Species diversity and phylogeny of the green macroalga *Ulva* (Ulvophyceae, Chlorophyta) in Singapore

YU FEI NG1,5* & DANWEI HUANG1,2,3,4,6*

1Department of Biological Sciences, National University of Singapore, Singapore 117558, Singapore
2Lee Kong Chian Natural History Museum, National University of Singapore, Singapore 117377, Singapore
3Tropical Marine Science Institute, National University of Singapore, Singapore 119227, Singapore
4Centre for Nature-based Climate Solutions, National University of Singapore, Singapore 117558, Singapore
5ng.yu_fei@u.nus.edu; https://orcid.org/0009-0002-1259-0119
6huangdanwei@nus.edu.sg; https://orcid.org/0000-0003-3365-5583

*Corresponding authors
tel: (+65) 6516-2699

Abstract

The green macroalgal genus *Ulva* (Ulvaceae, Ulvophyceae, Chlorophyta) is commonly found in coastal ecosystems and confers many important marine services. Despite prior research interest and recent advancements in *Ulva* taxonomy, in many parts of the world, its species diversity remains unresolved due to a high degree of morphological variation and phenotypic plasticity. DNA barcoding and phylogenetic approaches can potentially assist in identifying *Ulva* species precisely. Here, we analysed chloroplast tufA, rbcL and nuclear ITS from 49 *Ulva* specimens from Singapore. Gross and microscopic morphological examinations were also performed to characterise the morphological range of each species. Our results confirm the presence of three *Ulva* species in Singapore, all of which are new local records: *U. meridionalis*, *U. ohnoi*, and *U. tepida*. Although 10 *Ulva* species have been historically recorded in Singapore, none of them were found in the present study, likely due to prior misidentification, limited seasonal sampling, or local extirpations. *Ulva ohnoi* shows high morphological variation with five morphotypes. This study demonstrates that molecular analyses combined with morphological examinations are useful for characterising *Ulva* diversity.

Key words: Blue carbon, DNA barcoding, Green seaweed, Marine, Morphology, Southeast Asia

Introduction

The green macroalga *Ulva* Linnaeus (1753: 1163) is commonly found in coastal ecosystems within tropical and temperate zones. *Ulva* comprises many species with a high degree of phenotypic plasticity and many unresolved taxonomic issues (Hayden et al. 2003, Tran et al. 2022). The morphological plasticity can result in misidentification when using only morphological characteristics to determine *Ulva* species (Gao et al. 2016, Lee et al. 2019). Recent studies have discovered several new species and records of *Ulva* using molecular approaches (Bast et al. 2014, Kang et al. 2019, Lagourgue et al. 2022, Tran et al. 2023). For example, identification of *Ulva* species in USA, including four new records, was achieved with the sequencing and analysis of the following phylogenetic markers: elongation factor Tu (tufA), internal transcribed spacer (ITS), and ribulose biphosphate carboxylase large subunit (rbcL) (Melton III & Lopez-Bautista 2021).

*Ulva* confers many vital ecological services and can have significant impacts on the marine environment (Levine 2018, Macreadie et al. 2017). For example, some species form green tides, a form of harmful algal bloom (HAB), due to excessive growth (Wang et al. 2012) in many countries (Ye et al. 2011, Yoshida et al. 2015, Kwon et al. 2017). Indeed, the decay of *Ulva* species, such as *U. prolifera* O.F.Müller (1778: 7), could cause acidification and hypoxia, adversely affecting local ecosystems (Zhang et al. 2019). Due to anthropogenic climate change, the frequency of macroalgal blooms is projected to rise in coastal environments as ocean temperatures increase (Green-Gavrielidis & Thornber 2022). Therefore, it is vital to study *Ulva* diversity and distributions to better understand the potential impacts of macroalgal blooms on coastal environments.
Despite the ecological problems caused by such blooms, *Ulva* has multiple benefits for humanity. For example, *U. lactuca* Linnaeus (1753: 1163) can inhibit HABs by impeding the growth of seven other locally common species that cause such blooms (Tang & Gobler 2011). Epiphytes found on *U. lactuca* also exhibit antifouling activities to prevent invertebrate larvae and algal spores from settling onto the alga (Egan *et al.* 2000). Human consumption of *Ulva* has been shown to potentially improve gut health (Charoensiddhi *et al.* 2022). Ulvan, a cell wall polysaccharide of *Ulva* is of research interest in the field of biomaterial products, agriculture and healthcare due to the beneficial properties from its antioxidant, antiviral and anticoagulant activities (Kidgell *et al.* 2019).

