

Research Article

Xuan-Vy Nguyen*, Nhu-Thuy Nguyen-Nhat, Xuan-Thuy Nguyen, Trung-Hieu Nguyen, Si Hai Trinh Truong, Viet-Ha Dao, Anh-Duy Do and Karla J. McDermid*

New record of *Halymenia malaysiana* (Halymeniaceae, Rhodophyta) from Viet Nam, and its genetic diversity in the western Pacific

<https://doi.org/10.1515/bot-2022-0062>

Received October 4, 2022; accepted February 7, 2023;
published online March 14, 2023

Abstract: *Halymenia malaysiana*, one of the foliose species of *Halymenia* was first reported from Malaysia and showed some morphological features that distinguish it from sister species, such as *Halymenia dilatata*, *Halymenia maculata*, and *Halymenia porphyraeformis*. In this study, *Halymenia* samples were collected from nine locations along the coast of Viet Nam (8°–17°N) in the South China Sea and Gulf of Thailand. Morphological observations indicated that almost all samples were *Halymenia malaysiana* which was supported by two genetic markers, *rbcL* and *COI-5P*. However, the presence of *Halymenia dilatata* in Viet Nam is still unresolved. Based on *rbcL*, the common haplotype in Viet Nam was R1 as in Malaysia and three new haplotypes were added to *H. malaysiana* for Southeast Asia. Genetic differences were evident between the Sunda Shelf (Viet Nam and Malaysia) populations and those in Philippine waters. We suggest that a combination of morphology and molecular data would be useful to accurately assess the species diversity and phylogeography of *Halymenia* in Viet Nam and Southeast Asia region.

Keywords: diversity; *Halymenia malaysiana*; haplotype; new record; Viet Nam.

1 Introduction

Halymenia, a marine red algal genus of the family Halymeniaceae, is one of several species-rich red algal genera with 80 known species (Guiry and Guiry 2022). Chang (1970) proposed that the generic concepts of *Halymenia* should be based primarily on the structure of auxiliary cell ampullae. However, the presence of anticinal medullary filaments was used as a key characteristic to identify the genus *Halymenia* by Abbott (1967). Vegetative features seem to be more helpful to delineate species of *Halymenia* (Smedt et al. 2001; Hernández-Kantún et al. 2012). The foliose species of *Halymenia* have been taxonomically problematic due to their immense morphological plasticity and few distinctive morphological features (Tan et al. 2015). Therefore, current studies of the systematics of *Halymenia* are based on a combination of vegetative features, reproductive characters, and molecular analyses. Previously, Phang et al. (2016) listed 10 species of *Halymenia* in South East Asian countries. In recent years, three species including *H. johorensis* P.-L. Tan, P.-E. Lim, S.-M. Lin et S.-M. Phang from Malaysia, *H. hawaiiensis* Hernández-Kantún et A.R.Sherwood from the Hawaiian Islands, and *H. tondoana* De Clerck et Hernández-Kantún from the Philippines were described (Hernández-Kantún et al. 2012; Tan et al. 2018b). Rodríguez-Prieto et al. (2022) proposed the new species *H. taiwanensis* Showe M. Lin, Rodríguez-Prieto et Huisman from Taiwan based on a combination of morphological features and molecular evidence.

Halymenia malaysiana P.-L.Tan, P.-E.Lim, S.-M.Lin et S.-M.Phang was first reported from Malaysia based on a mixed collection of foliose *Halymenia* specimens resembling *H. dilatata*, *H. maculata*, and other foliose species of *Halymenia* collected from Malaysia, Indonesia, and the Philippines (Tan et al. 2015). The authors indicated that *H. malaysiana* could be distinguished from other similar foliose species, such

*Corresponding authors: Xuan-Vy Nguyen, Institute of Oceanography, Viet Nam Academy of Science and Technology, 01 Cau Da, Nha Trang City, Viet Nam; and Graduate University of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Ha Noi, Viet Nam, E-mail: nguyenxuanvi@gmail.com; and Karla J. McDermid, Marine Science Department, University of Hawaii-Hilo, 200 W. Kawili St., Hilo, HI 96720, USA, E-mail: mcdermid@hawaii.edu

Nhu-Thuy Nguyen-Nhat, Xuan-Thuy Nguyen, Trung-Hieu Nguyen and Si Hai Trinh Truong, Institute of Oceanography, Viet Nam Academy of Science and Technology, 01 Cau Da, Nha Trang City, Viet Nam

Viet-Ha Dao, Institute of Oceanography, Viet Nam Academy of Science and Technology, 01 Cau Da, Nha Trang City, Viet Nam; and Graduate University of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Ha Noi, Viet Nam

Anh-Duy Do, Research Institute for Marine Fisheries, 224 Le Lai, Ngo Quyen, Hai Phong, Viet Nam

as *H. dilatata* Zanardini by several morphological characters. For example, blades of *H. malaysiana* are oblong or suborbicular, whereas the blades of *H. dilatata* are irregular in outline. The blade margin and blade surface also show differences between *H. malaysiana* and *H. dilatata*. Notably, the stipe is absent in *H. malaysiana*, but is present in *H. dilatata* (Tan et al. 2015). Based on the nucleotide sequence of *rbcL*, Tan et al. (2018a) revealed that the *H. malaysiana* populations from Peninsular Malaysia were genetically homogenous and showed a low level of genetic divergence from populations from East Malaysia. Among eight haplotypes, haplotype R1 is common in Peninsular Malaysia, but was not found in the Philippines.

In Viet Nam, four species of *Halymenia* have been previously recorded (Nguyen et al. 2013). Two foliose species, *H. dilatata* and *H. maculata* were reported by Dawson (1954) based on material collected at Nha Trang Bay. Specimens of *H. dilatata* collected from Japan and Viet Nam were observed and compared by Kawaguchi and Lewmanomont (1999), and the authors concluded that the specimens collected from Okinawa were more similar to the illustration given by Zanardini (1858) than the Vietnamese samples. Morphological observations of *H. maculata* collected in Nha Trang, Viet Nam were carried out by Kawaguchi et al. (2002). The authors concluded that the Vietnamese specimens clearly fall within the range of *H. maculata*, and are distinct from *H. stipitata* I.A.Abbott in several morphological characters. Two species collected later from Vietnamese waters, including *Halymenia* cf. *ulvoidea* and *H. harveyana* J. Agardh were added to the checklist of marine algae for Viet Nam (Phạm-Hoàng 1969; Le 2000).

The combination of morphology with molecular techniques has advanced our understanding of species and genetic diversity, as well as the biogeographical ranges of individual algal species (Tan et al. 2018a; Tronholm et al. 2012). The present study documents a new record of *H. malaysiana* from Vietnamese waters, its genetic diversity, and haplotype distribution within the western Pacific Ocean.

