BIODIVERSITAS
Volume 20, Number 3, March 2019
Pages: 688-695

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d200311

New record of *Grateloupia taiwanensis* S.-M. Lin et H.-Y. Liang in Vietnam: Evidence of morphological observation and *rbc*L sequence analysis

XUAN-VY NGUYEN[1,2,♥], TRUNG-HIEU NGUYEN[1], VIET-HA DAO[2,3], LAWRENCE LIAO[4]
[1]Department of Marine Botany, Institute of Oceanography, Vietnam Academy of Science and Technology. 01 Cau Da, Nha Trang City 650000, Vietnam. Tel./fax. +84-258-3590394, ♥email: nguyenxuanvi@gmail.com
[2]Faculty of Marine Science and Technology, Graduate University of Science and Technology. 18 Hoang Quoc Viet, Ha Noi 100000, Vietnam
[3]Department of Hydro-Geochemistry, Institute of Oceanography, Vietnam Academy of Science and Technology. 01 Cau Da, Nha Trang City 650000, Vietnam
[4]University of Hiroshima. 1-4-4 Kagamiyama, Higashi-Hiroshima City Hiroshima, 739-8528, Japan

Manuscript received: 5 January 2019. Revision accepted: 16 February 2019.

Abstract. *Nguyen XV, Nguyen TH, Dao VH, 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: 688-695.* Members of *Grateloupia* show highly diverse morphological traits, and this makes species classification more difficult. Samples were found growing with other marine algae nearshore, 3–5 m depth at Da Nang City. Morphological observation of vegetative and reproductive structures as well as phylogenetic analysis based on the large subunit of ribulose-1,5-bisphosphate-carboxylase-oxygenase (*rbc*L) sequence confirmed its identification. Phylogeny of members of *Grateloupia* inferred from Bayesian Inference, Maximum Likelihood, Maximum Parsimony and Neighbour Joining indicated materials collected in Vietnam compared well with known *G. taiwanensis* from the type locality, with very little sequence divergence. *Grateloupia taiwanensis* S.-M. Lin et H.-Y. Liang is therefore reported for the first time from Vietnam.

Keywords: *Grateloupia taiwanensis*, morphology, new record, *rbc*L, Vietnam

INTRODUCTION

Among the members of Halymeniaceae, *Grateloupia* shows the largest number in species. These red benthic macroalgae are found in the warm temperate to tropical waters worldwide (DePriest et al. 2013). *Grateloupia* spp. are naturally collected and cultivated as food in Eastern Asia, and serve as shelter and substrate to several species of marine invertebrates. To date, there are 96 species of this genus recorded around the world (Guiry and Guiry 2019). In the Northwestern Pacific waters, approximately 25 *Grateloupia* species are found (Yang and Kim. 2015). In the South China Sea, bordered by six countries including Indonesia, Malaysia, Philippines, Singapore, Thailand, and Vietnam, 13 species of the genus *Grateloupia* have been reported (Phang et al. 2016). The recent study of Nguyen et al. (2013a) showed 13 species of *Grateloupia* in the checklist of marine algae of Vietnam. One species, *Grateloupia taiwanensis* S.-M. Lin et H.-Y. Liang was first described by Lin et al. (2008) based on materials collected at Taiwan. Afterward, the species was also found in the Western Atlantic (Wynne, 2017). So far, there are no records of *G. taiwanensis* in the Southeast Asia. According to Lin et al. (2008), *G. taiwanensis* showed the distinct patterns of development of the auxiliary cell ampullae, with an ampullae consisting of three orders of unbranched filaments that branch after diploidization of the auxiliary cell, with a pericarp formed with surrounding secondary medullary filaments.

Members of *Grateloupia* show highly diverse morphological traits, and it is one of the genera that presents a difficult species classification (Yu et al. 2013). In fact, Yang and Kim (2015) indicated that *Grateloupia* is one of the most taxonomically complex within the family Halymeniaceae. Moreover, classification based on morphological traits is difficult due to the highly variable terete to blade-like thalli among member of this genus that usually leads to misidentification (Zhao et al. 2012). Morphological variations within species have been found in several species including *Grateloupia doryphora* (Montagne) M. Howe (Villalard-Bohnsack and Harlin 2001), *Grateloupia filicina* (J.V. Lamouroux) C. Agardh (Baweja and Sahoo 2002), or frequent misidentification between species such as *Grateloupia turuturu* Yamada and *Grateloupia lanceola* (J. Agardh) J. Agardh (Verlaque et al. 2005) are common. *Grateloupia* species are notoriously difficult to identify due to their similarity of morphology and the lack of diagnostic characters (Figueroa et al. 2007). Hence, it presents one of the most complex taxonomic challenges.

Molecular systematics have been applied to classify *Grateloupia* so that the taxonomist acquire a better understanding of the species diversity in general. The plastidic gene encoding the large subunit of ribulose-1,5-