There is a general lack of macroalgal diversity research in Southeast Asia, widely acknowledged as a marine biodiversity hotspot (Roberts *et al.* 2002). In Singapore, until recently, only morphological characteristics were used to identify species (Noiraksar *et al.* 2012). Within the past six years, genetic tools have been utilised to assess brown seaweed diversity in Singapore (Yip *et al.* 2018, Kwan *et al.* 2021). Yet, no genetic studies have been conducted for green macroalgae. To date, a total of 10 *Ulva* species have been reported in Singapore (Lee *et al.* 2009, Pham *et al.* 2011, Noiraksar *et al.* 2012). Chuang (1961) and Lee (1966) first reported the presence of *U. reticulata* Forsskal (1755: 187). Subsequently, *Ulva australis* Areschoug (1854: 370), *U. beytensis* Thivy & Sharma (1966: 150), *U. clathrata* (Roth) Agardh (1811: 23), *U. flexuosa* Wulfen (1803: 1), *U. intestinalis* Linnaeus (1753: 1163), *U. lactuca*, and *U. ovata* (Thivy & Visalakshmi ex Joshi & Krishnamurthy) Kumar & Palanishamy (2021: 219) were recorded by Teo & Wee (1983). The presence of *U. compressa* Linnaeus (1753: 1163) was reported by Pham *et al.* (2011) while *U. rigida* Agardh (1823: 410) was discovered more recently by Noiraksar *et al.* (2012). However, none of these records have been validated with detailed morphological examination and molecular phylogenetic analysis. Therefore, this study aims to investigate the species diversity of *Ulva* in Singapore using morphological observations and molecular analyses from three genes: *tufA*, nuclear ITS, and *rbcL*.

### Materials and methods

#### Sampling sites and collection

*Ulva* specimens were collected by hand, during low tides, at 10 intertidal sites in Singapore (Fig. 1). At each site, two to four specimens of each *Ulva* morphotype were collected. Preliminary identification of *Ulva* morphotypes was performed on site according to Noiraksar *et al.* (2012) and Tran *et al.* (2023), and specimens were photographed in-situ. In the laboratory, epiphytes were removed from all specimens. For DNA sequencing, two to six 0.5 cm × 0.5 cm tissue fragments were cut from each specimen, preserved in 100% ethanol, and stored at -80°C. Two 0.5 cm × 0.5 cm tissue fragments were cut from each specimen and preserved in 5% formalin and filtered seawater for microscopic examination. All specimens were pressed as herbarium vouchers and deposited at the Singapore Herbarium (SING). Catalogue numbers are identical to the specimen numbers used in this study.

#### DNA extraction, PCR amplification, and sequencing

Tissue samples were digested in 20 μl of proteinase K and 900 μl of cetyltrimethylammonium bromide (CTAB) (Doyle & Doyle 1987) at 55°C for 14 to 20 hours. Phenol-chloroform-isooamyl alcohol (25:24:1) was used to extract DNA from digested tissue following Doyle & Doyle (1987). The genes *tufA*, ITS, and *rbcL* were PCR amplified using published primers and thermocycling protocols described in Supplementary File 1. Polymerase chain reaction (PCR) was conducted using GoTaq DNA polymerase (Promega). ITS primers were unable to amplify samples of *U. ohnoi* M.Hiraoka & S.Shimada in Hiraoka *et al.* (2004: 20). Agarose gel (1%) electrophoresis was used to verify PCR amplification success. PCR products were purified using Beckman Coulter AMPure XP beads in conjunction with a magnetic plate. Cycle sequencing was performed using BigDye Terminator Sequencing v3.1 chemistry (Applied Biosystems, Waltham). CleanSEQ Dye-Terminator Removal Kit (Beckman Coulter, Brea) was utilised for DNA precipitation according to the manufacturer’s instructions before automated capillary electrophoresis in an ABI 3130XL DNA Analyzer (ThermoFisher Scientific).

