# Genetic and Morphological Diversity in the Green Algal Genus *Caulerpa* J.V.Lamouroux (Bryopsidales, Chlorophyta) in Thailand

**Kattika Pattarach**

**A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology (International Program)**

**Prince of Songkla University**

**2019**

# Genetic and Morphological Diversity in the Green Algal Genus *Caulerpa* J.V.Lamouroux (Bryopsidales, Chlorophyta) in Thailand

**Kattika Pattarach**

**A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology (International Program)**

**Prince of Songkla University**

**2019**

| | |
|---|---|
| **Thesis Title** | Genetic and Morphological Diversity in the Green Algal Genus *Caulerpa* J.V.Lamouroux (Bryopsidales, Chlorophyta) in Thailand |
| **Author** | Miss Kattika Pattarach |
| **Major Program** | Biology (International Program) |

---

**Major Advisor**

....................................................

(Dr. Jaruwan Mayakun)

**Co-advisor**

....................................................

(Dr. Stefano G.A. Draisma)

**Examining Committee:**

...........................................Chairperson

(Prof. Khanjanapaj Lewmanomont)

.............................................Committee

(Dr. Jaruwan Mayakun)

.............................................Committee

(Dr. Stefano G.A. Draisma)

.............................................Committee

(Assoc. Prof. Dr. Anchana Prathep)

.............................................Committee

(Dr. Singtoe Boonrotpong)

The Graduate School, Prince of Songkla University, has approved this thesis as partial fulfillment of the requirements for the Master of Science Degree in Biology (International Program)

............................................................

(Prof. Dr. Damrongsak Faroongsarng)

Dean of Graduate School

This is to certify that the work here submitted is the result of the candidate's own investigations. Due acknowledgement has been made of any assistance received.

......................................Signature

(Dr. Jaruwan Mayakun)

Major Advisor

......................................Signature

(Dr. Stefano G.A. Draisma)

Co-advisor

......................................Signature

(Miss Kattika Pattarach)

Candidate

I hereby certify that this work has not been accepted in substance for any degree, and is not being currently submitted in candidature for any degree.

..............................................Signature

(Miss Kattika Pattarach)

Candidate

**ชื่อวิทยานิพนธ์** ความหลากหลายทางพันธุกรรมและสัณฐานวิทยาของสาหร่ายสีเขียวสกุล *Caulerpa* J.V.Lamouroux (Bryopsidales, Chlorophyta) ในประเทศไทย

**ผู้เขียน** นางสาวกัตติกา พัฒราช

**สาขาวิชา** ชีววิทยา (นานาชาติ)

**ปีการศึกษา** 2561

## บทคัดย่อ


สาหร่ายสีเขียวสกุล *Caulerpa* มีการกระจายในบริเวณเขตน้ำขึ้นน้ำลงและน้ำท่วมถึงของทะเลเขตร้อนและกึ่งเขตร้อน สาหร่ายบางชนิดมีความแปรผันทางลักษณะสัณฐานวิทยา ซึ่งความสามารถในการเปลี่ยนแปลงรูปร่างทางสัณฐานวิทยา และความซ้อนทับกันของลักษณะทางสัณฐานวิทยาเป็นอุปสรรคต่อการศึกษาทางด้านอนุกรมวิธานของสาหร่ายสกุลนี้ ซึ่งการศึกษาอนุกรมวิธานของสาหร่ายสกุลนี้ในประเทศไทยใช้เพียงข้อมูลทางสัณฐานวิทยาเท่านั้น ดังนั้นข้อมูลทางด้านชีวโมเลกุลจึงมีความจำเป็นในการจัดจำแนกสาหร่ายสกุลนี้ ในการศึกษาครั้งนี้เพื่อศึกษาความหลากหลายของสาหร่ายสกุล *Caulerpa* ในประเทศไทย โดยใช้ข้อมูลทางด้านสัณฐานวิทยาและชีวโมเลกุล (ยีน *tuf*A และ ITS rDNA sequences) จากตัวอย่างแห้งจากพิพิธภัณฑ์และตัวอย่างสด จากการศึกษาพบ สาหร่าย *Caulerpa* ทั้งสิ้น 8 ชนิด ในน่านน้ำไทย คือ *C. chemnitzia C. lentillifera C. macrodisca C. racemosa C. serrulata C. sertularioides C. taxifolia* และ *C. verticillata* และพบสาหร่ายชนิด *C. corynephora* ที่เคยรายงานนั้น เป็นเพียงความแปรผันทางลักษณะสัณฐานวิทยาของสาหร่ายชนิด *C. macrodisca* ดังนั้น *C. macrodisca* จึงมีสามลักษณะทางสัณฐานวิทยา คือ *C. macrodisca* ecad *ashmeadii C. macrodisca* ecad *corynephora* และ *C. macrodisca* ecad *macrodisca* และสาหร่าย *C. racemosa* จะมีสองลักษณะทางสัณฐานวิทยา คือ *C. racemosa* ecad *chemnitzia* และ *C. racemosa* ecad *racemosa* ดังนั้นการศึกษาในครั้งนี้สามารถสรุปได้ว่าลักษณะทางสัณฐานวิทยาเพียงอย่างเดียวไม่เพียงพอต่อการจัดจำแนกชนิดของสาหร่ายสกุล *Caulerpa* ได้ โดยยีน *tuf*A สามารถใช้ในการระบุชนิดของสาหร่ายสกุลนี้ได้ดี และ ITS rDNA sequence สามารถใช้ประกอบการยืนยันการจัดจำแนกได้ ดังนั้น ควรใช้ทั้งข้อมูลทางสัณฐานวิทยาร่วมกับข้อมูลทางพันธุกรรมในการศึกษาความ

หลากหลายของสาร่ายสกุล *Caulerpa* นอกจากนี้ในงานวิจัยครั้งนี้ไม่พบสาหร่ายสกุล *Caulerpa* บางชนิดที่เคยรายงานไว้ ในการสำรวจภาคสนาม และไม่สามารถจัดจำแนกชนิดได้เนื่องจากไม่สามารถสกัดสารพันธุกรรมจากตัวอย่างแห้งได้

**Thesis Title** Genetic and morphological diversity in the green algal genus *Caulerpa* J.V.Lamouroux (Bryopsidales, Chlorophyta) in Thailand

**Author** Kattika Pattarach

**Major Program** Biology (International Program)

**Academic Year** 2018

## ABSTRACT

*Caulerpa* is a coenocytic green algal genus that is widely distributed in the intertidal and subtidal zones of tropical and subtropical seas. Some species show a complex external morphology. A high morphological plasticity and overlapping morphologies hamper the taxonomy of the genus tremendously. All previous taxonomic studies of *Caulerpa* in Thailand were based on morphological observations. Since then the taxonomic status of several *Caulerpa* species has been challenged by molecular studies. The present study aims to reassess the *Caulerpa* diversity in Thailand using morphological and molecular data (*tuf*A gene and ITS rDNA sequences) from herbarium and fresh specimens. The result confirmed the occurrence of eight *Caulerpa* species in Thai waters, *i.e.*, *C. chemnitzia, C. lentillifera, C. macrodisca, C. racemosa, C. serrulata, C. sertularioides, C. taxifolia* and *C. verticillata*. Specimens previously identified as *C. corynephora* in Thailand actually are a morphological variety of *C. macrodisca*. Then, there were three morphological groups of *C. macrodisca*; *C. macrodisca* ecad *ashmeadii, C. macrodisca* ecad *corynephora* and *C. macrodisca* ecad *macrodisca*. In addition, *C. racemosa* is represented by two distinct morphologies; *C. racemosa* ecad *chemnitzia and C. racemosa* ecad *racemosa*. It is concluded that *Caulerpa* species identification cannot rely on morphology alone. The *tuf*A gene was useful for specific identification of *Caulerpa*, and ITS rDNA sequence was supported data. So, this study suggested that morphological and molecular data were good for *Caulerpa* diversity. In addition, several morphological *Caulerpa* species previously recorded for Thailand were not found during our field survey and their identity could not be confirmed with DNA sequence data obtained from herbarium specimens.

# ACKNOWLEDGEMENTS

I would like to express my gratitude to the contributed persons in this work.

I sincerely appreciate to Prof. Khanjanapaj Lewmanomont (Kasetsart University, Thailand) for her invaluable help of KUMF herbarium dried specimens.

I appreciate to Assoc. Prof. Dr Shao-Lun Liu (Tunghai University, Taiwan) for molecular research methodologies.

I deeply appreciate to Dr Jaruwan Mayakun (my advisor) and Dr Stefano G.A. Draisma (my co-advisors) for their opportunity to do this study. I most appreciated any supporting and comments, which advice throughout this work.

I also thank to Mr. Watana Chaithongkaew, Mr. Jatdilok Titioatchasai, Mr. Nutdanai Putthisawongand, Miss Surangkana Phandee and the Seaweed and Seagrass Research Unit (SSRU) team for their help in any field collection.

Finally, thanks to the Science Achievement Scholarship of Thailand (SAST) for my financial support, Faculty of Science, Prince of Songkla University (PSU) (Contact No. SCI610575S) and Graduate School, PSU for our grant.

Kattika Pattarach

# CONTENT

# LIST OF TABLES

# LIST OF FIGURES

## **LIST OF FIGURES** (CONTINUOUS)

**Lists** **Page**

# **LIST OF FIGURES** (CONTINUOUS)

## **LIST OF FIGURES** (CONTINUOUS)

**LIST OF FIGURES** (CONTINUOUS)

# INTRODUCTION

## Background and rationale

*Caulerpa* is one of the most common and widely distributed green algal genera in subtidal tropical and subtropical marine waters (de Senerpont Domis *et al.*, 2003; Belleza and Liao, 2007; Lee, 2008). The thallus of *Caulerpa* consists of a green creeping rhizome (stolon) with colorless downward growing rhizoids and upward growing photosynthetic fronds (assimilators). These assimilators usually bear many determinate branchlets termed ramuli. These ramuli come in a variety of shapes such as flattened, cylindrical, globular, or disk-shaped. The shape of the assimilators is the major key for species identification in this genus.

At present, there are 382 species (and infraspecific) names in the database, of which 97 species and 110 varieties and forms have been indicated as currently taxonomically accepted name (Guiry and Guiry, 2019). *Caulerpa* species show infraspecific morphological variation hampering delimiting species boundaries. This morphological plasticity may be induced by environmental factors, such as light intensity (Calvert, 1976) and temperature that affect the morphology of assimilators on *Caulerpa chemnitzia* (Esper) J.V.Lamouroux (as *Caulerpa racemosa* var. *peltata* (J.V.Lamouroux) Eubank) (Ohba *et al.*, 1992; Komatsu *et al.*, 1997). Other environmental factors; salinity, wave action and seasonal variations also influence the morphology of *Caulerpa* spp. (Calvert, 1976; Carruthers *et al.*, 1993; Meinesz *et al.*, 1995; Robledo and Freile-Pelegrín, 2005). Morphological plasticity has resulted in phenotypic variation within species of this genus and has been a long-standing source of uncertain taxonomy and causes the taxonomic havoc in *Caulerpa* (Famà *et al.*, 2002; Belleza and Liao, 2007; Sauvage *et al.*, 2013; Belton *et al.*, 2014) and the morphological characters only are not enough for the identification and classification of several species in this genus.

Recently, researchers applied molecular tools to identify some morphologically plastic species (Verbruggen *et al.*, 2009; Mahakham, 2011; de Clerck *et al.*, 2013). Molecular studies used the chloroplast-encoded *tuf*A gene and the Internal Transcribed Spacers (ITS) of the nuclear ribosomal cistron to infer phylogenetic relationships and a subgeneric classification in the genus *Caulerpa* in various regions (Olsen *et al.*, 1998; Famà *et al.*, 2002; Stam *et al.*, 2006; Kazi *et al.*, 2013; Sauvage *et al.*, 2013; Belton *et al.*, 2014; Draisma *et al.*, 2014). *Caulerpa* species from the Mediterranean region, California, India and Australia have been exhaustively investigated and determined (Verlaque, 2000; Zaleski and Murray, 2006; Kazi *et al.*, 2013; Belton *et al.*, 2014).

Phang *et al.* (2016) listed 27 *Caulerpa* species and 60 taxa including varieties and formae in the South China Sea, including eight countries; Brunei, Cambodia, Indonesia, Malaysia, Philippines, Singapore, Vietnam and Thailand. In Thailand, only Lewmanomont (2008) published on the taxonomy of *Caulerpa* using morphological data and reported 16 *Caulerpa* species in Thai waters (the Andaman Sea and the Gulf of Thailand coast). Morphological variation was recorded in many herbarium specimens of *Caulerpa racemosa* (Forsskål) J.Agardh collected from various areas, such as Phuket, Surat Thani, Trang and Satun provinces, but intraspecific names were not reported (Personal Observation). However, there is no data on genetic diversity and distribution patterns of *Caulerpa* in Thailand. The present study aims to investigate the genetic diversity and distribution patterns of *Caulerpa* in Thailand using the *tuf*A gene and ITS rDNA sequence data and morphological characters. This study can answer these questions; 1) how many species of *Caulerpa* are there along the coasts of Thailand? and 2) what is the extent of infraspecific morphological and genetic variation in *Caulerpa* species from Thailand? Then, the results were presented into 2 parts; 1. Diversity and distribution of the genus *Caulerpa* in Thailand and 2. An enigmatic *Caulerpa macrodisca* Decaisne (Chlorophyta) from the mangrove channels on the Andaman Sea coast of Thailand.

