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Diversity and phylogeny of the brown alga *Lobophora* (Dictyotales, Phaeophyceae) in Singapore

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Abstract

The brown macroalgal genus *Lobophora* (Phaeophyceae: Dictyotaceae) plays an ecologically significant role in many marine ecosystems, but their diversity and taxonomy remain poorly studied. Until 2012, six *Lobophora* species had been recognised globally based on morphological features. Yet, with more than 100 evolutionary taxonomic units characterised to date, it is now acknowledged that *Lobophora* comprises many cryptic species and its diversity was vastly underestimated. In light of a growing body of research integrating molecular and morphological data to delimit cryptic species, this study assessed the diversity and phylogeny of *Lobophora* in Singapore. A combination of molecular data and morphological observations were used to delimit and identify species from 33 specimens collected at eight sites in the southern islands of Singapore. The mitochondrial *cox3* and chloroplast *psbA* genes were amplified and sequenced for phylogenetic analysis. Three formally described species, *L. challengeriae*, *L. lamourouxii*, *L. pachyventera* (comprising two morphotypes), as well as one undescribed putative species, *Lobophora* sp61, were recovered. These findings replace the record of the Atlantic species *L. variegata* in Singapore and suggest that there are more species to be discovered in the biodiverse region of Southeast Asia. Precise understanding of *Lobophora* diversity is critical for ongoing and future work on coral–macroalgal ecological relationships.

Keywords: Brown macroalgae, DNA Barcoding, Morphology, Southeast Asia, *cox3*, *psbA*

Introduction

The brown macroalgal genus *Lobophora* J.Agardh (1894: 21) (Dictyotaceae, Phaeophyceae) is widely distributed across tropical and subtropical marine regions. It is ecologically significant as an important food source for reef herbivores, with herbivory rates by rabbitfish *Siganus lineatus* and sea urchin *Diadema setosum* on *Lobophora monticola* reaching up to 116 (± SD 42) mg per day (Vieira *et al.* 2019b). *Lobophora* has also been shown to be an allelopathic macroalga, exerting negative effects on the settlement and survivability of coral larvae, thereby limiting the resilience and recovery of degraded reefs (Fong *et al*., 2019). It is estimated that *Lobophora* first appeared in the Upper Cretaceous 60–75 million years ago in the Tethys Sea, subsequently dispersing and diversifying in the Atlantic and Indo-Pacific Oceans (Vieira *et al.* 2017). The ranges of most species are limited to their respective marine realms (Spalding *et al.* 2007), and more numerous historical speciation events within the Central Indo-Pacific have led to this marine realm having the highest species diversity (Vieira *et al.* 2017). According to Vieira (2020), there are 47 taxonomically accepted *Lobophora* species (see also Guiry & Guiry 2019), but at least another 60 putative species discovered in the last decade remain undescribed (Vieira *et al.* 2016, 2017).

Early studies described a limited diversity of *Lobophora*. Only six species were recognised prior to 2012 (Vieira *et al.* 2019a), with *L. variegata* (J.V.Lamouroux) Womersley ex E.C.Oliveira (1977: 217) being the most commonly

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documented species in historical records. Many studies had supported the wide distribution of *L. variegata* in temperate and tropical regions of the Atlantic and Indo-Pacific Oceans. Consistently, the general morphology of *Lobophora* is a fan-shaped thallus, either crustose, decumbent, or erect, ranging from brown to orange in colour, with the distinctly large medulla cell layer considered a diagnostic vegetative character for the genus (Abbas & Shameel 2010).

Due to the lack of distinctive morphological features among *Lobophora* algae, recent studies have utilised DNA analysis to delimit species. Integrative systematic research based on molecular and morphological data have unveiled surprisingly large cryptic species diversity in the genus. Sun *et al.* (2012) pioneered DNA analysis of *Lobophora* by sequencing the mitochondrial cytochrome c oxidase subunit III (*cox3*) and chloroplast ribulose-1,5-bisphosphate carboxylase (*rbcL*) genes, reporting nine *Lobophora* clades, including four novel species, from the western Pacific region and southeastern Australia. This work highlighted the severe underestimation of species diversity in *Lobophora*, and the potential of molecular tools for establishing phylogenetic relationships and to delimit species. Since then, there have been more studies using these new approaches to resolve the taxonomy and diversity of *Lobophora*. For example, four and 16 species have recently been described from the South China Sea (Sun *et al.* 2017) and Bismarck Sea off Papua New Guinea (Vieira *et al.* 2019a), respectively. However, with only one study conducted along the western Pacific coast (Sun *et al.* 2012), such research remains geographically patchy and poorly represented at regional scales.

In Singapore, *L. variegata* has been recorded as the sole species under genus *Lobophora* based on morphological observations (Lee *et al.* 2009a, b, Pham *et al.* 2011, Noiraksa *et al.* 2012, Ng *et al.* 2014, Lee *et al.* 2015, Phang *et al.* 2016). A preliminary study by Fong *et al.* (2019) detected *L. challengeriae* C.W.Vieira in Vieira *et al.* (2019a: 231) or *Lobophora* sp16 prior to formal description in Vieira *et al.* (2019a), in samples collected from Pulau Subar Darat (1°12'53"N 103°49'55"E). Given the location of Singapore within the South China Sea and proximity to Papua New Guinea in Central Indo-Pacific, it is possible that some of the species described in Sun *et al.* (2017) and Vieira *et al.* (2019a) are found in Singapore. Moreover, recent studies have shown that *L. variegata* originated from the Caribbean Sea and is restricted to the Atlantic Ocean (Schultz *et al.* 2015, Vieira *et al.* 2016). In light of the large taxonomic deficit of *Lobophora*, there is an urgent need to reassess the diversity of *Lobophora* in Singapore.