#### Sequence alignment and phylogenetic analysis

Geneious Prime v.2022.0.1 (https://www.geneious.com) was utilised to assemble and check DNA sequences. Preliminary identification of assembled sequences was based on the Basic Local Alignment Search Tool (BLAST) against GenBank sequences at the National Centre for Biotechnology Information (NCBI) (Clark *et al.* 2016, Sayers *et al.* 2019). Newly generated and previously published DNA sequences (Supplementary File 2) were assembled in
Mesquite v3.70 (Maddison & Maddison 2021) and aligned with MAFFT v7.49 (Katoh & Standley 2013) under default parameters.

Maximum likelihood (ML) and Bayesian inference (BI) were used for phylogenetic analysis. For ML, RAxML v8.2.4 (Stamatakis 2014) was run under the GTRGAMMA model with 50 random starting trees. Clade stability was tested utilising 1000 bootstrap pseudoreplicates. For BI, alignments were put through jModelTest v2.1.10 (Guindon & Gascuel 2003, Darriba et al. 2012) to obtain the optimal nucleotide substitution model, which was found to be GTR+I+G for all genes. BI analyses were conducted using MrBayes v3.2.7 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003). Two runs of four Markov chains and 10 million generations were performed with one tree logged after every 100 generations. Tracer v1.7.2 (Rambaut et al. 2018) was used to determine that the initial 10001 trees would have to be discarded as burn-in. All phylogenetic trees derived from ML and BI analysis were visualised in FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree).

FIGURE 1. Map of Ulva collection sites in Singapore for the present study.

Morphological examination
An Olympus CX41 compound microscope was used to examine the characteristics of the thallus’ margins, surface, and cells in transverse sections, while a Leica M205C dissecting microscope was used to examine second order branching of tubular specimens. Species morphological features were compared to published descriptions (Hiraoka et al. 2004, O’Kelly et al. 2010, Horimoto et al. 2011, Xie et al. 2020, Tran et al. 2023).

Results
Molecular phylogenetic analysis
Of the 75 collected Ulva specimens, a total of 49 were successfully sequenced, consisting of 47 specimens for tufA (Fig. 2), 12 specimens for ITS (Supplementary File 3), and 17 specimens for rbcL (Supplementary File 4). The phylogenetic trees produced by ML and BI analyses were mostly congruent in terms of species placements. Based on our molecular results, three Ulva species were confirmed for Singapore: U. ohnoi, U. meridionalis, and U. tepida.

Morphological examination
Ulva ohnoi has expanded, reticulate, small lobe-shaped thalli (Figs. 3A–C, 3F–H). Ulva ohnoi is distinguished from the tubular filamentous thalli of U. meridionalis (Fig. 4A–F) and U. tepida (Figs. 5A–B). A novel morphotype of U. ohnoi was discovered containing filamentous forms (Figs. 3D–E). This new morphotype can be distinguished from...
other local *Ulva* species as the filaments are non-tubular compared to the tubular filaments of *U. meridionalis* and *U. tepida*. Furthermore, there are reticulations among some filaments of *U. ohnoi*’s filamentous morphotype. *Ulva tepida* is generally smaller in filament length and width (Fig. 5B) compared to the filament of *U. meridionalis* (Figs. 4C–F). However, the filament length of *U. meridionalis* was as small as *U. tepida*. Masakiyo & Shimida (2014) distinguished *U. tepida* from *U. meridionalis* by estimating the proportion of surface cells that contain three pyrenoids (25.9% for *U. tepida* versus 3.1–3.4% for *U. meridionalis*). In Singapore, however, only 0–2 pyrenoids were found in *U. tepida* compared to 1–3 pyrenoids in *U. meridionalis*.

