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Two newly recorded species of *Lobophora* (Dictyotales, Phaeophyceae) for Viet Nam

Nguyen Quynh Nga[1,*], Dam Duc Tien[2,3,4,*], Christophe Vieira[5]

[1]*National Institute of Medicinal Materials (NIMM), 3B Quang Trung street, Cua Nam ward, Hanoi, Viet Nam*
[2]*Institute of Marine Environment and Resources (IMER), Vietnam Academy of Science and Technology (VAST), 246 Da Nang street, Ngo Quyen ward, Hai Phong city, Viet Nam*
[3]*Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet street, Nghia Do ward, Ha Noi, Viet Nam*
[4]*Hai Phong University of Medicine and Pharmacy, 72A Nguyen Binh Khiem street, Gia Vien ward, Hai Phong city, Viet Nam*
[5]*Research Institute of Basic Science, Jeju National University, Jeju 63243, Korea*

[*]Emails: *nguyenquynhnganimm@gmail.com; tiendd@imer.vast.vn*

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Abstract. *Lobophora providenceae* C.W.Vieira and *Lobophora challengeriae* C.W.Vieira are newly recorded from Co To island, Quang Ninh province, Viet Nam. Collected specimens are similar to *Lobophora providenceae* C.W.Vieira and *Lobophora challengeriae* C.W.Vieira recently described from Bismarck Sea (Papua New Guinea), not only in morphology but also in *rbc*L and *cox*3 gene sequences. The *Lobophora providenceae:* Thallus flabellate shaped, predominantly ruffled, dark brown, weakly attached to the hard substratum. The Thallus base is narrow, attached to the substratum by a basal rhizoids-margin entire. The Thallus is 91.2 – 93.1 µm thick, composed of 5 cell layers (single-cell layered medulla, two-cells layered cortex on the dorsal side, and one or two-cells layered cortex on the ventral side); Reproductive structures are undetected. *Lobophora challengeriae*: Thallus flabellate shaped, predominantly ruffled, light to dark brown, base narrow, and attached to the substratum by basal rhizoids. The thickness of the thallus is 108.3 – 115.9 µm and composed of 8–9 cell layers including four cell layers on the dorsal side, single-cell layer medulla, and three or four cells layer on the ventral side. Both species were found on dead coral reefs in the subtidal zone in Co To. Reproductive structures were undetected.

Keywords: *Lobophora*, Vietnam Algal flora, new record, morpho-genetic taxonomy; *rbc*L; *cox*3.

Classification numbers: 3.1.2, 3.4.4.

1. 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 rhodoliths beds [1]. The genus is characterized by a marginal row of meristematic cells and a single-layered large central medulla [2]. 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 [3-14]. At present, approximately 93 specific epithets of *Lobophora* are listed in AlgaeBase [15], and it was assumed that more than 100 species are present [16].

Taxonomic efforts are catching up with the number of molecular taxonomic units (MOTUs) identified [7, 16], 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.

Viet Nam has a long tropic coast, however, only one species *Lobophora variegata* has been documented based on rough morphological character so far [17-22]. In recent years, the results of some authors have added several of *Lobophora* species: [23] (*L. tsengii* D.Tien et Z.Sun); [24] (*L. obscura* (Dickie) C.W.Vieira, De Clerck et Payri); [25] (*L. papenfussii*); [26] (*L. asiatica*, *L. boussoleae*, *L. lamourouxii*, *L. obscura*1, *L. pachyventera*, *L. thailandensis*, *L. quangtriensis*, *L. vietnamensis*). Until 2024, the total number of *Lobophora* species recorded in Viet Nam is 12 species.

In the present study, we report two newly recorded species of *Lobophora* (Dictyotales, Phaeophyceae) from Co To island, Quang Ninh province, Viet Nam based on analysis of the *rbc*L and *cox*3 gene sequences and morphological observations.