bisphosphate-carboxylase-oxygenase (*rbc*L) was the focus of numerous marine algal studies concerning phylogeny and molecular evolution (Kawaguchi et al. 2001). Recently, several studies used *rbc*L as DNA barcode to identify the closely related species and found the new records of members of *Grateloupia*. For examples, *G. ramosa* H. W. Wang et R. X. Luan (Cao et al., 2016a), *G. variata* Cao, C. C., Li, Y. Z. et H. W. Wang (Cao et al. 2016b), *Grateloupia huanghaiensis* Wang, H. W., Guan, Y., Zhao, F. Q. et Zhao, D. (Wang et al. 2015), *Grateloupia tenuis* L. Yu, H. W. Wang et R. X. Luan (Yu et al. 2013), *Grateloupia dalianensis* H. W. Wang et D. Zhao, and *G. yinggehaiensis* H. W. Wang et R. X. Luan (Zhao et al. 2012) were recorded as new species in China, and *Grateloupia jejuensis* S. Y. Kim, E. G. Han et S. M. Boo (Kim et al. 2013) as a new species from Korea. In Vietnam, molecular systematics was limited to the taxonomy of seagrass (Nguyen et al. 2013b; 2015). Therefore, the use of phylogenetic analysis based on *rbc*L sequences and morphological observation is tested in resolving the taxonomic uncertainties among specimens of *Grateloupia*, and clarify the status of *Grateloupia* species in Vietnam. This study documents a new record of *Grateloupia taiwanensis* in Vietnam. The morphology, location, and habitats of *G. taiwanensis* are described, and a molecular phylogeny is presented showing the phylogenetic position of *G. taiwanensis* from Vietnam.

MATERIALS AND METHODS

Study area

The algae samples were collected at Da Nang City ($16^0$08'N; $108^0$07'), Vietnam (Figure 1) in February 2017. Snorkelling was used to collect the samples in the shallow water (3-5 m). Algal materials were washed with seawater in the field to remove the epiphytes and debris that were commonly attached to the algae. Each specimen was placed in a single plastic bag and kept on ice. Materials were transferred to the laboratory on the same day. In the laboratory, materials were re-washed with de-ionized water to remove seawater. One specimen was divided into two parts, one part was pressed as a herbarium voucher specimen (CS18-DN006) deposited in the Museum of Oceanography, Nha Trang City, Vietnam and a small blades of herbarium voucher specimen were used for DNA extraction and morphological analysis. Information of the sample is presented in Table 1.

Morphological observation and analysis

Sections were made by hand and were stained with 1% aniline blue acidified with 1% HCl. Photomicrographs were taken on CH30 (Olympus, Tokyo, Japan) with digital camera GP-KS162 (Panasonic, Osaka, Japan). For a comparison, morphological traits of two forms: foliose and terete were analysis. Data of morphological measurements of materials *Grateloupia taiwanensis* collected in Vietnam were compared with group of foliose form including *G. taiwanensis* (Lin et al. 2008), *G. sparsa* (Kawabata 1963, Verlaque et al. 2005, Yoshida 1998), *G. livida* (Yoshida 1998), *G. turuturu* (John et al. 2004, Verlaque et al. 2005, Yoshida 1998), *G. lanceolata* (Yoshida 1998), *G. belangeri* (De Clerck et al. 2005), and terete form including *G. orientalis* S.-M. Lin et H.-Y. Liang (Lin et al. 2008), *G. filicina* (Kawaguchi et al. 2001), *G. asiatica* Kawaguchi et H.W. Wang (Kawaguchi et al. 2001), *G. minima* P. et H. Crouan (De Clercket al. 2005a), *G. subpectiana* Holmes (Faye et al. 1994). The selection of parameters included in this study was adapted from the most widely used key characters in identification guides and was limited by the completeness of data sets (Table 2). Phylogenetic analyses of morphological data of thirteen species of *Grateloupia* were carried out by Mesquite for Window, version 3.51 (Maddison and Maddison 2018)

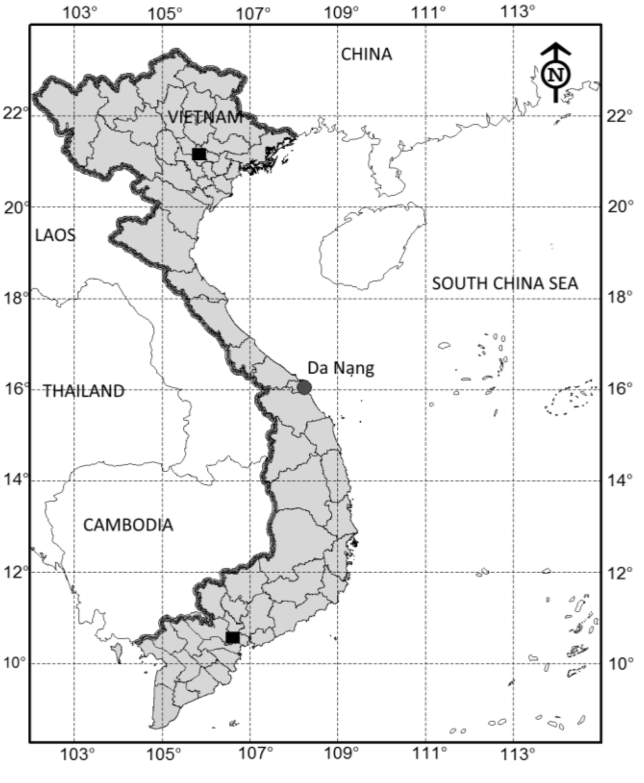

Figure 1. The map of Vietnam coast and sampling site at Da Nang (solid round). Source: The National Oceanic and Atmospheric Administration (NOAA), USA, public domain data. The map was processed by MapInfor Pro^{TM}, version 12.5.5 (Pitney Bowes Software Inc., NY, USA)

Table 1. Voucher specimens and GenBank assession numbers for *Grateloupia* taxa used in the phylogenetic analysis. *: new record in Vietnam; Bold: samples collected in the present study

