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NEW RECORD OF *Lobophora rosacea* (Dictyotales, Phaeophyceae) FROM BACH LONG VI ISD. HAI PHONG PROVINCE, VIETNAM

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ABSTRACT

Lobophora rosacea is newly reported from Bach Long Vi Island, Hai Phong province, Vietnam. Our specimens are very similar to *L. rosacea* recently described from New Caledonia and Hainan Island, China, not only in morphology but also in *rbcL* gene sequences. The plant grows clustered and attached to the substrate by a basal holdfast. Thallus has fan-shaped to circular, up to 10 cm wide and 7 cm tall, 117–123 µm thick, lax, predominantly erect, light orange. Thalli are attached to the substratum by basal rhizoids on the ventral surface. Thallus margin entire and composed of single cell layered medulla, 14.88 µm (11.28 - 19.16 µm) wide, 35.34 µm (31.49 - 38.74 µm) high, 82.39 µm (75.76 - 88.72 µm) long; three cell layered cortex on the dorsal, 9.85 µm (5.96 - 14.92 µm) wide, 8.03 µm (5.99 - 10.00 µm) high and ventral side respectively, 12.20 µm (8.79 - 15.14 µm) wide, 9.52 µm (8.10 - 12.14 µm) high. Reproductive structures are undetected.

Keywords: Bach Long Vi Island, *Lobophora rosacea*, molecular phylogeny, morphological, *rbc*L.

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INTRODUCTION

Lobophora is a common tropical to temperate genus of brown algae found in a plethora of habitats, including shallow and deep-water coral reefs, rocky shores, mangroves, seagrass beds, and rhodolith beds (Vieira, 2020a). The genus is characterized by a marginal row of meristematic cells and a single-layered large central medulla (Womersley, 1967, 1987). Because it is difficult to identify species based on morphological criteria alone, recent research on genetic analysis has suggested that the species level divergence was considerably underestimated, and a plethora of new species were described (Sun et al., 2012; Vieira et al., 2014; Schultz et al., 2015; Vieira et al., 2016a, Vieira et al., 2016b; Camacho et al., 2019; Vieira et al., 2019a,b; Vieira et al., 2020b; Vieira et al., 2020c; Vieira et al., 2021; Vieira et al., 2023). At present, approximately 93 specific epithets of *Lobophora* are listed in AlgaeBase (Guiry & Guiry, 2024), and it is assumed that more than 100 species are represented (Vieira et al., 2017).

Taxonomic efforts are catching up with the number of molecular taxonomic units (MOTUs) identified (Vieira et al., 2016b; Vieira et al., 2017), with the number of currently accepted getting closer and closer to the number of MOTUs. A study showed that among the 13 MOTUs identified in the Bismarck Sea in Papua New Guinea, 10 have already been described.

Vietnam has a long tropical coast. However, only one species *Lobophora variegata* has been documented based on rough morphological character so far (Pham Hoang Ho, 1969; Nguyen Huu Dinh et al., 1993; Dam Duc Tien, 2004; Titlyanov & Titlyanova, 2012; Nguyen Van Tu, 2013; Phang S. M. et al., 2016). In recent years, the results of some authors have added several of the *Lobophora* species: Sun et al. (2021) (*Lobophora tsengii* D.Tien et Z.Sun); Nguyen Van Tu et al. (2021) (*Lobophora obscura* (Dickie) C.W.Vieira, De Clerck et Payri)); Do Anh Duy et al. (2022) (*Lobophora papenfussii*); Dam Duc Tien et al. (2023) (*Lobophora asiatica*, *Lobophora boussoleae*, *Lobophora lamourouxii*, *Lobophora obscura*1, *Lobophora pachyventera*, *Lobophora thailandensis*, *Lobophora quangtriensis*, *Lobophora vietnamensis*). Until 2024, the total number of *Lobophora* species recorded in Vietnam is 12 species.