**Review of literature**

Lamouroux (1809) erected the genus *Caulerpa* and reported eight species representing five currently accepted species; *Caulerpa prolifera* (Forsskål) J.V.Lamouroux (including *Caulerpa ocellata* J.V. Lamouroux), the type of the genus that was designated in Trevisan (1849), *C. chemnitzia* (including *Caulerpa peltata* J.V.Lamouroux), *Caulerpa flexilis* J.V.Lamouroux ex C.Agardh (as *Caulerpa hypnoides* C.Agardh), *C. racemosa* (as *Caulerpa obtusa* J.V.Lamouroux, uncertain synonymy), *Caulerpa sertularioides* (S.G. Gmelin) M. Howe (as *Caulerpa myriophylla*) and *Caulerpa taxifolia* (M.Vahl) C.Agardh (as *Caulerpa pennata* J.V.Lamouroux). Kützing (1843) classified this genus into the family Caulerpaceae. *Caulerpa* consists of a creeping green stolon with colorless rhizoids and frond-like erect shoots. The upright shoots present considerable variation in morphology of the ramuli, such as sickle-shaped, disc-shaped, mushroom-shaped and globular. (Lee, 1999; Draisma *et al.*, 2014). A characteristic feature of this family is the presence of trabeculae, which are internal β-1,3 linked xylan cell wall outgrowths that give structural support the thallus (Lamouroux, 1809; Bold and Wynne, 1985; Famà *et al.*, 2002; Lee, 2008; Draisma *et al.*, 2014).

In 1899–1900, the first record of *Caulerpa* was from the vicinity of Ko Chang, the Gulf of Thailand during the Danish expedition to Siam (Rathbun, 1919). Reinbold (1901) found 11 species; *C. chemnitzia* (as *C. peltata*), *Caulerpa fastigiata* f. *minor* Weber-van Bosse, *Caulerpa lentillifera* J.Agardh (as *Caulerpa lentillifera* var. *longistipitata* Weber-van Bosse), *Caulerpa plumaris* var. *longipes* Weber-van Bosse, *C. racemosa* var. *uvifera* Weber-van Bosse, *Caulerpa scalpelliformis* (R.Brown ex Turner) C.Agardh, *Caulerpa sedoides* C.Agardh, *Caulerpa serrulata* var. *pectinata* (Weber-van Bosse) W.R.Taylor (as *Caulerpa freycinettii* var. *pectinata* Weber-van Bosse), *Caulerpa tongaensis* Papenfuss (as *Caulerpa filiformis* J.Agardh), *Caulerpa urvilleana* f. *tristicha* (J.Agardh) Weber-van Bosse and *Caulerpa verticillata* J.Agardh that grow on rocks in shallow water at Ko Chick, Ko Samae San and Ko Kahdat. In addition, Dawson (1954) found *C. lentillifera* in the northern part of Saen Soek, the Gulf of Thailand. Egerod (1974) published a second report including *Caulerpa mexicana* Sonder ex Kützing, *Caulerpa microphysa* (Weber-van Bosse) Feldmann, *Caulerpa racemosa* var. *macrophysa* (Sonder ex Kützing)

W.R.Taylor and *C. chemnitzia* (as *Caulerpa racemosa* var. *peltata* (J.V.Lamouroux) Eubank) from the Andaman Sea. The Fifth Thai Danish Expedition (1966), the third report (Seidenfaden *et al.,* 1968) showed five species of *Caulerpa*; *C. ambigua, Caulerpa fastigiata* Montagne, *C. mexicana, C. racemosa* and *C. sertularioides* at Phuket (Egerod, 1971, 1974, 1975). Lewmanomont (1978) studied some algae that are used as food in Thailand. She reported that *C. racemosa* var. *macrophysa* and *Caulerpa corynephora* Montagne (as *Caulerpa racemosa* var. *corynephora* (Montagne) Weber-van Bosse) are commonly used as food for people in the South. The Algae Checklist of Thailand (Lewmanomont *et al.*, 1995) lists 19 *Caulerpa* species, seven varieties and two formae. The most recent taxonomic morphology based treatment of the genus *Caulerpa* in Thailand lists 16 species including *Caulerpa ashmeadii* Harvey, *Caulerpa cupressoides* (Vahl) C.Agardh, *Caulerpa fastigiata* Montagne, *C. lentillifera*, *C. racemosa* var. *macrophysa* (as *Caulerpa macrophysa* (Sonder ex Kützing) G.Murray), *Caulerpa manorensis* Nizamuddin, *Caulerpa mexicana* Sonder ex Kützing, *C. microphysa*, *C. chemnitzia* (as *C. peltata*), *Caulerpa macrodisca* (Forsskål) J. Agardh (as *C. peltata* var. *macrodisca*), *C. corynephora* (as *C. racemosa* var. *corynephora*), *C. serrulata*, *C. sertularioides*, *C. taxifolia*, *C. verticillata* and *C. ambigua* currently recognized species (Lewmanomont, 2008).

A revision of the *Caulerpa* herbarium specimens in the Princess Maha Chakri Sirindhorn Natural History Museum (NHM) in Hat Yai revealed phenotypic variations of *Caulerpa* specimens (Pattarach, 2016; Personal Observation) and identification at species and subspecies level remains problematic. Seaweed expert Eric Coppejans also found *Caulerpa* with different shapes and arrangements of ramuli from the same study site in Krabi province, Thailand (personal communication). The *Caulerpa racemosa-peltata* complex along the Thai coast showed a variety of morphologies.

The branching of photosynthetic fronds exhibits a diverse range of ramuli morphology. Light intensity and temperature affect the morphology of assimilators on *C. chemnitzia* (as *C. racemosa* var. *peltata*) (Ohba *et al.*, 1992). Other environmental factors; salinity, wave action and seasonal variations also influence the morphology of *Caulerpa* spp. (Calvert, 1976; Carruthers *et al.*, 1993; Meinesz *et al.*, 1995; Robledo and Freile-Pelegrín, 2005). Morphological plasticity has resulted in phenotypic variation within species of this genus and has been a long-standing source of uncertain taxonomy (Famà *et al.*, 2002; Belleza and Liao, 2007; Sauvage *et al.*, 2013; Belton *et al.*, 2014).

DNA barcoding is recently developed molecular tool to assist the identification of *Caulerpa* species. This method analyzes a nucleotide sequence of a standard DNA region of an unknown specimen and compares it with the nucleotide sequences of identified specimens. This can give an accurate and reliable identification in a short time (Mahakham, 2011). This technique was used to clearly identify cryptic species in the bryopsidalean genus *Codium* that showed morphological plasticity (Lee and Kim, 2015).

The *tuf*A gene is a conserved sequence in green algae, located in the chloroplast genome. It also is a common DNA markers used in phylogenetic studies because it can present high variation at the species level (Famà *et al.*, 2002; Pečnikar and Buzan, 2014). Currently 1,115 *Caulerpa tuf*A sequences in the GenBank nucleotide database representing 70 *Caulerpa* species (78 % of the 90 currently recognized species) (see Draisma *et al.*, 2014, suppl. table S2). In addition, the Internal Transcribed Spacers (ITS1 and ITS2) of the nuclear ribosomal cistron have also been used frequently in *Caulerpa* taxonomy (Kazi *et al.*, 2013; Yeh and Chen, 2014; Stam *et al.*, 2006). There are many reasons to use ITS rDNA as a barcoding marker. First, the nucleotide sequence of this gene is highly variable at the inter- and infraspecific level in green algae (Bakker *et al.*, 1995; Olsen *et al.* 1994, 1998). Second, the variation in ITS nucleotide sequences is about 3–4 times higher than that in chloroplast genes (Mahakham, 2011). Third, there are many ITS rDNA sequences available in GenBank, than can be used for the development of PCR primers.

Many molecular studies of *Caulerpa* have been done in many countries. For example, Olsen *et al.* (1998) used ITS rDNA sequences for the identification of *C. taxifolia* and *C. mexicana* in the Mediterranean. These two taxa have similar morphologies, but maximum parsimony and maximum likelihood phylogenies clearly separate the two taxa. The two species are thus not conspecific.

Famà *et al.* (2002) presented a molecular phylogeny of 23 *Caulerpa* taxa from tropical and subtropical zones of the world using the *tuf*A gene. Their phylogenetic tree showed that the traditional morphology based infrageneric classification into 12 sections does not represent evolutionary history as most sections are polyphyletic. Sixteen clades could be discerned in their maximum likelihood phylogeny. *C. mexicana, C. sertularioides, C. webbiana, C. prolifera, C. taxifolia* and *C. racemosa* were sampled multiple times and each formed a monophyletic clade, except the latter two species. *C. distichophylla* was nested inside *C. taxifolia* and *C. racemosa* was polyphyletic divided into four clades.

Stam *et al.* (2006) did a phylogenetic survey of *Caulerpa* in the Caribbean using ITS rDNA and *tuf*A. Their phylogeny showed that *C. brachypus* was sister to *C. scalpelliformis*. In addition, *C. cupressoides* and *C. serrulata* could not unambiguously be distinguished from each other and *C. racemosa* and *C. scalpelliformis* were both polyphyletic. Moreover, *C. taxifolia* and *C. racemosa* were considered as alien species record for the first time in Florida, USA.

Sauvage *et al.* (2013) studied the molecular diversity of the *C. racemosa-peltata* complex in New Caledonia and Australia using the *tuf*A gene. The result showed that five lineages were identified in New Caledonia, and the main diversity may be restricted to the Indo-Pacific region. In addition, *Caulerpa cylindracea* Sonder (as *C. racemosa* var. *cylindracea*) was found as new record at Lizard Island on the Great Barrier Reef, Australia.

Kazi *et al.* (2013) studied molecular phylogeny and DNA barcoding of *Caulerpa* in India using the *tuf*A, *rbc*L, 18S rDNA and ITS rDNA. The result showed that the classification of *Caulerpa* using multiple markers is more efficient than using just one gene. The molecular phylogeny result was consistent with the morphological study.

Belton *et al.* (2014) studied the character of phenotypic plasticity and identification of the *C. racemosa-peltata* complex in Australia using *tuf*A and *rbc*L gene sequences. The result revealed 11 *Caulerpa* lineages within the complex, and five of them, such as *Caulerpa lamourouxii* (Turner) C.Agardh and *Caulerpa oligophylla* Montagne showed a high degree of phenotypic plasticity and some overlap with others.

Draisma *et al.* (2014) revised the subgeneric classification of *Caulerpa* based on a time-calibrated molecular phylogeny. The phylogenetic tree based on *rbc*L and *tuf*A gene sequences revealed six main clades that were each given subgenus status. Three of the six subgenera are currently monotypic, albeit *Caulerpella ambigua* (Okamura) Prud'homme van Reine & Lokhorst showed high diversity. It was concluded that *Caulerpella ambigua* represents multiple cryptic species nested inside the genus *Caulerpa*. So, *Caulerpella ambigua* was transferred back to the genus *Caulerpa* called that *Caulerpa ambigua* Okumura.

In conclusion, the phylogenetic analysis using some specific nucleotide sequences is able to identify morphological plastic species in the genus *Caulerpa*. This has been demonstrated in several regions, e.g. the Caribbean, the Mediterranean, India, New Caledonia and Australia. However, a comprehensive molecular study of *Caulerpa* in Thailand is still lacking. The present research aims to study the genetic diversity and distribution patterns of *Caulerpa* in Thai waters.

**Questions of this research**

1. How many species of *Caulerpa* are there along the coasts of Thailand?
2. What is the extent of infraspecific morphological and genetic variation in *Caulerpa* species from Thailand?

**Objectives**

1. To identify the species of *Caulerpa* using morphological and molecular evidence.
2. To assess the genetic diversity of *Caulerpa* using *tuf*A and ITS rDNA sequences.
3. To confirm the identification of *Caulerpa*.

For answering the question, I begin with 1. Diversity and distribution of the genus *Caulerpa* in Thailand, followed by 2. An enigmatic *Caulerpa macrodisca* Decaisne (Chlorophyta) from the mangrove channels on the Andaman Sea coast of Thailand.

# RESEARCH METHODOLOGY

## Collection sites

The study sites were chosen along the Thai coast in 15 provinces following Lewmanomont (2008) (Fig. 1)

### The Gulf of Thailand

- Losin, Pattani
- Kao Seng and Ko Kham, Songkhla
- Sichon, Nakhon Si Thammarat
- Ko Samui and Ko Tan, Surat Thani
- Mu Ko Chumphon, Chumphon
- Ao Manao, Prachuap Khiri Khan
- Ko Kram and Ko Samae San, Chonburi
- Ko Chik, Chanthaburi
- Saoson Beach, Rayong
- Ko Chang, Ko Kradat, Ao Cho and Leam Sok, Trat

### The Andaman Sea

- Tang Khen Bay, Phuket
- Ko Phra Thong and Ko Similan, Phang Nga
- Ko Siboya, Khlong Yang, Ko Lanta, Krabi
- Ko Libong, Leam Yong Lam and Khao Bae Na, Trang
- Ko Lidee, Ko Lipe, Thung Wa and Che Bilang, Satun