This study aims to investigate the diversity of *Lobophora* in Singapore and reconstruct the phylogenetic relationships among local species in the context of the global *Lobophora* phylogeny. Based on prior evidence that *L. variegata* is constrained to the Atlantic Ocean and that many cryptic species have been detected in this region, we hypothesise that the *Lobophora* macroalgae found in Singapore are not conspecific with *L. variegata* and comprise multiple Indo-Pacific lineages.

Materials and methods

Sampling sites and collection

A total of 33 *Lobophora* specimens of different representative morphologies were collected from eight sites in the southern offshore islands of Singapore (Fig. 1; see Supplementary File 1). Collection was carried out from May 2019 to January 2020 by intertidal surveys and SCUBA from 1 to 10 m deep. Fresh samples were processed and a 1 cm^2 subsample was taken from each specimen for preservation in 100% molecular-grade ethanol. The remaining algae were stored in -80˚C for subsequent morphological observations.

DNA extraction, amplification and sequencing

Subsamples were digested in 900 µl of cetyltrimethylammonium bromide (CTAB) and 20 µl of proteinase K, and subsequently incubated at 55˚C overnight for 14 h. DNA was extracted from the digested tissue using phenol-chloroform-isoamyl alcohol. Genomic DNA Clean & Concentrator™ kit (Zymo Research, Singapore) was used according to manufacturer's instructions to remove inhibitors.

Using published primers, two genes were amplified: (1) mitochondrial encoded cytochrome c oxidase III gene (*cox3*, 610 base pairs, or bp) (Silberfeld *et al.* 2010), and (2) chloroplast encoded photosystem II protein D1 gene (*psbA*, 919 bp) (Yoon *et al.* 2002). We initially also targeted the *rbcL* gene (Sun *et al.*, 2012, Vieira *et al*., 2014, 2016), but amplifications were largely unsuccessful and therefore this locus was omitted from subsequent analyses. Polymerase chain reaction (PCR) mixtures consisted of 1.0 µl DNA, 12.5 µl GoTaq DNA polymerase (Promega), 1.0 µl each of

forward and reverse primers, and 9.5 µl nuclease-free water. Following Vieira *et al.* (2016), PCR was carried out with an initial denaturation at 94°C for 3 min, 35 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds and 72°C for 45 seconds, and a final extension at 72°C for 5 min. PCR products were purified with SureClean Plus (Bioline, Singapore).

Cycle sequencing of purified products was carried out using BigDye Terminator v3.1 (Applied Biosystems, Foster City, California) according to the manufacturer's instructions, and sequencing was performed on an ABI 3130XL DNA Analyzer (ThermoFisher Scientific). Geneious v9.1.6 (Kearse *et al.* 2012) was used for assembly of consensus sequences under default parameters.

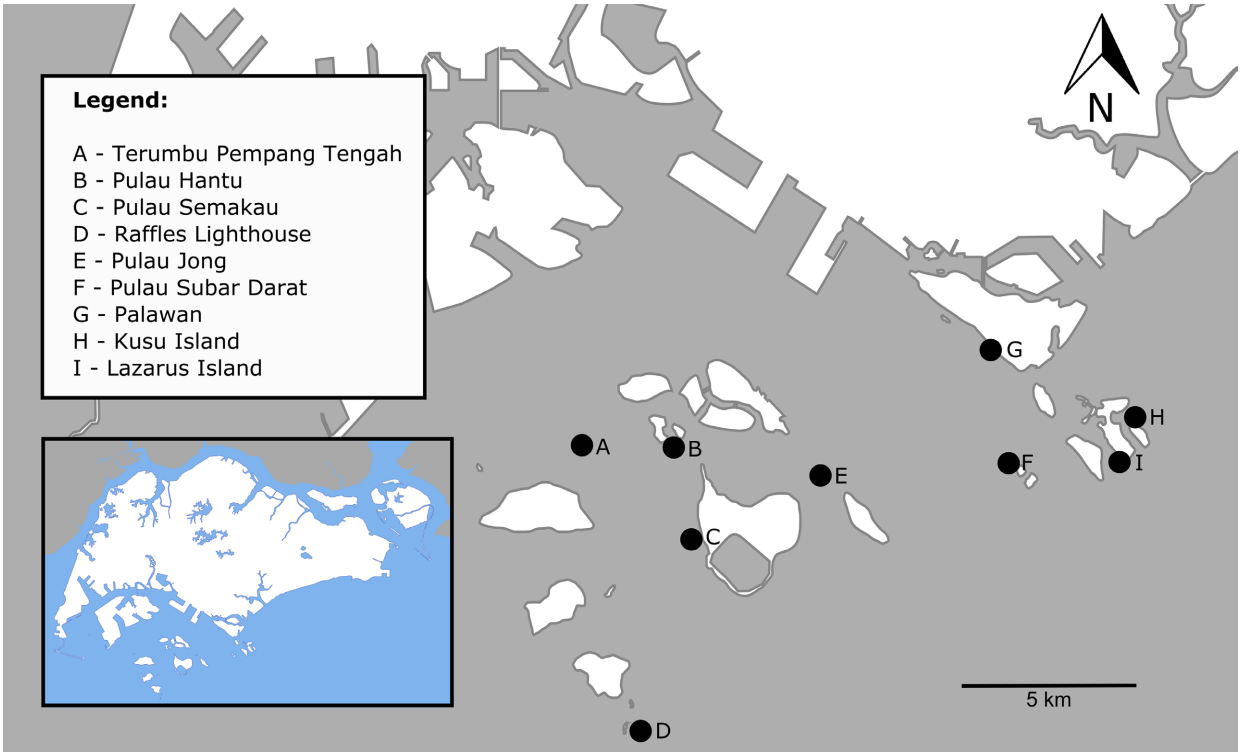

FIGURE 1. Map of collection sites (denoted by circles), with inset showing the map of Singapore.