2 Materials and methods

2.1 Sample collection

Halymenia samples were collected at nine different locations by snorkeling and SCUBA diving in Viet Nam including Con Co Island (17.151°N; 107.337°E), Nui Thanh (15.500°N; 108.691°E), Ly Son Island (15.375°N; 109.133°E), Van Phong Bay (12.409°N; 109.337°E), Nha Trang (12.208°N; 109.215°E), Ninh Hai (11.658°N; 109.176°E), Phu Quy Island (10.527°N; 108.960°E), Con Dao Island (08.684°N; 106.625°E) and Nam Du

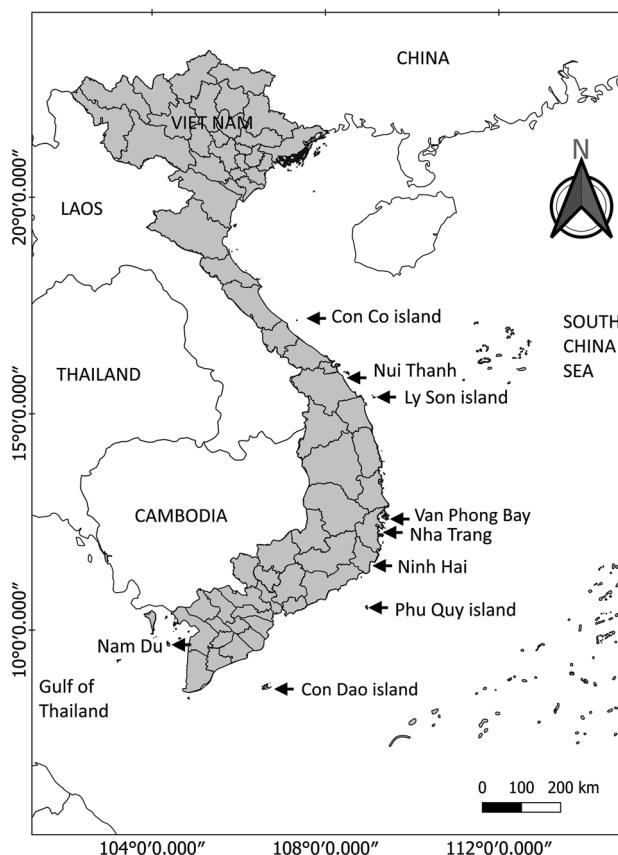


Figure 1: Map of Viet Nam coast and sampling sites (arrows). See Supplementary Table S1 for more information.

archipelago (09.649°N; 104.388°E) (Figure 1). At each site, one to five different thalli were collected. For the morphological study, algal samples were preserved in 5% formalin seawater or pressed on herbarium sheets, whereas materials used for DNA extraction were preserved in DESS solution (dimethyl sulfoxide, disodium EDTA, and saturated NaCl) (Yoder et al. 2006). Voucher specimens are deposited in the Institute of Oceanography (VMO), Nha Trang City, Viet Nam. Details of collection time and other information for each voucher specimen are listed in Supplementary Table S1.

2.2 Molecular data acquisition

The fragments fixed in DESS solution were rinsed in sterilized water before homogenizing each sample with a bead mill MM400 (Retsch, Germany) at 22 Hz, 2 min. The Quick-DNA™ Miniprep Plus Kit (Zymo Research, CA, USA) was used for DNA extraction following the manufacturer's instructions. The regions selected for PCR amplifications were the portions of plastid genes encoding the large subunit of ribulose-1,5-bisphosphate-carboxylase-oxygenase (*rbcL*) and cytochrome oxidase subunit 1 (COI-SP). For *rbcL*, PCRs consisted of an initial denaturation step at 94 °C for 3 min, 35 cycles consisting of denaturation at 94 °C for 45 s, annealing at 51 °C for 60 s, and elongation at 72 °C for 70 s. The 35 cycles were followed by a final extension at 72 °C for 5 min. In this study, the following primer pairs F8/R646, F481/R1150 (Wang et al. 2000), and

F577/R1381-ii (Hommersand et al. 1994; Wang et al. 2000), were used to obtain the length about 1250 bp. For COI-5P, the primer pairs COI1F/COI1R (Tan et al. 2015) and M13LF3/M13Ri were used to amplify the COI-5P, and the PCR condition followed Saunders and Moore (2013). The total volume of 25 μL included 2x OneTag[®] Master Mix (NewEngland Biolabs, Ipswich, MA, USA), 10–30 ng template DNA, and 1 pmol of each primer. PCRs was performed in MasterCycler Nexus SX1 (Eppendorf, Germany) with a heated lid. PCR products were cleaned using a GenElute[™] PCR Clean-Up kit (SigmaAldrich, St.Louis, MI, USA) following the manufacturer's instructions. All PCRs were repeated two to four times independently to reduce errors in the final consensus sequence to a minimum. Direct sequencing of the PCR products was done by 1ST BASE (Selangor, Malaysia). The consensus sequence was achieved by Clone Manager 9 (Sci-Ed, Cary, NC, USA).

2.3 Phylogenetic analysis

The *rbcL* sequences included 14 newly generated sequences obtained in this study (only distinct sequences per sampling site were used), 46 sequences of known *Halymenia* species, and 2 *rbcL* sequences of *Glyphyrosiphon intestinalis* and *Polyopes constrictus* (out-groups) retrieved from GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>) (Supplementary Table S1). The COI-5P dataset contained 28 known sequences of *Halymenia* spp. (Tan et al. 2018a), one newly generated sequence obtained in this study (PQ-H17), and one sequence of *Pachymenia cariosa* (out-group). The sequences were aligned by MAFFT algorithm with the selection of the q-ins-i option (Katoh and Standley 2013). jModelTest version 2.1.6 (Darriba et al. 2012) and the corrected AIC (Akaike Information Criterion) were used to select the best model for the analysis. Phylogenetic analyses were performed using maximum likelihood (ML) in RAxML version 8.1 (Stamatakis 2014) with 1000 bootstrap replications, and Bayesian Inference (BI) (Metropolis-coupled Markovchain Monte-Carlo method) in MrBayes v.3.2.2 (Ronquist et al. 2012). Two parallel runs with four chains each (three heated and one cold) were performed for 2 million generations, sampling the tree every 1000 generations. The consensus tree based on two different trees (achieved from the two methods) was constructed by Dendro Scope software, version 3.2.10 (Huson and Scornavacca 2012).