2. MATERIAL AND METHOD

2.1. Sampling and morphological analysis

Samples of *Lobophora* were collected during a sampling campaign led by the Vietnam Academy of Science and Technology (VAST), in 2018, 2022, 2023 and 2024 in Co To (Co To 1, Co To 2, Thanh Lan 1, Thanh Lan 2, Thanh Lan 3, Co To con and Hon Ca Chep) (Table 1 and Figure 1).

A total of 15 specimens were collected by SCUBA diving. Seaweed material was preserved in silica gel for molecular analyses, and each specimen was mounted for a herbarium. Herbarium specimens were deposited in the Marine Biological Museum of the Institute of Marine Environment and Resources (IMER) – VAST at Hai Phong city, Viet Nam.

Table 1. Samples of *Lobophora* collected from Co To island district, Quang Ninh province, Viet Nam.

N	Locations	Date	Coordinates	Collector	Sample symbol
1	Co To 1	Mar. 14, 2019	21° 00'11.25"N - 107°46'33.93"E	Dam Duc Tien	IMER190036 (2)
2	Co To 2	Mar. 16, 2019	20°57'31.33"N - 107°45'48.65"E	Pham Ngoc Lam	IMER190042 (4)
3	Thanh Lan 1	Oct. 22, 2022	20°58'51.92"N - 107°48'22.48"E	Dam Duc Tien	IMER2200277 (3)
4	Thanh Lan 2	Oct. 22, 2022	21° 00'52.77"N - 107°48'57.68"E	Nguyen Van Minh	IMER220084 (6)
5	Thanh Lan 3	Jun. 5, 2023	21° 02'24.25"N - 107°51'0.22"E	Dam Duc Tien	IMER230093 (2)
6	Co To con	Jun, 3, 2023	21° 02'03.51"N - 107°46'19.60"E	Pham Van Chien	IMER230092 (5)