**FIGURE 2.** Phylogenetic tree of *Ulva* with *Umbraulva kaloukalaula* as outgroup based on tufA DNA sequences. Bootstrap values ≥ 50 for maximum likelihood and posterior probability values ≥ 0.8 for Bayesian phylogenetic analyses are stated above branches. Samples sequenced from this study are coloured in green (*U. ohnoi*), blue (*U. meridionalis*), and red (*U. tepida*).
**Taxonomic account**

*Ulva ohnoi* M.Hiraoka & S.Shimada in Hiraoka *et al.* (2004: 20)

Material examined:—SINGAPORE. Changi: Changi Beach Carpark 1, 16 July 2022, NYF220701, NYF220702, NYF220703, NYF220704, NYF220705, NYF220707 (SING); Changi: Changi Point Ferry Terminal, 16 July 2022, NYF220708, NYF220711, NYF220712, NYF220713 (SING); Pulau Ubin: Chek Jawa, 17 July 2022, NYF220724, NYF220726, NYF220731, NYF220732 (SING); Pulau Ubin: Sensory Trail, 17 July 2022, NYF220735 (SING); East Coast: East Coast Park Carpark G, 18 July 2022, NYF220738, NYF220739, NYF220740, NYF220741, NYF220743, NYF220745, NYF220746, NYF220755 (SING); Changi: Changi Beach Carpark 7, 12 August 2022, NYF220811, NYF220812, NYF220813, NYF220814, NYF220815, NYF220816, NYF220821 (SING); Pulau Hantu, 14 August 2022, NYF220829, NYF220834, NYF220840 (SING); Sentosa: Tanjong Rimau, 23 December 2022, NYF221209 (SING); Changi: Changi Beach Carpark 6, 24 December 2022, NYF221211 (SING).

Description:—*U. ohnoi* comprises five morphotypes: reticulate, expanded, expanded with some reticulations, lobe-shaped, and filamentous thalli. The reticulate morphotype has reticulated ribbon-shaped thallus without perforations, 5–50 cm in length and 0.2–2.5 cm in width (Figs. 3A, 3F). The morphotype with expanded thalli shows foliose, irregularly expanded thallus with no reticulations, but sometimes with perforations and/or tears, 5–30 cm in length and 5–30 cm in width (Fig. 3C). The morphotype with expanded thalli possessing some reticulations shows foliose thalli expanding irregularly at the base along with some reticulations and perforations; sometimes with ribbon-like structures with reticulations towards the tips, 8–20 cm in length and 20–50 cm in width (Fig. 3G). The small lobe-shaped morphotype has foliose thallus forming small tufts, without reticulations or perforations, 0.5–1.5 cm in length and 1.2–3 cm in width (Fig. 3H). The filamentous morphotype has long, filamentous, non-tubular thallus with wrinkles and some reticulations, sometimes with secondary branches, 17–53.5 cm in length and 0.3–0.9 cm in width (Figs. 3D–E). Surface cells are irregular, polygonal, or rectangular, 7–19 μm in length, 7–12 μm in width (n = 20). Chloroplast with 1 to 2 pyrenoids (Fig. 3I). Various microscopic teeth present at margin (Figs. 3J–M), but lacking teeth of the *Ulva* morphotype found attached to hard substrates like rocks or logs (Fig. 3B) while filamentous thallus morphotype found attached to hard substrates like rocks or logs (Fig. 3B) while filamentous thallus attaches to sandy substrate (Fig. 3D).

