

A new species *Lobophora tsengii* sp. nov. (Dictyotales; Phaeophyceae) from Bach Long Vy (Bailongwei) Island, Vietnam*

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Received Jul. 31, 2020; accepted in principle Sep. 28, 2020; accepted for publication Nov. 30, 2020

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Abstract A new species *Lobophora tsengii* is described from Bach Long Vy (Bailongwei) Island, Haiphong Province, Vietnam. The plants inhabit the subtidal zone and have predominantly erect fan-shaped thalli that attach to the substrate by a basal holdfast. The thallus is commonly composed of a single layer of large medullary cells with four layers of cortical cells on either side of the medulla. The newly collected specimens from the island are morphologically similar to those from Hainan Island, China, but differ from the New Caledonian ones in having thicker thallus. In molecular phylogenetic analyses based on concatenated *rbcL* and *cox3* sequences, the specimens from the island were 100% identical to those from Hainan Island, China, and they formed a clade separating from other *Lobophora* species. Additionally, based on analyses of numerous *cox3* sequences, our specimens were also distinguished from the closely related taxa occurring in New Caledonia, Kenya, and West Australia. Combining the morphological and molecular analyses, we conclude that our specimens represent a new species of *Lobophora*, which is apparently endemic to the Beibu Gulf (Gulf of Tonkin) and adjacent waters.

Keyword: Bach Long Vy (Bailongwei) Island; *cox3*; *Lobophora*; molecular phylogeny; morphology; *rbcL*

1 INTRODUCTION

The brown alga *Lobophora* (Dictyotales, Phaeophyceae) occurs in tropical and subtropical seas around the world. The genus is characterized by a marginal row of meristematic cells and a single-layered large central medulla in the thallus (Womersley, 1967, 1987). It is difficult to identify species based on morphological criteria alone, and the recent molecular phylogenetic analyses suggest that the species level divergence is considerably underestimated; as consequence, a dozen new species have been described (Sun et al., 2012; Vieira et al., 2014, 2016, 2019; Schultz et al., 2015; Camacho et al., 2019). So far, 45 specific epithets of *Lobophora*

are listed in AlgaeBase (Guiry and Guiry, 2020), and it is estimated that more than 100 species are present in this genus (Vieira et al., 2017).

Previously, a single species of *Lobophora*, i.e. *L. variegata* (J. V. Lamouroux) Womersley ex Oliveira, was reported from Bach Long Vy (Bailongwei) Island, a small island located in the

* Supported by the Vietnam Ministry of Science and Technology (No. KC.09.23/16-20), the Scientific Research Activities for Senior Researchers in 2021 (No. NCVCC23.05/21-21), the Chinese National Projects, the Science and Technology Basic Resources Investigation Program of China (No. 2018FY100205), and the Strategic Priority Research Program of the Chinese Academy of Sciences (No. XDA23050304)

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center of the Beibu Gulf (Gulf of Tonkin) (Dam, 1997). This species with erect fan-shaped from Vietnam was identified as *L. variegata* using gross morphological characters (Pham, 1969; Nguyen et al., 1993; Dam, 1997, 2004; Van Nguyen et al., 2013); however, *L. variegata* is not geographically distributed in the Pacific (Schultz et al., 2015; Vieira et al., 2016). In recent years, some new species have been reported from Vietnam and the neighbor waters (Sun et al., 2012; Phang et al., 2016; Vieira et al., 2016), and therefore in the present study, we conducted a taxonomic study of *Lobophora* found in Gulf of Tonkin, Vietnam, using morphological and molecular data.