Species	Coordinates		Locations	Voucher specimens	GenBank assession no
	Latitude (degree N)	Longitude (degree E)			
Grateloupia acuminata	na	na	Japan	SAP088107[a]	AB055480
Grateloupia americana	na	na	USA	na	AF488814
Grateloupia asiatica	na	na	South Korea	HAL003[b]	KF475728
Grateloupia angusta	na	na	South Korea	HAL001[b]	KF475727
Grateloupia belangeri	na	na	South Africa	ODC879[c]	AY772026
Grateloupia belangeri	na	na	South Africa	ODC865[c]	AY772027
Grateloupia dichotoma	na	na	Brasil	na	AF488823
Grateloupia doryphora	na	na	Peru	na	AF488817
Grateloupia elliptica	na	na	Japan	MI46[b]	JX475036
Grateloupia filicina	na	na	Italy	Cormaci s.n.[c]	AY772028
Grateloupia filiformis	na	na	Brasil	na	AF488822
Grateloupia huangiae	na	na	Taiwan	NTOU-MK30iv2002[d]	HM590410
Grateloupia huangiae	na	na	Taiwan	NTOU-LP17i2005[d]	HM590409
Grateloupia gibbesii	na	na	USA	na	JX645160
Grateloupia kurogii	na	na	South Korea	G128[b]	KJ648564
Grateloupia lanceola	na	na	South Korea	GT068[b]	JX475031
Grateloupia livida	na	na	Japan	na	AF488815
Grateloupia longifolia	na	na	South Africa	na	AY178765
Grateloupia orientalis	na	na	Taiwan	na	EU292744
Grateloupia orientalis	na	na	China	LNU092102[e]	JF803828
Grateloupia phuquocensis	na	na	Hawaii, USA	ODC905[c]	AY772022
Grateloupia ramossissima	na	na	Taiwan	na	EU292741
Grateloupia somalensis	na	na	Madagascar	HEC15214[c]	AY772021
Grateloupia stipitata	na	na	New Zealand	Na	AF488816
Grateloupia subpectinata	na	na	South Korea	HAL004[b]	KJ648566
Grateloupia subpectinata	na	na	South Korea	HAL029[b]	KJ648567
Grateloupia taiwanensis	na	na	Taiwan	20.iv.2003[d]	EU292742
Grateloupia taiwanensis	na	na	Taiwan	30.iv.2002[d]	EU292743
Grateloupia taiwanensis	na	na	USA	UA130	JX645159
Grateloupia taiwanensis	na	na	USA	na	KC894740
*Grateloupia taiwanensis**	16.140	108.133	Vietnam	CS18-DN006[f]	MK167364
Grateloupia turuturu	na	na	South Korea	HAL032[c]	KJ648568
Grateloupia versicolor	na	na	Mexico	na	AF212197
Halymenia durvillei			(Out-group)		AY772020

Note: na = non-available; [a] = Hokkaido University, Japan; [b]= Jeju National University, South Korea; [c]= Ghent University, Belgium; [d]= Liaoning Normal University, China, [e] = National Taiwan Ocean University, Taiwan, [f]= Institute of Oceanography, Vietnam

Table 2. Morphological characters of *Grateloupia* spp. used for the analyses. The data are taken from various sources as cited in the Materials and Methods section

Species	Form	Thallus habit	Margins	High (cm)	Thickness (µm)	Layers	Texture
Grateloupia taiwanensis	1	1	1	1, 2	3, 4	2, 3	1
G. taiwanensis	1	1	1	1, 2	3, 4	2, 3	1
G. sparsa	1	2	2	3	3	1	1
G. livida	1	3	2	2, 3	nk	2, 3, 4	2
G. turuturu	1	1	3	3	1	2	1
G. lanceolata	1	2	3	4	4	5	1, 2
G. belangeri	1	4	1	2, 3	1	2, 3, 4	nk
G. phuquocensis	1	1	2	1	3, 4	1, 2	nk
G. orientalis	2	5, 6	2	1, 2	2, 3, 4	2, 3	1, 3
G. filicina	2	5	2	1, 2	4	1, 2, 3	1, 3
G. asiatica	2	5	1	1, 2, 3	4	2, 3, 4	1
G. minima	2	5	1	1	4	2, 3, 4	3
G. subpectinata	2	6	1	1, 2, 3	4	4	1

Note: Forms (foliose = 1; terete = 2), thallus habits (linear to lanceolate = 1; lanceolate = 2; flattened = 3; cuneate to lanceolate = 4; compressed = 5; terete = 6), margins (frequent bladelets = 1; irfrequent bladelets; entire margins = 3), high (less than 10 cm = 1; between 12-16 cm = 2; between 25-40 cm = 3, between 60 and 100 cm = 4), thickness (<260 µm = 1; between 260-400 µm = 2; between 400-500 µm = 3, between 500-1300 µm = 4), layers (4-5 layers = 1; 5-6 layers = 2; 7-8 layer = 4; 9-10 layers = 4; 10 layers = 5), texture (gelatinous = 1; leathery = 2; cartilaginous = 3), nk = not known. Bold = material collected in Vietnam.