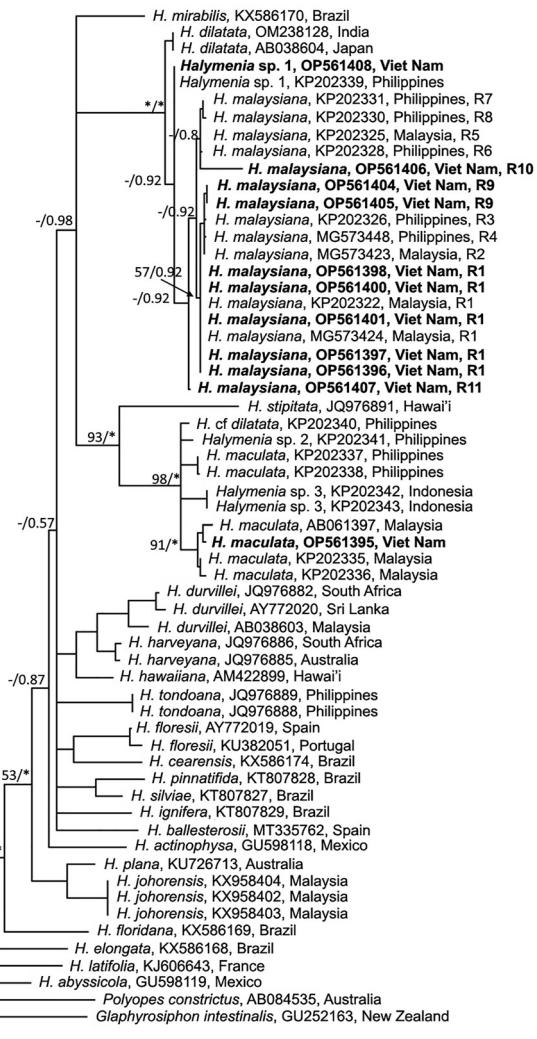
2.4 Morphological observation

Sections were made by hand and were treated with acetone-hematoxylin-chloral hydrate and mounted in 50% Hoyer's mounting medium. Photomicrographs were taken on Olympus BX53, (Olympus, Tokyo, Japan) with a Q-imaging digital camera (Burnaby, BC, Canada), and habit views were reproduced with an Epson scanner (Tokyo, Japan) at Institute of Oceanography, Viet Nam.

2.5 Genetic diversity and haplotype distributions

For the genetic diversity and haplotype distributions, all sequences of haplotypes of *H. malaysiana* from Peninsular Malaysia obtained by Tan et al. (2018a) (S1: Pulau Merambong, Johor; S2: Pulau Besar, Malacca; S3: Pulau Tinggi, Johor), East Malaysia (S4: Tun Mustapha Park, Sabah; S5: Pulau Karindingan, Sabah), Philippines (S6: Busuanga Island, Calamian Islands; S7: Grande Island, Subic Bay, Luzon; and S8:

Catanduanes Island; S9: Siargao Island), and Viet Nam (this present study, S10) were included in the analysis. Three locations including S1-3, S4-5, and S10 are in the Sunda Shelf, whereas two other locations, including S6-7 and S8-9 are located in the Philippines – out of the Sunda Shelf. In total, 57 *rbcL* sequences including 43 sequences from the Philippines, Malaysia (Tan et al. 2015, 2018a), and all new 14 *rbcL* sequences from Viet Nam were used for the analyses. The number of haplotypes (*N*), haplotype diversity (*h*), and nucleotide diversity (π) were measured by using DnaSP version 6 (Rozas et al. 2017). The genetic diversities were calculated for each region (Sunda Shelf and Philippine waters) and all data. Haplotype data were also used to construct a TCS network (Clement et al. 2000) by PopART (Leigh and Bryant 2015). Significant genetic differences among two regions (Φ_{CT}),



five representative populations (S1–S3, S4–5, S6–7, S8–9 and S10) (Φ_{SC}) (Figure 1), and within populations (Φ_{ST}) were calculated by non-parametric analysis of molecular variance (AMOVA) to examine the hierarchical population genetic structure by grouping the samples of *H. malaysiana* with Arlequin version 3.5 (Excoffier and Lischer 2010).

3 Results

3.1 Phylogenetic analysis of *rbcL* and COI-5P

The *rbcL* dataset for phylogenetic analyses consisted of 1207 characters and 59 taxa. The alignment showed 1076 bp (89.1%) of conserved sites, 131 bp (10.9%) of variable sites, 88 bp (7.4%) of parsimony informative characters, and 43 bp (3.6%) of singletons. Both phylogenetic analyses (ML and BI) grouped the new sequences of samples recently collected in Viet Nam into the clade with species of the *Halymenia* clade. The collections of “*H. dilatata*” from different localities in Viet Nam grouped with the *H. malaysiana* clade. Samples collected in Nam Du Island grouped with *Halymenia* sp. 1 (PSM12856, collected in Siquijor, Philippines). *Halymenia maculata* collected from Nha Trang Bay grouped with the known clade of *H. maculata* (Figure 2). The intraspecific pairwise distance (*p*-distance) among all collections of *H. malaysiana* from the Philippines, Malaysia, and Viet Nam based on *rbcL* sequence analyses ranged from 0% (0 bp) to 2.07% (25 bp) (Table 1). The intraspecific pairwise distance (*p*-distance) among *H. maculata* ranged from 0.1% to (1 bp) – 1.7% (21 bp) (data not shown). In the same way, results of ML and BI based on COI-5P also placed the sequences of samples collected in Viet Nam in the clade of *H. malaysiana* (Supplementary Figure S1). Based on COI-5P DNA sequences, the divergence between *H. malaysiana* from Viet Nam and

other locations (i.e., Philippines and Malaysia) were from 0.16 (1 bp) to 1.44% (9 bp) (Supplementary Table S2).

3.2 *Halymenia malaysiana* P.-L. Tan, P.-E. Lim, S.-M. Lin et S.-M. Phang

Order Halymeniales, Rhodophyta.

Halymenia malaysiana P.-L. Tan, P.-E. Lim, S.-M. Lin et S.-M. Phang 2015.

References: Tan et al. (2015: 207–210, figs: 2–9).

Description: Thalli are foliose, pink to pinkish brown, gelatinous in texture, arising from a small discoid holdfast; stipe is not present; blades are oblong or suborbicular, broad with smooth surface, undulated margins; 10–30 cm long and 15–40 cm wide (Figure 3A, B). Cortex consists of 4–6 layers, and the cortical cells are subspherical to elongated or rounded, ellipsoidal to stellate in shape (Figure 3C). Medulla is filamentous, commonly consisting of refractive ganglionic cells, mostly with 6 arms (Figure 3D, E). Reproductive structures are distributed over the thallus. Cystocarps are deeply embedded in the medulla of the fertile blades. The auxiliary cell cuts off a gonimoblast initial terminally from a cup-shaped depression (Figure 4C, D). Gonimolobes differentiate into ellipsoidal or ovoid carposporangia (Figure 4E). Mature carposporophytes are spherical, 100–120 µm in diameter, bearing several gonimolobes (Figure 4F).

Type locality: Pulau Merambong, Johor, southern Peninsular Malaysia.

Distribution: Distributed in Pulau Besar, Malacca; Pulau Merambong, Johor; Tun Mustapha Park, Sabah (Malaysia). Siargao Island, Catanduanes Island, Subic Bay, Busuanga Island (Philippines), South Gam (Indonesia).