2 MATERIAL AND METHOD

2.1 Sampling and morphological analysis

New specimens were collected by snorkeling or Self-Contained Underwater Breathing Apparatus (SCUBA) diving from three sites (site 1: 20°08'15"N, 107°43'36"E; site 2: 20°08'01"N, 107°44'06"E; site 3: 20°07'39"N, 107°44'03"E) around Bach Long Vy (Bailongwei) Island (Fig.1). Specimens for molecular studies were desiccated immediately in silica gel. Other specimens were dried and mounted on herbarium sheets. All the herbaria were deposited in the Marine Biological Museum of the Institute of Marine Environment and Resources (MBMIMER), Haiphong, Vietnam. For morphological observations, thalli were sectioned manually using a razor blade and mounted on glass slides in Karo Syrup/seawater. Photographs were taken with a Motic BA300 microscope (Taiwan, China) equipped with a ZEISS A-Plan 40X/0, 65 N. A. Phase contrast 2 objective (Zeiss, Germany) and an Olympus TG 5 digital camera (Japan).

2.2 DNA sequencing and phylogenetic analysis

Genomic DNA was extracted from the silica gel-dried specimens and herbarium specimens (morphology analyzed later) using a DNeasy® Plant Mini Kit (Qiagen, Hiden, Germany), according to the manufacturer's instructions. The *rbcL* and *cox3* genes were PCR amplified with TaKaRa Ex Taq enzyme in 25- μ L reaction column (TaKaRa, Japan). Primers and PCR conditions were as those described by Sun et al. (2012). The DNA sequencing was performed by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China).

Eight specimens from Bach Long Vy Island were newly sequenced. The sequences of specimens from

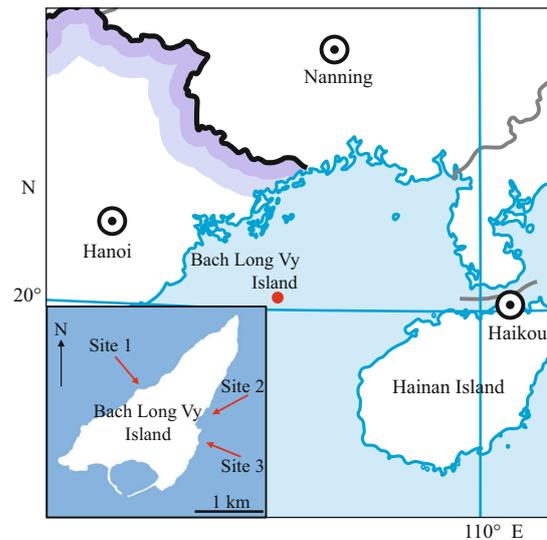


Fig.1 Map showing the collection sites around Bach Long Vy (Bailongwei) Island (red arrows) in the present study

Map review No. GS(2019)1679.

Hainan Island (Sun et al., 2012) were also added to analyze. A concatenated data set consisting of 24 *rbcL* and 24 *cox3* sequences from GenBank, respectively corresponding to 21 specimens, included 15 species of *Lobophora* and two species of *Zonaria* (outgroup). A second *cox3* data set consisting of sequences from 47 specimens of *L. rosacea*, 6 specimens of *Lobophora* sp. 43 (Vieira et al., 2017, 2019), and 3 specimens of the new species in this study.

The concatenated nucleotide matrices were generated with PhyloSuite (Zhang et al., 2020). The new sequences and the previously published ones for phylogenetic analyses were aligned with MAFFT v7.313 (Katoh and Standley, 2013) and then manually adjusted. The maximum likelihood (ML) and Bayesian inference (BI) analysis were carried out to construct phylogenetic trees by using the software of IQ-TREE v1.6 (Nguyen et al., 2015) and MrBayes v3.2.6 (Ronquist et al., 2012), respectively. The best substitution model for each analysis was evaluated by Partitionfinder 2.0 (Lanfear et al., 2017). For BI analysis, two independent analyses were run with four chains each for 100 million generations, with the first 25% of the resulting trees was discarded as burn-in. FigTree v.1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>) was used to visualize the trees.