Remarks:—New species record for Singapore. In the *tufA* tree, *Ulva ohnoi* DNA sequences from Singapore formed a well-supported clade with five other species: *Ulva pseudo-ohnoi, U. lactuca, U. spinulosa, U. reticulata* and *U. taeniata* (ML bootstrap / BI posterior probability: 97/1) (Fig. 2). There was also strong support for the same clade in the ITS and rbcL trees (Supplementary Files 3, 4). Based on morphological data, we excluded *U. taeniata, U. reticulata,* and *U. spinulosa* from our sampled collections. *Ulva taeniata* thalli have wrinkles (Setchell & Gaedner 1920), *U. spinulosa* has either smooth or heavily denticulate margins, and *U. reticulata* has smooth microscopic margins (Tran *et al.* 2023). These morphological characteristics are not found in the *Ulva* samples collected in this study.

*Ulva ohnoi* cell sizes measured at surface view and transverse section in our specimens were within the range reported for the *U. ohnoi* holotype (Hiraoka *et al.* 2004). *U. ohnoi* intraspecific DNA sequence divergences observed in this study were 0.0–0.79% and 0.0–0.36% for *tufA* and *rbcL,* respectively. When comparing our sequences with that of the *U. ohnoi* holotype (Hiraoka *et al.* 2004), the genetic distances were 0.0–0.53% based on *rbcL,* supporting conspecificity. There appear to be no phylogenetic patterns associated with the five *U. ohnoi* thallus morphotypes, indicating that all non-tubular *Ulva* samples collected in this study are of the same species.

Three of five *U. ohnoi* morphotypes reported here have similar gross morphology to *U. ohnoi* reported in other studies (Hiraoka *et al.* 2004, O’Kelly *et al.* 2010, Tran *et al.* 2023). Two new morphotypes have been uncovered, one with lobe-shaped thallus that attaches to hard objects and the other with filamentous thallus found on sandy substrate. However, the lobe-shaped thallus is smaller in length and width when compared to other morphotypes suggesting that these could be young *U. ohnoi.* There are gross and microscopic variations within the *U. ohnoi* species (Hiraoka *et al.* 2004, O’Kelly *et al.* 2010, Lee *et al.* 2019) that may be driven by various environmental factors. For example, pollution...
from wastewater discharge and surface runoffs could affect Ulva morphology as Zeroual et al. (2020) reported that U. intestinalis and U. rigida thickened their cell wall and had a reduction of thalli size in the presence of heavy metals. Such environmental variations could result in morphological variation among U. ohnoi specimens. Hiraoka et al. (2004) recommended distinguishing U. ohnoi from other Ulva species by examining the life history and morphology such as the number of perforations in the middle of the thallus. As U. ohnoi has not been recorded in Singapore until this study, the newly discovered morphotypes in this study expand upon the morphological range described for the species thus far (Hiraoka et al. 2004). We note that the filamentous morphotype is represented by a single specimen (NYF221211), so its identity requires further validation. Ulva ohnoi has similar gross morphology to some Ulva species which were previously recorded in Singapore, including U. beytensis, U. reticulata, U. lactuca, U. australis, and U. rigida.

Ulva meridionalis R.Horimoto & S.Shimada in Horimoto et al. (2011: 161)

Material examined:—SINGAPORE. Pulau Ubin: Chek Jawa, 17 July 2022, NYF220716, NYF220717, NYF220718, NYF220719, NYF229720, NYF220722, NYF220725 (SING); East Coast: East Coast Park Carpark G, 18 July 2022, NYF220742 (SING); Sentosa: Tanjong Rimau, 23 August 2022, NYF220805, NYF220807 (SING); Pulau Hantu, 14 August 2022, NYF220832 (SING).

Description:—Thallus in the shape of tubular filaments, smooth or wrinkled (Figs. 4C–F, 4H). Thallus width decreases towards the base (Fig. 4G). Second-order branching restricted to the thallus base. Some thalli have one to multiple transverse constrictions (Fig. 4I). Thallus filaments are 0.6–19.5 cm in length; apex/median of filament are 3–100 mm in width (n = 6); base of filament are 40–672 μm in width (n = 6). Surface cells are mostly cuboidal, polygonal, or irregular. Cells in surface view are 6–15 μm in length and 5–11 μm in width (n = 20). In surface view, chloroplast covers the periphery of cell with 1 to 3 pyrenoids (Fig. 4J). In transverse sections, thallus 28–33 μm in width (n = 5), cell shape is rectangular, 20–23 μm in length and 13–17 μm in width (n = 6), with parietal chloroplast (Fig. 4L).