Sequence alignment and phylogenetic analysis

Newly-generated DNA sequences (GenBank accession numbers MW658779–MW658818; see Supplementary File 1), along with 548 *cox3* and 298 *psbA* sequences published by Vieira *et al.* (2016), Camacho *et al.* (2019), Vieira *et al.* (2019a), Vieira *et al.* (2019d), Fong *et al.* (2019) and Vieira *et al*. (2020), were compiled in Mesquite v3.61 (Maddison & Maddison 2019). For *cox3*, *Dictyota dichotoma* and *Padina arborescens* were designated as outgroup taxa; for *psbA*, *Dictyota dichotoma* and *Padina australis* were designated as outgroup taxa. *cox3* and *psbA* sequences were separately aligned in MAFFT 7.453 (Katoh & Standley 2013) under default '--auto' parameters.

Analyses were conducted for each locus based on maximum likelihood (ML), Bayesian inference (BI), and maximum parsimony (MP). ML analysis was conducted using RAxML 8.2.4 (Stamatakis *et al.* 2005, Stamatakis 2006, 2014) under the GTRGAMMA model and with 50 random starting trees. Clade stability was tested using 1000 bootstrap replicates (Stamatakis *et al.* 2008). For Bayesian inference, the best-fit molecular evolutionary model for Bayesian analysis was determined using jModelTest 2.1.10 (Guindon & Gascuel 2003, Posada 2008, Darriba *et al.* 2012), testing for a total of 24 models based on the Akaike Information Criterion (*cox3*: GTR + I + G, *psbA*: GTR + I + G). Bayesian analysis was conducted using MrBayes v3.2.6 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003, Ronquist *et al.* 2012) with four Markov chains of 12 million generations implemented in two runs, logging one tree per 100 generations. The first 20001 trees were discarded as burn-in following run convergence assessed using TRACER v1.7.1 (Rambaut *et al.* 2014). Finally, MP analysis was conducted using TNT v.1.5 (Goloboff *et al.* 2003, 2008, Goloboff & Catalano 2016) with 1000 random addition sequence replicates, each with 100 cycles of sectorial searches, tree fusing, ratcheting and drifting. Clade stability was tested using 1000 bootstrap replicates.

Morphological observations

The external morphology of each *Lobophora* specimen was recorded *in situ* prior to collection. Growth forms were recorded based on Vieira (2020). The internal morphology was examined from longitudinal and transverse sections of the thallus obtained manually with a razor blade. Images were taken with a Leica MC170 HD (5 M pixel) camera mounted on a Leica DMi1 compound microscope. The size of the thallus, size and number of cortical and medulla cells, and presence of reproductive structures were recorded. Species were identified based on published morphological descriptions (Sun *et al.* 2012, Vieira *et al.* 2014, Vieira *et al.* 2017, Vieira *et al.* 2019a, Vieira *et al.* 2020).

Results

Phylogenetic analyses

From 33 *Lobophora* specimens collected, 16 *cox3* and 18 *psbA* sequences were generated from 22 samples. Both genes were successfully sequenced from 12 samples.

The topologies of *cox3* (Fig. 2) and *psbA* (Fig. 3) trees were largely similar. Maximum likelihood (ML), Bayesian inference (BI) and maximum parsimony (ML) were generally congruent and clades were strongly supported at the same positions on the trees, so the ML trees are presented here (for detailed phylogenetic data and results, see Supplementary Files 2 and 3). The designated outgroups, *Padina* and *Dictyota*, were recovered unequivocally as sister groups to a moderately supported *Lobophora* clade (*cox3*: ML/BI/MP: 57/1/87, *psbA*: ML/BI/MP: 73/1/52). *Lobophora* consisted of two major sister clades, a moderately supported smaller clade (*cox3*: ML/BI/MP: 61/0.5/59, *psbA*: ML/BI/MP: 78/1/52) and a largely unsupported bigger clade (*cox3*: ML/BI/MP: 10/0.5/57, *psbA*: ML/BI/MP: 52/0.99/32).

There was generally low support for deep nodes closer to the root of the phylogeny, but shallow nodes at the species level were well supported. Within the smaller major clade, *L. challengeriae* and newly obtained sequences formed a well-supported clade with *cox3* (ML/BI/MP: 95/0.72/42) and *psbA* (ML/BI/MP: 100/1/100). *Lobophora lamourouxii* Payri & C.W.Vieira in Vieira *et al.* (2020: 597) and newly obtained sequences formed a strong clade with *cox3* (ML/BI/MP: 97/1/100) and *psbA* (ML/BI/MP: 100/1/99). Within the larger major clade, *L. pachyventera* Z.Sun, P.E.Lim, J.Tanaka & H.Kawai in Sun *et al.* (2012: 507), and newly obtained sequences formed a strong clade with *cox3* (ML/BI/MP: 96/1/98) and *psbA* (ML/BI/MP: 94/1/89). *Lobophora* sp61 and the newly obtained sequence LOBO29 formed a strong clade with *cox3* (ML/BI/MP: 100/1/100) and *psbA* (ML/BI/MP: 100/1/100). *Lobophora pachyventera* and *Lobophora* sp61 were sister clades but this relationship was only supported on the *psbA* tree (ML/BI/MP: 72/0.94/58), and not on the *cox3* tree (ML/BI/MP: 13/0.5/5).