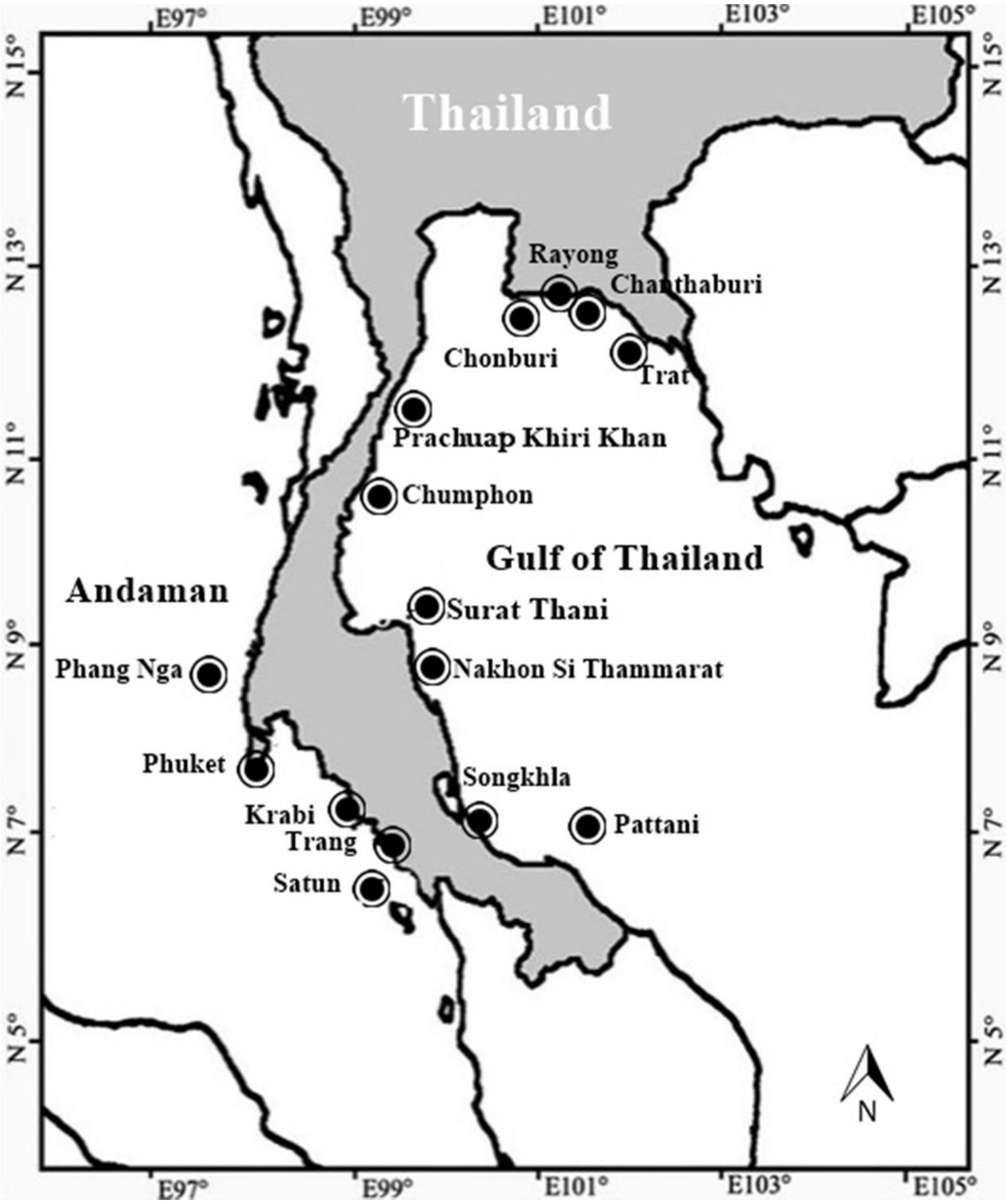


**Figure 1** Map of study sites at 15 provinces in Thailand.

**Environmental factors**

Four environmental factors were recorded in this study, *i.e.*, habitat type, water temperature, light intensity, and salinity (Table 1). Water temperature was estimated in some sites using either a laboratory thermometer or the Onset-Hobo data logger (Onset Computer Corporation, Contoocook, NH, USA). Light intensity was also measured using the Onset-Hobo data logger. More than 800 µmol photons $m^{-2}$ $s^{-1}$ was defined as high light intensity and less than 800 µmol photons $m^{-2}$ $s^{-1}$ as low intensity. Salinity was determined in some sites using a salinity reflectometer (ATC, 0–100 ppt, XHO RHS-10ATC, ATACO, China).

**Table 1** Summary of environmental factors used in this study. n.m., not measured.

| Study sites | GPS coordinates | Environmental factors | | | |
|---|---|---|---|---|---|
| | | **Habitat types** | **Water temperature** (°C, mean±SE) | **Light intensity** | **Salinity** (‰) |
| **Pattani** | | | | | |
| Losin | 7°19'N, 101°51'E | Dead coral in coral reef of subtidal zone | n.m. | n.m. | 35 |
| **Songkhla** | | | | | |
| Kao Seng | 7°10'N, 100°37'E | Rocky shore in intertidal zone | 34±0.12 | High light | 33 |
| Ko Kham | 6°58'N, 100°51'E | Rocky shore in intertidal zone | 33±0.47 | High light | 33 |
| **Nakhon Si Thammarat** | | | | | |
| Sichon | - | Sandy bottom and rock in intertidal zone | n.m. | n.m. | n.m. |
| **Surat Thani** | | | | | |
| Ko Samui | 9°34'N, 100°03'E | Dead coral, sandy bottom and rock in intertidal zone | 32±0.32 | High light | 33 |
| Ko Tan | 9°23'N, 99°57'E | Dead coral, sandy bottom and rock in intertidal and subtidal zone | 32±0.45 | High light | 33 |

| Study sites | GPS coordinates | Environmental factors | | | |
|---|---|---|---|---|---|
| | | **Habitat types** | **Water temperature** (°C, mean±SE) | **Light intensity** | **Salinity** (‰) |
| **Chumphon** | | | | | |
| Mu Ko Chumphon | 10°19'N, 99°18'E<br>10°19'N, 99°16'E<br>10°17'N, 99°14'E | Dead coral, sandy bottom and rock in intertidal and subtidal zone | 30 | Low light (monsoon) | 33 |
| **Prachuap Khiri Khan** | | | | | |
| Ao Manao | 11°45'N, 99°47'E | Sandy bottom and rock in intertidal zone | 34 | High light | 33 |
| **Chonburi** | | | | | |
| Ko Kram | - | Dead coral, sandy bottom and rock in intertidal and subtidal zone | n.m. | n.m. | n.m. |
| Ko Samae San | - | Dead coral, sandy bottom and rock in intertidal and subtidal zone | n.m. | n.m. | n.m. |
| **Chanthaburi** | | | | | |
| Ko Chik | 12°17'N, 102°14'E | Dead coral and rock in intertidal and subtidal zone | 35 | High light | 33 |

| **Study sites** | **GPS coordinates** | **Environmental factors** | | | |
|---|---|---|---|---|---|
| | | **Habitat types** | **Water temperature** (°C, mean±SE) | **Light intensity** | **Salinity** (‰) |
| **Rayong** | | | | | |
| Saoson Beach | 12°37'N, 101°28'E | Sandy bottom in intertidal zone | 34 | High light | 33 |
| **Trat** | | | | | |
| Ko Chang | 12°04'N, 102°16'E | Dead coral and rock in intertidal zone | 35 | High light | 34 |
| Ko Kradat | - | Dead coral and rock in intertidal zone | n.m. | n.m. | n.m. |
| Ao Cho | 12°33'N, 101°27'E | Sandy bottom and rock in intertidal zone | n.m. | n.m. | 33 |
| Leam Sok | 12°02'N, 102°35'E | Rocky shore in intertidal zone | 35 | High light | 33 |
| **Phuket** | | | | | |
| Tang Khen Bay | - | Dead coral in intertidal zone | n.m. | n.m. | 33 |

| **Study sites** | **GPS coordinates** | **Environmental factors** | | | |
|---|---|---|---|---|---|
| | | **Habitat types** | **Water temperature** (°C, mean±SE) | **Light intensity** | **Salinity** (‰) |
| **Phang Nga** | | | | | |
| Ko Phra Thong | 9°07'N, 98°15'E | Dead coral and rock in intertidal zone | n.m. | n.m. | 33 |
| Ko Similan | 8°38'N, 97°39'E | Dead coral and rock in intertidal zone | n.m. | n.m. | n.m. |
| **Krabi** | | | | | |
| Ko Siboya | 7°53'N, 98°58'E | Sandy bottom and rock in intertidal zone | n.m. | n.m. | 33 |
| Khlong Yang | 7°48'N, 99°07'E | Muddy bottom and fish cages of mangrove channels | 27±0.45 | Low light | ~28 (27–30) |
| Ko Lanta | 7°28'N, 99°05'E | Tide pool of rocky shore in intertidal zone | 29±1.25 | High light | 33 |
| **Trang** | | | | | |
| Ko Libong | 7°15'N, 99°26'E | Sandy bottom of subtidal zone | 30±0.19 | High light | 33 |
| Leam Yong Lam | 7°23'N, 99°20'E | Sandy bottom of subtidal zone | 32±0.47 | High light | 33 |
| Khao Bae Na | 7°24'N, 99°20'E | Sandy bottom of subtidal zone | 32±0.37 | High light | 33 |

| Study sites | GPS coordinates | Environmental factors | | | |
|---|---|---|---|---|---|
| | | **Habitat types** | **Water temperature** (°C, mean±SE) | **Light intensity** | **Salinity** (‰) |
| **Satun** | | | | | |
| Ko Lidee | 6°47'N, 99°45'E | Sandy bottom and rock in intertidal zone | 33±0.04 | High light | ~33 (31–34) |
| Ko Lipe | 6°29'N, 99°18'E | Sandy bottom and rock in intertidal zone | 35 | High light | 35 |
| Thung Wa | 6°37'N 100°01'E | Muddy bottom and fish cages of mangrove channels | n.m. | Low light | ~30 (27–30) |
| Che Bilang | - | Muddy bottom and fish cages of mangrove channels | n.m. | Low light | ~30 (27–30) |

## Specimen collection

At least three specimens (complete thalli; rhizoid, stolon and frond of each morphological species from each study site) without epiphyte were collected using scuba diving and snorkeling from the intertidal to around 5–10 m depth. The samples were preserved on herbarium sheets for morphological study. Specimens were pressed and labels with collection date, locality, collector name, and habitat type were added to the herbarium following Coppejans *et al*. (2010). All specimens were photographed using the camera Canon 60D with Canon EF-S 18–55mm f/3.5–5.6 IS. The fragments (1–2 cm) of each sample were quickly dried and preserved in silica gel for molecular studies. In addition, herbarium-dried specimens from the Princess Maha Chakri Sirindhorn Natural History Museum (NHM) in Prince of Songkla University (PSU) and Kasetsart University Museum of Fisheries (KUMF) in Kasetsart University (KU) were also sampled for nucleotide analysis.

## The morphological study

Twenty-seven morphological characters (Table 2, Fig. 2) such as assimilator length, stolon and ramuli diameter (adapted from de Senerpont Domis *et al.*, 2003) were measured on each fresh specimen using a caliper, before it is processed into a herbarium specimen. Once herbarium dried specimens were measured using NIH ImageJ software (Rasband, 1997). Each specimen was investigated using field guides of seaweed (Trono, 1997; de Clerck *et al.*, 2005; Phang *et al.*, 2008; Coppejans *et al.*, 2010; 2017).

**Table 2** Summary of characters to be used in morphometric analysis (adapted from de Senerpont Domis *et al.*, 2003)

| No. | Morphological structures | Unit | Description |
|---|---|---|---|
| 1 | Number of stolon branches per cm stolon | no./cm | taken by counting the number of stolon branching and dividing it by the stolon length |
| 2 | Diameter of Stolon | cm | measured as thickest part of the stolon near the growing tip |
| 3 | Number of rhizoid clusters per cm stolon | no./cm | taken by counting the number of rhizoid clusters and dividing it by the stolon length |
| 4 | Spacing of rhizoid clusters | cm | measured as distance between rhizoid clusters |
| 5 | Length of rhizoid cluster | cm | measured as the base to the apical end of rhizoid of the thallus |
| 6 | Number of assimilators per cm stolon | no./cm | taken by counting the number of assimilator and dividing it by the stolon length |
| 7 | Spacing of assimilators | cm | measured as distance between assimilators |
| 8 | Length of assimilator | cm | measured from the base to the apical end of the upright of the thallus |
| 9 | Width of assimilator | cm | measured from the right side to the left side at the widest assimilator of the thallus |
| 10 | Assimilator shape | none | flat, feather-like (distichous), irregulary and thread-like (filiform) |
| 11 | Assimilator margin | none | entire, serrate and spiny |
| 12 | Rachis length | cm | measured from the base to the first ramulus |
| 13 | Diameter of rachis | cm | measured as the thickest part of the main axis of the assimilator near the apex |
| 14 | Diameter of a constricted of rachis | cm | measured a narrow part of rachis |
| 15 | Number of laterals per cm assimilator | no./cm | taken by counting the number of assimilator branching and dividing it by the assimilator length |
| 16 | Spacing of ramuli | cm | measured as distance between ramuli on the assimilator |

| No. | Morphological structures | Unit | Description |
|---|---|---|---|
| 17 | Arrangement of ramuli | none | opposite, alternate, whorled and irregularly |
| 18 | Length of ramulus | cm | measured from the base to the apical end of ramulus |
| 19 | Diameter of ramulus | cm | measured as the broadest part of ramulus |
| 20 | Shape of ramulus | none | turbinate shape, subspherical shape, clavate shape, serrate shape, falcate shape, cylindrical shape, peltate shape, mushroom-liked and filiform shape |
| 21 | Shape of ramulus tip | none | acute, acuminate, rounded, oval, mucronate, teeth and three spines |
| 22 | Shape of ramulus base | none | cuneate, obtuse, cordate and oblique |
| 23 | Diameter of a constricted of ramulus | cm | measured a narrow part of ramulus |
| 24 | Length of stalk | cm | measured from the base of ramuli to the axes of assimilator |
| 25 | Diameter of stalk | cm | measured as the broadest part of the stalk |
| 26 | Diameter of a constricted of stalk | cm | measured a narrow part of stalk |
| 27 | Shape of tip | none | acute, acuminate, rounded, mucronate, teeth and disc |

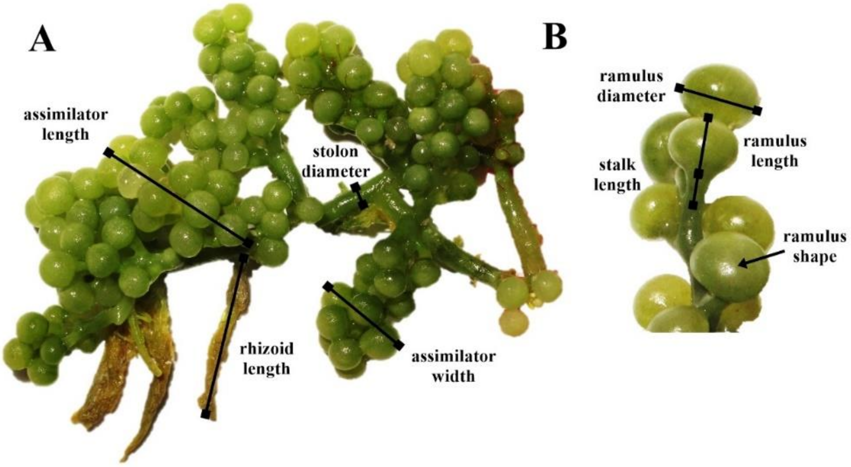


**Figure 2** Morphological characters were used for morphometric measurement (A., thallus and B., ramuli of *C. racemosa* (KP40A) from Ko Lipe, Satun)

## Molecular study

### DNA extraction, amplification and sequencing

DNA was extracted from Silica gel dried specimens using ZR plant/Seed DNA MiniPrep™ Kit (Zymo Research Corporation, New York, USA). A small sample was gridded and added 750 µl Lysis Solution into the ZR BashingBead™ Lysis tube. The tube was processed with vortex meter at maximum speed for 40 minutes and centrifuged at 10,000 x g for 3 minutes. The supernatant (400 µl) was transferred to a Zymo-Spin™ IV Spin Filter tube and centrifuge at 7,000 x g for 3 minutes. The solution was mixed with Plant/Seed DNA Binding Buffer (1,200 µl). The mixture was filtrated by Zymo-Spin™ IIC Column tube and centrifuged at 10,000 x g for 3 minutes. DNA Pre-Wash Buffer (200 µl) was added to the Zymo-Spin™ IIC Column tube and centrifuged at 10,000 x g for 3 minutes. Plant/Seed DNA Wash Buffer (500 µl) was added to the Zymo-Spin™ IIC Column tube and centrifuged at 10,000 x g for 3 minutes (2 time). DNA Elution Buffer (80 µl) was added into the column matrix and centrifuged at 10,000 x g for 3 minutes. The eluted DNA was filtrated by Zymo-Spin™ IV-HRC Spin Filter tube and centrifuge at exactly 8,000 x g for 3 minutes. The DNA product was finally extracted and stored at -20 ºC.