7	Hon Ca Chep	Jun. 6, 2023	21° 03'00.15"N - 107°45'14.45"E	Dam Duc Tien	IMER230098 (2)

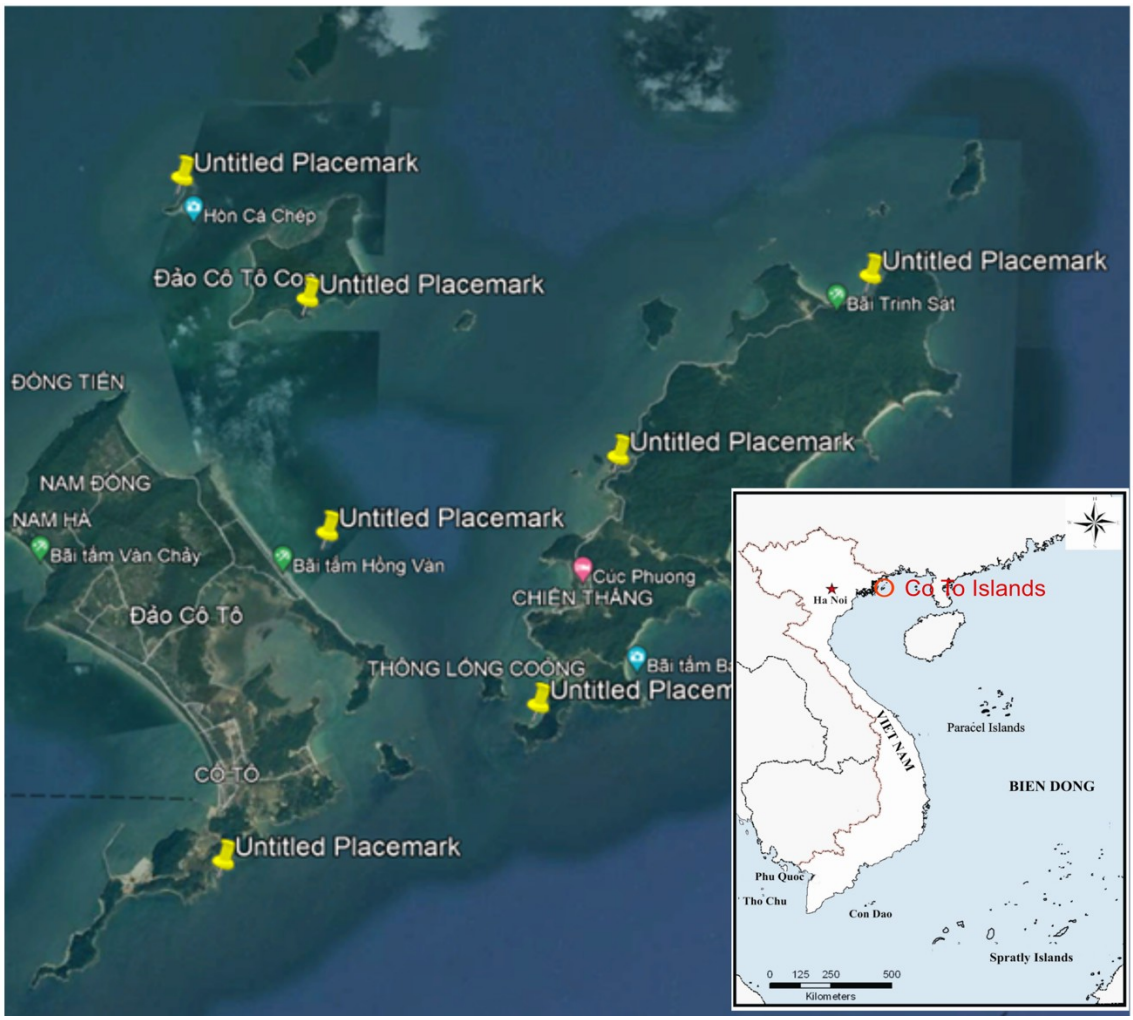


Figure 1. Map of the collection sites of the two newly recorded species.

2.2. Molecular analyses

Two samples of the two *Lobophora* species (Vietnam4-D5910 from voucher specimen IMER190042 and Vietnam5-D5911 from voucher specimen IMER220084) were collected and dried in sealed bags with silica-gel. Total genomic DNA was extracted from tissue samples using the MagPurix® Plant DNA Extraction Kit (ZP02014) and automated DNA extraction by MagPurix 12s Automated Nucleic Acid Purification System (Zinexts Life Science Corp. Taiwan); with a final elution volume 100 µL sequences were generated for the cox3 and *rbc*L genes. Generated sequences were added to the species level dataset for the genus *Lobophora* [14]. Specimen level phylogenies were generated based on *cox*3 (724 bp) and *rbc*L (1249 bp) sequences individually using Bayesian inference (BI) and maximum likelihood (ML) analyses. For the reconstruction of the phylogenetic tree, the longest sequences from representative specimens were selected for each species and each gene (cox3, *rbc*L). The BI analyses were run using MrBayes v.3.2.2 [27], with the implementation of the GTR + I + G nucleotide substitution model, initiated