Habitat:—Anchors onto sand, in between pebbles, or on rocks (Figs. 4A–B).

Remarks:—New species record for Singapore. Ulva meridionalis has similar gross morphology to the previously recorded U. compressa, U. flexuosa and U. intestinalis due to its filamentous thalli. There was generally strong support for the Ulva meridionalis clade in the tufA, ITS and rbcL trees (Fig. 2; Supplementary Files 3, 4). The Vietnamese Ulva spp. 3 and 4 (Tran et al. 2023) are closely related to U. meridionalis. However, they can be distinguished from U. meridionalis because there are no pyrenoids in Ulva sp. 3 and 3–6 pyrenoids in Ulva sp. 4 (Tran et al. 2023).

Ulva tepida Y.Masakiyo & S.Shimada (2014: 11)

Material examined:—SINGAPORE. Changi: Changi Beach Carpark 1, 16 July 2022, NYF220710 (SING); East Coast: East Coast Park Carpark G, 18 July 2022, NYF220747, NYF220754 (SING).

Description:—Thallus monostromatic, in the shape of tubular filaments, smooth with the width decreasing towards the base (Fig. 5B). Thallus base with second-order branching (Fig. 5C). Filaments 1–14 mm in length, filament apices 0.2–0.9 mm in width (n = 6), filament bases 8–72 μm in width (n = 6). Surface cells are rectangular or irregularly shaped, 7–19 μm in length and 4–11 μm in width (n = 20) (Figs. 5D–E). Chloroplast occupies most of the cell periphery, with 0–2 pyrenoids. In transverse section, thallus 16–18 μm in width (n = 5), cell shape is rectangular, 12–14 μm in length and 8–11 μm in width (n = 6), with parietal chloroplasts (Fig. 5F).

Distribution in Singapore: Changi Point Ferry Terminal and East Coast Park Carpark G.

Habitat:—Attached to upper intertidal rocks (Fig. 5A).

Remarks:—New species record for Singapore. Because U. tepida is smaller than most other Ulva species, it is likely that this species had been missed during previous surveys and collections. Ulva tepida has similar gross morphology to the previously recorded U. compressa, U. flexuosa and U. intestinalis due to its filamentous thalli. The tufA phylogeny showed moderate ML and full BI support for the U. tepida / Ulva sp. 5 (Vietnam) clade (85/1) (Fig. 2), while the U. tepida clade was weakly supported in the ITS tree (67/0.67) (Supplementary File 3). There was no phylogenetic support for the U. tepida clade in the rbcL tree (Supplementary File 4). Even though Ulva sp. 5 from Vietnam is nested within the Ulva tepida clade, the former is distinguishable from U. tepida as it lacks pyrenoids (Tran et al. 2023).

Discussion

All three Ulva species discovered in this study represent new records for Singapore. Interestingly, none of the 10 Ulva species recorded by Pham et al. (2011) and Noiraksar et al. (2012) were found. Unlike other studies such as Lagourgue et al. (2022) and Tran et al. (2023), we recorded lower local Ulva diversity using DNA sequencing tools. Previous Ulva checklists in Singapore were limited to morphology-based taxonomic identifications. Due to the high degree of phenotypic plasticity (Gao et al. 2016), unresolved taxonomic issues (Hayden et al. 2003), and the lack of Ulva research in Southeast Asia, it is likely that Ulva species previously recorded in Singapore solely on the basis of comparative morphological analyses were misidentified.