3 RESULT

3.1 Molecular phylogenetic analysis

Although numerous specimens were obtained from

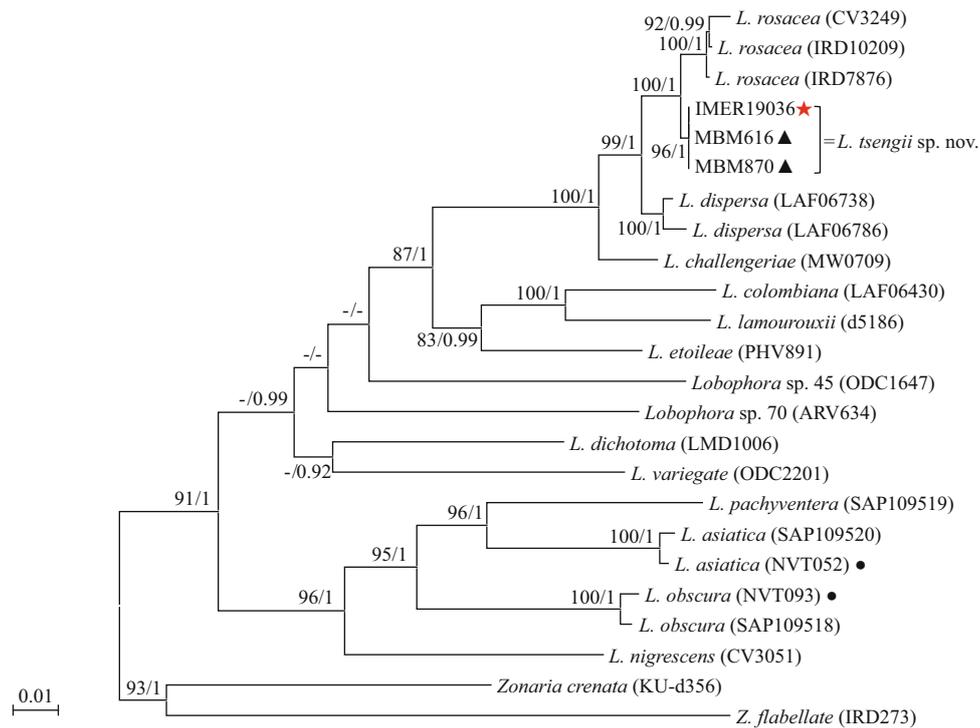


Fig.2 Maximum-likelihood tree based on *rbcL* and *cox3* gene sequences

The type specimen of *Lobophora tsengii* from Bach Long Vy Island was marked with a red star and two exemplar specimens from Hainan Island were marked with black triangles. Two other specimens from Vietnam respectively identified as *L. asiatica* and *L. obscura* were marked with black circles. Voucher numbers were added in the parentheses. Bootstrap values and posterior probabilities were indicated at branches (ML/BI), and above 80% or 0.9 were shown.

the three sites of Bach Long Vy Island and from two sides of Hainan Island (Changjiang in the west coast, Changpo in the east coast), a single identical sequence was obtained for the *rbcL* gene (1 348 bp) and the *cox3* gene (690 bp) respectively. The specimens from Bach Long Vy Island, Vietnam were 100% identical to those from Hainan Island, China, but differed from *Lobophora rosacea* Vieira, Payri et De Clerck from New Caledonia. In the phylogenetic tree based on concatenated *rbcL* and *cox3* sequences data set (Fig.2), the two species of *Zonaria* were designated the outgroup, and the remaining 13 species plus several unnamed *Lobophora* spp. formed a large *Lobophora* clade. An Atlantic species *L. dispersa* (LAF06738, LAF06786) Camacho, Freshwater et Fredericq showed the closest relation with the two taxa of *Lobophora* from Vietnam and China mentioned above.

In the phylogenetic tree based on *cox3* sequences data set (Fig.3), most specimens of *Lobophora rosacea* shared the same sequence, including the type specimens IRD10213 and one specimen from Kenya (ODC1571). In contrast, the specimens from Hainan and Bach Long Vy Islands had a long genetic distance from other ones and showed a closer relation to those identified as *Lobophora* sp. 43 from West Australia.