Table 1: Estimates of evolutionary divergence (%) and number of different nucleotides (shaded cells) between 11 haplotypes of *Halymenia malaysiana* in South East Asia based on *rbcL*.

	R1	R9	R10	R11	R2	R3	R4	R5	R6	R7	R8
R1		0.25	1.82	0.33	0.17	0.25	0.25	0.25	0.33	0.33	0.41
R9	3		2.07	0.58	0.08	0.17	0.17	0.33	0.41	0.41	0.50
R10	22	25		1.99	1.99	2.07	2.07	1.74	1.82	1.82	1.91
R11	4	7	24		0.50	0.58	0.58	0.41	0.50	0.50	0.58
R2	2	1	24	6		0.08	0.08	0.25	0.33	0.33	0.41
R3	3	2	25	7	1		0.17	0.33	0.41	0.41	0.50
R4	3	2	25	7	1	2		0.33	0.41	0.41	0.50
R5	3	4	21	5	3	4	4		0.08	0.08	0.17
R6	4	5	22	6	4	5	5	1		0.17	0.25
R7	4	5	22	6	4	5	5	1	2		0.08
R8	5	6	23	7	5	6	6	2	3	1	

R1–R8: names of haplotypes (Tan et al. 2018a). R9–R11: new haplotypes. Italic type indicates haplotypes from Vietnamese waters.

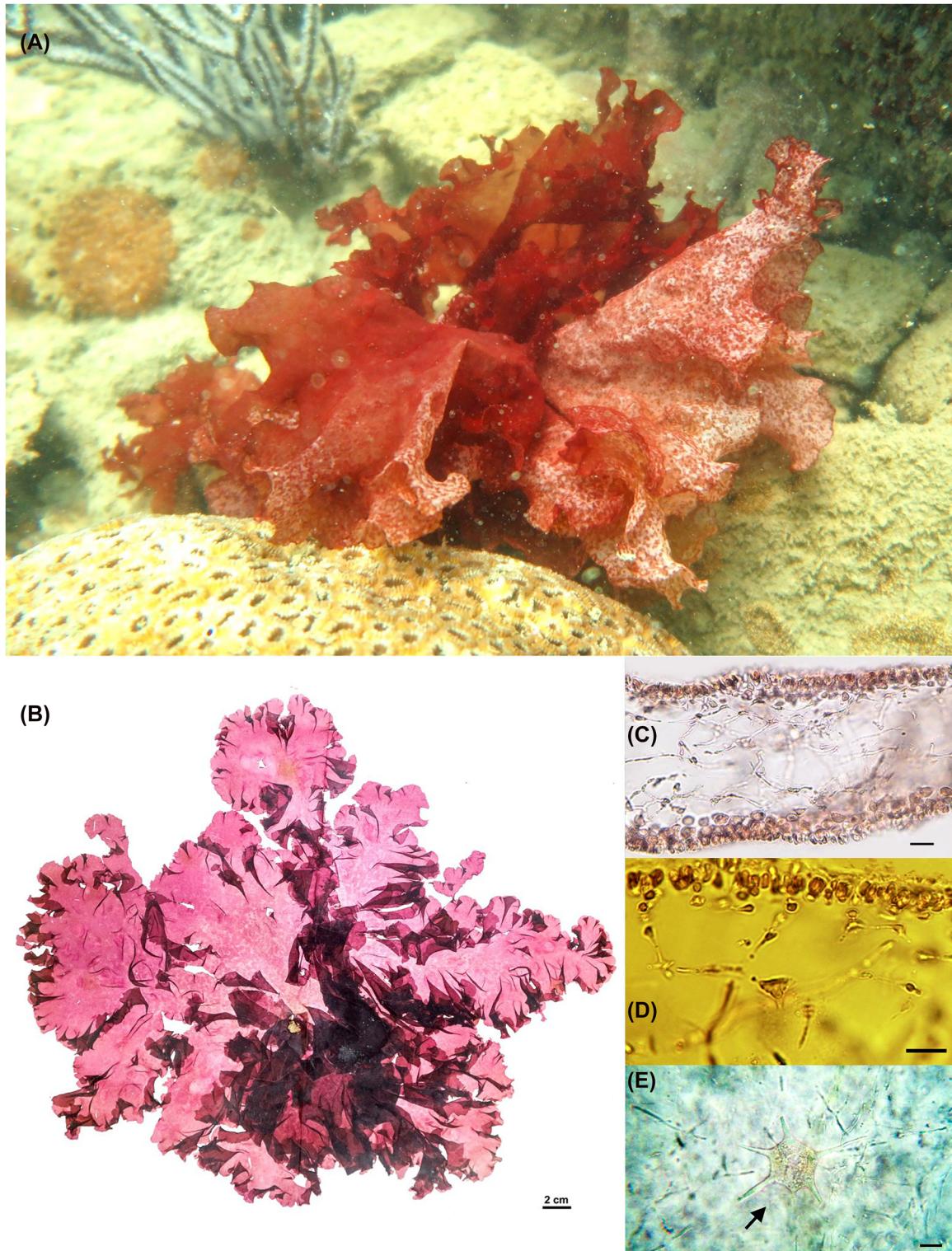


Figure 3: *Halymenia malaysiana* from Viet Nam showing habit and vegetative morphology. (A) Thallus in Van Phong Bay (VPKH-H2). (B) Herbarium voucher specimen CDNT-H15 collected at Nha Trang Bay. (C) Cross section through middle part of a young blade showing densely arranged cortical cells and sparse, loosely arranged, filamentous medullary cells of CDNT-H15. (D) Magnification of cortex layers from (C). (E) Anomalous refractive ganglionic cell (arrow) with 6 arms from CDNT-H15. Scale bars in (C–E) = 20 µm.