Bach Long Vi is a small island located in the Gulf of Tonkin, about 130 kilometers off the northern coastal city of Hai Phong. Research results on seaweed on Bach Long Vi Island of Dam Duc Tien (1997) also indicated that there is only one species of the *Lobophora* genus, which is *L. variegata*.

In the present study, we report a new Vietnamese record species of *Lobophora rosacea* from Bach Long Vi Island, based on analysis of the *rbc*L gene sequences and morphological observations.

MATERIALS AND METHODS

Sampling and morphological analysis

New specimens were collected from three sites of Bach Long Vi Island by snorkelling, SCUBA diving, and immediately desiccated in silica gel. Some specimens were dried and mounted on herbarium sheets. All specimens were deposited in the Marine Biological Museum of the Institute of Science and Technology for Energy and Environment (ISTEE) (Table 1 & Fig. 1).

Table 1. Samples of *Lobophora* collected from Bach Long Vi Isd. Hai Phong, Vietnam

No.	Locations	Date	Coordinates	Collector	Sample symbol
1	Bach Long Vi 1	July. 14.2021	21°00’11.25”N - 107°43’57.63”E	Dam Duc Tien	IMER 210046 (3)
2	Bach Long Vi 2	May. 16.2022	20°7’43.89”N - 107°44’7.10”E	Dam Duc Tien	IMER 220042 (2)
3	Bach Long Vi 3	Oct. 4.2023	20°7’37.74”N - 107°43’7.63”E	Nguyen Quynh Nga, Dam Duc Tien, Nguyen Thi Mai Anh	IMER 230059 (6)

Figure 1. Map of the collection sites of the newly recorded species

Molecular analyses

Three samples of *L. rosacea* from voucher specimens IMER 210046, IMER 220042, and IMER 230059 were collected and dried in sealed bags with silica gel.

Total genomic DNA was extracted from the tissue samples dried in silica gel using the MagPurix® Plant DNA Extraction Kit (ZP02014) and the automated DNA extraction system MagPurix 12s Automated Nucleic Acid Purification System (Zinexts Life Science Corp., Taiwan) following the manufacturer's protocol, with a final elution volume of 100 µL. Sequences were generated for the chloroplast-encoded ribulose-1,5-bisphosphate carboxylase/oxygenase (*rbc*L) gene. The newly generated sequences were incorporated into the most recent species-level dataset for the genus *Lobophora* (Vieira et al., 2022b). Specimen-level phylogenies were generated based on the rbcL (1249 bp) sequences using Bayesian inference (BI) and maximum likelihood (ML) analyses. For phylogenetic tree reconstruction, the longest sequences from representative specimens were selected for each species and gene (*rbc*L). BI analyses were performed using MrBayes v.3.2.2 (Ronquist et al., 2012) with the GTR + I + G nucleotide substitution model, initiated with a random starting tree. Four Markov chain Monte Carlo (MCMC) chains were run simultaneously for 250 million generations, with trees sampled every 1000 generations. The first 25% of trees were discarded as burn-in, based on the stationarity of log-likelihood values assessed using Tracer v.1.7.1 (Rambaut et al., 2014). The ML phylogenetic tree was reconstructed using the IQ-TREE v.1.6.8 platform, with ultrafast bootstrapping (1000 replicates) using the GTR + F + I + G4 nucleotide substitution model. The best-fitting model was identified using ModelFinder (Nguyen et al., 2015).