3.2 Morphological analysis

Fan-shaped thalli grew on the substrate in the subtidal zone, attached with a basal holdfast. Plants frequently overlapped each other and formed a dense rosette (Fig.4a). Larger thalli were predominantly erect, spirally arranged, and up to 9-cm wide and 8-cm high. Fresh specimens were yellow to light brown in color but became dark brown when dried (Fig.4b). Anatomically, the mid-region of the thallus was commonly composed of nine layers, including a single layer of large medullary cells and four layers of small cortical cells on dorsal and ventral sides (Fig.4c & d). Sporangial sori were scattered on ventral surface of the mature thallus (Fig.4e), and sporangia were sessile and ovate without paraphyses in section view (Fig.4f). Male and female reproductive structures were undetected. Some mature specimens with sporangia had seven layers, the medullary layer surrounded by dorsal and ventral layers of cortical cells with three layers (Fig.4f).

3.3 Taxonomy

Lobophora tsengii D. Tien et Z. Sun sp. nov.

=*Lobophora* sp. 67 Vieira et al. (2016)

=*Lobophora rosacea* Sun et al. (2017)

Table 1 Comparison of morphological characters among the specimens from Bach Long Vy Island, Hainan Island, and New Caledonia

Character	<i>Lobophora tsengii</i> sp. nov.		<i>L. rosacea</i>
	Specimens from Bach Long Vy Island	Specimens from Hainan Island	Specimens from New Caledonia
Holdfast	Basal rhizoids	Basal rhizoids	Basal rhizoids
Erect or prostrate	Predominantly erect	Predominantly erect	Predominantly erect
Shape	Fan-shaped, to circular	Fan-shaped, to circular	Fan-shaped, reniform to circular
Thallus width (cm)	To 9	To 9	To 10
Thallus height (cm)	To 8	To 8	To 8
Thallus thickness (μm)	90–190	110–165	80–130 or 110–170
Dorsal cortical layers' number	3–4	3–4	2–4
Ventral cortical layers' number	3–4	3–4	2–3
Diameter of sporangia (μm)	50–75	60–75	Unknown
Reference	This study	Sun et al., 2017	Vieira et al., 2014

from those of other conspecific taxa in more than 8% base substitution. The specimens of *L. obscura* Vieira, De Clerck et Payri (NVT093) and *L. asiatica* Sun, Tanaka et Kawai (NVT052), which were also present in the phylogenetic tree.

Additionally, the phylogenetic tree based on all published *cox3* sequences closely related to *L. rosacea*, *L. tsengii*, and *Lobophora* sp. 43 (Fig.3), demonstrated that *L. tsengii* was separated from the related taxa. Vieira et al. (2016, 2017) had conducted species delimitation analyses with *cox3* sequences of *Lobophora* and treated the specimen (MBM616) from Hainan Island as a separate species (*Lobophora* sp. 67), instead of merging it into *L. rosacea*. Almost simultaneously, Sun et al. (2017) reported the same specimens from Hainan Island as a population of *L. rosacea* without the references of Vieira et al. (2016, 2017). Subsequently, another closely related *Lobophora* sp. 43 was detected from Coral Bay, Western Australia based on *cox3* sequences, without morphological description (Vieira et al., 2017, 2019).

Vieira et al. (2017) suggested that most *Lobophora* species have small ranges limited to marine realms. It seems *L. rosacea* is endemic in New Caledonia, except one specimen (ODC1571) collected from Kenya, probably due to the equator warm current in the Indian Ocean. *Lobophora* sp. 43, which showed a closer genetic distance to *L. tsengii* than *L. rosacea*, may be endemic in West Australia. *L. tsengii* is distributed around Hainan Island, rare in the south coast and abundant in the west coast and Beibu Gulf. Since the morphological and molecular characters and geographic distribution of our specimens are different from the previously described species, it is reasonable to identify them as a separate species.

Considering Bach Long Vy Island where this species grows abundantly, we took this offshore Island as the type locality.