Indeed, U. ohnoi, U. meridionalis and U. tepida observed in this study have similar gross morphology to other Ulva species which were previously recorded in Singapore. For example, the number and/or percentage of pyrenoids have been thought to be helpful for distinguishing U. tepida from other filamentous Ulva species like U. flexuosa (Masakiyo & Shimada 2014). However, the use of morphology alone can lead to misidentification as our U. tepida samples have 0–2 pyrenoids compared to the holotype (1–5 pyrenoids) (Masakiyo & Shimada 2014). Nevertheless, it is possible to differentiate the three Ulva species detected in Singapore. Ulva ohnoi has reticulated/expanded(filamentous thalli while U. meridionalis and U. tepida have tubular filamentous thalli. Ulva meridionalis has generally larger thalli and 1–3 pyrenoids, while U. tepida has smaller thalli and 0–2 pyrenoids.

Apart from misidentification, other reasons for the absence of previously recorded Ulva species, despite the large spatial representation of our collection, include limited seasonal sampling and local species loss. The biomass of Ulva varies considerably due to seasonal blooms (Kwan et al. 2022), and some Ulva species could have been missed due to their low biomass during our sampling period. Nevertheless, it remains uncertain whether seasonal climatic fluctuations significantly affect Ulva biomass and diversity in Singapore to preclude our collection of other Ulva species as the annual seawater temperature fluctuation is only 1°C (Sin et al. 2016). Despite the limited temporal sampling in this study, we cannot discount the possibility that Ulva species previously recorded in Singapore could be locally extinct (Poquita-Du et al. 2019). Within the past few decades, land reclamation and other coastal development activities have resulted in habitat loss across all marine ecosystems in Singapore (Tay et al. 2018, Chou et al. 2019). Some Ulva species could also have been replaced by other macroalgal species. For example, U. ohnoi could have displaced other Ulva species as it has been found at almost every surveyed site (see Nakamura et al. 2020).

Overall, the molecular markers used in this study were effective in helping to delimit and identify macroalgal species (Hayden et al. 2003, Saunders & Kucera 2010, Yip et al. 2018, Kwan et al. 2021, Lagourgue et al. 2022, Tran
et al. 2023). However, some of our Ulva specimens could not be PCR amplified or sequenced. The resolution and taxonomic coverage of molecular markers vary, and primer efficacy can be inconsistent among species (Bast et al. 2014, Kang et al. 2019). Therefore, future studies should also aim to optimise primers for commonly used Ulva DNA sequencing markers.

Conclusion

Three new Ulva species records to Singapore have been uncovered using molecular phylogenetic analyses of tufA, ITS, and rbcL genes. None of the Ulva species previously recorded in Singapore have been found potentially due to prior misidentification, limited seasonal sampling here, or local extirpation. Results from this study improve Ulva taxonomy in Southeast Asia and demonstrate the importance of using both molecular data and morphology to study macroalgal diversity. Better understanding of Ulva diversity is important for understanding its ecological effects on Singapore’s intertidal ecosystem and blue carbon sequestration (Liu et al. 2020, Kwan et al. 2022). Specifically, Ulva green tides have been known to cover seagrass meadows and other coastal ecosystems, impacting diversity and blue carbon processes along shorelines (Zhang et al. 2019, Liu et al. 2020). Findings from diversity studies would thus enable coastal ecosystems and resources to be better managed.

Acknowledgements

We thank Zhi Ting Yip, Yi Feng Choo, Zick Soh, Xavier Wong, Shalyn Tan, WeiKiat Quek, Jian Kai Low, Roxanne Lau, Xue Shen Teo, Lumin Ong, Nicholas Yap, Regina Yeo, Rochelle Chan, Marc Chang and Randolph Quek for field and laboratory assistance. We also acknowledge National Parks Board Singapore, for granting the research and collection permit (NP/RP20-096). This research was financially supported by Temasek and the Lee Kong Chian Natural History Museum.

References

https://doi.org/10.1371/journal.pone.0109295
https://doi.org/10.1016/j.jff.2022.105204
https://doi.org/10.1016/B978-0-08-100853-9.00031-2
https://doi.org/10.1093/nar/gkv1276
https://doi.org/10.1038/nmeth.2109