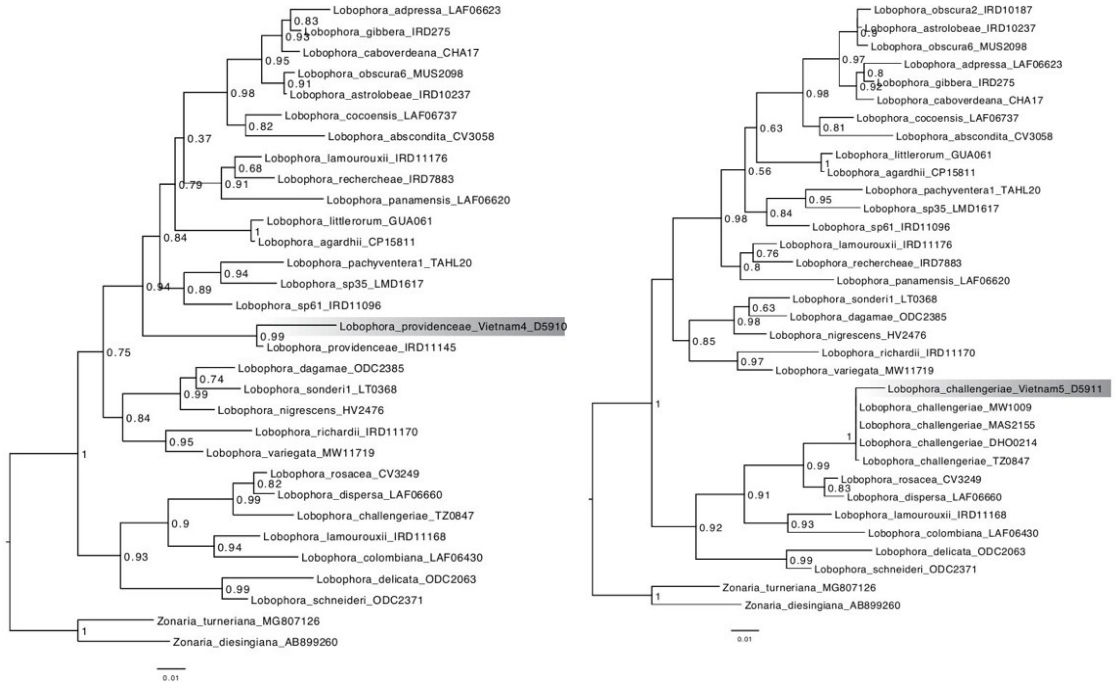
with a random starting tree and four chains of Markov chain Monte Carlo iterations simultaneously for 250.000.000 generations, keeping one tree every 1000 generations. The first 25% of trees were discarded as burn-in, based on the stationarity of log-likelihood as assessed using Tracer v.1.7.1 [28]. The ML phylogenetic tree was reconstructed using online IQ-TREE v.1.6.8 platform under ultrafast bootstraps with 1000 replicates [29] under the GTR + F + I + G4 nucleotide substitution model.

2.3. Morphological analyses

Lobophora samples (listed in Table 1) were analyzed for external and anatomical structures. Thalli were transverse and longitudinal sectioned using a razor blade. The cutting slides were stained in 1% acidified aniline-blue solution and mounted on glass slides in 20% Karosyrup [4]. Microscopic photographs of cutting slides were taken by using a camera (TG 50) attached to a compound microscope (Nikon YS 100), in situ photographs were taken by an underwater camera (Olympus TG5).

3. RESULTS AND DISCUSSION

3.1. Molecular phylogenetic analysis



A: Lobophora providenceae C.W.Vieira B: Lobophora challengeriae C.W.Vieira

Figure 2. Maximum-likelihood tree based on *rbc*L and *cox*3 gene sequences.

Sequences of *rbc*L and *cox*3 genes were generated from samples of two species collected in Co To (Vietnam4-D5910, Vietnam5-D5911). The Maximum-likelihood tree based on *rbc*L and *cox*3 gene sequences is presented in Figure 2. On the phylogenetic tree, studied samples are positioned in *Lobophora* groups equating to species in line with previous studies [7, 14].

Vietnam5-D5910 and Vietnam5-D5911 are respectively identical to *Lobophora providenceae* and *Lobophora challengeriae* samples in previous studies with high value of Bayesian PP (0.99 – 1 respectively). As a result, two new records have been identified for the algal flora of Viet Nam: *Lobophora providenceae* C.W.Vieira and *Lobophora chanlengeriae* C.W.Vieira.

3.2. Morphological analysis and taxonomy

***Lobophora providenceae* C.W.Vieira, 2019. (Figure 3)**

Morphological observations: Thallus flabellate shaped, up to 3.5 cm wide and 3.2 cm tall, predominantly ruffled, dark brown, weakly attached to the hard substratum. Thallus base narrow, attached to the dead coral by a basal rhizoids, margin entire. The Thallus 91.2 -93.1 µm thick, 4 - 5 cell layers including single-cell layered medulla (cell size: 20.2 – 28.5 µm width x 31.3 - 38.9 µm height x 67.5 – 81.1 µm length); 2 cell layered cortex on the dorsal side (cell size: 6.3 – 21.5 µm width x 8.9 – 10.2 µm height) and 1-2 cell layered cortex on the ventral side (cell size: 18.7 – 26.2 µm width x 10.2 – 11.0 µm height).