Dam (1997) had misidentified *Lobophora tsengii* as *L. variegata* based on the specimens collected from Bach Long Vy Island. Luan et al. (2013) also misidentified this species from Hainan Island as *L. variegata*. However, *L. variegata* is not distributed in this region and geographically restricted to the Caribbean Sea (Schultz et al., 2015; Vieira et al., 2016). The misidentification may be due to the morphological resemblance in the erect fan-shaped thallus. As to the species with prostrate thallus, *Lobophora* sp. reported from Nha Trang, Vietnam (Tsutsui et al., 2005) should be identified as *L. obscura* (= *L. crassa* Sun, Lim et Kawai in Sun et al., 2012), based on the distinguished morphological feature. Vieira et al. (2016) detected two prostrate species, *L. obscura* and *L. asiatica* from Vietnam based on molecular phylogenetic analyses. To date, three species of *Lobophora* were reported from Vietnam, including *L. asiatica*, *L. obscura*, and *L. tsengii* (= *Lobophora* sp. 67 in Vieira et al., 2016; = *L. rosacea* in Sun et al., 2017).

In our recent survey on the algal flora in Vietnam, several *Lobophora*-like taxa were detected, and a further investigation should be carried out based on morphological and molecular phylogenetic analyses in the future.

5 CONCLUSION

A new species of *Lobophora tsengii* was described from Bach Long Vy Island, which had been mistakenly identified as *L. variegata* and *L. rosacea* in China and Vietnam. Our study shows that *L. tsengii* is different