It is the sequence of primers for rbc of *Lobophora rosacea*:

GGAGAATCTTCAACTGCTACATGGA
CTGTCGTTTGGACTGACTTATTAACAG
CCTGCGACATTTATCGAGCAAAAGCCT
ATCGTGTGGATCCTGTACCAGGAACAA
ACGATCAATTCTTTGCCTATGTTGCGTA
TGATTGTGACTTATTTGAAGAAGGATC
TTTAGCTAATTTAACTGCTTCGATTATC
GGAAACGTATTTGGATTCAAAGCCGTT
AAAGCTTTACGTTTAGAAGATATGAGA
ATTCCATTTGCATATTTAAAAACGTTCC
AAGGCCCAGCTACTGGGGTAATCGTAG
AGAGAGAAAGATTAGATAAATTTGGT
AAACCATTATTAGGTGCAACTGTGAAA
CCTAAATTAGGACTTTCAGGGAAGAAC
TATGGACGTGTAGTTTATGAAGGTCTA
CGAGGTGGTCTTGACTTCTTAAAGGAT
GACGAAAACATTAATTCGCAGCCTTTT
ATGCGTTGGAAAGAACGTTTTCTATAT
TGTATGGAAGGTGTAAATCGTGCTGCA
GCTGCAACAGGAGAAGTTAAAGGTTC

Morphological analyses

Morphological observations of *Lobophora* species included analyses of the external and internal structures of the specimens listed. For morphological observations, thalli were sectioned manually using a razor blade and mounted on glass slides in Karo Syrup/seawater (Vieira et al., 2014). The cross sections were cut by hand, stained in 1% acidified aniline-blue solution and mounted on glass slides in 20% Karosyrup. Microscopic photographs of cross sections were taken by using a camera (TG 50) attached to a compound microscope (Nikon YS 100), and in situ photographs were taken by an underwater camera (Olympus TG5).

RESULTS AND DISCUSSION

Molecular phylogenetic analysis

A total of three algal samples from a single species collected at Bạch Long Vĩ (IMER 210046, IMER 220042, and IMER 230059) were successfully sequenced for the *rbc*L gene, and their sequences were found to be 100% identical to other *L. rosacea*. These sequences were compared with 17 published *Lobophora* species sequences available on GenBank. A phylogenetic tree based on *rbc*L gene sequences was constructed using the Maximum-likelihood method. In the phylogenetic tree, the studied samples were positioned within *Lobophora* clades, corresponding to species described in previous studies (Vieira et al., 2016a; Vieira et al., 2022b). Phylogenetic analysis revealed that the studied samples were identical to *L. rosacea* based on comparisons with previously reported sequences. The identification was strongly supported by a high Bayesian posterior probability (PP) value, ranging from 0.99 to 1, indicating robust statistical confidence in the assignment. Furthermore, the analysis clearly distinguished *L. rosacea* from species of other genera, notably *Zonaria*, which was placed as an outgroup in the phylogenetic tree. As a result, *L. rosacea* was confirmed as a new record for Vietnam's algal flora. (Fig. 2), contributing to the biodiversity knowledge of the region's marine flora.

Morphological analysis and taxonomy

Specimens examined

Dam Duc Tien, 14th Jul, 2021 (IMER 210046 - 3); Dam Duc Tien, 26th May, 2022 (IMER 220042 -2); Nguyen Quynh Nga, Dam Duc Tien, Nguyen Thi Mai Anh, 4th Oct 2023 (IMER 230059 - 6).

Habitat: Hard substrates in the subtidal zone 2-5 m deep in the marine natural areas of Bach Long Vi Island.

Morphology

Fan-shaped thalli grow on the substrate in the subtidal zone by a basal holdfast, with a holdfast made from a mound of rhizoid. Larger ones are predominantly erect, spirally arranged, up to 10 cm wide and 8 cm high, brown dark or yellow. Numerous thalli overlap each other and form a dense rosette. Dried specimens become dark brown. The middle part of the thallus is 117–123 μm thick, composed of nine cell layers, a single layer of large medullary cells and four layers of cortical cells on the dorsal and ventral sides. Reproductive structures are undetected (Fig. 3).

Distribution in the world: Papua New Guinea, Tuvalu.

Distribution in Vietnam: Bach Long Vi Isd. 1 (21°00'11.25"N - 107°43'57.63"E), Bach Long Vi Isd.2 (20°7'43.89"N - 107°44'7.10"E) and Bach Long Vi Isd.3 (20°7'37.74"N - 107°43'7.63"E).