DNA extraction, amplification and sequencing

The dried materials were rehydrated in sterile water for one hour. The materials were homogenized by a mortar and pestle in liquid nitrogen, and 100 mg of the finely powdered algal material was used for DNA extraction. DNA extraction was carried out using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instruction with slight modifications according to Yang and Kim (2015). Briefly, 400 µL buffer AP1 and 4 µL RNase were added to the samples. After incubating at 63^0C for 2 h, 130 µL buffer P3 added to the lysate. The tubes were incubated on ice for 5 min and centrifuged at 1,400 rpm for 5 minutes. 430 µL lysate was transferred to QIAshredder column. The remaining steps were followed manufacturer's instruction. DNA quality was checked on agarose gels stained with Midori Green Advance (Nippon Genetics Europe GmbH, Düren) and the concentration was measured by a spectrophotometer U-2900 (Hitachi, Tokyo, Japan). The region selected for PCR amplification was the partial *rbc*L gene. In this study, two combinations of primer were used to obtain two overlapping fragments of the *rbc*L gene. The first primer pairs of F7 (5'-AACTCTGTAGAACGNACAAG-3') (Gavio and Fredericq 2002) and R898 (5'-GACGAGAATAAGTTGARTTACC-3') (Kim et al. 2010), and the second primer pairs of F762 (5'-GTATGAAAGAGCTGAATTTG-3') (Kim et al. 2010) and R1381 (5'-ATCTTTCCATAGATCTAAAGC-3') (Freshwater and Rueness 1994) were used. The total volume of 25 µl included 2x OneTag® Master Mix (New England Biolabs, Ipswich, MA, USA), 10-30 ng template DNA, and 1 pmol of each primer. PCR was performed in an Applied Biosystems 2720 thermocycler (Applied Biosystems, Foster, CA, USA) with a heated lid under the following conditions: initial denaturation for 4 min at 96°C followed by 35 cycles of denaturation for 60 s at 95°C, primer annealing for 2 min at 50°C, and extension for 2 min at 72°C, and a final extension at 72°C for 7 min. All PCR reactions were repeated two to four times independently with the same individual to keep errors (possibly created by the *Taq* polymerase) in the final consensus sequence to a minimum. PCR products were cleaned using a GenElute^{TM} PCRClean-Up kit (Sigma-Aldrich, St. Louis, MI, USA) following manufacturer's instruction. Direct sequencing of PCR product was done by 1ST BASE (Selangor, Malaysia) from both directions. The consensus sequence was achieved by Clone Manager 9 (Sci-Ed, Cary, NC, USA). For comparison, known *rbc*L sequences of *G. taiwanensis* (DePriest et al. 2013, Lin et al. 2008) and other *Grateloupia* species were added to the dataset (Table 1).

Phylogenetic analysis

In total, 34 *rbc*L sequences obtained during this study and retrieved from the GenBank (www.ncbi.nlm.nih.gov) were used for the analysis. These sequences were aligned by CLUSTAL W using MEGA7 (Kumar et al. 2016), and the alignment was further modified by eye. jModelTest version 2.1.6 (Darriba et al. 2012, Guindon and Gascuel 2003) and the corrected AIC was 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 (Rodriguez et al. 1990), Neighbour Joining (NJ), Maximum Parsimony (MP) (Felsenstein 1992) in MEGA7 and Bayesian Inference (BI) (Metropolis-coupled Markov-chain Monte-Carlo method, GTR model) performed in MrBayes v.3.2.2 (Ronquist et al. 2012). In the BI, the two parallel runs with four chains each (three heated and one cold) were performed for 1 million generations, sampling a tree every 100 generations. The posterior probability values in each node were calculated by FigTree software (version 1.4.2). In the analyses, trees were tested by the bootstrapping method with 1,000 replications. Sequence divergences and nucleotide differences were also calculated in MEGA7. The consensus tree based on four different trees (achieved from the four methods) was constructed by Dendro Scope software, version 3.2.10 (Huson and Scornavacca 2012). *Halymenia durvillei* Bory de Saint-Vincent was used as an out-group.

RESULTS AND DISCUSSION

Morphology

Thallus foliose, arising from a small discoid holdfast, dark brown, gelatinous and soft, up to 15 cm high, usually consisting of two to four simple linear to lanceolate blades 8-12 cm long and 2-3 cm wide, which often bear marginal, lanceolate bladelets 4-20 mm long by 3-4 mm wide from the upper and basal parts (Figure 2.A); thallus thickness 400 - 500 µm (Figure 2.B); cortex 8-10 layers (Figure 2.C); medulla filamentous with the filaments lax (Figure 2E); basal cluster surrounding the gonimoblasts during cystocarp development (Figure 2.D). Morphological characteristics of *G. taiwanensis* collected in Vietnam are very similar to the known *G. taiwanensis* in Taiwan. The result of analysis of morphological traits among *Grateloupia* members indicated that our material is superficially similar to some other foliose species including *Grateloupia livida, G. sparsa, G. phuquocensis, G. lanceolata, G. turututu*, and *G. belangeri* (Table 2, Figure 3).

Phylogenetic tree

A final alignment of 1257 bp without gap was generated for partial *rbc*L, of which 890 (70.8%) were conserved sites, 367 (29.2%) were variable sites, 271 (21.6%) were parsimony informative characters, 96 (7.6%) were singleton. Results of the four algorithms applied (ML, NJ, MP, BI) showed that sequence of *G. taiwanensis* collected in Vietnam was positioned into a clade consisting of the known *G. taiwanensis*. The bootstrap support values of ML, NJ, MP and posterior probability value of BI are high, 100% and 1.0, respectively (Figure 4). Only one nucleotide was different between *G. taiwanensis* collected in Vietnam and the published sequence data for *G. taiwanensis* (data not shown). In addition, evolutionary divergence between our sequence data and published *G. taiwanensis* sequences is very low (0.001) (data not shown).

Discussion

Several empirical studies indicated that the family Halymeniaceae exhibits high species diversity, especially within *Grateloupia*, which is well-known as a complex taxonomic challenge mainly due to their high morphological plasticity making species identification difficult. Moreover, within Halymeniaceae, the formation of auxiliary cell ampullae is a notably important characteristic for identification.