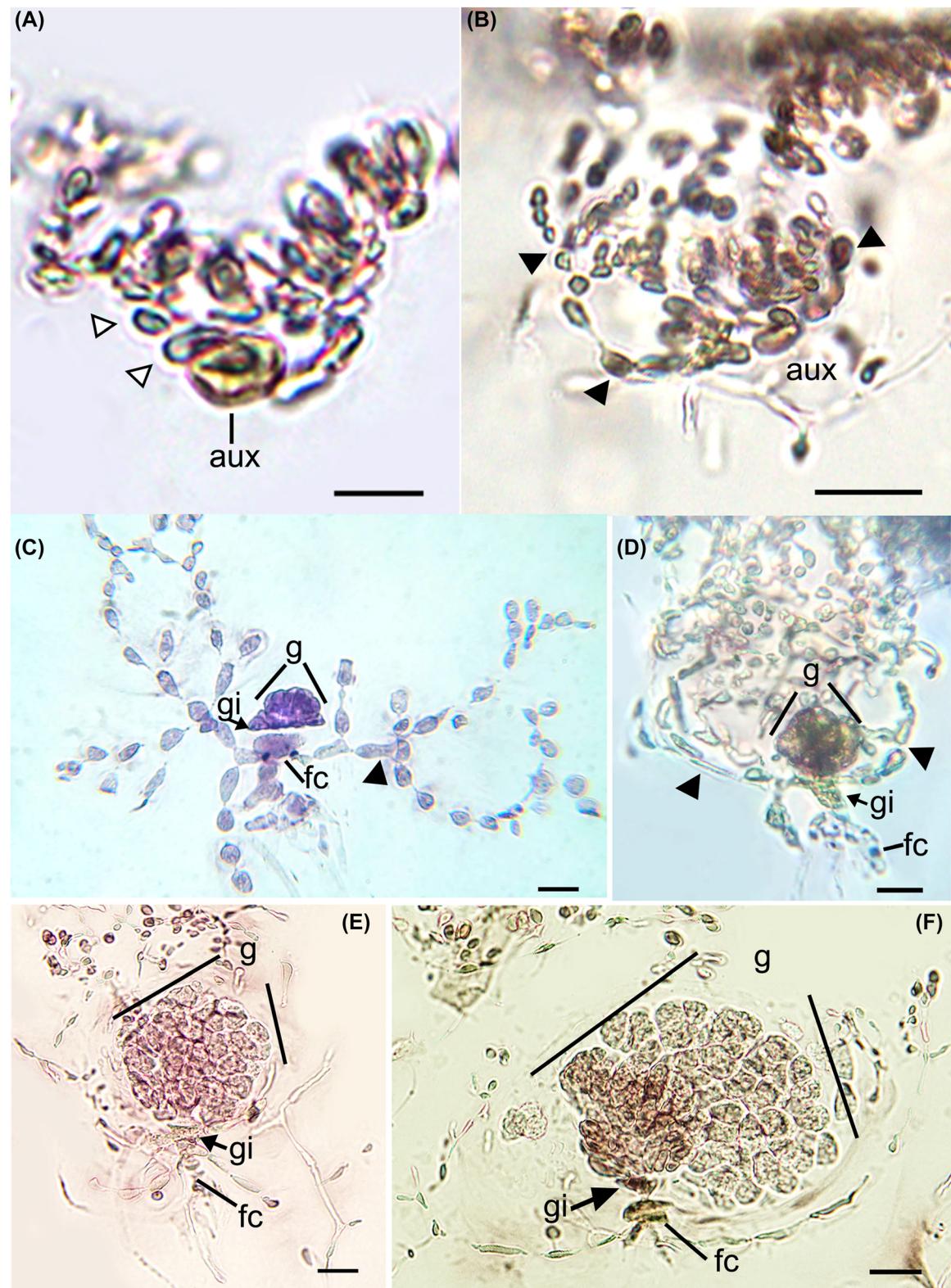


Figure 4: *Halymenia malaysiana* from Viet Nam (CDNT-H15) showing cystocarp development. (A) Auxiliary cell and ampullar filaments (white arrowheads). (B) Young cystocarp showing auxiliary cell and medullary filaments (black arrowheads). (C-E) Development of other young cystocarps showing a basal fusion cell, gonimoblast initial (black arrow) and gonimolobe enveloped by elongated ampullar filaments and secondary medullary filaments. (F) Mature cystocarp showing gonimoblast initial (gi, black arrow), basal fusion cell (fc), and gonimolobe (g). Scale bars = 20 µm; aux, auxiliary cell; g, gonimolobe; gi, gonimoblast initial; fc, fusion cell.

Vouchers: QTCC01, 06 July 2018, 3 m depth at Con Co Island, Quang Tri province ($17^{\circ}15'1''N$; $107^{\circ}34'4''E$), Viet Nam. NTh-H82, 28 September 1999, 8 m depth at Nui Thanh, Quang Nam province ($15^{\circ}49'9''N$; $108^{\circ}69'1''E$), Viet Nam. LSQN-H3, 17 February, 2022, 3 m depth at Ly Son Island, Quang Ngai province ($15^{\circ}39'3''N$; $109^{\circ}12'6''E$), Viet Nam. CDNT-H15, 25 February, 2022, 4 m depth at Cau Da, Nha Trang Bay, Khanh Hoa province ($12^{\circ}20'8''N$; $109^{\circ}21'6''E$), Viet Nam. NH-H16, 01 March 2022, 4 m at Thai An, Ninh Hai, Ninh Thuan province ($11^{\circ}66'9''N$; $109^{\circ}17'9''E$), Viet Nam. PQ-H17, 15 April 2022 at 5 m at Phu Quy Island, Binh Thuan Province ($10^{\circ}55'4''N$; $109^{\circ}95'5''E$), Viet Nam. CDVT-H4, 20 April 2022 4 m at Con Dao Island, Ba Ria Vung Tau province, ($08^{\circ}68'4''N$; $106^{\circ}62'5''E$), Viet Nam.

Remarks: The herbarium voucher specimens were deposited in the Museum of Oceanography, Nha Trang City. Specimens at the Research Institute for Marine Fisheries, Hai Phong City, Institute of Marine Environment and Resources, Hai Phong City, Viet Nam were misidentified as *H. dilatata*.

3.3 Genetic diversity and phyogeography

A total of 57 *rbcL* sequences (including 14 new sequences from the present study) of *H. malaysiana* could be separated into two geographic regions: Sunda Shelf and Philippine waters, and 11 haplotypes were generated, three of which were newly detected in this study (R9–R11) and the remaining (R1–R8) were deduced from previous studies. Within all regions, the nucleotide diversity (π) and haplotype diversity (Hd) of *rbcL* were 0.0024 and 0.59, respectively. Samples from Philippine waters revealed higher genetic diversity ($\pi = 0.0030$ and $Hd = 0.86$) than those of the Sunda Shelf samples ($\pi = 0.0017$ and $Hd = 0.36$) (Table 2). The results of AMOVA based on the two regions explained 14% of the variation (or fixation index $\Phi_{CT} = 0.141$, p -value <0.001) (Table 3). Haplotype analysis showed that six haplotypes (R1, R2, R5, R9–R11) were found in the Sunda Shelf region (sites S1–5 and S10), and the other five haplotypes (R3, R4, R6, R7

Table 2: Number of sequences used and estimates of genetic diversity for *Halymenia malaysiana*.

Regions	N	h	Hd	π	S
Sunda shelf	48	6	0.36	0.0017	28
Philippines	8	5	0.86	0.0030	8
Overall	56	11	0.59	0.0024	33

N, number of isolates sequenced; *h*, number of haplotypes; *Hd*, haplotype diversity; π , nucleotide diversity; *S*, number of segregating sites. For regions, see Figure 1.

Table 3: Analysis of molecular variance (AMOVA) results for *rbcL* variation of *Halymenia malaysiana* collected from the two regions: Sunda Shelf and the Phillipines.