Habitat: growing on dead coral reefs in the subtidal zone, 2–5 m depth.

Specimens examined: Dam Duc Tien, 14th Jul, 2021 (IMER 210046 - 3); Dam Duc Tien, 26th May, 2022 (IMER 220042 -2); Nguyen Quynh Nga, Dam Duc Tien, Nguyen Thi Mai Anh, 4th Oct 2023 (IMER 230059 - 6) (Table 2).

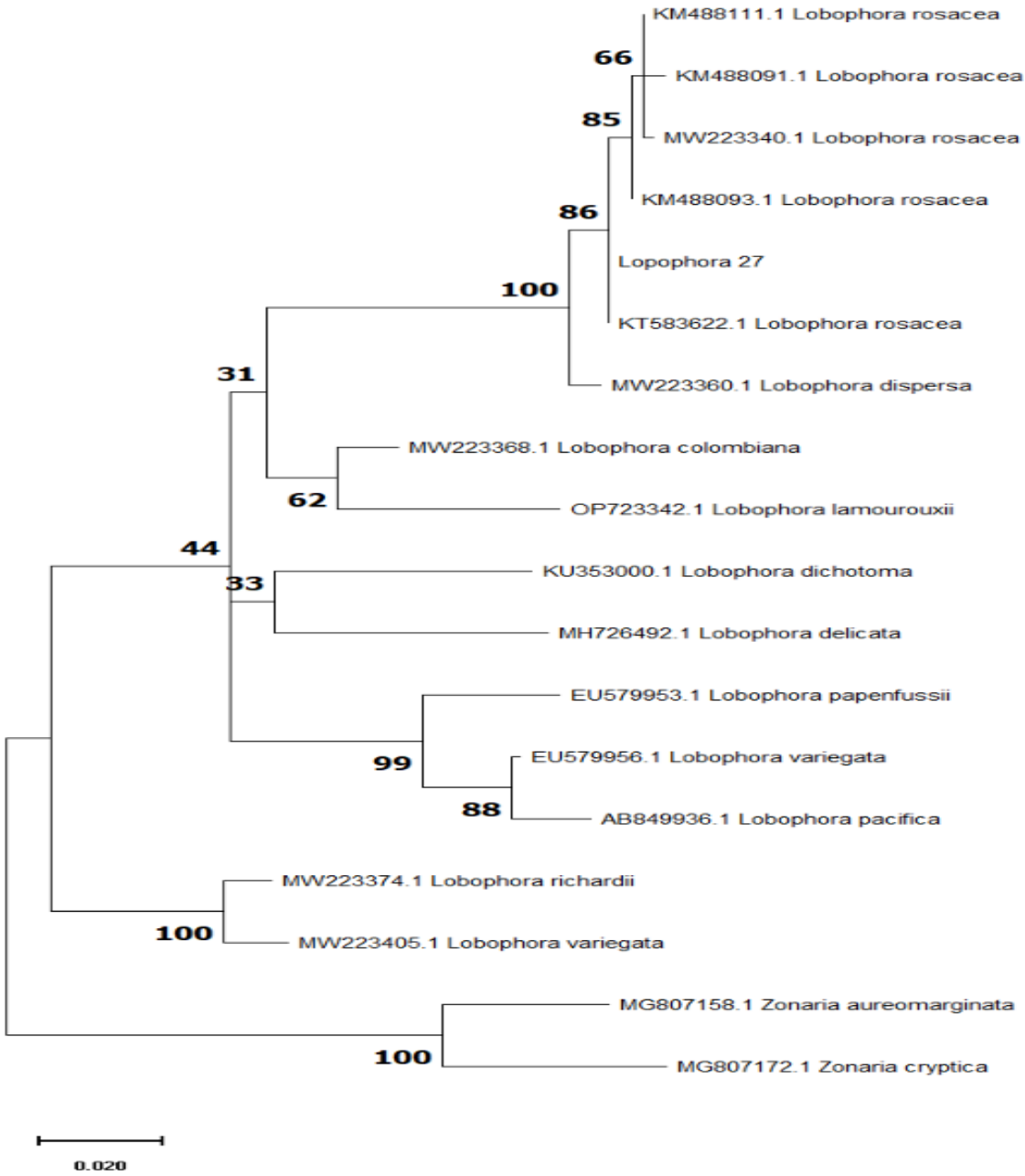

Figure 2. Maximum-likelihood tree based on *rbc*L gene sequences

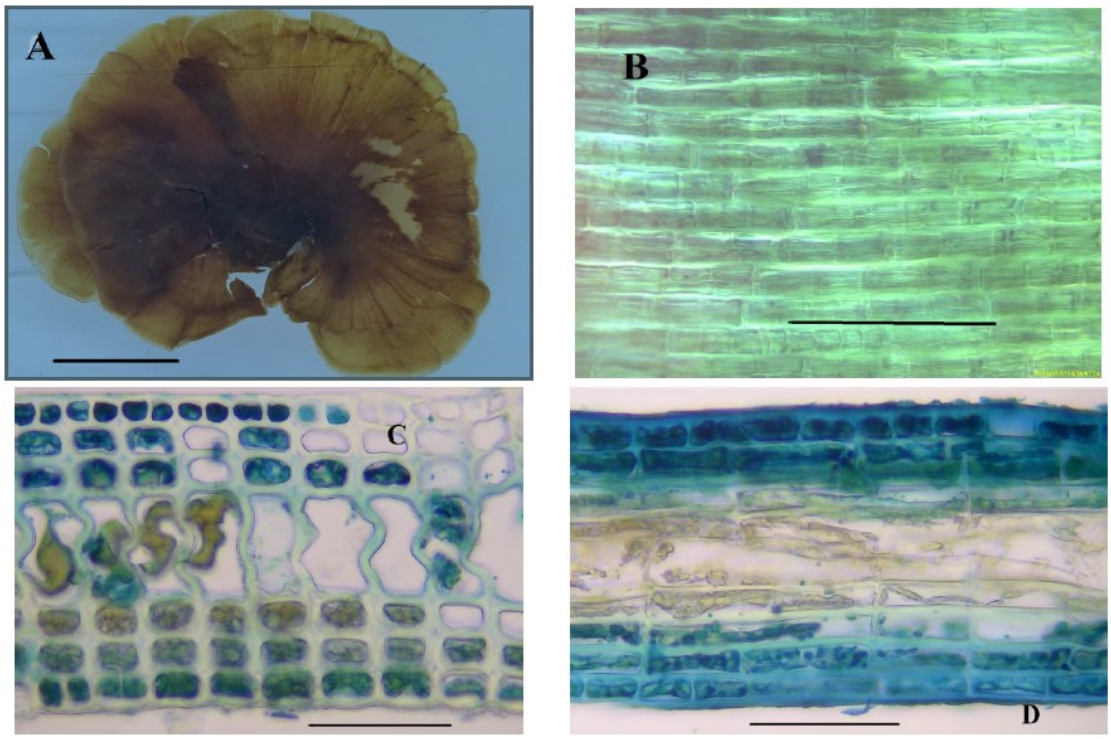

Figure 3. Morphology of *Lobophora rosaceae* C.W.Vieira, Payri & De Clerck, 2019 from Bach Long Vi island district, Hai Phong, Vietnam. A: Ex situ photographs, Scale bar = 3 cm; B: Cells from the thallus surface, Scale bar = 50 µm; C: Cross section, Scale bar = 50 µm; D: Longitudinal section, Scale bar = 50 µm

Table 2. Comparison of morphological characters between specimens from Bach Long Vi Island, Hainan Island and New Caledonia