Morphological observation of samples collected in Vietnam reveals the new record of *G. taiwanensis*. In the present study, morphological characters of member *Grateloupia* also analyzed. There are no much differences between Vietnamese sample and known *G. taiwanensis* in Taiwan (Lin et al. 2008) in terms of thallus habits and anatomy. Hence, the result of evolutionary histories of key morphological characters also indicated that material collected in Vietnam is allied to *G. taiwanensis* in Taiwan, and both of them join the foliose clade that includes other foliose species such as *Grateloupia sparsa, G. livida, G. turuturu, G. phuquocensis, G. lanceolata* and *G. belangeri*. However, there are no distinct clades of foliose and terete based on molecular analysis. For example, *Grateloupia subpectinata* groups to other foliose species include *G. taiwanensis, G. turuturu, G. phuquocensis, G. huangiae*.

Previously, the total species of *Grateloupia* in Vietnam is 10 (Phạm-Hoàng 1969) much higher compared to other countries in SE Asia (Phang et al. 2016). The occurrence of *G. taiwanensis* is an added evidence of high species diversity of marine algae seen in Vietnam. So far, *G. phuquocensis* was originally described and collected in Phu Quoc Island, Vietnam (Tanaka and Phạm-Hoàng 1963). This species was also found in other regions of the Pacific islands latter (N'Yeurt and Payri 2010). In Vietnam, two subspecific taxa namely, *G. filicina* f. *prolongata* (J. Agardh) C.K. Tseng and *G. filicina* f. *porracea* (Kützing) M Howe were recorded in the northern Vietnam (Nguyen et al. 1993). However, the latter studies indicated that the two above entities were recognized as distinct species as *Grateloupia prolongata* J. Agardh and *G. porracea* Kützing (De Clerck et al. 2005b, Wang et al. 2000). Hence, *G. taiwanensis* is the eleventh species of *Grateloupia* in Vietnam.

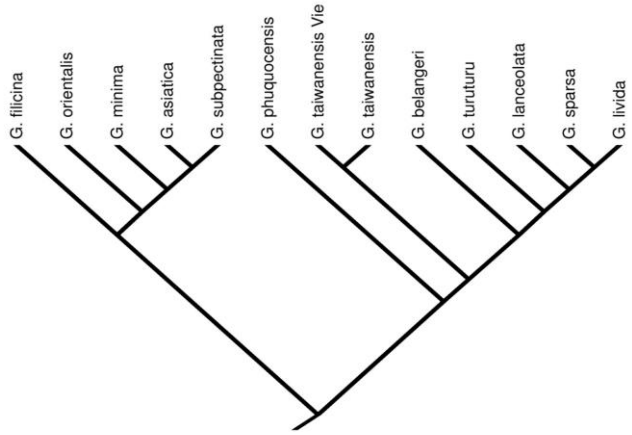

Figure 3. Evolutionary histories of key morphological characters for *Grateloupia* using UPGMA reconstruction method in Mesquite 3.51 (Maddison and Maddison 2018). Vie = Vietnam. Ancestral character states are referred to in Table 2

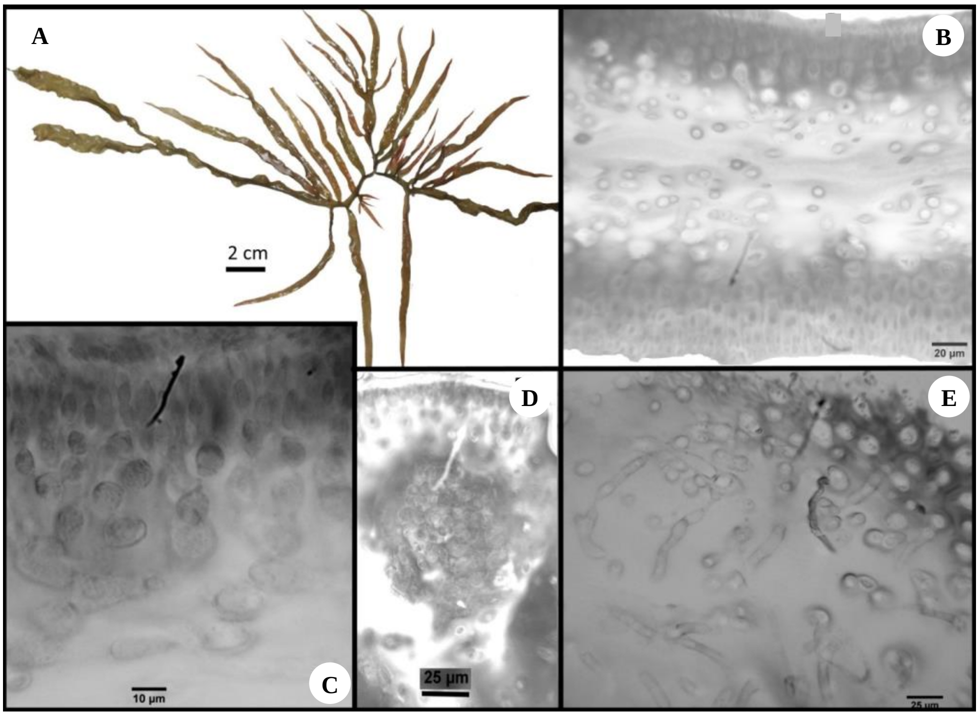

Figure 2. *Grateloupia taiwanensis* Showe M. Lin et H.Y. Liang collected in Da Nang, Vietnam. A. Fresh sample of herbarium specimen (CS18-DN006) from Da Nang, Vietnam, B. Cross-section through a bladelet showing cortex and medulla, C. Detail of inner cortex, D. Cystocarp, E. Medulla filamentous with the filaments lax