Overall, three formally described species, *L. challengeriae*, *L. lamourouxii*, and *L. pachyventera,* as well as one undescribed putative species, *Lobophora* sp61, were recovered in four distinct lineages.

Morphological analysis

Five morphotypes of *Lobophora*, belonging to four species, were recognised from the collection here (Table 1, Fig. 4). *Lobophora pachyventera* accounted for two of the five morphotypes.

Lobophora challengeriae was distinguished from the other taxa by their characteristically fasciculate growth form, versus decumbent and/or crustose growth form in the remaining species. One *L. challengeriae* specimen measured 9 cm tall and 7 cm wide, which exceed the descriptions of 3 cm tall and 4 cm wide in Vieira *et al.* (2019a), suggesting a larger range of thallus size for the species. *Lobophora* sp61 was found to have considerably larger thallus than the remaining taxa. The crustose growth forms of *L. pachyventera* and *L. lamourouxii* were similar, both strongly adhering to the substrate, with small thalli that were orange to reddish brown.

Thallus thickness and medulla size were variable and overlapped between species (Table 1). The range in the number of dorsal cortical cells differed slightly among species: *L. challengeriae* had 2–4 layers of dorsal cortical cells, whereas *L. pachyventera*, *L. lamourouxii* and *Lobophora* sp61 had 2–3 layers of dorsal cortical cells. *Lobophora challengeriae* and *L. pachyventera* had two to four layers of ventral cortical cells, while *Lobophora* sp61 had 2–3 layers of ventral cortical cells. *Lobophora lamourouxii* consistently had 2 layers of ventral cortical cells, the smallest among the four species examined. Although not a diagnostic trait, sporangial sori were found attached to the surface of *L. lamourouxii* samples only.

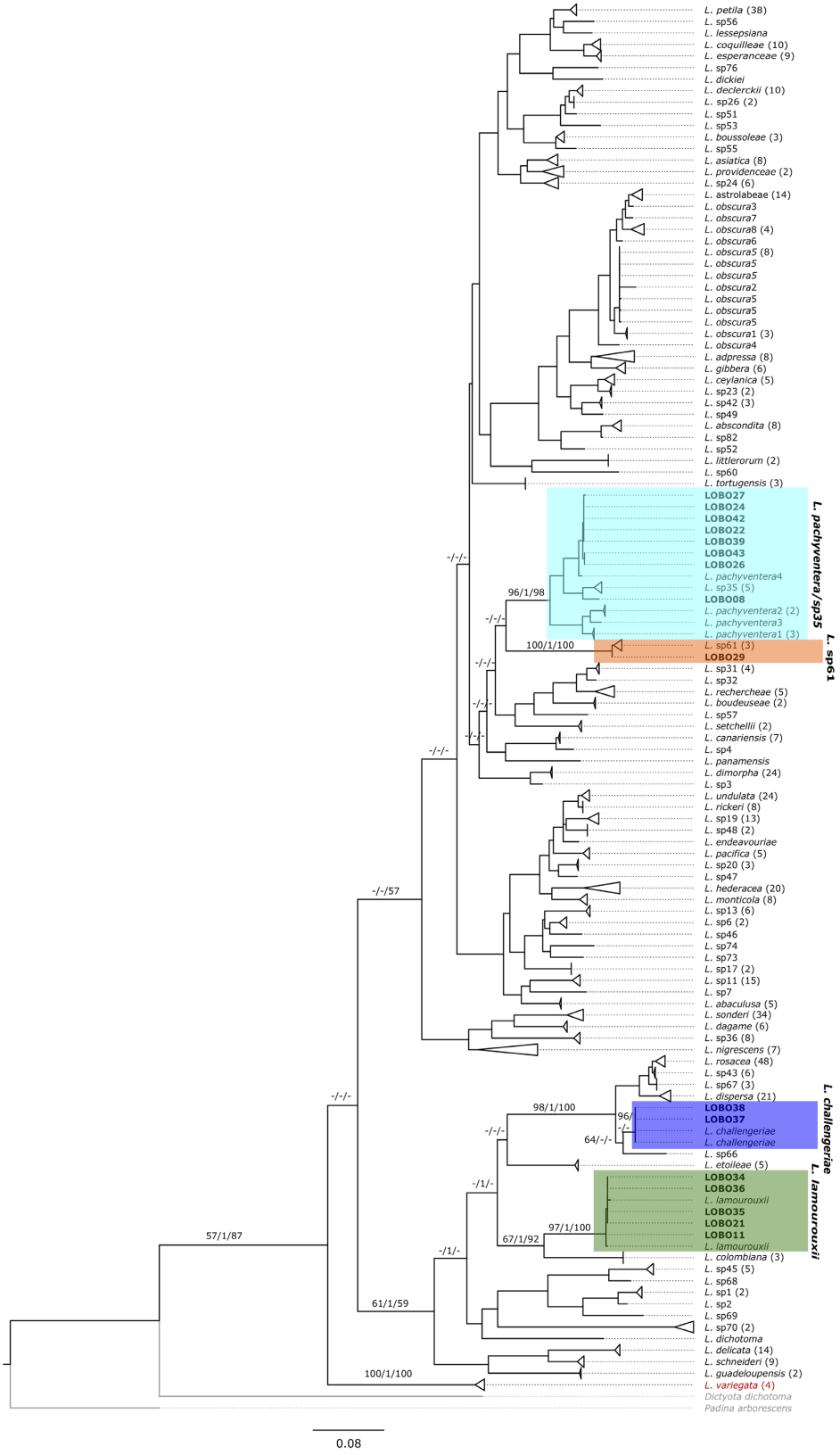

FIGURE 2. Phylogenetic reconstruction of *Lobophora* with *Dictyota dichotoma* and *Padina arborescens* as outgroups, based on maximum likelihood (ML) analysis of *cox3* alignment. Numeral in parentheses represents number of individuals of the particular species recovered in a clade. Nodal support values indicated as ML/BI/MP when ML or maximum parsimony (MP) bootstrap >0.5, or when posterior probabilities for Bayesian inference (BI) >0.8, at species level and above. Sequences from Singapore are in bold. *Lobophora variegata* is in red.