Distribution in the world: Papua New Guinea, Tuvalu, Viet Nam.

Distribution in Viet Nam: Co To 1 Isd. (21° 00'11.25"N - 107°46'33.93"E), Co To 2 Isd. (20°57'31.33"N - 107°45'48.65"E); Hon ca Chep Isd. (21° 03'00.15"N - 107°45'14.45"E) (Co To island district, Quang Ninh province, Viet Nam).

Habitat: Growing on dead coral reefs in the subtidal zone, 4-5 m depth.

Specimens examined: Co To Isd. 1 (IMER 190036) Dam Duc Tien, Co To Isd. 2 (IMER 190042) Pham Ngoc Lam and Hon ca Chep Isd. (IMER2300 98) Dam Duc Tien (Table 2).

Lobophora challengeriae C.W.Vieira (Figure 4)

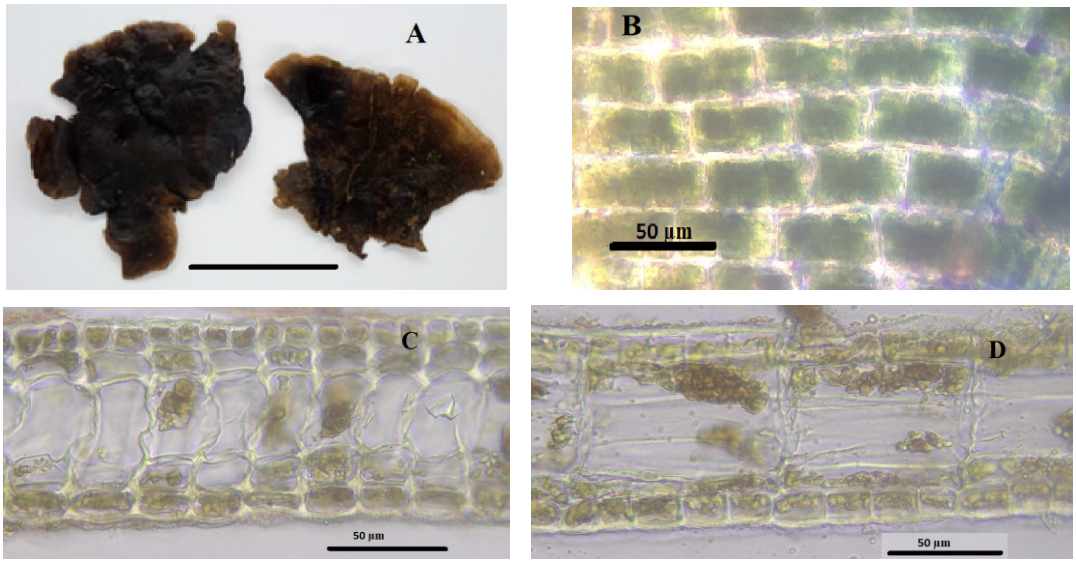
Morphological observations: Thallus flabellate shaped, up to 3.8 cm wide and 3.4 cm tall, predominantly ruffled, light to dark brown in color. The Thallus base narrow, attached to the substratum by a basal rizhoids, margin entire. The Thallus 108.3 -115. 9 µm thick, 8 - 9 cell layers including single-cell layered medulla (cell size: 19.7 – 24.2 µm width x 25.8 - 31.8 µm height x 63.6 – 77.3 µm length); 4 cell layered cortex on the dorsal side (cell size: 9.1 – 22.7 µm width x 7.0 – 10.9 µm height) and 3 - 4 cell layered cortex on the ventral side (cell size: 7.9 – 22.8 µm width x 7.5 – 10.6 µm height).