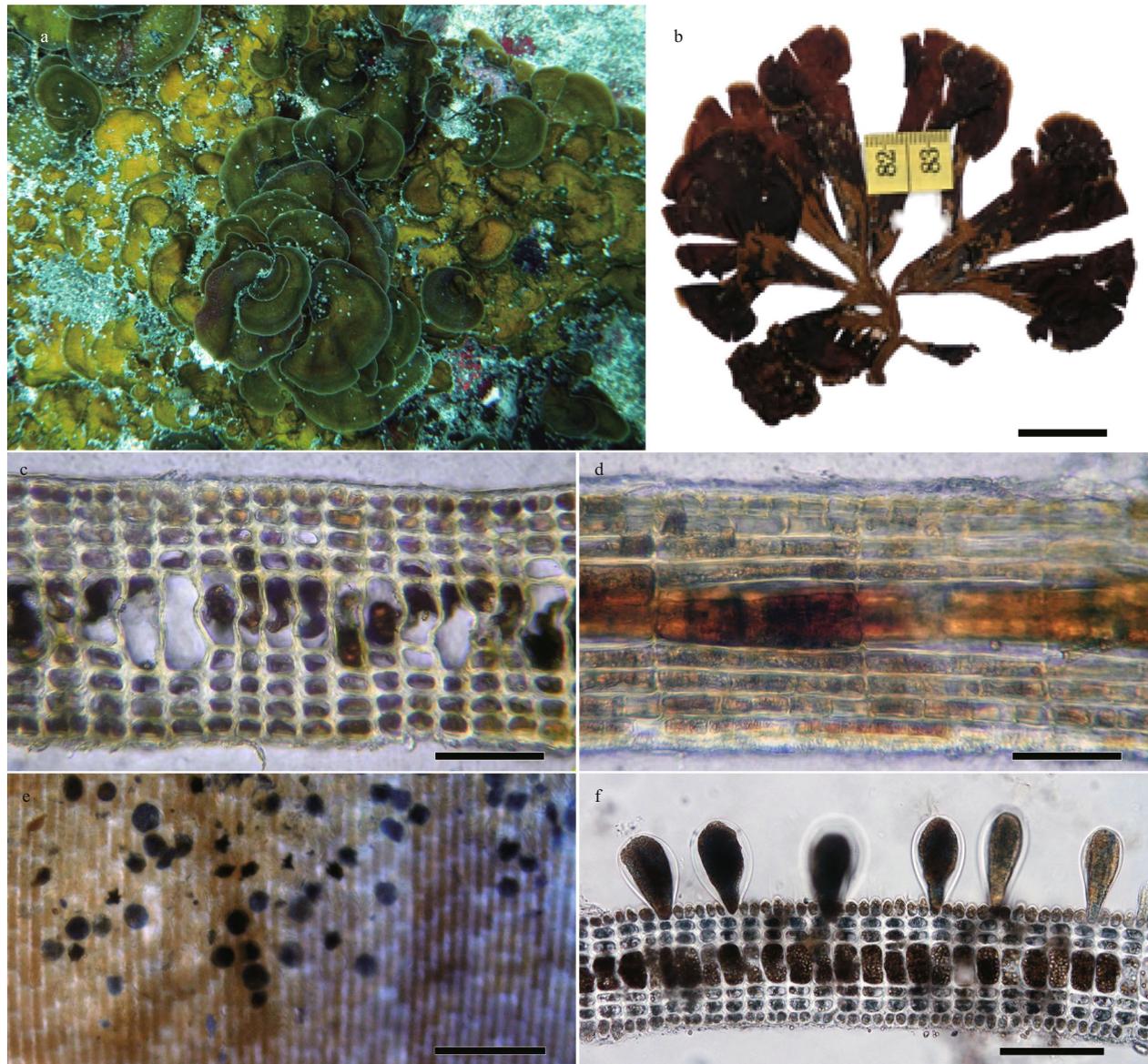


Fig.4 Morphology of *Lobophora tsengii* sp. nov. from the Bach Long Vy Island, Haiphong, Vietnam

a. plants growing underwater (Bach Long Vy Island, 14 July 2019); b. holotype specimen (IMRE 19036), showing the fan-shaped thallus and the basal holdfast (scale bar=2 cm); c–d. transverse and longitudinal sections of the middle portion of the type specimen, showing obvious medullary layer and four-layered cortex on both sides of the medulla (scale bar=30 μ m); e. Surface view of sporangial sori (scale bar=200 μ m); f. transverse section of a mature thallus, showing a three-layered cortex on both sides of medulla and sporangia without paraphyses (scale bar=100 μ m).

from the related *L. rosacea* based on both morphological and molecular data, and the later should be endemic to the Southern Hemisphere.

6 DATA AVAILABILITY STATEMENT

All datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

References

Camacho O, Fernández-García C, Vieira C, Gurgel C F D,

- Norris J N, Freshwater D W, Fredericq S. 2019. The systematics of *Lobophora* (Dictyotales, Phaeophyceae) in the western Atlantic and eastern Pacific oceans: eight new species. *Journal of Phycology*, **55**(3): 611-624.
- Dam D. 1997. Marine algae from Bach Long Vy Island. *Marine Resources and Environment J.*, IV. p.244-252.
- Dam D. 2004. Species composition and distribution of marine algae from the North of Vietnam. *In: Proceeding of Workshop on Natural Environment, Sustainable protection and Conservation. Italy-Vietnam Cooperation Perspective.* 2004. p.85-101.
- Guiry M D, Guiry G M. 2020. AlgaeBase. Worldwide electronic publication. National University of Ireland,