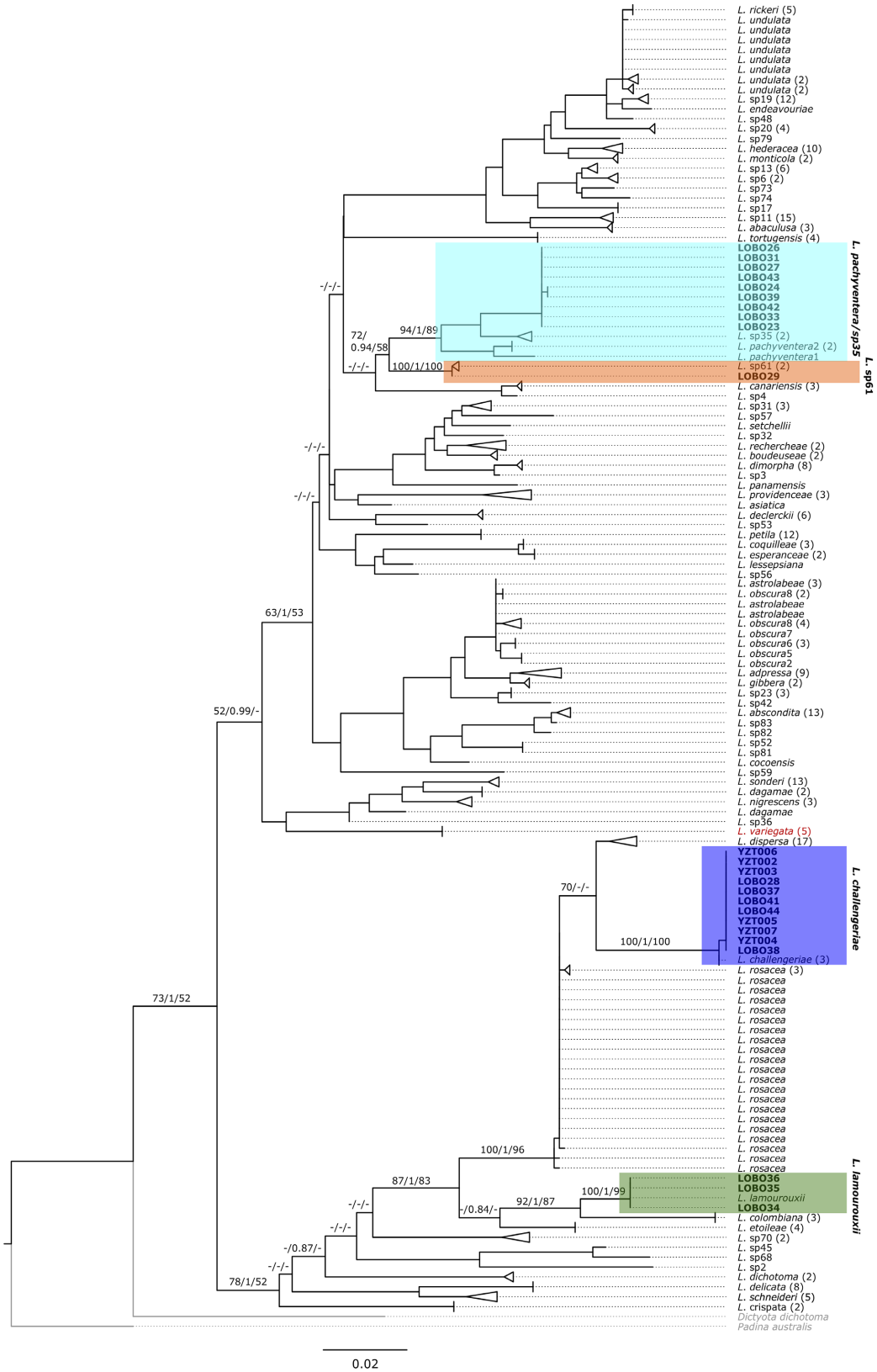

FIGURE 3. Phylogenetic reconstruction of *Lobophora* with *Dictyota dichotoma* and *Padina australis* as outgroups, based on maximum likelihood (ML) analysis of *psbA* alignment. Numeral in parentheses represents number of individuals of the particular species recovered in a clade. Nodal support values indicated as ML/BI/MP when ML or maximum parsimony (MP) bootstrap >0.5, or when posterior probabilities for Bayesian inference (BI) >0.8, at species level and above. Sequences from Singapore are in bold. *Lobophora challengeriae* sequences from Fong *et al.* (2019) are prefixed with 'YZT'. *Lobophora variegata* is in red.