### PCR amplification

**Primers for *tuf*A gene** (Famà *et al.*, 2002)

Forward primer: *tuf*A (5´-TGAAACAGAAMAWCGTCATTATGC-3´)

Reverse primer: *tuf*AR (5´-CCTTCNCGAATMGCRAAWCGC-3´)

**Primers for ITS sequence** (Kooistra *et al.*, 2002 and White *et al.*, 1990)

Forward primer: H1F (5´-CTCTGAACCTTCGCACGTAGA-3´)

Reverse primer: ITS4R (5´-TCCTCCGCTTATTGATAGATGC-3´)

PCR amplification of *tuf*A gene was performed in 20 µL of master mix contained 0.4 µL of each 0.2 µM primer, 0.4 µL of 0.2 mM dNTPs, 0.12 µL of 0.1X Taq DNA polymerase and 2 µL of 10X buffer (Clontech Laboratories Inc., a Takara Bio company, CA, USA) and 1 µL DNA template. Double-stranded DNA amplifications were performed in a S1000™ thermal cycler (Bio-Rad Laboratories, California, USA). An initial denaturation at 96ºC for 4 minutes followed by 40 cycles of 94ºC for 30 second, 52ºC for 30 s and 72°C for 1 min, a final extension at 72°C for 6 min. For an annealing of ITS rDNA sequence, 48°C for 30 s (Fig. 3).

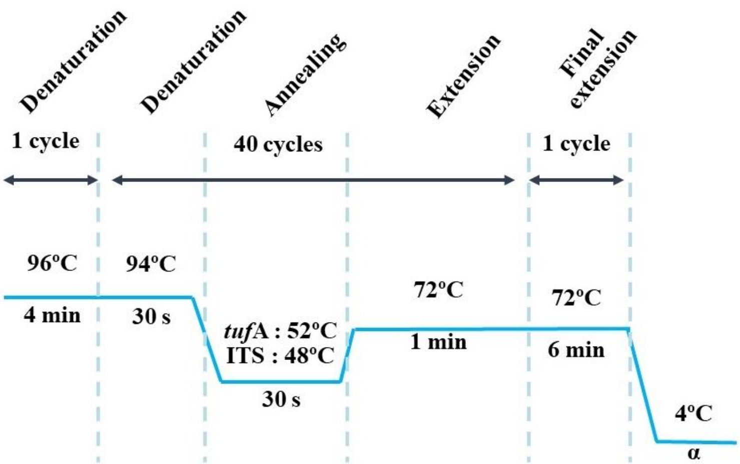


**Figure 3** This is PCR amplification condition of this study following Taq DNA polymerase protocol (Clontech Laboratories Inc., a Takara Bio company, CA, USA).

## Nucleotide sequencing

PCR products were purified and sequenced by Genedragon Inc. (Taiwan) and Macrogen Inc. (Seoul, Korea).

**Nucleotide sequence alignment analysis**

A total of 270 *Caulerpa tuf*A sequences from GenBank together with 45 newly *tuf*A *Caulerpa* sequences (Table 3) were used with *Dichotomosiphon tuberosus* A.Braun ex Kützing) A.Ernst (GenBank MH591082) as outgroup. For ITS sequences, 59 GenBank sequences with 56 newly generated ITS *Caulerpa* sequences (Table 3) were analyzed together with outgroup, *Rhipiliopsis reticulata* (C.Hoek) Farghaly & Denizot (GenBank AF416386). All sequences were aligned using the MUSCLE software (Edgar, 2004). Maximum Likelihood (ML) phylogenetic tree was inferred in MEGA v7 (Kumar *et al.*, 2016) using GTR+G+I model. Branch support was assessed with bootstrap (1,000 replications). Maximum Likelihood bootstrap percentages (BP) values were considered as weak (below 60%), moderate (70%–79%) and strong (80%–100%). Bayesian Inference (BI) was performed in MrBayes (Huelsenbeck and Ronquist, 2001) using Markov Chain Monte Carlo chains (MCMC) of 40,000,000 generations, sampled every 4,000 generations with 10% burnin period (Draisma *et al.*, 2014). Bayesian Inference posterior probabilities (PP) were considered as no supported (below 0.90), weak (0.90–0.94) and strong (0.95–1.00).

**Table 3** Collection data, GenBank accession numbers (**bold**, indicates the sequence was determined in the present study, and ✔ as not submitted to GenBank), and references for specimens used in the present study. n.a., not available.

| *tuf*A NCBI accession | ITS NCBI accession | GenBank name | Location | Updated name | References | Voucher specimens | Note |
|---|---|---|---|---|---|---|---|
| - | AJ007817 | *C. mexicana* | San Blas Island, Panamá | - | Olsen *et al.* (1998) | RH JUNI 98 01821 | |
| - | AJ007818 | *C. mexicana* | Florida, USA | - | Olsen *et al.* (1998) | RH JUNI 98 01943 | |
| - | AJ007819 | *C. taxifolia* | Sicily, Italy | - | Olsen *et al.* (1998) | RH JUNI 98 01941 | |
| - | AJ007823 | *C. taxifolia* | Townsville, Australia | - | Olsen *et al.* (1998) | RH JUNI 98 01946 | |
| - | AY205288 | *C. racemosa* var. *peltata* | Green Island, Taiwan | *C. chemnitzia* | Yeh and Chen (2004) | n.a. | |
| - | AY205289 | *C. racemosa* var. *peltata* | Dali, Taiwan | *C. chemnitzia* | Yeh and Chen (2004) | n.a. | |
| - | AY205290 | *C. racemosa* var. *laetevirens* | Fengchueisha, Taiwan | *C. chemnitzia* | Yeh and Chen (2004) | n.a. | |
| - | AY205291 | *C. racemosa* var. *laetevirens* | Kenting, Taiwan | *C. chemnitzia* | Yeh and Chen (2004) | n.a. | |
| - | AY206420 | *C. racemosa* var. *macrophysa* | Green Island, Taiwan | *C. racemosa* | Yeh and Chen (2004) | n.a. | |
| - | AY206421 | *C. racemosa* var. *macrophysa* | Nanwan, Taiwan | *C. racemosa* | Yeh and Chen (2004) | n.a. | |
| - | AY206422 | *C. microphysa* | Green Island, Taiwan | - | Yeh and Chen (2004) | n.a. | |
| - | AY206423 | *C. serrulata* | Green Island, Taiwan | - | Yeh and Chen (2004) | n.a. | |
| - | AY206426 | *C. webbiana* | Green Island, Taiwan | - | Yeh and Chen (2004) | n.a. | |
| - | AF416386 | *Rhipiliopsis reticulata* | Isla Galeta, Panamá | - | Kooistra *et al.* (2002) | n.a. | |
| - | KF840135 | *C. corynephora* | Tamil Nadu, India | - | Kazi *et al.* (Unpublished) | BHAV CSIR-CSMCRI 20130329003 | |
| - | KR478535 | *C. racemosa* var. *macrophysa* | India | *C. racemosa* | Karthick *et al.* (2015) | n.a. | |
| AJ417928 | - | *C. cupressoides* var. *lycopodium* | Uken, Japan | - | Famà *et al.* (2002) | n.a. | |
| AJ417929 | - | *C. cupressoides* | St. Barthélemy, Lesser Antilles | - | Famà *et al.* (2002) | n.a. | |
| AJ417930 | - | *C. cupressoides* var. *flabellata* | Bocas del Toro, Panamá | - | Famà *et al.* (2002) | n.a. | |
| AJ417931 | - | *C. serrulata* | Dahab, Egypt | - | Famà *et al.* (2002) | n.a. | |
| AJ417932 | - | *C. serrulata* | Pangasinan, Philippines | - | Famà *et al.* (2002) | n.a. | |

| *tuf*A NCBI accession | ITS NCBI accession | GenBank name | Location | Updated name | References | Voucher specimens | Note |
|---|---|---|---|---|---|---|---|
| AJ417933 | - | *C. serrulata* | Colón, Panamá | - | Famà *et al.* (2002) | n.a. | |
| AJ417934 | - | *C. brachypus* | Pangasinan, Philippines | - | Famà *et al.* (2002) | n.a. | |
| AJ417936 | - | *C. taxifolia* | Moreton Bay, Australia | - | Famà *et al.* (2002) | n.a. | |
| AJ417937 | - | *C. taxifolia* | Safaga, Egypt | - | Famà *et al.* (2002) | n.a. | |
| AJ417938 | - | *C. taxifolia* | Puerto-Rico, USA | - | Famà *et al.* (2002) | n.a. | |
| AJ417939 | - | *C. taxifolia* | N. Kwa-Zulu Natal, South Africa | - | Famà *et al.* (2002) | n.a. | |
| AJ417941 | - | *C. ashmeadii* | Florida, USA | - | Famà *et al.* (2002) | n.a. | |
| AJ417942 | - | *C. prolifera* | Bali, Indonesia | - | Famà *et al.* (2002) | n.a. | |
| AJ417944 | - | *C. sertularioides* | Martinique, Lesser Antilles | - | Famà *et al.* (2002) | n.a. | |
| AJ417945 | - | *C. sertularioides* | Colón, Panamá | - | Famà *et al.* (2002) | n.a. | |
| AJ417946 | - | *C. sertularioides* | Melones Island, Panamá | - | Famà *et al.* (2002) | n.a. | |
| AJ417947 | - | *C. racemosa* var. *macrophysa* | Galeta, Panamá | *C. racemosa* | Famà *et al.* (2002) | n.a. | |
| AJ417948 | - | *C. racemosa* var. *peltata* | Panamá | *C. chemnitzia* | Famà *et al.* (2002) | n.a. | |
| AJ417949 | - | *C. racemosa* var. *peltata* | Isla Naos, Panamá | *C. chemnitzia* | Famà *et al.* (2002) | n.a. | |
| AJ417950 | - | *C. racemosa* | Galeta, Panamá | *C. chemnitzia* | Famà *et al.* (2002) | n.a. | |
| AJ417951 | - | *C. mexicana* | Cuba | - | Famà *et al.* (2002) | n.a. | |
| AJ417953 | - | *C. mexicana* | Dahab, Red Sea | - | Famà *et al.* (2002) | n.a. | |
| AJ417954 | - | *C. racemosa* var. *lamourouxii* | Uken, Japan | *C. oligophylla* | Famà *et al.* (2002) | n.a. | |
| AJ417955 | - | *C. racemosa* var. *occidentalis* | Livorno, Italy | *C. cylindracea* | Famà *et al.* (2002) | n.a. | |
| AJ417956 | - | *C. racemosa* var. *macrophysa* | Florida, USA | *C. racemosa* | Famà *et al.* (2002) | n.a. | |
| AJ417957 | - | *C. racemosa* var. *turbinata* | Dahab, Egypt | *C. lamourouxii* | Famà *et al.* (2002) | n.a. | |
| AJ417958 | - | *C. webbiana* | Dahab, Egypt | - | Famà *et al.* (2002) | n.a. | |
| AJ417959 | - | *C. lanuginosa* | Florida, USA | - | Famà *et al.* (2002) | n.a. | |
| AJ417960 | - | *C. geminata* | Cape Bank, Australia | - | Famà *et al.* (2002) | n.a. | |
| AJ417961 | - | *C. microphysa* | Texas Flower Gardens, USA | - | Famà *et al.* (2002) | n.a. | |
| AJ417963 | - | *Caulerpella ambigua* | Texas Flower Gardens, USA | *C. ambigua* | Famà *et al.* (2002) | n.a. | |
| AJ417964 | - | *C. filiformis* | Bronte Beach, Australia | - | Famà *et al.* (2002) | n.a. | |