Characters	Specimens from Bach Long Vi Island	Specimens from Hainan Island	Specimens from New Caledonia
Holdfast	Made of basal rhizoids	Made of basal rhizoids	Made of basal rhizoids
Erect or prostrate	Erect	Erect	Predominantly erect
Shape	Fan-shaped, to circular	Made of basal rhizoids	Made of basal rhizoids
Thallus width (cm)	To 10	To 9	To 10
Thallus height (cm)	To 8	To 8	To 8
Number of cell layers	7	3–5	7–9
Thickness (µm)			
Min - Max	117–123	110–165	80–170
Average value	120.65	-	-
± S.D	1.79	-	-
Dorsal cells			
Number of cell layers	3	3-4	3 - 4
Width (µm)			
Min - Max	5.96–14.92	-	-
Average value	9.85	-	-

Characters	Specimens from Bach Long Vi Island	Specimens from Hainan Island	Specimens from New Caledonia
± S.D	3.59	-	-
Height (µm)		-	-
Min - Max	5.99 - 10.00	-	-
Average	8.03	-	-
± S.D	1.33	-	-
Medulla cells			
Number of cell layers	1	1	1
Width (µm)			
Min - Max	11.28–19.16	-	-
Average value	14.88	-	-
± S.D	2.41	-	-
Height (µm)			
Min - Max	31.49–38.74	-	-
Average	35.34	-	-
± S.D	2.21	-	-
Length (µm)			
Min - Max	75.76–88.72	-	-
Average	82.39	-	-
± S.D	4.55	-	-
Ventral cells			
Number of cell layers	3	3–4	2–4
Width (µm)			
Min - Max	8.79–15.14	-	-
Average value	12.20	-	-
± S.D	1.98	-	-
Height (µm)		-	-
Average value	9.52	-	-
± S.D	1.50	-	-
Min - Max	8.10–12.14	-	-
Coloration	Brown dark or yellow	Brown	Light to dark brown
Habitat	Growing in dead coral reefs in the subtidal zone, 2–5 m depth	Growing in shallow lagoon, attached within branched corals or substratum	Growing in dead coral reefs in the subtidal zone, 4–5 m depth
Diameter of sporangia (µm)	Unknown	60–75	Unknown
References	This study	Sun et al. (2017)	Vieira et al. (2014)

DISCUSSION

Morphologically, the Vietnamese specimens are similar to those from Chinese and New Caledonian in an erect, fan-shaped thallus and a basal mound of rhizoids. However, the thalli from Vietnam and China are thicker than those from New Caledonia, because of one or two cortical layers being reduced in some specimens from the Southern Hemisphere (Table 2). Vieira et al. (2014) documented two *L. rosacea* morphotypes,

including the thinner type with less than seven-cell-layers and the thicker type with nine cell layers. Thus, the newly discovered *L. rosacea* species in Bach Long Vi is the first morphological type.

Moreover, it seems hard to identify *L. rosacea*, *L. dispersa* and *L. challengeriae* without molecular data. Reproductive structures have not been found on the surface of our Vietnamese specimen, probably due to being out of reproductive season. Sun et al. (2017) collected specimens in spring, while the present specimens were collected in summer.

It is the first time that *L. rosacea* is described from Bach Long Vi Island in the center of Tonkin Gulf. To date, eleven species of *Lobophora* were reported from the BIEN DONG (South China Sea), including *L. asiatica*, *L. boussoleae*, *L. lamourouxii*, *L. obscura* (= *L. crassa*), *L. pachyventera*, *L. papenfussii*, *L. thailandensis*, *L. quangtriensis*, *L. tsengii*, *L. variegate*, *L. vietnamensis* and *L. rosacea*. (Dam et al., 2023).

CONCLUTION

Using morphological methods combined with molecular biology, we have identified a Lobophora species *L. rosacea*. This is a new record species for Vietnam's algal flora.

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