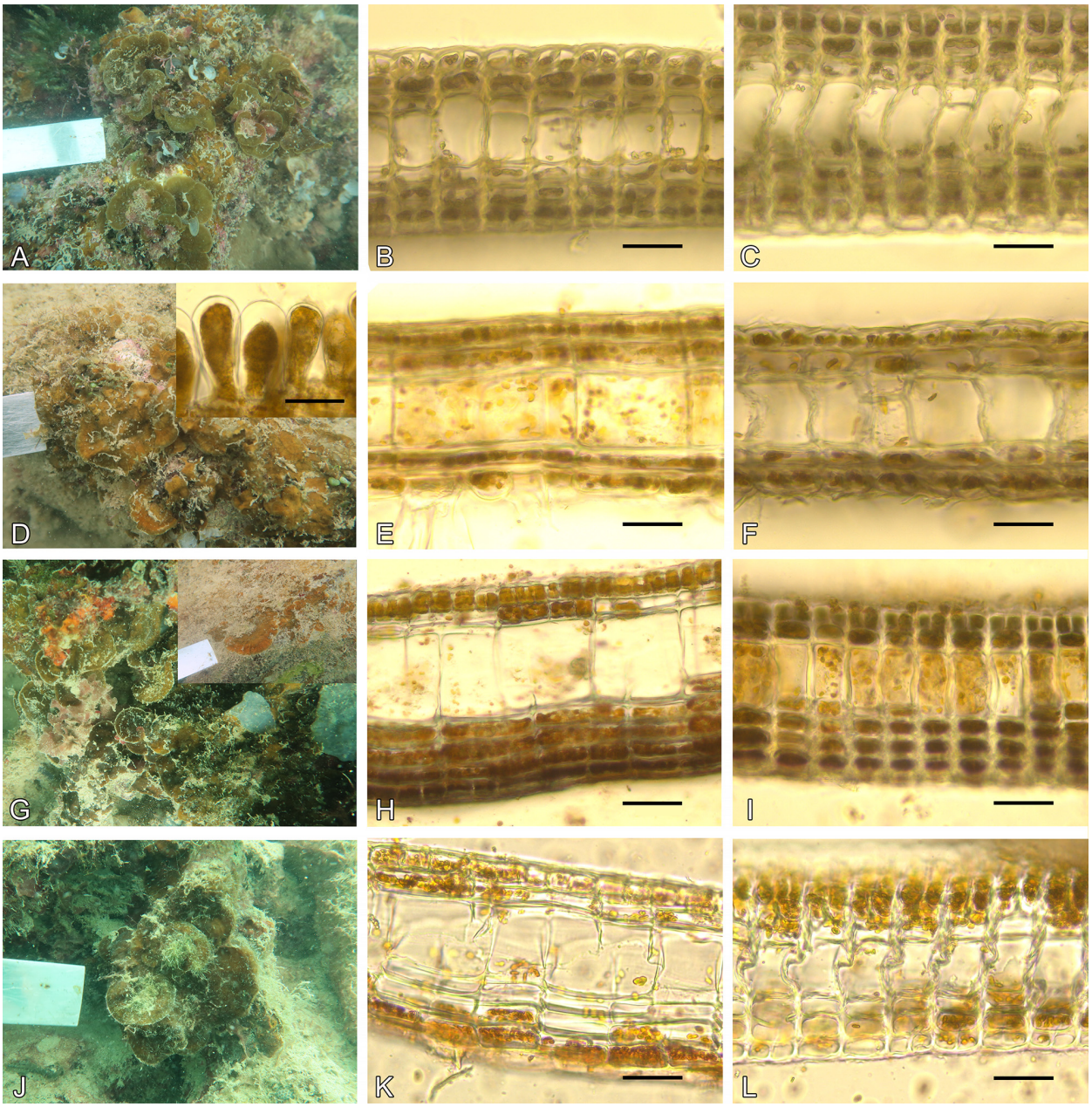

FIGURE 4. Morphological observations of *Lobophora* species found in Singapore. *Lobophora challengeriae in situ* (A), *L. challengeriae* longitudinal section (B), *L. challengeriae* transverse section (C). *Lobophora lamourouxii in situ* (D) (inset: sori), *L. lamourouxii* longitudinal section (E), *L. lamourouxii* transverse section (F). *Lobophora pachyventera* morphotype 1 *in situ* (G) (inset: morphotype 2), *L. pachyventera* longitudinal section (H), *L. pachyventera* transverse section (I). *Lobophora* sp61 *in situ* (J), *Lobophora* sp61 longitudinal section (K), *Lobophora* sp61 transverse section (L). The metallic scale is 2.5 cm in width (A, D, G, J). Scale bars represent 0.1 mm.

Discussion

This study is the first assessment of *Lobophora* diversity in Singapore based on both molecular phylogenetic analyses and morphological examinations. The discovery of four species of *Lobophora*, all previously assumed to be *L. variegata* by external morphological traits, underscores that the species richness of *Lobophora* is greater than previously known. *Lobophora challengeriae* and *Lobophora* sp61 were recorded by Vieira *et al.* (2019a) from the Bismarck Sea, off Papua New Guinea. *Lobophora lamourouxii* and *L. pachyventera* have been recorded by Sun *et al.* (2012) and Vieira *et al.* (2016) respectively from Malaysia. No *L. variegata* has been found, further supporting its restriction to the Atlantic Ocean. Overall, Singapore has a lower diversity of *Lobophora* in terms of species richness as compared to the northwestern Pacific coast and Bismarck Sea.

TABLE 1. Morphological observations of the four *Lobophora* species found in Singapore.