| *tuf*A NCBI accession | ITS NCBI accession | GenBank name | Location | Updated name | References | Voucher specimens | Note |
|---|---|---|---|---|---|---|---|
| AJ417965 | - | *C. paspaloides* | Florida, USA | - | Famà *et al.* (2002) | n.a. | |
| AJ417967 | - | *C. verticillata* | Florida, USA | - | Famà *et al.* (2002) | n.a. | |
| AJ417968 | - | *C. geminata* | Coffs Harbour, Australia | - | Famà *et al.* (2002) | n.a. | |
| AJ417969 | - | *C. cactoides* | Jervis Bay, Australia | - | Famà *et al.* (2002) | n.a. | |
| AJ417970 | - | *C. flexilis* | Jervis Bay, Australia | - | Famà *et al.* (2002) | n.a. | |
| AJ417971 | - | *C. scalpelliformis* | Cape Banks, Australia | - | Famà *et al.* (2002) | n.a. | |
| AJ417972 | - | *C. scalpelliformis* | Damour, Lebanon | - | Famà *et al.* (2002) | n.a. | |
| AJ417973 | - | *C. selago* | Abu Dhiab, Egypt | - | Famà *et al.* (2002) | n.a. | |
| AJ512413 | - | *C. racemosa* var. *racemosa* | Bolinao reef, Philippines | *C. oligophylla* | de Senerpont Domis *et al.* (2003) | n.a. | |
| AJ512415 | - | *C. racemosa* var. *laetevirens* | Bolinao reef, Philippines | *C. chemnitzia* | de Senerpont Domis *et al.* (2003) | n.a. | |
| AJ512417 | - | *C. racemosa* var. *mucronata* | Bolinao reef, Philippines | *C. racemosa* | de Senerpont Domis *et al.* (2003) | n.a. | |
| AJ512418 | - | *C. racemosa* var. *mucronata* | Bolinao reef, Philippines | *C. racemosa* | de Senerpont Domis *et al.* (2003) | n.a. | |
| AJ512426 | - | *C. flexilis* | Jerbis Bay, Australia | - | de Senerpont Domis *et al.* (2003) | n.a. | |
| DQ652330 | - | *C. cupressoides* | Florida, USA | - | Stam *et al.* (2006) | FL018 | |
| DQ652332 | DQ652237 | *C. cupressoides* | US Virgin Islands2, USA | - | Stam *et al.* (2006) | FL046 | |
| DQ652345 | - | *C. cupressoides* | Florida, USA | - | Stam *et al.* (2006) | FL246 | |
| DQ652346 | DQ652302 | *C. serrulata* | California, USA | - | Stam *et al.* (2006) | CA033 | |
| DQ652348 | DQ652304 | *C. serrulata* | California, USA | | Stam *et al.* (2006) | CA042 | |
| DQ652349 | DQ652301 | *C. serrulata* | California, USA | - | Stam *et al.* (2006) | CA068 | |
| DQ652353 | - | *C. brachypus* | California, USA | - | Stam *et al.* (2006) | CA018 | |
| DQ652354 | - | *C. brachypus* | Indiana, USA | - | Stam *et al.* (2006) | FL145 | |
| DQ652355 | - | *C. brachypus* | eBay, Union, OH | - | Stam *et al.* (2006) | FL101 | |
| DQ652360 | DQ652297 | *C. taxifolia* | California, USA | - | Stam *et al.* (2006) | CA067 | |
| DQ652361 | DQ652298 | *C. taxifolia* | Florida, USA | - | Stam *et al.* (2006) | FL131 | |
| DQ652362 | DQ652537 | *C. ashmeadii* | California, USA | - | Stam *et al.* (2006) | CA007 | |
| DQ652363 | DQ652533 | *C. ashmeadii* | Florida, USA | - | Stam *et al.* (2006) | FL064 | |
| DQ652372 | DQ652249 | *C. prolifera* | California, USA | - | Stam *et al.* (2006) | CA008 | |

| *tuf*A NCBI accession | ITS NCBI accession | GenBank name | Location | Updated name | References | Voucher specimens | Note |
|---|---|---|---|---|---|---|---|
| DQ652373 | DQ652250 | *C. prolifera* | California, USA | - | Stam *et al.* (2006) | CA031 | |
| DQ652376 | DQ652247 | *C. prolifera* | Florida, USA | - | Stam *et al.* (2006) | FL085 | |
| DQ652393 | DQ652290 | *C. sertularioides* | California, USA | - | Stam *et al.* (2006) | CA037 | |
| DQ652400 | DQ652271 | *C. sertularioides* | Florida, USA | - | Stam *et al.* (2006) | FL033 | |
| DQ652409 | DQ652283 | *C. sertularioides* | Florida, USA | - | Stam *et al.* (2006) | FL136 | |
| DQ652420 | DQ652265 | *C. racemosa* | Aquarium trade, California, USA | - | Stam *et al.* (2006) | CA014 | |
| DQ652422 | DQ652263 | *C. racemosa* | Aquarium trade, California, USA | - | Stam *et al.* (2006) | CA060 | |
| DQ652424 | - | *C. racemosa* | Florida, USA | - | Stam *et al.* (2006) | FL030 | |
| DQ652425 | - | *C. racemosa* | US Virgin Islands2, USA | - | Stam *et al.* (2006) | FL045 | |
| DQ652426 | - | *C. racemosa* | Florida, USA | *C. nummularia* | Stam *et al.* (2006) | FL108 | |
| DQ652428 | - | *C. racemosa* | Aquarium shop (Florida, USA) | *C. macrodisca* | Stam *et al.* (2006) | FL158 | |
| DQ652429 | - | *C. racemosa* | Aquarium shop (Florida, USA) | *C. macrodisca* | Stam *et al.* (2006) | FL159 | |
| - | ✔ | *C. racemosa* | Aquarium trade, California, USA | *C. elongata* | Stam *et al.* (2006), This study | CA049 | for ITS: clone 1 |
| DQ652433 | DQ652238 | *C. mexicana* | Florida, USA | - | Stam *et al.* (2006) | FL003 | |
| DQ652437 | DQ652239 | *C. mexicana* | Florida, USA | - | Stam *et al.* (2006) | FL017 | |
| DQ652446 | DQ652241 | *C. mexicana* | Florida, USA | - | Stam *et al.* (2006) | FL077 | |
| DQ652457 | DQ652244 | *C. mexicana* | Florida, USA | - | Stam *et al.* (2006) | FL199 | |
| DQ652467 | DQ652321 | *C. racemosa* | California, USA | *C. chemnitzia* | Stam *et al.* (2006) | CA053 | |
| DQ652468 | DQ652323 | *C. racemosa* | California, USA | *C. chemnitzia* | Stam *et al.* (2006) | CA056 | |
| DQ652470 | - | *C. racemosa* | Florida, USA | *C. chemnitzia* | Stam *et al.* (2006) | FL011 | |
| DQ652474 | DQ652311 | *C. racemosa* | Florida, USA | *C. chemnitzia* | Stam *et al.* (2006) | FL055 | |
| DQ652494 | DQ652259 | *C. racemosa* | California, USA | *C. oligophylla* | Stam *et al.* (2006) | CA040 | |
| DQ652495 | DQ652251 | *C. racemosa* | California, USA | *C. cylindracea* | Stam *et al.* (2006) | CA034 | |
| DQ652496 | - | *C. lanuginosa* | Florida, USA | - | Stam *et al.* (2006) | FL166 | |
| DQ652499 | - | *C. paspaloides* | Florida, USA | - | Stam *et al.* (2006) | FL013 | |
| DQ652513 | DQ652326 | *C. microphysa* | California, USA | - | Stam *et al.* (2006) | CA041 | |
| DQ652514 | DQ652327 | *C. microphysa* | California, USA | - | Stam *et al.* (2006) | CA045 | |
| DQ652525 | - | *C. verticillata* | Florida, USA | - | Stam *et al.* (2006) | FL006 | |
| DQ652530 | - | *C. verticillata* | Florida, USA | - | Stam *et al.* (2006) | FL175 | |
| FJ432655 | - | *Caulerpella ambigua* | Hawaii, USA | *C. ambigua* | Draisma *et al.* (2014) | TS0024 | |

| *tuf*A NCBI accession | ITS NCBI accession | GenBank name | Location | Updated name | References | Voucher specimens | Note |
|---|---|---|---|---|---|---|---|
| FJ810424 | - | *C. freycinetii* var. *integerrima* | Eilat, Israel | *C. integerrima* | Wynne *et al.* (2009) | n.a. | |
| FJ810425 | - | *C. freycinetii* var. *integerrima* | Eilat, Israel | *C. integerrima* | Wynne *et al.* (2009) | n.a. | |
| FJ810426 | - | *C. bartoniae* | South Africa | - | Wynne *et al.* (2009) | n.a. | |
| FM956011 | - | *C. biserrulata* | Papua, Indonesia | - | Draisma and Prud'homme van Reine (Unpublished) | L SGAD0712179 | |
| FM956012 | - | *C. brachypus* | Thousand Islands, Indonesia | - | Draisma and Prud'homme van Reine (Unpublished) | L SGAD0509301 | |
| FM956013 | - | *C. brachypus* | Papua, Indonesia | - | Draisma and Prud'homme van Reine (Unpublished) | L SGAD0712020 | |
| FM956014 | - | *C. cupressoides* | Thousand Islands, Indonesia | - | Draisma and Prud'homme van Reine (Unpublished) | L SGAD0509213 | |
| FM956015 | - | *C. serrulata* cf. var. *pectinata* | Thousand Islands, Indonesia | *C. serrulata* var. *pectinata* | Draisma and Prud'homme van Reine (Unpublished) | L SGAD0509189 | |
| FM956016 | - | *C. lessonii* | Thousand Islands, Indonesia | - | Draisma and Prud'homme van Reine (Unpublished) | L SGAD0509589 | |
| FM956017 | - | *C. serrulata* f. *spiralis* | Thousand Islands, Indonesia | - | Draisma and Prud'homme van Reine (Unpublished) | L SGAD0509248 | |
| FM956018 | - | *C. serrulata* f. *spiralis* | Thousand Islands, Indonesia | - | Draisma and Prud'homme van Reine (Unpublished) | L SGAD0509249 | |
| FM956020 | ✔ | *C. serrulata* f. *spiralis* | Red Sea, Egypt | - | Draisma and Prud'homme van Reine (Unpublished), This study | L SGAD1033 | |
| FM956021 | ✔ | *C. lentillifera* | Java, Indonesia | - | Draisma *et al.* (2014), This study | L SGAD 0509343 | for ITS: clone 1 |
| - | ✔ | *C. lentillifera* | Papua, Indonesia | | Draisma and Prud'homme van Reine (Unpublished), This study | L SGAD 0712132 | for ITS: clone 1 |
| FM956024 | - | *C. lentillifera* | Papua, Indonesia | - | Draisma *et al.* (2014) | L SGAD 0712757 | |
| FM956025 | ✔ | *C. microphysa* | Tanzania | - | Draisma *et al.* (2014), This study | GENT TZ0270 | for ITS: clone 1 |
| FM956026 | ✔ | *C. opposita* | Borneo, Indonesia | - | Draisma *et al.* (2014), This study | L 03-038 | for ITS: clone 1 |
| FM956027 | - | *C. microphysa* | Sri Lanka | - | Draisma *et al.* (2014) | GENT HEC11838 | |
| FM956029 | - | *C. parvifolia* | Papua, Indonesia | - | Draisma and Prud'homme van Reine (Unpublished) | L SGAD0712794 | |
| FM956031 | - | *C. fastigiata* | Borneo, Indonesia | - | Draisma *et al.* (2014) | L 03-36 | |
| FM956034 | - | *C. filicoides* | Borneo, Indonesia | - | Draisma *et al.* (2014) | L 03-039 | |
| FM956035 | - | *C. filiformis* | South Africa | - | Draisma *et al.* (2014) | L 2005-31 | |
| FM956036 | - | *C. flexilis* | New Zealand | - | Draisma *et al.* (2014) | L 1998 | |
| FM956037 | - | *C. brownii* | New Zealand | - | Draisma *et al.* (2014) | L 81 (5) | |
| FM956038 | - | *C. trifaria* | Tasmania, Australia | - | Draisma *et al.* (2014) | L L14 | |

| *tuf*A NCBI accession | ITS NCBI accession | GenBank name | Location | Updated name | References | Voucher specimens | Note |
|---|---|---|---|---|---|---|---|
| FM956039 | - | *C. sedoides* f. *sedoides* | New Zealand | *C. sedoides* | Draisma *et al.* (2014) | L 6 | |
| FM956040 | - | *C. longifolia* | Tasmania, Australia | - | Draisma *et al.* (2014) | L L15 | |
| FM956041 | ✔ | *C. lanuginosa* var. *delicatula* | Tanzania | *C. antoensis* | Draisma and Prud'homme van Reine (Unpublished), This study | GENT TZ0503 | for ITS: clone 1 |
| FM956042 | - | *C. prolifera* | Ischia, Italy | - | Draisma and Prud'homme van Reine (Unpublished) | L SGAD 0706105 | |
| FM956043 | ✔ | *C. racemosa* f. *requieni* | Thousand Islands, Indonesia | *C. oligophylla* | Sauvage *et al.* (2013), This study | SGAD 0509242 | for ITS: clone 1 |
| FM956044 | ✔ | *C. racemosa* f. *requieni* | Berau delta, Indonesia | *C. oligophylla* | Draisma and Prud'homme van Reine (Unpublished), This study | L 03-040 | for ITS: clone 1 |
| FM956045 | **MK481938** | *C. racemosa* f. *clavifera* | Papua, Indonesia | *C. racemosa* | Sauvage *et al.* (2013), This study | SGAD 0712247 | for ITS: Clone 1 |
| FM956046 | ✔ | *C. racemosa* var. *macra* | Papua, Indonesia | *C. macra* | Sauvage *et al.* (2013), This study | SGAD 0509331 | for ITS: Clone 1 |
| FM956048 | ✔ | *C. racemosa* f. *cylindracea* | Gulf of Naples, Italy | *C. cylindracea* | Sauvage *et al.* (2013), This study | SGAD 0706109 | for ITS: Clone 1 |
| FM956051 | **MK481937, MK481943** | *C. racemosa* var. *occidentalis* | Thousand Islands, Indonesia | *C. racemosa* | Sauvage *et al.* (2013), This study | SGAD 0509638 | for ITS: Clone 1, 2 |
| FM956052 | - | *C. racemosa* f. *macrophysa* | Berau delta, Indonesia | *C. racemosa* | Sauvage *et al.* (2013) | L 03–342 | |
| FM956053* | - | *C. racemosa* var. *chemnitzia* | Thousand Islands, Indonesia | *C. macrodisca* | Sauvage *et al.* (2013) | SGAD 0509359 | |
| FM956054 | - | *C. racemosa* var. *chemnitzia* | Berau delta, Indonesia | *C. macrodisca* | Sauvage *et al.* (2013) | L 03–341 | |
| FM956055 | **MK481944-MK4819449** | *C. peltata* var. *peltata* | Papua, Indonesia | *C. macrodisca* | Sauvage *et al.* (2013), This study | SGAD 0712635 | for ITS: Clone 1-6 |
| FM956056 | - | *C. nummularia* | Thousand Islands, Indonesia | *C. chemnitzia* | Sauvage *et al.* (2013) | SGAD 0509640 | |
| FM956057 | ✔ | *C. nummularia* | Berau delta, Indonesia | *C. chemnitzia* | Sauvage *et al.* (2013), This study | L 03–227 | |
| FM956058 | - | *C. nummularia* | Ko Kham, Songkhla, Thailand | *C. chemnitzia* | Draisma *et al.* (2014) | GENT *Caulerpa* peltata 11-12-2006 | |
| FM956059 | ✔ | *C. nummularia* | Papua, Indonesia | *C. chemnitzia* | Draisma and Prud'homme van Reine (Unpublished), This study | L SGAD 0712145 | for ITS: Clone 1 |
| FM956060 | - | *C. peltata* f. *peltata* | Philippines | *C. chemnitzia* | Draisma and Prud'homme van Reine (Unpublished) | GENT FL1176 | |