Distribution in the world: Papua New Guinea, Tuvalu, Viet Nam.

Distribution in Viet Nam: Thanh Lan 1 Isd. (20°58'51.92"N - 107°48'22.48"E), Thanh Lan 2 Isd.(21° 00'52.77"N - 107°48'57.68"E), Thanh Lan 3 Isd.(21° 02'24.25"N - 107°510'0.22"E), Co To con Isd. (21° 02'03.51"N - 107°46'19.60"E). (Co To island, Quang Ninh province, Viet Nam).

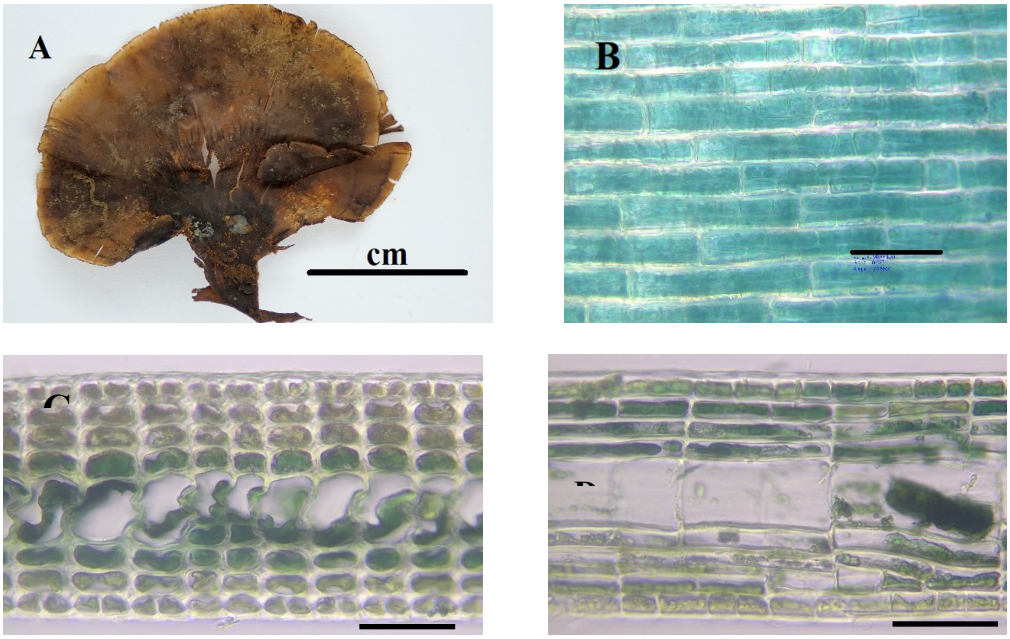
Habitat: Growing on dead coral reefs in the subtidal zone, 4-5 m depth.

Specimens examined: Thanh Lan 1 Isd. (IMER2200277) Dam Duc Tien, Thanh Lan 2 Isd. (IMER220084) Nguyen Van Minh, Thanh Lan 3 Isd. (IMER230093 (2)) Dam Duc Tien and Co To con Isd. (IMER2300 92) Pham Van Chien (Table 2).



A. *Ex situ* photographs, Scale bar = 2 cm; B. Cells from the thallus surface; C. Thallus transverse section; D. Thallus longitudinal section

Figure 3. Morphology of *Lobophora providenceae* C.W.Vieira from Co To island.



A. *Ex situ* photographs, Scale bar = 1 cm; B. Cells from the thallus surface; C. Thallus transverse section; D. Thallus longitudinal section

Figure 4. Morphology of *Lobophora challengeriae* C.W.Vieira, 2019 from Co To island.

Table 2. Comparison of some anatomical, morphological, and ecological features between studied samples collected from Co To with the original descriptions.

