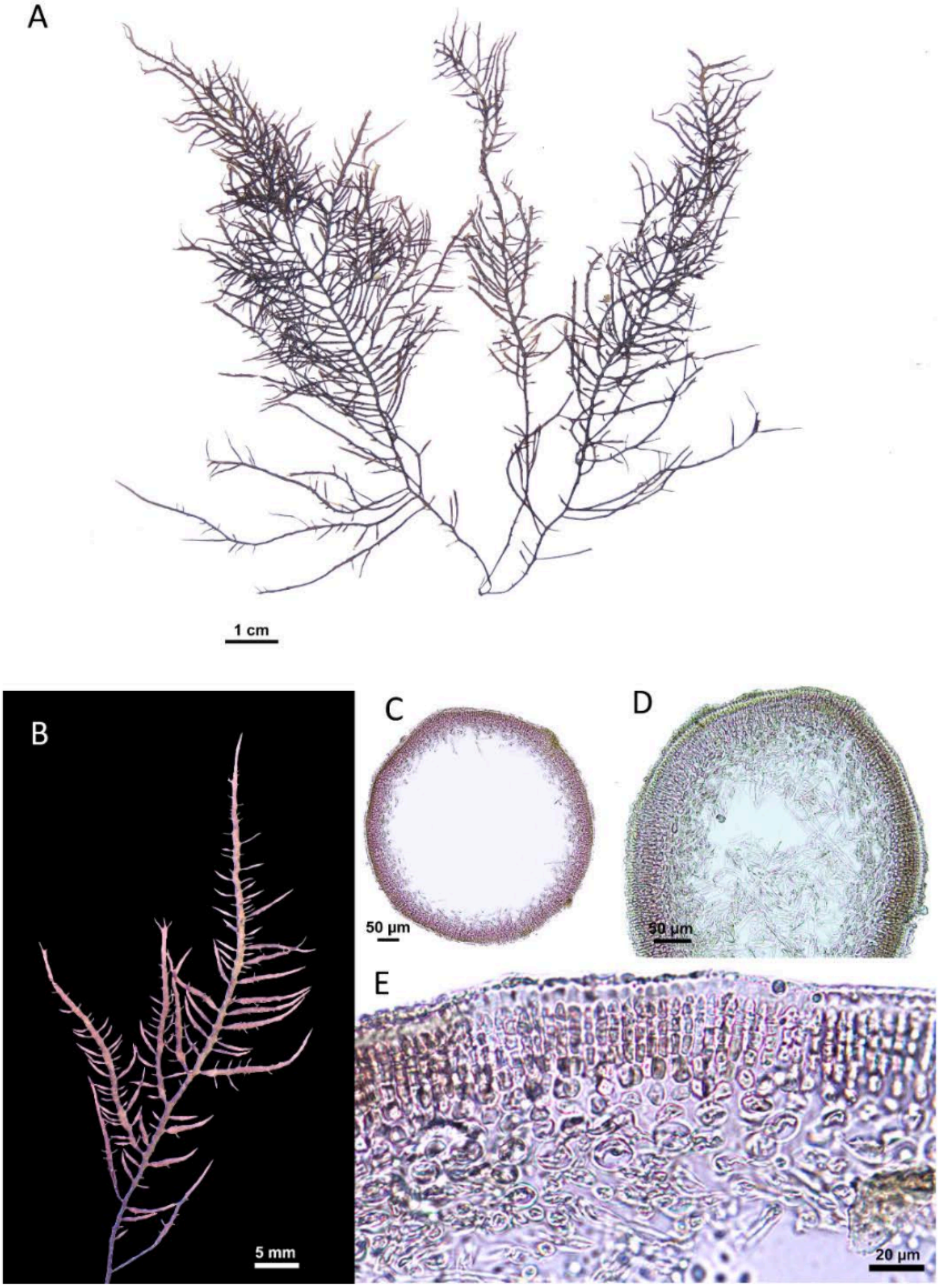

Figure 3. Grateloupia yangjiangensis from Vietnam showing habit and vegetative morphology. **A**: Thallus in Xom Con, Nha Trang (VMO-G42); **B**: Close up of irregularly arranged, pinnate branchlets; **C**: Cross-section of branchlet showing cellular cortex and hollow center; **D**: Cross-section of main blade showing cellular cortex and filamentous medulla; **E**: Close up of inner cortex and medulla