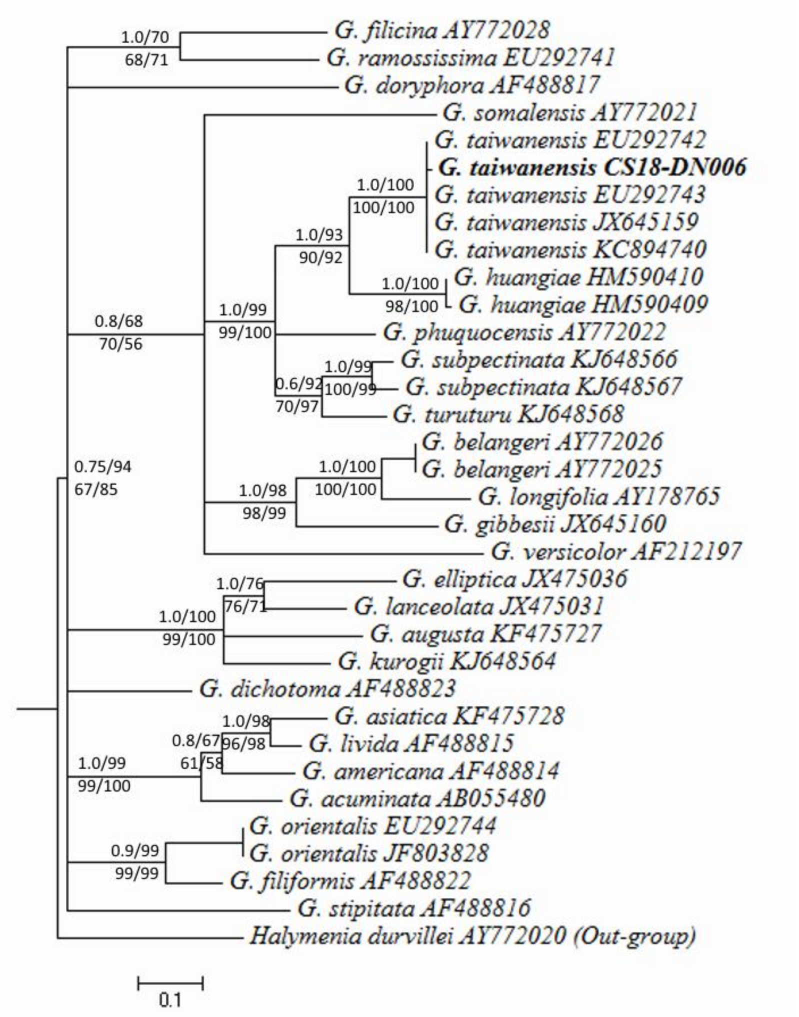

Figure 4. Phylogeny of members of *Grateloupia* inferred from Bayesian Inference, Maximum Likelihood, Maximum Parsimony and Neighbour Joining. The data set based on 1257 bp of *rbc*L. The posterior probability and bootstrap values of each method are shown in each node: above nodes, left: Bayesian Inference, right: Maximum Likelihood; below nodes, left: Maximum Parsimony, right: Neighbour Joining. **Bold**, sample collected at Vietnam. The consensus tree was constructed by Dendro Scope software, version 3.2.10 (Huson and Scornavacca 2012)

Misidentification is the main issues within the genus *Grateloupia* in particular and marine red algae in general. Well-known examples may be found in two species: *G. turuturu* and *G. doryphora*. So far, *G. doryphora* was the name applied to the most records of introduced *Grateloupia* in Europe and North America (Marston and Villalard-Bohnsack 2002), and the authors indicated that the alga previously reported as *G. doryphora* could be a misidentification of the Japanese taxon *G. turuturu*. However, latter studies showed that *G. doryphora* and *G. turuturu* are distinct species based on morphological anatomy and molecular analysis (Verlaque et al. 2005). Citing another macrophyte, a seagrass species *Halophila major* (Zollinger) Miquel was misidentified as *Halophila ovalis* (R. Brown) J.D. Hooker in Vietnam due to overlapping morphological characters (Nguyen et al. 2013b).

The result of the present study indicated that there are no geographical relationship among *G. taiwanensis* collected in Vietnam, Taiwan and USA. However, in another species *Grateloupia subpectiana,* some geographical patterns can be inferred. Samples from the Pacific side of Japan were grouped together as one clade, and the other samples Korea, China, and the west side of Japan, were grouped as another clade (Nelson et al. 2013). The authors also found that the unique clone of *Grateloupia subpectiana* collected in Australia and New Zealand is an invasive species from China or Korea. On another hand, the new record of *G. doryphora* in the Egyptian Mediterranean Sea is a non-native species that was most properly originated from Peru and Chile and may have been introduced as spores transported in the ballast water of ships (El Din and Aboul-Ela 2017). In the same way, *G. filicina* and *G. turuturu* are considered introduced species in Atlantic coast of northern Europe from East Asian (Montes et al. 2017). El Din and Aboul-ela (2017) indicated that *G. turuturu* have also been shown to be highly invasive after its introduction through aquaculture activities (D'Archino et al. 2007; Mathieson et al. 2008).