Source of variation	d.f.	SS	σ^2	% of variation	Fixation indices
Among regions	1	87.662	3.432	14.067	$\Phi_{CT} = 0.141^{**}$
Among populations	3	94.716	1.343	5.503	$\Phi_{SC} = 0.064$
Within populations	50	981.259	19.626	80.430	$\Phi_{ST} = 0.195^*$
Total	54	1163.673	24.401		

d.f., degrees of freedom; SS, sum of squares. For regions, see Figure 5.

* $p < 0.05$, ** $p < 0.001$.

and R8) were found in the Philippines (sites S6–9). Notably, R1 is the most common haplotype, which was shared by Viet Nam (S10), Peninsular Malaysia (S1–S3), and East Malaysia (S1–5). Haplotypes R2 and R5 were found in East Malaysia only, whereas haplotypes R9–11 were found only in Viet Nam (Figure 5). The haplotype network based on the *rbcL* sequences failed to yield any clear phylogeographical separation between the two regions: Sunda Shelf and Philippine waters. There were 22 mutations between R1 and R10, but only 1–7 mutations between R1 and the remaining haplotypes (R1–8 and R11) (Figure 6).

4 Discussion

Previously, the distribution of *H. malaysiana* was restricted to a few specific countries, including Malaysia, the Philippines, and Indonesia (Tan et al. 2015). Our results revealed the presence of *H. malaysiana* as a new record in Vietnamese waters, which is a remarkable range extension for this species. Our morphological observations and phylogenetic analyses were based on our new collections from several sites along the coast of Viet Nam (8° – $17^{\circ}N$) and previous specimens labelled as “*H. dilatata*” collected at Nha Trang Bay, the site of specimens used by Kawaguchi and Lewmanomont (1999). The morphological characteristics of “*H. dilatata*” from Viet Nam were quite similar to the illustration of *H. malaysiana* given by Tan et al. (2015), such as oblong or suborbicular blade shape, sinusoidal undulated margins, rare orbicular proliferations on blade surface, and no stipe. Remarkably, Kawaguchi and Lewmanomont (1999) indicated that the specimens of *H. dilatata* collected in Okinawa, Japan, and India and the “*H. dilatata*” collected in Nha Trang, Viet Nam were in agreement with the original and additional descriptions by Zanardini (1858), and that the vegetative morphologies of material from Japan and India are extremely

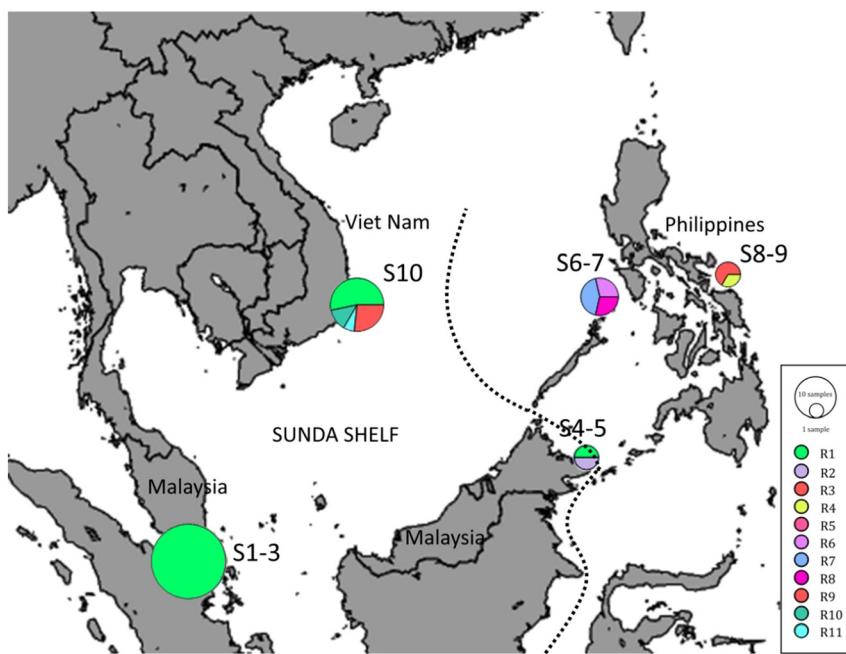


Figure 5: Distribution of haplotypes of *Halymenia malaysiana* in Sunda Shelf (Malaysia and Viet Nam) and the Philippines. 11 haplotypes (R1–R11) are defined by different colors. Sites 1–9 (S1–S9) were defined by Tan et al. (2018a). S10 is defined in this study. Dotted line represents the border of the Sunda Shelf. The data were processed by PopART software.

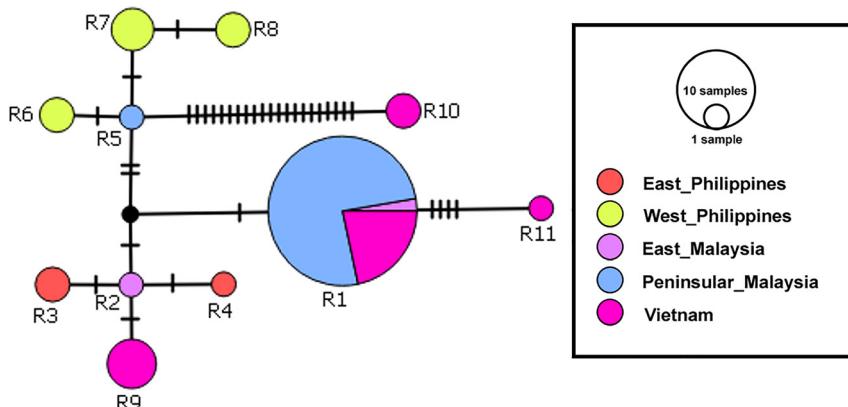


Figure 6: Haplotype network of 11 haplotypes of *Halymenia malaysiana* and their distribution. Haplotype names are written beside the circles. Each cross in the lines between two haplotypes is a single mutation. The data were processed by PopART software.

similar. Cystocarp development of *H. malaysiana* was observed in detail (Tan et al. 2015). Our present study also provided more detail of cystocarp features and development, especially the auxiliary cell, young gonimolobe and basal fusion cell (Figure 4).

The result of phylogenetic analyses based on both *rbcL* and COI-5P sequences indicated that “*H. dilatata*” collected from Vietnamese waters grouped within the *H. malaysiana* clade. Interestingly, the phylogenetic analyses also revealed that *H. dilatata* from Japan and India form a distinct clade which may support the morphological observations that were previously made by Kawaguchi and Lewmanomont (1999). In the present study, the single specimen of “*H. dilatata*” collected in Nam Du Archipelago (Gulf of Thailand) is

identical with *Halymenia* sp. 1 from the Philippines. Unfortunately, we have only a single sample, and not enough for morphological observation. Therefore, more material from the Gulf of Thailand should be added in further studies.