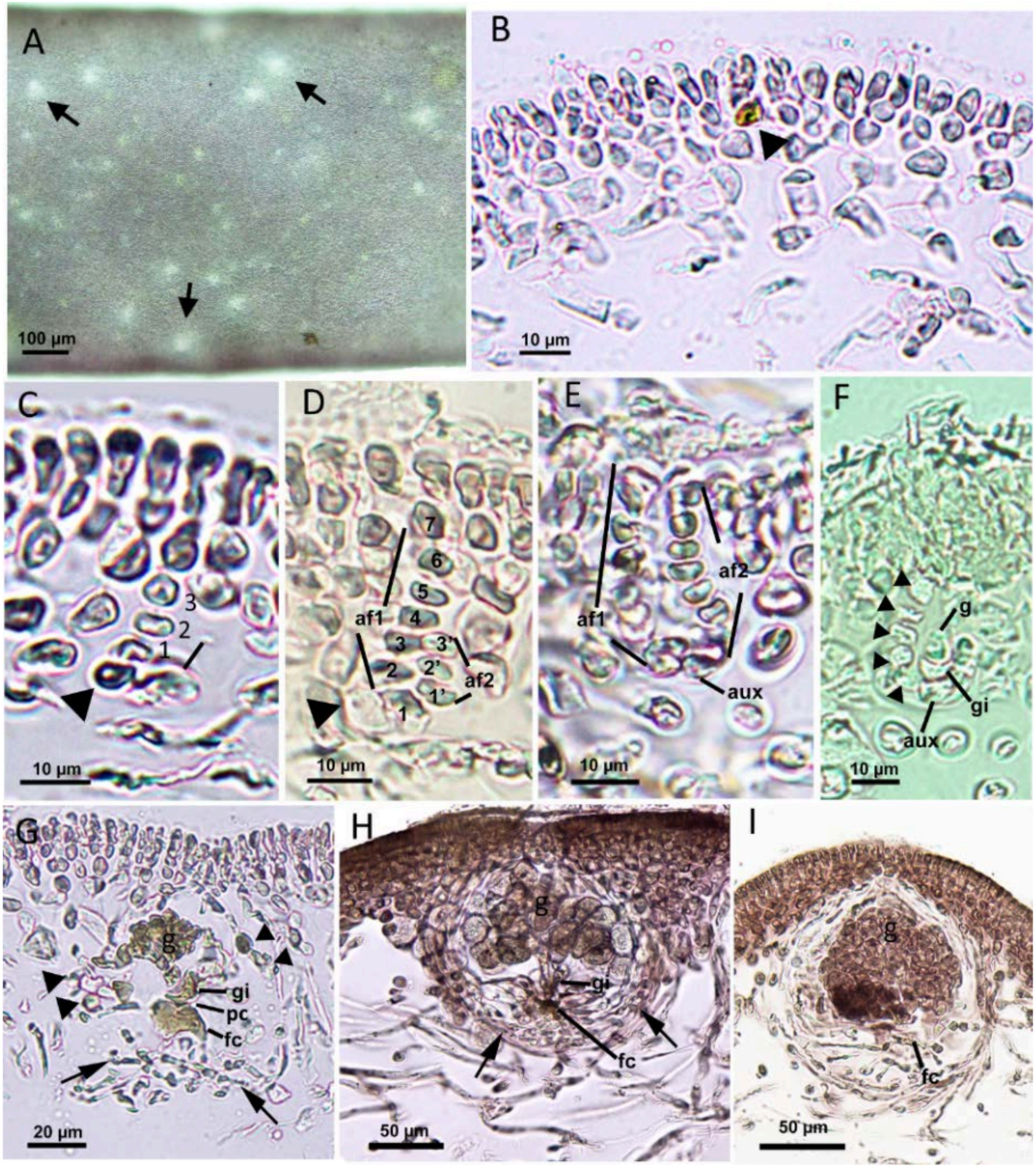

Figure 4. Grateloupia yangjiangensis from Vietnam showing auxiliary cell (*aux*) and cystocarp development. **A**: A closer view of the fertile blade showing carposporophytes (arrows); **B**: Auxiliary cells in the inner; **C**: The first-order auxiliary ampullar filament with 3 cells; **D**: Growth of the first-order (*af1*, labeled 1–7) and second-order (*af2*, labeled 1'–3') ampullar filaments borne on a basal inner cortical cell (arrowhead); **E**: Development of two ampullar filaments and *aux*; **F**: An auxiliary cell bearing the primary gonimolobe (*g*) borne on the gonimoblast initial (*gi*); **G**: Early stage of gonimoblast development showing ampullar filaments (arrowheads), *aux*, pit-connection (*pc*), fusion cell (*fc*), *gi*, and *g*; **H**: Detail of *fc* and *g* surrounded by highly branched ampullar filaments (arrows); **I**: A closer view of mature *g* composed of gonimolobes

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Appendix 1. GenBank accession and Herbarium voucher specimens (bold and italic) of the sequences used in the alignment. Bold in face: samples collected in Vietnam; -: As above; #: Out-group; *,**: Previously identified as *Grateloupia filicina,* and *Grateloupia* sp.