Character	Species			
	Lobophora providenceae		*Lobophora challengeriae*	
	Studied samples	[10]	Studied samples	[10]
Thallus				
Number of cell layers	4 - 5	3 - 5	8 - 9	6 - 10
Thickness (µm)				
Average value	92.3		112.1	
± S.D	6.2		2.5	
Min - Max	91.2 – 93.1	70 - 112	108.3 - 115.9	106 - 188
Dorsal cells				
Number of cell layers	2	1 - 2	3 - 4	3 - 5
Width (µm)		-		-
Average value	15.1		17.2	
± S.D	6.7		7.2	
Min - Max	6.3 - 21.5		9.1 - 22.7	
Height (µm)		-		-
Average	9.4		9.1	
± S.D	0.7		1.4	
Min - Max	8.9 - 10.2		7.0 - 10.9	
Medulla cells				
Number of cell layers	1	1	1	1
Width (µm)		-		-
Average value	22.9		21.9	
± S.D	3.8		1.9	
Min - Max	20.2 - 28.5		19.7 – 24.2	
Height (µm)		-		-
Average	34.6		28.4	
± S.D	3.8		2.6	
Min - Max	31.8 - 38.9		25.8 - 31.8	
Length (µm)		-		-
Average	73.9		70.5	
± S.D	5.6		5.9	
Min - Max	67.5 – 81.1		63.6 - 77.3	
Ventral cells				
Number of cell layers	1 - 2	1 - 2	3 - 4	2 - 4
Width (µm)		-		-
Average value	21.9		15.2	
± S.D	3.4		5.9	
Min - Max	18.7 - 26.2		7.9 - 22.8	
Height (µm)		-		-
Average value	10.4		8.7	
± S.D	0.41		1.5	
Min - Max	10.2 - 11.0		7.5 - 10.6	
Coloration	Brown	Brown	Light to dark brown	Light to dark brown

Habitat	Growing in dead coral reefs in the subtidal zone, 4-5m depth	Growing in shallow lagoon, attached within branched corals or substratum	Growing in dead coral reefs in the subtidal zone, 4-5m depth	Growing in shallow waters near mangrove habitat attached to the substratum

3.3. Discussion

The specimens of *Lobophora providenceae* and *L. challengeriae* collected from Co To are similar to those from the Bismarck Sea [8] in morphology (flabellate shaped thallus, base narrow, attached to the substratum by a basal rizhoids, margin entire) as well as *cox*3 and *rbc*L sequences. Both species were found on dead coral reefs in the subtidal zone in Co To. Reproductive structures have not been observed from collected specimens, probably because they are not being in reproductive season.

It is the first time *Lobophora providenceae* C.W.Vieira and *Lobophora challengeriae* C.W.Vieira are recorded from Co To island, Quang Ninh province, Viet Nam. To date, fourteen species of *Lobophora* were reported from Viet Nam, including *L. variegata* (J.V.Lamouroux) Womersley ex E.C.Oliveira; *L. tsengii* D.Tien et Z.Sun, *L. obscura* (Dickie) C.W.Vieira, De Clerck et Payri, *L. papenfussii* (W.R.Taylor) Farghaly, *L. asiatica* Z.Sun, Ji.Tanaka & H.Kawai, *L. boussoleae* C.W.Vieira & Payri, *L. lamourouxii* Payri & C.W.Vieira, *L. obscura* (Dickie) C.W.Vieira, De Clerck & Payri, *L. pachyventera* Z.Sun, P.-E.Lim, Ji.Tanaka & H.Kawai, *L. thailandensis* A.M.Klomjit and C.W.Vieira, *L. quangtriensis* D.T.Dam, M.L.Nguyen et C.W.Vieira, *L. vietnamensis* D.T.Dam, M.L.Nguyen et C.W.Vieira, *L. providenceae* C.W.Vieira and *L. challengeriae* C.W.Vieira.

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CRediT authorship contribution statement. Nguyen Quynh Nga: Methodology, Investigation, Morphological and DNA barcode analysis. Dam Duc Tien: Investigation, Morphological analysis Formal analysis, Funding acquisition, Supervision. Christophe Vieira: Formal analysis, DNA barcode analysis.

Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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