Results of the present study provided the morphological and molecular evidence in documenting the first record in *G. taiwanensis* in Vietnam as well as Southeast Asia, the first such record in a truly tropical locality. Further molecular marker studies and detail morphological observation may show whether this and other *Grateloupia* species are recent immigrant to Vietnam or were just not recognized as such by previous collectors. There is certainly an urgent need to re-examine the species of *Grateloupia* occuring in Vietnam using combined morphological and molecular approach

ACKNOWLEDGEMENTS

We are deeply indebted to all staff of the Department of Marine Ecology, Department of Biochemistry, Institute of Oceanography and VAST Keylab on Food and Environmental Safety (Central Vietnam) for the ability to use their equipment. The Graduate School of Biosphere Science, Hiroshima University, Japan is gratefully acknowledged for the travel grant provide to LML. The comments of the two anonymous reviewers helped improve the manuscript.

REFERENCES

Baweja P, Sahoo D. 2002. Structure and reproduction of *Grateloupia filicina* (Halymeniaceae, Rhodophyta) from Indian Coast. Algae 17: 161-170.

Cao C, Liu M, Guo S, Zhao D, Luan R, Wang H. 2016a. *Grateloupia ramosa* Wang & Luan sp. nov. (Halymeniaceae, Rhodophyta), a new species from China based on morphological evidence and comparative *rbc*L sequences. Chin J Oceanol Limn 34: 283-294.

Cao C, Li YZ, Wang HW. 2016b. Morphological observation, life history and *rbc*L gene sequence analysis of a new species, *Grateloupia variata* sp. nov. (Halymeniaceae, Rhodophyta) from Qingdao, China. Mar Biol Res 12: 443-453.

D'Archino RWA, Zuccarello GC. 2007. Invasive marine red alga introduced to New Zealand waters: First record of *Grateloupia turuturu* (Halymeniaceae, Rhodophyta). N Z J Mar Fresh 41: 35-42.

Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat Meth 9: 772.

De Clerck O, Gavio B, Fredericq S, Bárbara I, Coppejans E. 2005a. Systematics of *Grateloupia filicina* (Halymeniaceae, Rhodophyta), based on *rbc*L sequence analyses and morphological evidence, including the reinstatement of *G. minima* and the description of *G. capensis* sp. nov.1. J Phycol 41: 391-410.

De Clerck O, Gavio B, Fredericq S, Cocquyt E, Coppejans E. 2005. Systematic reassessment of the red algal genus *Phyllymenia* (Halymeniaceae, Rhodophyta). Eur J Phycol 40: 169-178.

DePriest MS, Bhattacharya D, López-Bautista JM. 2013. The plastid genome of the red macroalga *Grateloupia taiwanensis* (Halymeniaceae). PloS One 8: e68246. DOI: 10.1371/journal.pone.0068246.

El Din NGS, Aboul-Ela HM. 2017. The new record of *Grateloupia doryphora* (Halymeniaceae, Rhodophyta) algae in the Egyptian Mediterranean Sea recognized by morphological and molecular intergrative approach. Plant Cell Biotechnol Mol Biol 18: 972-2025.

Faye EJ, Wang HW, Kawaguchi S, Shimada S, Masuda M. 2004. Reinstatement of *Grateloupia subpectinata* (Rhodophyta, Halymeniaceae) based on morphology and *rbc*L sequences. Phycol Res 52: 59-67.

Felsenstein J. 1992. Phylogenies from restriction sites: A maximum-likelihood approach. Evolution 46: 159-173.

Figueroa FL, Korbee N, De Clerck O, Bárbara I, Gall EAR. 2007. Characterization of *Grateloupia lanceola* (Halymeniales, Rhodophyta), an obscure foliose *Grateloupia* from the Iberian Peninsula, based on morphology, comparative sequence analysis and mycosporine-like amino acid composition. Eur J Phycol 42: 231-242.

Freshwater DW, Rueness J. 1994. Phylogenetic relationships of some European *Gelidium* (Gelidiales, Rhodophyta) species, based on *rbc*L nucleotide sequence analysis. Phycologia 33: 187-194.

Gavio B, Frederic S. 2002. *Grateloupia turuturu* (Halymeniaceae, Rhodophyta) is the correct name of the non-native species in the Atlantic known as *Grateloupia doryphora*. Eur J Phycol 37: 349-359.

Guindon S, Gascuel O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 52: 696-704.

Guiry MD, Guiry GM. 2019. AlgaeBase. Worldwide electronic publication. National University of Ireland. http://www.algaebase.org. [4 February 2019].

Huson DH, Scornavacca C. 2012. Dendroscope 3: An interactive tool for rooted phylogenetic trees and networks. Syst Biol 61: 1061-1067.

John MD, Price HJ, Lawson GW, van Reine WP, Kostermans PT. 2004. A taxonomic and geographical catalogue of the seaweeds of the western coast of Africa and adjacent islands. Nova Hedwigia Bieh 127: 1-339.

Kawabata S. 1963. A contribution to the systematic study of Grateloupiaceae from Japan (2). J. Hokkaido Gakugei Univ 13: 190-210.

Kawaguchi S, Wang HW, Horiguchi T, Sartoni G, Masuda M. 2001. A comparative study of the red alga *Grateloupia filicina* (Halymeniaceae) from the Northwestern Pacific and Mediterranean with the description of *Grateloupia asiatica*, sp. nov. J Phycol 37: 433-442.

Kim MS, Kim SY, Nelson W. 2010. *Symphyocladia lithophila* sp. nov. (Rhodomelaceae, Ceramiales), a new Korean red algal species based on morphology and *rbc*L sequences. Bot Mar 53: 233-241.