| *tuf*A NCBI accession | ITS NCBI accession | GenBank name | Location | Updated name | References | Voucher specimens | Note |
|---|---|---|---|---|---|---|---|
| FM956061 | ✔ | *C. selago* cf. var. *selago* | Berau delta, Indonesia | *C. chemnitzia* | Draisma and Prud'homme van Reine (Unpublished), This study | L 03-128 | for ITS: Clone 1 |
| FM956062 | - | *C. scalpelliformis* | Tasmania, Australia | - | Draisma *et al.* (2014) | L L13 | |
| FM956063 | - | *C. scalpelliformis* | South Africa | - | Draisma *et al.* (2014) | L 2005-12 | |
| FM956064 | - | *C. sertularioides* f. *brevipes* | Thousand Islands, Indonesia | - | Draisma and Prud'homme van Reine (Unpublished) | L SGAD 0509214 | |
| FM956065 | - | *C. taxifolia* | Thousand Islands, Indonesia | - | Draisma and Prud'homme van Reine (Unpublished) | L SGAD 0509244 | |
| FM956066 | - | *C. taxifolia* | Philippines | - | Draisma and Prud'homme van Reine (Unpublished) | GENT FL1126 | |
| FM956067 | - | *C. urvilleana* | Papua, Indonesia | - | Draisma and Prud'homme van Reine (Unpublished) | L SGAD 0712203 | |
| FM956070 | ✔ | *C. verticillata* | Berau delta, Indonesia | - | Draisma *et al.* (2014), This study | L 03-130 | |
| FM956072 | - | *C. verticillata* | Java, Indonesia | - | Draisma *et al.* (2014) | L 0509287 | |
| FM956073 | - | *C. webbiana* f. *disticha* | Azores, Portugal | - | Draisma *et al.* (2014) | n.a. | |
| FM956074 | - | *C. webbiana* | Tanzania | - | Draisma *et al.* (2014) | GENT TZ0486 | |
| FM956075 | - | *C. racemosa* cf. var. *corynephora* | Khlong Yang, Krabi, Thailand | *C. macrodisca* | Sauvage *et al.* (2013) | HEC161156 | |
| FN667648 | - | *C. webbiana* f. *disticha* | Velasco, Palau | - | Draisma *et al.* (2014) | L V2.25.05 | |
| FN667649 | - | *C. manorensis* | Hall Island, Palau | *Caulerpa* sp. | Draisma *et al.* (2014) | L H6-25-04 | |
| FN667650 | - | *C. okamurae* | Japan | - | Draisma *et al.* (2014) | L SGAD 0909013, L:H6-25-04 | |
| FR848331 | - | *C. corynephora* | Western Australia | - | Draisma *et al.* (2014) | PERTH 6.10.9.2.6 | |
| FR848332 | - | *C. elongata* | Northern Reef, Palau | - | Draisma *et al.* (2014) | L V2.25.04 | |
| FR848333 | - | *C. simpliciuscula* | Victoria, Australia | - | Draisma *et al.* (2014) | L 09.10.048 | |
| FR848334 | - | *C. remotifolia* | Victoria, Australia | - | Draisma *et al.* (2014) | L 09.10.074 | |
| FR848335 | - | *C. obscura* | Victoria, Australia | - | Draisma *et al.* (2014) | L 09.10.052 | |
| FR848337 | - | *C. antoensis* | Northern Reef, Palau | - | Draisma and Prud'homme van Reine (Unpublished) | L NR03-25-08 | |
| FR848338 | - | *C. longifolia* | Victoria, Australia | - | Draisma *et al.* (2014) | L 09.10.042 | |
| FR848339 | - | *C. parvifolia* | Rottnest Island, Australia | - | Draisma *et al.* (Unpublished) | n.a. | |
| FR848340 | - | *C. cliftonii* | Victoria, Australia | - | Draisma *et al.* (2014) | L 09.10.051 | |
| FR848341 | - | *C. brownii* | Victoria, Australia | - | Draisma *et al.* (2014) | L 09.10.057 | |

| *tuf*A NCBI accession | ITS NCBI accession | GenBank name | Location | Updated name | References | Voucher specimens | Note |
|---|---|---|---|---|---|---|---|
| FR848342 | - | *C. racemosa* cf. var. *macra* | Palau | *C. macra* | Draisma *et al.* (Unpublished) | L C18-05-23 | |
| FR848343 | - | *C. sedoides* f. *geminata* | Western Australia | *C. sedoides* | Draisma *et al.* (2014) | PERTH - | |
| FR848344 | - | *C. filicoides* var. *andamanensis* | Chuuk, Federated States of Micronesia | *C. andamanensis* | Draisma *et al.* (2014) | L C7-05-09 | |
| GU592621 | - | Ulvophyceae | Lizard Island, Australia | *C. nummularia* | Händeler *et al.* (2010) | n.a. | |
| GU592622 | - | Ulvophyceae | Lizard Island, Australia | *C. nummularia* | Händeler *et al.* (2010) | n.a. | |
| JN645149 | - | *C. racemosa* | Grande Terre, New Caledonia | *C. megadisca* | Sauvage *et al.* (2013) | IRD5639 | |
| JN645150 | - | *C. racemosa* | Porquerolles, France | *C. cylindracea* | Sauvage *et al.* (2013) | TS0133 | |
| JN645152 | - | *C. racemosa* | Fiji | *C. macra* | Sauvage *et al.* (2013) | IRD1878 | |
| JN645153 | - | *C. taxifolia* | Goeland Island, New Caledonia | - | Sauvage *et al.* (2013) | IRD5637 | |
| JN645154 | - | *C. racemosa* | Ile des Pins, New Caledonia | *C. megadisca* | Sauvage *et al.* (2013) | IRD5636 | |
| JN645160 | - | *C. racemosa* | Ile des Pins, New Caledonia | *C. chemnitzia* | Sauvage *et al.* (2013) | IRD5627 | |
| JN645162 | - | *C. racemosa* | Grande Terre, New Caledonia | *C. chemnitzia* | Sauvage *et al.* (2013) | IRD5638 | |
| JN645163 | - | *C. racemosa* | Grande Terre, New Caledonia | *C. cylindracea* | Sauvage *et al.* (2013) | IRD5629 | |
| JN645164 | - | *C. racemosa* | Ile des Pins, New Caledonia | *C. macra* | Sauvage *et al.* (2013) | IRD5624 | |
| JN645165 | - | *C. racemosa* | Grande Terre, New Caledonia | *C. macrodisca* | Sauvage *et al.* (2013) | IRD5635 | |
| JN645167 | - | *C. racemosa* | Grande Terre, New Caledonia | *C. macra* | Sauvage *et al.* (2013) | IRD5640 | |
| JN645168 | - | *C. racemosa* | Ile des Pins, New Caledonia | *C. oligophylla* | Sauvage *et al.* (2013) | IRD5633 | |
| JN645169 | - | *C. racemosa* | Grande Terre, New Caledonia | - | Sauvage *et al.* (2013) | IRD5641 | |
| JN645170 | - | *C. racemosa* | Grande Terre, New Caledonia | *C. oligophylla* | Sauvage *et al.* (2013) | IRD5642 | |
| JN645173 | - | *C. racemosa* | Minnie Waters, NSW, Australia | *C. nummularia* | Sauvage *et al.* (2013) | HV2132 | |
| JN645174 | - | *C. racemosa* | Hopetoun Beach, SWA, Australia | *C. cylindracea* | Sauvage *et al.* (2013) | HV2537 | |
| JN645175 | - | *C. racemosa* | Leander Reef, Australia | *C. cylindracea* | Sauvage *et al.* (2013) | HV2634 | |
| JN817646 | - | *C. obscura* | Australia | - | Belton *et al.* (2014) | AD A90909 | |
| JN817647 | - | *C. opposita* | Lizard Island, Australia | - | Draisma *et al.* (2014) | AD A92544 | |

| *tuf*A NCBI accession | ITS NCBI accession | GenBank name | Location | Updated name | References | Voucher specimens | Note |
|---|---|---|---|---|---|---|---|
| JN817653 | - | *C. corynephora* | Australia | - | Belton *et al.* (2014) | PERTH 08292590 | |
| JN817655 | - | *C. fergusonii* | Western Australia | - | Draisma *et al.* (2014) | AD A91682 | |
| JN817656 | - | *Caulerpa* sp. | Lizard Island, Australia | *C. megadisca* | Belton *et al.* (2014) | AD-A92609 | |
| JN817657* | - | *Caulerpa* sp. | Lizard Island, Australia | *C. megadisca* | Belton *et al.* (2014) | AD-A90107 (holotype) | |
| JN817659 | - | *C. racemosa* | Long Reef, Kimberley, Australia | - | Belton *et al.* (2014) | PERTH 08292736 | |
| JN817661 | - | *C. racemosa* | Lizard Island, Australia | - | Belton *et al.* (2014) | AD-A92441 | |
| JN817665* | - | *C. racemosa* | Montgomery Reef, Kimberley, Australia | - | Belton *et al.* (2014) | PERTH 08292728 | |
| JN817666 | - | *C. macrodisca* | Lizard Island, Australia | - | Belton *et al.* (2014) | AD-A88056 | |
| JN817667 | - | *C. peltata* | Montgomery Reef, Australia | *C. chemnitzia* | Belton *et al.* (2014) | PERTH 08292671 | |
| JN817668 | - | *C. peltata* | Montgomery Reef, Australia | *C. chemnitzia* | Belton *et al.* (2014) | PERTH 08292698 | |
| JN817669 | - | *C. chemnitzia* | Cassini Island, Australia | - | Belton *et al.* (2014) | PERTH 08292701 | |
| JN817673 | - | *C. cylindracea* | Ningaloo Reef, Australia | - | Belton *et al.* (2014) | AD-A88236 | |
| JN817675 | - | *C. cylindracea* | Ningaloo Reef, Australia | - | Sauvage *et al.* (2013) | AD-A91736 | |
| JN817681 | - | *C. racemosa* | Long Reef, Australia | *C. lamourouxii* | Belton *et al.* (2014) | PERTH 08292647 | |
| JN817683 | - | *C. racemosa* | Ningaloo Reef, Australia | *C. lamourouxii* | Belton *et al.* (2014) | AD-A90154 | |
| JN817685* | - | *C. nummularia* | Heron I., Australia | - | Belton *et al.* (2014) | AD-A91369 | |
| JN851135 | - | *C. cactoides* | Australia | - | Belton *et al.* (2014) | GWS024470 | |
| JN851137 | - | *C. fergusonii* | Australia | - | Belton *et al.* (2014) | GWS025259 | |
| JN851138 | - | *C. nummularia* | Lord Howe Island, Australia | - | Belton *et al.* (2014) | GWS023933 | |
| JN851139 | - | *C. nummularia* | Lord Howe Island, Australia | - | Belton *et al.* (2014) | GWS023932 | |
| JN851140 | - | *C. nummularia* | Lord Howe Island, Australia | - | Belton *et al.* (2014) | GWS023180 | |
| JN851143* | - | *C. cylindracea* | Point Peron, WA, Australia | - | Sauvage *et al.* (2013) | GWS025471 | |
| JN851146 | - | *C. flexilis* | Australia | - | Belton *et al.* (2014) | GWS015249 | |
| KC153492 | JF932264 | *C. scalpelliformis* | Veraval, Gujarat, India | - | Kazi *et al.* (2013) | 19102009001 | |
| KC153493 | JF932265 | *C. verticillata* | Okha, Gujarat, India | - | Kazi *et al.* (2013) | 17102009001 | |