In the present study, we used only *rbcL* sequences to analyze the genetic diversity and haplotype distributions. Two primers (COI1F and COI1R) proposed by Tan et al. (2018a) were applied to amplify COI-5P marker; however, they did not work for the specimens of *Halymenia* collected from Viet Nam. The AMOVA results regarding the *rbcL* sequences showed the presence of low genetic differentiation between Sunda Shelf (Viet Nam and Peninsular Malaysia) and the Philippines. Among the three haplotypes found in Viet Nam, R1 is most common, and occurs in four out of the

nine locations. Tan et al. (2018a) indicated that R1 was also found in West and East Malaysia, but not in the Philippines. Similarly, *Gracilaria salicornia* (C. Agardh) Dawson in the Philippines shows as a group distinct from other South East Asian countries, such as Malaysia and Thailand (Yang et al. 2013). For another red macroalga, *Phycocalidina acanthophora* (E.C.Oliveira et Coll) Santiañez, the dataset of *rbcL* indicated that there is no haplotype sharing among populations in the Philippines and other nearby areas including Taiwan, Japan, and Hong Kong (Dumilag and Aguinaldo 2017). However, these studies used *rbcL*, which is a very conserved gene. More samples and more genetic markers need to be added. Additionally, in our recent studies of the two green macroalgae, *Halimeda opuntia* (Linnaeus) J.V.Lamouroux and *H. macroloba* Decaisne, the haplotype network based on the elongation factor Tu gene (*tufA*) did not reveal haplotype sharing between Viet Nam and Philippines (Nguyen et al. 2022).

Haplotype sharing between Viet Nam and Peninsular Malaysia may be explained by the existence of the South China Sea western boundary current. From October to December, the current flows from the Vietnamese coast towards the Java Sea through the Karimata Strait, but in the summer, this current runs from the Karimata Strait to the coast of Central Viet Nam (Fang et al. 2012). The presence of populations of *H. malaysiana* from 17°N in Viet Nam to 2°N in Peninsular Malaysia documents a distribution of at least 1667 km. This long range raises new questions about the biogeography, ecology, physiology, and evolution of *H. malaysiana* and other South East Asian species of *Halymenia*.

In summary, the present study reveals a new record of *H. malaysiana* from Vietnamese waters. Our study also adds more photographic detail about cystocarp development in *H. malaysiana*. In both Malaysia and Viet Nam, haplotype R1 is the most common and suggests interesting biogeographic affinities. We also elucidated three new haplotypes for the regions. Future studies will focus on more foliose and branched species of *Halymenia* using both morphological observations and molecular analyses of multiple genes.

Acknowledgements: We are deeply indebted to all staff at the VAST Keylab on Food and Environmental Safety (Central Viet Nam) for the ability to use their equipment. We would like to thank the anonymous reviewers for their editing, suggestions and comments. We also thank projects TĐĐTBTB0.04/21–23 and KC.09.05/16–20 for material sharing.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript.

Research funding: This work was supported by Vietnam Academy of Science and Technology, grant coded CSCL.17.02/22–23.

Conflict of interest statement: The authors declare that they have no conflicts of interest regarding this article.

References

- Abbott, I.A. (1967). Studies in some foliose red algae of the Pacific coast. I. Cryptonemiaceae (I). *J. Phycol.* 3: 139–149.
- Chang, Y.M. (1970). *Morphological studies of red algae of the family cryptonemiaceae*. University of California Publications in Botany, Berkeley and Los Angeles, California.
- Clement, M., Posada, D., and Crandall, K.A. (2000). TCS: a computer program to estimate gene genealogies. *Mol. Ecol.* 9: 1657–1659.
- Darriba, D., Taboada, G.L., Doallo, R., and Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nat. Methods* 9: 772.
- Dawson, E.Y. (1954). Marine plants in the vicinity of the Institut Oceanographique de Nha Trang, Viet Nam. *Pac. Sci.* 8: 373–469.
- Dumilag, R.V. and Aguinaldo, Z.Z.A. (2017). Genetic differentiation and distribution of *Pyropia acanthophora* (Bangiales, Rhodophyta) in the Philippines. *Eur. J. Phycol.* 52: 104–115.
- Excoffier, L. and Lischer, H.E.L. (2010). Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under linux and windows. *Mol. Ecol. Resour.* 10: 564–567.
- Fang, G., Wang, G., Fang, Y., and Fang, W. (2012). A review on the South China Sea western boundary current. *Acta Oceanol. Sin.* 31: 1–10.
- Guiry, M.D. and Guiry, G.M. (2022). *AlgaeBase*. World-Wide Electronic Publication, National University of Ireland, Galway, Available at: <https://www.algaebase.org> (Accessed 26 December 2022).
- Hernández-Kantún, J.J., Sherwood, A.R., Riosmena-Rodríguez, R., Huisman, J.M., and De Clerck, O. (2012). Branched *Halymenia* species (Halymeniaceae, Rhodophyta) in the Indo-Pacific region, including descriptions of *Halymenia hawaiiensis* sp. nov. and *H. tondoana* sp. nov. *Eur. J. Phycol.* 47: 421–432.
- Hommersand, M.H., Fredericq, S., and Freshwater, D.W. (1994). Phylogenetic systematics and biogeography of the Gigartinaceae (Gigartianales, Rhodophyta) based on sequence analysis of *rbcL*. *Bot. Mar.* 37: 193–203.
- Huson, D.H. and Scornavacca, C. (2012). Dendroscope 3: an interactive tool for rooted phylogenetic trees and networks. *Syst. Biol.* 61: 1061–1067.
- Katoh, K. and Standley, D.M. (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.* 30: 772–780.
- Kawaguchi, S. and Lewmanomont, K. (1999). Morphology and culture study of a red alga, *Halymenia dilatata* Zanardini, from Vietnam and Japan. In: Abbott, I.A. (Ed.). *Taxonomy of economic seaweeds with reference to some Pacific species*, Vol. 7. California Sea Grant College Program, La Jolla, CA, pp. 147–161.
- Kawaguchi, S., Lewmanomont, K., and McDermid, K.J. (2002). Morphology of *Halymenia maculata* J. Agardh from Vietnam. In: Abbott, I.A. and McDermid, K.J. (Eds.), *Taxonomy of economic seaweeds with reference to some Pacific species*, Vol. 8. California Sea Grant College Program, La Jolla, pp. 259–266.
- Le, H.N. (2000). Notes on some new species of marine algae from Ninh Thuan province (South Vietnam). *Coll. Mar. Res. Works* 10: 141–148.