Kim SY, Han EG, Kim MS, Park JK, Boo SM. 2013. *Grateloupia jejuensis* (Halymeniales, Rhodophyta): A new species previously confused with *G. elata* and *G. cornea* in Korea. Algae 28: 233-240.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33: 1870-1874.

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

Maddison WP, Maddison DR. 2018. Mesquite: A modular system for evolutionary analysis. Version 3.51. http://www.mesquiteproject.org

Marston M, Villalard-Bohnsack M. 2002. Genetic variability and potential sources of *Gratelouia doryphora* (Halymeniaceae, Rhodophyta), an invasive species in Rhode Island waters (USA). J Phycol 38: 649-658.

Mathieson AC, Dawes CJ, Pederson J, Gladych RA, Carlton JT. 2008. The Asian red seaweed *Grateloupia turuturu* (Rhodophyta) invades the Gulf of Maine. Biol Invasions 10: 985-988.

Montes M, Rico JM, García-Vazquez E, Borrell-Pichs JI. 2017. Molecular barcoding confirms the presence of exotic Asian seaweeds (*Pachymeniopsis gargiuli* and *Grateloupia turuturu*) in the Cantabrian Sea, Bay of Biscay. Peer J 5: e3116. DOI: 10.7717/peerj.3116.

N'Yeurt DRA, Payri C. 2010. Marine algal flora of French Polynesia III. Rhodophyta, with additions to the Phaeophyceae and Chlorophyta. Cryptogamie Algol 31: 3-205.

Nelson WA, Kim SY, D'Archino R, Boo SM. 2013. The first record of *Grateloupia subpectinata* from the New Zealand region and comparison with *G. prolifera*, a species endemic to the Chatham Islands. Bot Mar 56: 507-513.

Nguyen HD, Huynh QN, Tran NB, Nguyen VT. 1993. Marine algae of North Vietnam (Rong biển phía Bắc Việt Nam). Sci. & Tech. Publ. House, Hanoi. [Vietnamese]

Nguyen VT, Le NH, Lin SM, Steen F, De Clerck O. 2013a. Checklist of the marine macroalgae of Vietnam. Bot Mar 56: 207-227.

Nguyen XV, Holzmeyer L, Papenbrock J. 2013b. New record of the seagrass species *Halophila major* (Zoll.) Miquel in Vietnam: Evidence from leaf morphology and ITS analysis. Bot Mar 56: 313-321.

Nguyen XV, Höfler S, Glasenapp Y, Thangaradjou T, Lucas C, Papenbrock J. 2015. New insights into DNA barcoding of seagrasses. Syst Biodivers 13: 496-508.

Phạm-Hoàng H. 1969. Marine alage in Vietnam: Southern part. Trung tâm học liệu, Saigon. [Vietnamese]

Phang SM, Yeong HY, Ganzon-Fortes ET, Lewmanomont K, Prathep A, Le NH, Gerung GS, Tan KS. 2016. Marine algae of the South China Sea bordered by Indonesia, Malaysia, Philippines, Singapore, Thailand and Vietnam. Raffles B Zool 34: 13-59.

Rodriguez F, Oliver JL, Marin A, Medina JR. 1990. The general stochastic model of nucleotide substitution. J Theor Biol 142: 485-501.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Hohna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61: 539-542.

Tanaka T, Phạm-Hoàng H. 1963. Notes of some marine algae from Viet-Nam - I. Mem Fac Fisher Kagoshima Univ 11: 24-40.

Verlaque M, Brannock PM, Komatsu T, Villalard-Bohnsack M, Marston M. 2005. The genus *Grateloupia* C. Agardh (Halymeniaceae, Rhodophyta) in the Thau Lagoon (France, Mediterranean): A case study of marine plurispecific introductions. Phycologia 44: 477-496.

Villalard-Bohnsack M. Harlin MM. 2001. *Grateloupia doryphora* (Halymeniaceae, Rhodophyta) in Rhode Island waters (USA): Geographical expansion, morphological variations and associated algae. Phycologia 40: 372-380.

Wang HW, Guan Y, Zhao FQ, Zhao D. 2015. *Grateloupia huanghaiensis* sp. nov. (Halymeniaceae, Rhodophyta), a peculiar new species from China. Mar Biol Res 11: 396-404.

Wang HW, Kawaguchi S, Horiguchi T, Masuda M. 2000. Reinstatement of *Grateloupia catenata* (Rhodophyta, Halymeniaceae) on the basis of morphology and *rbc*L sequences. Phycologia 39: 228-237.

Wynne MJ. 2017. A checklist of benthic marine algae of the tropical and subtropical western Atlantic: Four revision. Nova Hedwigia, Beihefte 145. J. Cramer, Stutgard.

Yang MY, Kim MS. 2015. Taxonomy of *Grateloupia* (Halymeniales, Rhodophyta) by DNA barcode marker analysis and a description of *Pachymeniopsis volvita* sp. nov. J Appl Phycol 27: 1373-1384.

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

Yu L, Wang H, Luan R. 2013. *Grateloupia tenuis* Wang et Luan sp. nov. (Halymeniaceae, Rhodophyta): A new species from South China Sea based on morphological observation and *rbc*L gene sequences analysis. BioMed Res Intl 2013: 560163. DOI: 10.1155/2013/560163.

Zhao D, Wang H, Sheng Y, Lu J, Luan R. 2012. Morphological observation and *rbc*L gene sequences studies of two new species, *Grateloupia dalianensis* H.W.Wang et D. Zhao, sp. nov. and *G. yinggehaiensis* H.W.Wang et R.X. Luan, sp. nov. (Halymeniaceae, Rhodophyta) from China. Acta Oceanol Sin 31: 109-120.