| *tuf*A NCBI accession | ITS NCBI accession | GenBank name | Location | Updated name | References | Voucher specimens | Note |
|---|---|---|---|---|---|---|---|
| KC153495 | JF932267 | *C. racemosa* | Veraval, Gujarat, India | - | Kazi *et al.* (2013) | 4122009001 | |
| KC153497 | JF932269 | *C. microphysa* | Okha, Gujarat, India | - | Kazi *et al.* (2013) | 2122009001 | |
| KC153499 | JF932271 | *C. sertularioides* f. *brevipes* | Porbandar, Gujarat, India | - | Kazi *et al.* (2013) | 3122009003 | |
| KC153503 | JF932275 | *C. scalpelliformis* var. *denticulata* | Okha, Gujarat, India | *C. denticulata* | Kazi *et al.* (2013) | 2122010002 | |
| KC153505 | JN034414 | *C. lentillifera* | Poshitra rocks, Gujarat, India | - | Kazi *et al.* (2013) | 20110419014 | |
| KC153508 | JQ745710 | *C. taxifolia* | Poshitra rocks, Gujarat, India | - | Kazi *et al.* (2013) | 20110420001 | |
| KC153509 | JQ745711 | *C. serrulata* | Sadamunian, Tamilnadu, India | - | Kazi *et al.* (2013) | 20101230001 | |
| KC153510 | JQ745712 | *C. peltata* | Manapadu, Tamilnadu, India | *C. chemnitzia* | Kazi *et al.* (2013) | 20110103001 | |
| KC153512 | JQ745714 | *C. scalpelliformis* | Mandapam, Tamilnadu, India | - | Kazi *et al.* (2013) | 20110104001 | |
| KC153513 | JQ745715 | *C. sertularioides* f. *longipes* | Poshitra rocks, Gujarat, India | - | Kazi *et al.* (2013) | 20110419019 | |
| KC153514 | JQ745716 | *C. veravalensis* | Okha, Gujarat, India | - | Kazi *et al.* (2013) | 20101202001 | |
| KC153515 | JQ745717 | *C. taxifolia* | Kunkeshwar, Maharashtra, India | - | Kazi *et al.* (2013) | 20110223001 | |
| KC153517 | JQ745719 | *C. verticillata* | Krusadai Island, Tamilnadu, India | - | Kazi *et al.* (2013) | 20110110001 | |
| KC153519 | JQ745721 | *C. peltata* | Ratnagiri, Maharashtra, India | *C. chemnitzia* | Kazi *et al.* (2013) | 20110225001 | |
| KF256081* | - | *C. racemosa* var. *lamourouxii* | Marsa Alam, Red Sea, Egypt | *C. lamourouxii* | Belton *et al.* (2014) | HV03477 | |
| KF256084 | - | *C. racemosa* var. *lamourouxii* | Cassini Island, Australia | *C. lamourouxii* | Belton *et al.* (2014) | PERTH 08428220 | |
| KF256085* | - | *C. oligophylla* | Heron Island, Australia | - | Belton *et al.* (2014) | AD-A95457 | |
| KF256086 | - | *C. oligophylla* | Thousand Islands, Indonesia | - | Belton *et al.* (2014) | SGAD0509292 | |
| KF256088 | - | *C. racemosa* var. *macra* | Northern Reefs, Palau | *C. macra* | Belton *et al.* (2014) | LNR13a0109 | |
| KF256089* | - | *C. racemosa* var. *macra* | Berau delta, Indonesia | *C. macra* | Belton *et al.* (2014) | L 03-453 | |
| KF256090 | - | *C. macrodisca* | Long Reef, Australia | - | Belton *et al.* (2014) | PERTH 08292663 | |
| KF256091 | - | *Caulerpa* sp. | Yaukuvelailai Island, Fiji | *C. megadisca* | Belton *et al.* (2014) | DML40342 | |
| KF256092 | - | *C. macrodisca* | Thousand Islands, Indonesia | - | Belton *et al.* (2014) | SGAD0509415 | |
| KF256093 | - | *C. macrodisca* | Java Sea, Indonesia | - | Belton et al. (2014) | SGAD0509390 | |

| *tuf*A NCBI accession | ITS NCBI accession | GenBank name | Location | Updated name | References | Voucher specimens | Note |
|---|---|---|---|---|---|---|---|
| KF256094 | - | *C. macrodisca* | Java Sea, Indonesia | - | Belton *et al.* (2014) | SGAD0509510 | |
| KF256095 | - | *C. macrodisca* | Papua, Indonesia | - | Belton *et al.* (2014) | SGAD0712405 | |
| KF256097 | - | *C. racemosa* | Papua, Indonesia | - | Belton *et al.* (2014) | L0789171 | |
| KF256098 | - | *C. nummularia* | Dravuni, Fiji | - | Belton *et al.* (2014) | DML40015 | |
| KF256099 | - | *C. chemnitzia* | St George's, Bermuda | - | Belton *et al.* (2014) | CWS008555 | |
| KF256100 | - | *C. chemnitzia* | Blue cut channel, Bermuda | - | Belton *et al.* (2014) | CWS008366 | |
| KF256101* | - | *C. chemnitzia* | Matara, Sri Lanka | - | Belton *et al.* (2014) | HEC15952 | |
| KF256105 | - | *C. chemnitzia* | Lizard Island, Australia | - | Belton *et al.* (2014) | AD-A92551 | |
| KF256106 | - | *C. chemnitzia* | Exumas, Bahamas | - | Belton *et al.* (2014) | DML70471 | |
| KF314138 | - | *C. corynephora* | Australia | - | Belton *et al.* (2014) | PERTH 08195803 | |
| KF314139 | - | *C. agardhii* | Australia | - | Draisma *et al.* (2014) | PERTH 08399697 | |
| KF314142 | - | *C. cupressoides* | Australia | - | Draisma *et al.* (2014) | AD A91732 | |
| KF314149 | - | *C. constricta* | Australia | - | Belton *et al.* (2014) | AD A94521 | |
| KF314158 | - | *C. brachypus* | Australia | - | Draisma *et al.* (2014) | AD A91664 | |
| KF649854 | - | *C. cliftonii* | Australia | - | Belton *et al.* (2014) | AD A79076 | |
| KF649856 | - | *C. brownii* | Australia | - | Belton *et al.* (2014) | AD A92244 | |
| KF649860 | - | *C. trifaria* | Australia | - | Belton *et al.* (2014) | AD A92257 | |
| KF649862 | - | *C. alternans* | Australia | - | Belton *et al.* (2014) | AD A88966 | |
| KF649865 | - | *C. flexilis* | Australia | - | Draisma *et al.* (2014) | AD A92238 | |
| KF649869 | - | *C. hedleyi* | Australia | - | Draisma *et al.* (2014) | AD A89143 | |
| KF649870 | - | *C. fergusonii* | Australia | - | Belton *et al.* (2014) | AD A93587 | |
| KF649873 | - | *C. simpliciuscula* | Australia | - | Draisma *et al.* (2014) | AD A89133 | |
| KF649874 | - | *C. simpliciuscula* | Australia | - | Belton *et al.* (2014) | GWS015874 | |
| KF649875 | - | *C. simpliciuscula* | Australia | - | Belton *et al.* (2014) | AD A92243 | |
| KF649876* | - | *Caulerpa* sp. | Australia | *C. lucasii* | Draisma *et al.* (2014) | AD A88572 | |
| KF649878 | - | *C. vesiculifera* | Kangaroo Island, Australia | - | Draisma *et al.* (2014) | AD A92234 | |
| KF649879 | - | *C. papillosa* | Kangaroo Island, Australia | - | Draisma *et al.* (2014) | AD A92242 | |
| KF649883 | - | *C. hodgkinsoniae* | Australia | - | Belton *et al.* (2019) | GWS032688 | |

| *tuf*A NCBI accession | ITS NCBI accession | GenBank name | Location | Updated name | References | Voucher specimens | Note |
|---|---|---|---|---|---|---|---|
| KF649891 | - | *C. remotifolia* | Australia | - | Belton *et al.* (2019) | AD A92285 | |
| KF649893 | - | *C. remotifolia* | Australia | - | Belton *et al.* (2019) | AD A93751 | |
| KF649900 | - | *C. taxifolia* | Australia | - | Draisma *et al.* (2014) | AD A89134 | |
| KF649902 | - | *C. filiformis* | Australia | - | Belton *et al.* (2014) | AD A95186 | |
| KF649914 | - | *C. longifolia* | Australia | - | Belton *et al.* (2014) | AD A92232 | |
| KF921072 | - | *C. floridana* | Florida | - | Sauvage *et al.* (2014) | WRT345 | |
| KM016916 | - | *C. sedoides* | Australia | - | Draisma *et al.* (2014) | AD A89135 | |
| KM186523 | - | *Caulerpella ambigua* | Hawaii, USA | *C. ambigua* | Draisma *et al.* (2014) | TS0252 | |
| KM186527 | - | *Caulerpella ambigua* | Kerama Island, Japan | *C. ambigua* | Draisma *et al.* (2014) | TS1180 | |
| KM186528 | - | *C. okamurae* | Japan | - | Draisma *et al.* (2014) | GENT HV1888 | |
| KM186529 | - | *C. filicoides* var. *andamanensis* | Hawaii, USA | *C. andamanensis* | Draisma *et al.* (2014) | ARS1611 /HS2004-140 | |
| **MK497059** | - | *C. macrodisca* | East Kalimantan, Indonesia | - | This study | L_03_344 | |
| **MK497060** | - | *C. macrodisca* | Java Sea, Indonesia | - | This study | SGAD0509332 | |
| **MK497061** | - | *C. macrodisca* | Java Sea, Indonesia | - | This study | SGAD0509539 | |
| **MK497062** | - | *C. macrodisca* | West Papua, Indonesia | - | This study | SGAD0712194 | |
| **MK497063** | - | *C. macrodisca* | West Papua, Indonesia | - | This study | SGAD0712198 | |
| **MK497064** | - | *C. macrodisca* | West Papua, Indonesia | - | This study | SGAD0712199 | |
| **MH591082** | - | *Dichotomosiphon tuberosus* | n.a. | - | Cremen *et al.* (2019) | Shimada 1401 | |
| **MK497053** | **MK481940** | *C. macrodisca* | Market, Krabi | - | This study | KP4D | |
| ✔ | ✔ | *C. verticillata* | Ko Lidee, Satun | - | This study | KP5A | |
| ✔ | ✔ | *C. racemosa* | Ko Tan, Surat Thani | - | This study | KP10A | |
| ✔ | ✔ | *C. serrulata* | Ko Tan, Surat Thani | - | This study | KP11B | |
| ✔ | ✔ | *C. verticillata* | Ko Tan, Surat Thani | - | This study | KP13A | |
| ✔ | ✔ | *C. serrulata* | Big Buddha, Ko Samui, Surat Thani | - | This study | KP14C | |
| ✔ | ✔ | *C. sertularioides* | Big Buddha, Ko Samui, Surat Thani | - | This study | KP15C | |
| ✔ | ✔ | *C. lentillifera* | Ko Siboya, Krabi | - | This study | KP21A | |
| ✔ | ✔ | *C. racemosa* | Ko Siboya, Krabi | - | This study | KP22A | |

| *tuf*A NCBI accession | ITS NCBI accession | GenBank name | Location | Updated name | References | Voucher specimens | Note |
|---|---|---|---|---|---|---|---|
| ✔ | ✔ | *C. taxifolia* | Ko Siboya, Krabi | - | This study | KP23A | |
| ✔ | ✔ | *C. racemosa* | Ko Kham, Songkhla | - | This study | KP28B | |
| ✔ | ✔ | *C. chemnitzia* | Ko Kham, Songkhla | - | This study | KP29A | |
| ✔ | ✔ | *C. racemosa* | Ko Kham, Songkhla | - | This study | KP30A | |
| ✔ | ✔ | *C. chemnitzia* | Ko Kham, Songkhla | - | This study | KP31A | |
| ✔ | ✔ | *C. chemnitzia* | Ko Kham, Songkhla | - | This study | KP32A | |
| ✔ | ✔ | *C. sertularioides* | Ko Lidee, Satun | - | This study | KP35A | |
| ✔ | ✔ | *C. racemosa* | Ko Lipe, Satun | - | This study | KP39C | |
| ✔ | ✔ | *C. racemosa* | Ko Lipe, Satun | - | This study | KP40A | |
| ✔ | ✔ | *C. serrulata* | Ko Lipe, Satun | - | This study | KP41A | |
| ✔ | - | *C. verticillata* | Ko Lipe, Satun | - | This study | KP42A | |
| ✔ | ✔ | *C. taxifolia* | Ko Libong, Trang | - | This study | KP43A | |
| ✔ | ✔ | *C. serrulata* | Taling Ngam, Ko Samui, Surat Thani | - | This study | KP45A | |
| ✔ | ✔ | *C. taxifolia* | Ko Matsum, Surat Thani | - | This study | KP46B | |
| ✔ | ✔ | *C. serrulata* | Ko Matsum, Surat Thani | - | This study | KP47B | |
| ✔ | ✔ | *C. racemosa* | Losin, Pattani | - | This study | KP49A | |
| ✔ | ✔ | *C. chemnitzia* | Losin, Pattani | - | This study | KP50A | |
| ✔ | ✔ | *C. racemosa* | Losin, Pattani | - | This study | KP51A | |
| ✔ | ✔ | *C. taxifolia* | Leam Yong Lam, Trang | - | This study | KP53A | |
| ✔ | ✔ | *C. taxifolia* | Leam Yong Lam, Trang | - | This study | KP55A | |
| ✔ | ✔ | *C. taxifolia* | Khao Bae Na, Trang | - | This study | KP57A | |
| **MK497054** | **MK481941** | *C. macrodisca* | Fish cage, Kilim river, Malaysia | - | This study | KP65A | |
| ✔ | ✔ | *C. racemosa* | Bakan Teang, Ko Lanta, Krabi | - | This study | KP68A | |
| ✔ | ✔ | *C. racemosa* | Bakan Teang, Ko Lanta, Krabi | - | This study | KP70A | |
| ✔ | ✔ | *C. serrulata* | Tangkhen Bay, Phuket | - | This study | KP72A | |
| **MK497055** | **MK481942** | *C. macrodisca* | Fish cage, Khlong Yang, Krabi | - | This study | KP73A | |
| **MK497056** | - | *C. macrodisca* | Fish cage, Khlong Yang, Krabi | - | This study | KP74A | |
| - | **MK481939** | *C. macrodisca* | Ao Cho, Trat | - | This study | KUMF04404 | |

| *tuf*A NCBI accession | ITS NCBI accession | GenBank name | Location | Updated name | References | Voucher specimens | Note |
|---|---|---|---|---|---|---|---|
| MK497057 | - | *C. macrodisca* | Tung Wa, Satun, Thailand | - | This study | KUMF06872 | |
| MK497058 | - | *C. macrodisca* | Che Bilang, Satun, Thailand | - | This study | KUMF06874 | |
| ✔ | ✔ | *C. serrulata* | Ko Rok, Krabi | - | This study | SP284 | |
| ✔ | ✔ | *C. serrulata* | Ko Rok, Krabi | - | This study | SP301 | |
| - | ✔ | *C. taxifolia* | Ko Rawi, Satun | - | This study | SP332 | |
| ✔ | ✔ | *C. taxifolia* | Ko Rawi, Satun | - | This study | SP343 | |
| ✔ | ✔ | *C. serrulata* | Ko Rok, Krabi | - | This study | SP358 | |
| - | ✔ | *C. serrulata* | Ko Rok, Krabi | - | This study | SP368 | |
| ✔ | - | *C. serrulata* | Ko Kradan, Trang | - | This study | SP372 | |
| ✔ | ✔ | *C. taxifolia* | Ko Rawi, Satun | - | This study | SP474 | |
| - | ✔ | *C. taxifolia* | Ko Rawi, Satun | - | This study | SP234 | |

* indicated as *tuf*A barcode sequences (Belton *et al.*, 2014)

# RESULTS AND DISCUSSION

The results and discussion are divided into two parts:

Part 1. Diversity and distribution of the genus *Caulerpa* in Thailand

Part 2. An enigmatic *Caulerpa macrodisca* Decaisne (Chlorophyta) from the mangrove channels on the Andaman Sea coast of Thailand.