Genera	Species	Locations	GB	Source
Democorynus	*dichotomus*	Italy	JX070628	Gargiulo et al., 2013
	horridus	Italy	JX070627	-
Grateloupia	*catenata*	South Korea	KF475737	Yang et al., 2015
	carnosa	Japan	AB038608	Kawaguchi et al., 2001
	filicina	Italy	AJ868476	De Clerck et al., 2005a
	filicina	Spain	AJ868475	-
	'filicina'	Papua New Guinea	AJ868485	-
	'filicina'	Venezuela	AJ868487	De Clerck et al., 2005b
	'filicina'	Curaçao	AJ868486	-
	'filicina'	Sri Lanka	AY772029	De Clerck et al., 2005a
	'filicina'	Madagascar	AJ868488	De Clerck et al., 2005b
	'filicina'	French Polynesia	AJ868479	De Clerck et al., 2005a
	'filicina'	Hawaii	AJ868480	-
	'filicina'	Floria, USA	AJ868478	-
	'filicina'	Texas, USA	AJ868483	-
	'hawaiiana'	Hawaii	AY772030	De Clerck et al., 2005a
	livida	Japan	AB055482	Wang et al., 2000
	orientalis	Taiwan	EU292744	Lin et al., 2008
	ramosa	China	JX974606	Cao et al, 2016
	ramosissima	Taiwan	EU292741	Lin et al., 2008
	ramosissima	**Quy Nhon, Viet Nam**	**OR099708** ***VMO-G32***	**This study**
	tenuis	China	KC918541	Yu et al., 2013
	turuturu	Japan	AF488820	Gavio and Fredericq, 2002
	yangjiangensis	Hainan, China	HQ324238	Wang et al., 2014
	yangjiangensis	Guangdong, China	HQ324236	-
	yangjiangensis[*]	**Nha Trang, Viet Nam**	**OR099703** ***VMO-G42***	**This study**
	yinggehaiensis	Hainan, China	HQ332513	Zhao et al., 2012
	'filicina'	**Hon Ne, Viet Nam**	**OR099704** ***VMO-G03***	**This study**
	'livida'	**Sam Son, Viet Nam**	**OR099705** ***VMO-G65***	-
	'filicina'	**Vinh Linh, Viet Nam**	**OR099706** ***VMO-G04***	-
	'filicina'	**Tu Hien, Viet Nam**	**OR099707** ***VMO-G71***	-
Mariaramirezia	*osornoensis*	Chile	MG191653	Schneider et al., 2018
Neorubra	*decipiens*	Peru	KJ561154	Calderon et al., 2014b
Pachymeniopsis	*gargiuloi*	Italy	AY651060	Manghisi et al., 2014
Prionitis	*simplex*	USA	MG191648	Schneider et al., 2018
Phyllymenia	*acletoi*	Peru	KF363926	Calderon et al., 2014a
	belangeri	South Africa	AY772035	De Clerck et al, 2005b
	capensis	South Africa	AJ868467	De Clerck et al, 2005a

	gibbesii	Egypt	MW148222	Rodriguez-Prieto et al, 2021
	huangiae	Taiwan	HM590409	Lin et al., 2011
	huangiae	Lang Co, Viet Nam	MT649256	Nguyen et al, 2021
	taiwanensis	Taiwan	EU292742	Lin et al., 2008
	taiwanensis	Lang Co, Viet Nam	MK167364	Nguyen et al, 2019
	taiwanensis[**]	**Cat Ba, Viet Nam**	**OR099709** **_CB22a_**	**This study**
	sp. 1	Chile	KF363930	Direct submission
	sp. 2	Chile	KF363932	Direct submission
Yonagunia	*formosana*	Viet Nam	AB116242	Kawaguchi et al., 2004
	taiwani-borealis	Taiwan	MT501502	Lin et al., 2020
Halymenia	*durvillei#*	South Africa	JQ976883	Hernandez-Kantun et al., 2012