- Galway, <http://www.algaebase.org>. Accessed on 2020-09-21.
- Katoh K, Standley D M. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution*, **30**(4): 772-780.
- Lanfear R, Frandsen P B, Wright A M, Senfeld T, Calcott B. 2017. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution*, **34**(3): 772-773.
- Luan R, Ding L, Lu B, Tseng C K. 2013. Flora Algarum Marinarum Sinicarum Tomus III Phaeophyta No. I (1): Ectocarpales Ralfsiales Sphaecariales Dictyotales. Science Press, Beijing.
- Nguyen H, Huynh Q, Tran N, Nguyen V. 1993. Marine macroalgae (In the North Vietnam). Science and Technology Publisher, Hanoi.
- Nguyen L T, Schmidt H A, von Haeseler A, Minh B Q. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution*, **32**(1): 268-274.
- Pham H. 1969. Marine Algae of South Vietnam. Saigon Learning Resource Center, 558p.
- Phang S M, Yeong H Y, Ganzon-Fortes E T, Lewmanomont K, Prathep A, Hau L N, Gerung G S, Tan K S. 2016. Marine algae of the South China Sea bordered by Indonesia, Malaysia, Philippines, Singapore, Thailand and Vietnam. *Raffles Bulletin of Zoology*, **34**: 13-59.
- Ronquist F, Teslenko M, van der Mark P, Ayres D L, Darling A, Höhna S, Larget B, Liu L, Suchard M A, Huelsenbeck J P. 2012. MrBayes 3.2: efficient bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, **61**(3): 539-542.
- Schultz N E, Lane C E, Le Gall L, Gey D, Bigney A R, de Reviers B, Rousseau F, Schneider C W. 2015. A barcode analysis of the genus *Lobophora* (Dictyotales, Phaeophyceae) in the western Atlantic Ocean with four novel species and the epitypification of *L. variegata* (J.V. Lamouroux) E. C. Oliveira. *European Journal of Phycology*, **50**(4): 481-500.
- Sun Z M, Hanyuda T, Lim P E, Tanaka J, Gurgel C F D, Kawai H. 2012. Taxonomic revision of the genus *Lobophora* (Dictyotales, Phaeophyceae) based on morphological evidence and analyses *rbcL* and *cox3* gene sequences. *Phycologia*, **51**(5): 500-512.
- Sun Z M, Wang Y Q, Yan P C, Guo H, Yao J T, Tanaka J, Kawai H. 2017. New record of *Lobophora rosacea* (Dictyotales; Phaeophyceae) from the South China Sea. *Chinese Journal of Oceanology and Limnology*, **35**(1): 192-197.
- Tsutsui I, Huynh O, Nguyen H, Arai S, Yoshida T. 2005. The Common Marine Plants of Southern Vietnam. Japan Seaweed Association, Kochi, Japan. 250p.
- Van Nguyen T, Le N H, Lin S M, Steen F, De Clerck O. 2013. Checklist of the marine macroalgae of Vietnam. *Botanica Marina*, **56**(3): 207-227.
- Vieira C, Camacho O, Sun Z M, Fredericq S, Leliaert F, Payri C, De Clerck O. 2017. Historical biogeography of the highly diverse brown seaweed *Lobophora* (Dictyotales, Phaeophyceae). *Molecular Phylogenetics and Evolution*, **110**: 81-92.
- Vieira C, Camacho O, Wynne M J, Mattio L, Anderson R J, Bolton J J, Sansón M, D'Hondt S, Leliaert F, Fredericq S, Payri C, De Clerck O. 2016. Shedding new light on old algae: matching names and sequences in the brown algal genus *Lobophora* (Dictyotales, Phaeophyceae). *Taxon*, **65**(4): 689-707.
- Vieira C, D'Hondt S, De Clerck O, Payri C E. 2014. Toward an inordinate fondness for stars, beetles and *Lobophora*? Species diversity of the genus *Lobophora* (Dictyotales, Phaeophyceae) in New Caledonia. *Journal of Phycology*, **50**(6): 1101-1119.
- Vieira C, De Clerck O, Millet L, Payri C E. 2019. Description of ten new *Lobophora* species from the Bismarck Sea (Papua New Guinea). *Phycological Research*, **67**(3): 228-238.
- Womersley H B S. 1967. A critical survey of the marine algae of southern Australia. II. Phaeophyta. *Australian Journal of Botany*, **15**(2): 189-270.
- Womersley H B S. 1987. The Marine Benthic Flora of Southern Australia. Part II. Australian Biological Resources Study, Australia. 484p.
- Zhang D, Gao F L, Jakovlić I, Zou H, Zhang J, Li W X, Wang G T. 2020. PhyloSuite: an integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. *Molecular Ecology Resources*, **20**(1): 348-355.