Trait	*L. challengeriae*	*L. lamourouxii*	*L. pachyventera* morphotype 1	*L. pachyventera* morphotype 2	*Lobophora* sp61
Growth forms	Fasciculate	Crustose	Decumbent	Crustose	Decumbent
Thallus size	3.5–7 cm wide, 3.5–9 cm tall	2 cm wide, 1.5 cm tall	3 cm wide, 2.7 cm tall	2 cm wide, 2 cm tall	7 cm wide, 5 cm tall
Thallus colour	Dark brown	Orange to reddish brown	Dark brown	Orange to reddish brown	Reddish brown
Thallus thickness	185–267 µm	165–195 µm	141–214 µm	179–305 µm	186–206 µm
Medulla size	30–151 µm wide, 51–85 µm thick	45–122 µm wide, 45–122 µm thick	34–148 µm wide, 43–93 µm thick	92–204 µm wide, 71–133 µm thick	48–67 µm wide, 56–87 µm thick
Number of dorsal cortical cells	2–4 layers	2–3 layers	2–3 layers	2–3 layers	2–3 layers
Number of ventral cortical cells	2–4 layers	2 layers	2–4 layers	2–4 layers	2–3 layers
Reproductive structures	Absent	Sori attached to surface, diameter 82 µm, length 193 µm	Absent	Absent	Absent

Here, the four novel *Lobophora* records in Singapore are discussed in relation to previous records in other regions. First, *L. challengeriae* specimens found in Singapore are similar to *L. challengeriae* (*Lobophora* sp16 Vieira *et al.* 2017) described from Papua New Guinea in Vieira *et al.* (2019a). Specimens from both localities have been observed to grow with mixed macroalgal assemblages, except that those from Papua New Guinea were found to grow near mangroves, whereas the study sites here comprise seawalls and reefs not in the vicinity of mangroves.

Second, Sun *et al.* (2012) described that the whole thallus of *L. pachyventera* from Hainan, Taiwan and Malaysia was firmly attached to the substratum (i.e. prostrate). However, for the specimens from Singapore, only the basal part of the thallus was attached to the substratum, while the rest of the structure curved upward and was not in contact with the substratum (i.e. decumbent). Otherwise, the morphology of this species is largely consistent throughout the Indo-Pacific region. *Lobophora pachyventera* specimens in this study exhibit two distinct morphologies as described in Sun *et al.* (2012)—prostrate/decumbent and dark brown in colour, or crustose and orange to reddish brown.

Third, Vieira *et al.* (2020) described that *L. lamourouxii* (*Lobophora* spWA03 Schultz *et al.* 2015 and *Lobophora* sp64 Vieira *et al.* 2016) had large, translucent, green thalli with prostrate growth form. Here, however, we have observed that its thalli are small and orange to reddish brown, with crustose growth form. Morphologically, it closely resembles the crustose growth form of *L. pachyventera*. However, the thallus of *L. lamourouxii* has 2 layers of ventral cortical cells and is 165–195 µm thick, whereas the thallus of *L. pachyventera* has 2–4 layers of ventral cortical cells and is thicker at 179–305 µm.

Finally, *Lobophora* sp61 is present in Papua New Guinea (Vieira *et al.* 2019a) but has yet to be formally described due to insufficient material globally (C. Vieira, pers. comm.). In Vieira *et al.* (2017) and Vieira *et al.* (2019a), *Lobophora* sp61 was an outgroup to the large major clade, but in the present study and Vieira *et al.* (2019c), *Lobophora* sp61 is nested within the clade. The sample here has been obtained from the reef slope at 5–8 m depth, but due to limited sampling, it remains inconclusive whether *Lobophora* sp61 is restricted to a deeper depth relative to other *Lobophora* species in Singapore.

A more comprehensive collection will help test the species identities obtained here. In particular, observations of just one specimen of *Lobophora* sp61 are inadequate for characterising the species' variability. Sun *et al.* (2012), Vieira *et al.* (2014, 2016) and Camacho *et al.* (2019) also combined their *cox3* and *psbA* data with sequences of the *rbcL* gene, for which we have been unable to successfully amplify across most of our samples. Here, we opt to interpret the relationships based on separate analyses in order to identify differences in tree topology concerning the species examined here. Our reconstructions show that species assignments based on the sample placements on both gene trees are robust, but continuing work on the *rbcL* marker would help verify our results.

Conclusion

The macroalgal genus *Lobophora* is formally assessed in Singapore with molecular data and morphological observations, showing that *Lobophora* here has hitherto been misidentified as *L. variegata*. We replace this past erroneous record with four species, *L. challengeriae*, *L. lamourouxii*, *L. pachyventera* and *Lobophora* sp61, which form well-supported clades with the respective conspecific sequences from previous studies. The high degree of morphological similarity between the crustose growth form of *L. pachyventera* and *L. lamourouxii* highlights the importance of DNA sequencing for identifying cryptic species.

Given the dearth of research on macroalgae in Southeast Asia, more thorough sampling needs to be done in the region in order to understand the diversity and phylogeny of *Lobophora* and macroalgae in general (Yip *et al.* 2018). In relation to the vast biomass and fundamental ecological roles of macroalgae in sustaining various marine ecosystems, taxonomic and phylogenetic studies on macroalgae such as *Lobophora* are crucial for more robust ecological inferences and better-informed conservation applications (Knowlton & Jackson 1994, Isaac *et al.* 2004, Thomson *et al.* 2018, Vieira 2020).

Taxonomy

Family
Dictyotaceae J.V.Lamouroux ex Dumortier (1822: 72, 101)

Genus
Lobophora J.Agardh (1894: 21)

Species
Lobophora challengeriae C.W.Vieira in Vieira *et al.* (2019a: 231)

Lobophora sp16 Vieira *et al.* 2017
Specimens observed:—LOBO28, LOBO37, LOBO38, LOBO41, LOBO44 (Reef Ecology Laboratory, National University of Singapore, NUS)

Description:—Thallus large, erect and flabellate, exhibits fasciculate growth form (Fig. 4A). Base of thallus narrow, attached to the substratum by basal rhizoids. Margin entire. Thallus 3.5–7 cm wide and 3.5–9 cm tall, dark brown. Thallus 185–267 μm thick, composed of a single layer of medulla cells, 2–4 layers of dorsal cortical cells, and 2–4 layers of ventral cortical cells (Fig. 4B and 4C). Medulla cells 30–151 μm wide and 51–85 μm thick. Dorsal and ventral cortical cell layers 60–86 μm and 57–104 μm thick respectively.