- Leigh, J.W. and Bryant, D. (2015). Popart: full-feature software for haplotype network construction. *Methods Ecol. Evol.* 6: 1110–1116.
- Nguyen, T.H., Nguyen-Nhat, N.T., Nguyen, X.T., and Nguyen, X.V. (2022). Morphological variation and haplotype diversity of *Halymenia macroloba* and *H. opuntia* (chlorophyta: halimedaceae) from southern Vietnam. *Vietnam J. Mar. Sci. Technol.* 22: 165–176.
- Nguyen, V.T., Le, N.H., Lin, S.M., Steen, F., and De Clerck, O. (2013). Checklist of the marine macroalgae of Vietnam. *Bot. Mar.* 56: 207–227.
- Phạm-Hoàng, H. (1969). *Marine algae of South Vietnam. Trung tâm Học liệu Sài Gòn, Việt Nam* (in Vietnamese).
- Phang, S.M., Yeong, H.Y., Ganzon-Fortes, E.T., Lewmanomont, K., Pratheepong, A., Hau, L.N., Gerung, G.S., and Tan, K.S. (2016). Marine algae of the South China Sea bordered by Indonesia, Malaysia, Philippines, Singapore, Thailand and Vietnam. *Raffles Bull. Zool.* 34: 13–19.
- Rodríguez-Prieto, C., Huisman, J.M., and Lin, S.M. (2022). Molecular phylogeny of foliose *Halymenia* and *Astroepiphloea* (Halymeniaceae, Rhodophyta) from the indo-pacific, with the description of *Halymenia taiwanensis* sp. nov. *Phycologia* 61: 384–395.
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., and Huelsenbeck, J.P. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* 61: 539–542.
- Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J.C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S.E., and Sánchez-Gracia, A. (2017). DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Mol. Biol. Evol.* 34: 3299–3302.
- Saunders, W.G. and Moore, E.T. (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.
- Smedt, G., de Clerck, O., Leliaert, F., Coppejans, E., and Liao, L.M. (2001). Morphology and systematics of the genus *Halymenia* C. Agardh (Halymeniales, Rhodophyta) in the Philippines. *Nova Hedwigia* 73: 293–322.
- Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313.
- Tan, P.L., Lim, P.E., Lin, S.M., Phang, S.M., Draisma, S.G.A., and Liao, L.M. (2015). Foliose *Halymenia* species (Halymeniaceae, Rhodophyta) from Southeast Asia, including a new species, *Halymenia malaysiana* sp. nov. *Bot. Mar.* 58: 203–217.
- Tan, P.L., Lim, P.E., Lin, S.M., Phang, S.M., Draisma, S.G.A., and Liao, L.M. (2018a). A genetic diversity assessment of *Halymenia malaysiana* (Halymeniaceae, Rhodophyta) from Malaysia and the Philippines based on COI-5P and rbcL sequences. *J. Appl. Phycol.* 30: 3445–3454.
- Tan, P.L., Lim, P.E., Lin, S.M., and Phang, S.M. (2018b). *Halymenia johorensis* sp. nov. (Halymeniaceae, Rhodophyta), a new foliose red algal species from Malaysia. *J. Appl. Phycol.* 30: 187–195.
- Tronholm, A., Leliaert, F., Sansón, M., Afonso-Carrillo, J., Tyberghein, L., Verbruggen, H., and De Clerck, O. (2012). Contrasting geographical distributions as a result of thermal tolerance and long-distance dispersal in two allegedly widespread tropical brown algae. *PLoS One* 7: e30813.
- Wang, H.W., Kawaguchi, S., Horiguchi, T., and Masuda, M. (2000). Reinstatement of *Grateloupia catenata* (Rhodophyta, Halymeniaceae) on the basis of morphology and rbcL sequences. *Phycologia* 39: 228–237.
- Yang, M.Y., Geraldino, P.J.L., and Kim, M.S. (2013). DNA barcode assessment of *Gracilaria salicornia* (gracilariaeae, Rhodophyta) from Southeast Asia. *Bot. Stud.* 54: 218–226.
- Yoder, M., De Ley, I.T., Wm King, I., Mundo-Ocampo, M., Mann, J., Blaxter, M., Poiras, L., and De Ley, P. (2006). DESS: a versatile solution for preserving morphology and extractable DNA of nematodes. *Nematology* 8: 367–376.
- Zanardini, G. (1858). *Plantarum in mari Rubro hucusque collectarum enumeratio (juvante A. Figari)*. Mem. R. Ist. Veneto Sci., Let. Ed Arti 7: 209–309.
- Supplementary Material:** This article contains supplementary material (<https://doi.org/10.1515/bot-2022-0062>).

Bionotes



Xuan-Vy Nguyen

Institute of Oceanography, Viet Nam Academy of Science and Technology, 01 Cau Da, Nha Trang City, Viet Nam
Graduate University of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Ha Noi, Viet Nam
nguyenxuanvi@gmail.com

Xuan-Vy Nguyen leads the Department of Marine Botany, Institute of Oceanography, Vietnam Academy of Science and Technology. He was awarded a Doctor rer. nat. in 2013 by the Gottfried-Wilhelm-Leibniz-Universität Hannover, Germany. His works focus on the taxonomy and ecology of marine macrophytes. He is interested in the species, genetic biodiversity and population structure of marine botany, interaction between marine macrophytes and environmental stressors under molecular points of view.



Nhu-Thuy Nguyen-Nhat

Institute of Oceanography, Viet Nam Academy of Science and Technology, 01 Cau Da, Nha Trang City, Viet Nam

Nhu-Thuy Nguyen-Nhat is a research academic at the Department of Marine Botany, Institute of Oceanography, Vietnam Academy of Science and Technology. She has studied taxonomy, biology of marine algae and mangroves for over 10 years. Recently, she focuses on the taxonomy of Halymeniales, Rhodophyta.



Xuan-Thuy Nguyen

Institute of Oceanography, Viet Nam Academy of Science and Technology, 01 Cau Da, Nha Trang City, Viet Nam

Xuan-Thuy Nguyen is a research academic at the Department of Marine Botany, Institute of Oceanography, Vietnam Academy of Science and

Technology. She works on the taxonomy of red algae. She is also interested in gene flows and seascape of marine plants.



Anh-Duy Do

Research Institute for Marine Fisheries, 224 Le Lai, Ngo Quyen, Hai Phong, Viet Nam

Anh-Duy Do is a research academic at the Research Institute for Marine Fisheries. He was awarded a Doctor in Biology (2022) by the Research Institute for Marine Fisheries. He is a marine biologist, focusing on macroalgae biodiversity, biology, ecology and taxonomy. He has a broad experience in marine biodiversity and conservation surveys in the South China Sea. He has

participated in more than 10 research projects on the marine environment, and participated in numerous symposia and workshops.



Karla J. McDermid

Marine Science Department, University of Hawaii-Hilo, 200 W. Kawili St., Hilo, HI 96720, USA
mcdermid@hawaii.edu

Karla J. McDermid came to the Hawaiian Islands in 1982 as a graduate student to work on *Laurencia* species with Dr. Izzie Abbott, and she never left. Now a Professor of Marine Science at the University of Hawai'i at Hilo, Karla teaches and encourages young scientists to study Hawaiian seaweeds.