Holotype:—PC0063047 (IRD11086). Kavieng, Papua New Guinea. 13 August 2014. Collected by C. Payri.

Distribution:—Kenya, Oman, Sri Lanka, Tanzania (Vieira *et al.* 2016), Papua New Guinea (Vieira *et al.* 2019a), Singapore (this study).

Habitat:—Reef flat (1–2 m depth), reef crest (3–4 m depth) and reef slope (8–10 m depth). Loosely attached to hard substratum, growing in dense clumps.

Lobophora lamourouxii Payri & C.W.Vieira in Vieira *et al.* (2020: 597)

Lobophora spWA03 Schultz *et al.* 2015 and *Lobophora* sp64 Vieira *et al.* 2016
Specimens observed:—LOBO11, LOBO21, LOBO34, LOBO35, LOBO36 (Reef Ecology Laboratory, NUS)

Description:—Thallus exhibiting crustose growth form (Fig. 4D). Base of thallus narrow, growing firmly attached to the substrate. Margin entire. Thallus 2 cm wide and 1.5 cm tall, orange to reddish brown. Thallus 165–195 μm thick, composed of a single layer of medulla cells, 2–3 layers of dorsal cortical cells, and 2 layers of ventral cortical cells (Fig. 4E and 4F). Medulla cells 45–122 μm wide and 45–122 μm thick. Dorsal and ventral cortical cell layers 49–56 μm and 47–56 μm thick respectively. Sporangial sori attached to surface, 82 μm in diameter and 193 μm in length (Fig. 4D, inset).

Holotype:—NOU 201665 [IRD11176, CP15166]. Saint Vincent. 26 April 2015. Collected by C. Payri.

Type locality:—Wallibou (13.333, 61.221617), Saint Vincent, Windward Islands, Lesser Antilles, West Indies.

Distribution:—Guadeloupe (Shultz *et al.* 2015), Malaysia (Vieira *et al.* 2016), Curacao, Saint Vincent (Vieira *et al.* 2020)**,** Singapore (this study).

Habitat:—Intertidal and reef crest (3–4 m depth). Attached to hard substratum growing with mixed turf algal assemblages, and also found strongly adherent on intertidal seawalls.

Lobophora pachyventera Z.Sun, P.E.Lim, J.Tanaka & H.Kawai in Sun *et al.* (2012: 507)

Specimens observed:—LOBO08, LOBO22, LOBO23, LOBO24, LOBO26, LOBO27, LOBO31, LOBO33, LOBO39, LOBO42, LOBO43 (Reef Ecology Laboratory, NUS)

Description:—Thallus large and flabellate with two distinct morphotypes. First morphotype (LOBO22, LOBO24, LOBO26, LOBO33, LOBO42) thallus exhibits decumbent growth form (Fig. 4G). Base of thallus narrow, growing with basal parts attached to substrate and distal parts curving upwards. Margin entire. Thallus 3 cm wide and 2.7 cm tall, dark brown. Thallus 141–214 μm thick, composed of a single layer of medulla cells, 2–3 layers of dorsal cortical cells, and 2–4 layers of ventral cortical cells (Fig. 4H and 4I). Medulla cells 34–148 μm wide and 43–93 μm thick. Dorsal and ventral cortical cell layers 37–81 μm and 38–81 μm thick respectively. Second morphotype (LOBO08, LOBO23, LOBO27, LOBO31, LOBO39, LOBO43) thallus exhibits crustose growth form (Fig. 4G, inset). Base of thallus narrow, strongly attached to the substratum. Margin entire. Thallus 2 cm wide and 2 cm tall, orange to reddish brown. Thallus 179–305 μm thick, composed of a single layer of medulla cells, 2–3 layers of dorsal cortical cells, and 2–4 layers of ventral cortical cells. Medulla cells 92–204 μm wide and 71–133 μm thick. Dorsal and ventral cortical cell layers 51–87 μm and 179–305 μm thick respectively.

Holotype:—SAP109519. Sunayama Beach, Miyakojima Island, Okinawa, Japan. 9 May 2009. Collected by J. Tanaka & Z. Sun.

Distribution:—China, Malaysia, Japan (Sun *et al.* 2012), New Caledonia (Vieira *et al.* 2014), Taiwan, Vanuatu, (Vieira *et al.* 2016), Singapore (this study).

Habitat:—Reef flat (1–2 m depth) and reef crest (3–4 m depth). Commonly growing on hard substratum and coral rubble, sometimes epiphytically on coralline algae.

***Lobophora* sp61** Vieira *et al.* 2019a

Specimen observed:—LOBO29 (Reef Ecology Laboratory, NUS)

Description:—Thallus exhibits decumbent growth form (Fig. 4J). Base of thallus narrow, growing with basal parts attached to substrate and distal parts curving upwards. Margin entire. Thallus large, 7 cm wide and 5 cm tall, reddish brown. Thallus 186–206 μm thick, composed of a single layer of medulla cells, 2–3 layers of dorsal cortical cells, and 2–3 layers of ventral cortical cells (Fig. 4K and 4L). Medulla cells 48–67 μm wide and 56–87 μm thick. Dorsal and ventral cortical cell layers 48–62 μm and 68–76 μm thick respectively.

Distribution:—Papua New Guinea (Vieira *et al.* 2019a), Singapore (this study).

Habitat:—Reef slope (5–8 m depth). Attached to hard substratum with other algae growing epiphytically on it.

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Compliance with Ethical Standards

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Ethical approval: This article does not contain any studies with animals performed by any of the authors.

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