# Part 1

# Diversity and distribution of the genus *Caulerpa* in Thailand

# Diversity and distribution of the genus *Caulerpa* in Thailand

## 1. *Caulerpa* diversity in Thai waters

### a. *Caulerpa* genetic diversity in Thailand

An alignment composed of 271 partial *tuf*A sequences (767 bp in length) and 60 partial ITS rDNA sequences (1,114 bp in length) from GenBank with 108 newly generated *Caulerpa* sequences (45 *tuf*A sequences and 63 ITS sequences) was analyzed using Maximum Likelihood (not shown) and Bayesian Inference (*tuf*A in Fig. 4 and ITS sequence in Fig. 5). Both topologies confirmed a congruent result of eight *Caulerpa* species in Thai waters including *C. chemnitzia*, *C. lentillifera*, *C. macrodisca*, *C. racemosa*, *C. serrulata*, *C. sertularioides*, *C. taxifolia* and *C. verticillata* (Fig. 6). Each species clade had strong branch support (BS>80, PP>0.95).

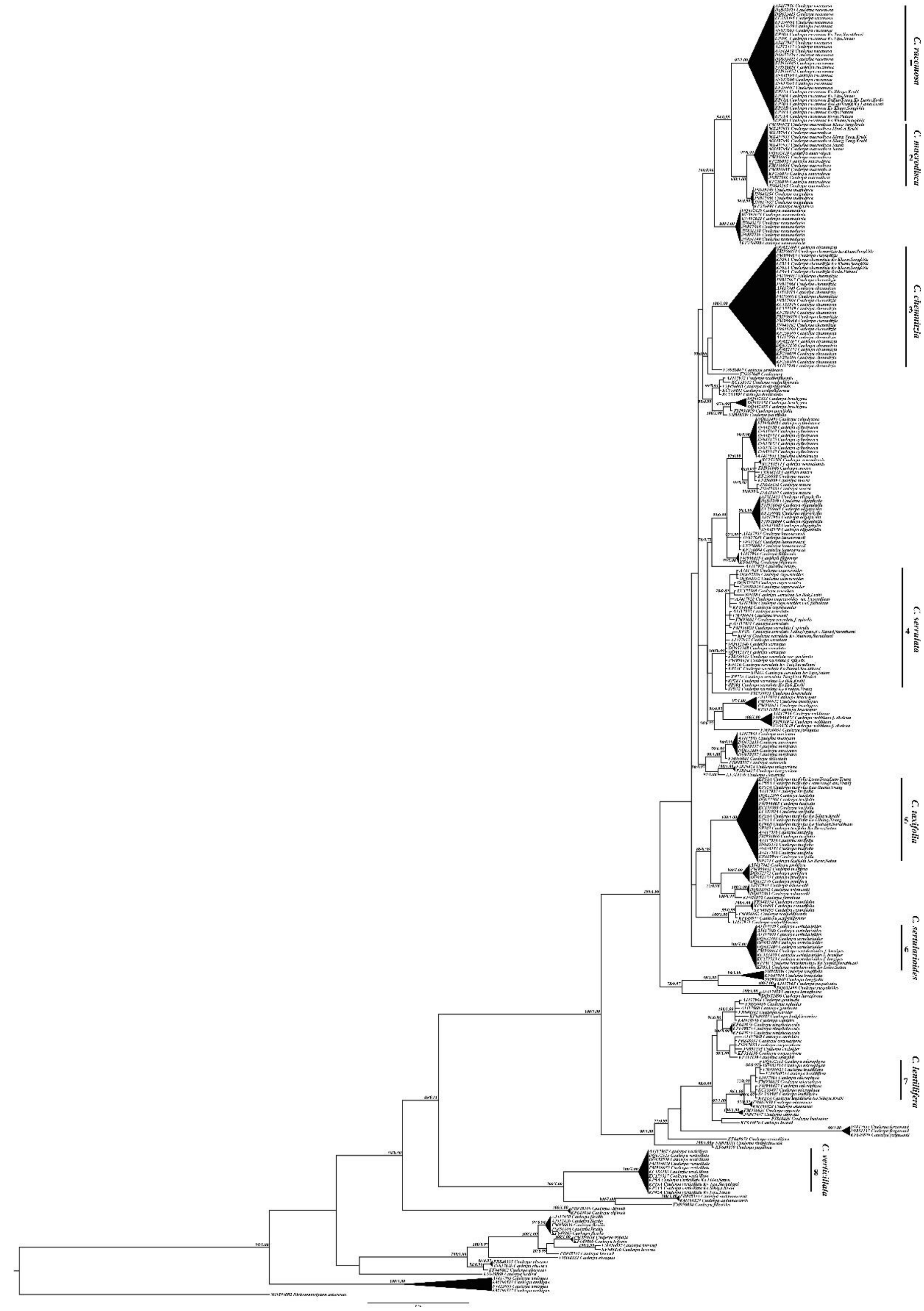


**Figure 4** Bayesian phylogenetic tree constructed from a total of 316 partial *tuf*A DNA sequences (alignment = 767 bp in length). Node Numbers indicate as BP and PP values. BP < 70% and PP < 0.7 are not shown. The scale is 0.04 expected changes per site.

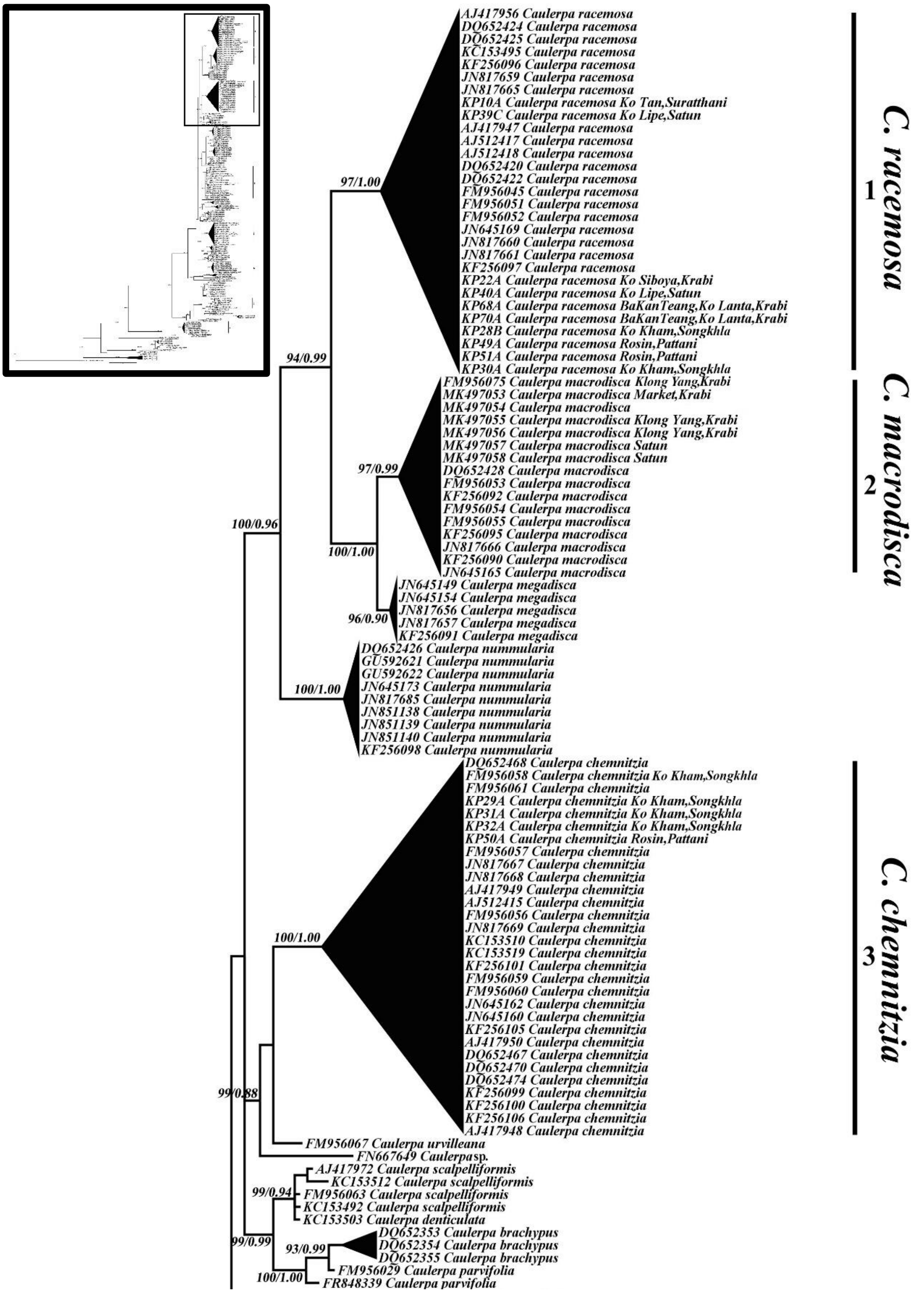


**Figure 4** A., Bayesian *tuf*A phylogenetic tree showed *Caulerpa* diversity from *C. racemosa* clade to *C. parvifolia*, of which there were 3 Thai *Caulerpa* clades, *i.e.*, *C. racemosa*, *C. macrodisca* and *C. chemnitzia*. (continous)

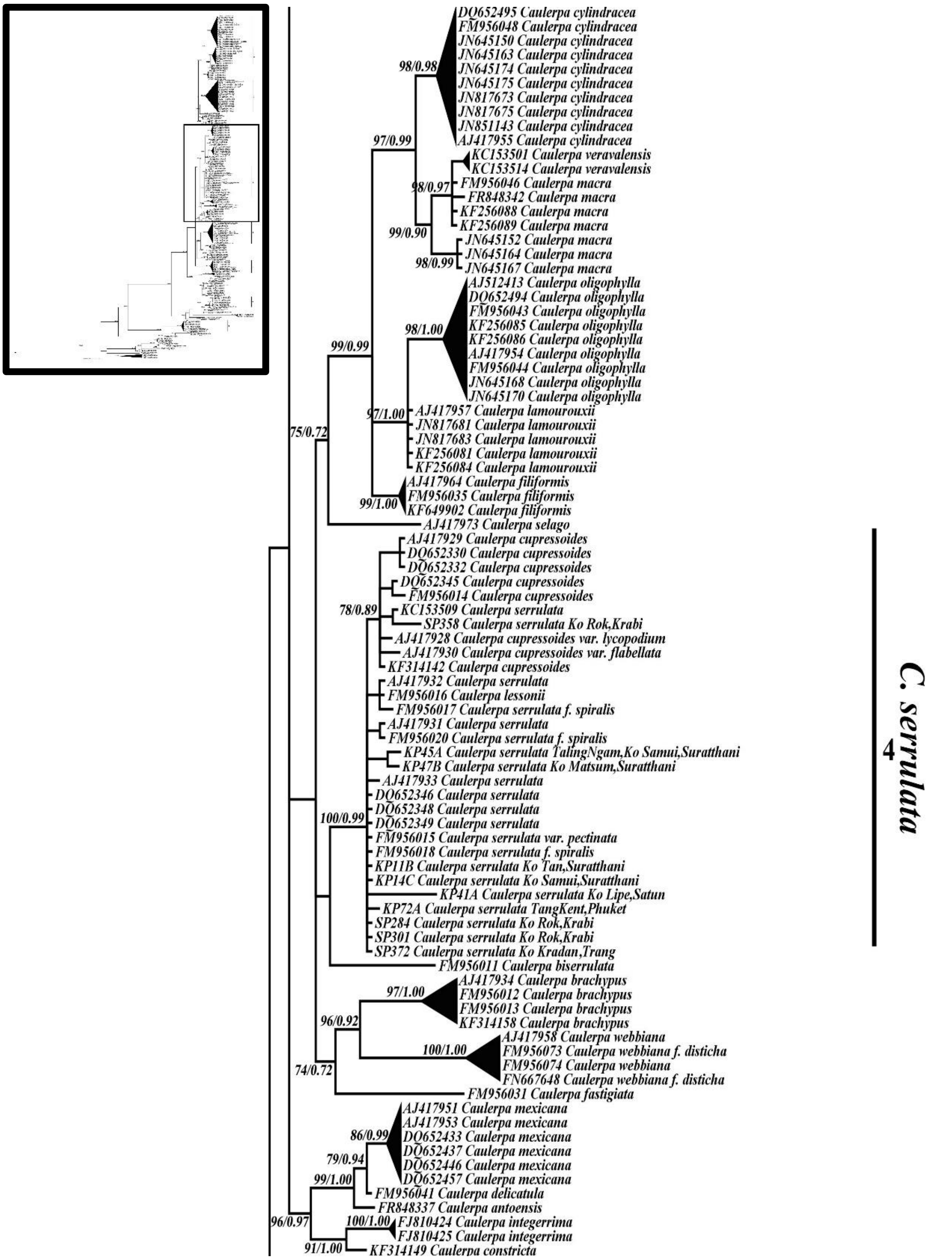


**Figure 4** B., Bayesian *tuf*A phylogenetic tree showed *Caulerpa* diversity from *C. cylindracea* clade to *C. constricta*, of which there was a Thai *Caulerpa* clades, *i.e.*, *C. serrulata*. (continuous)

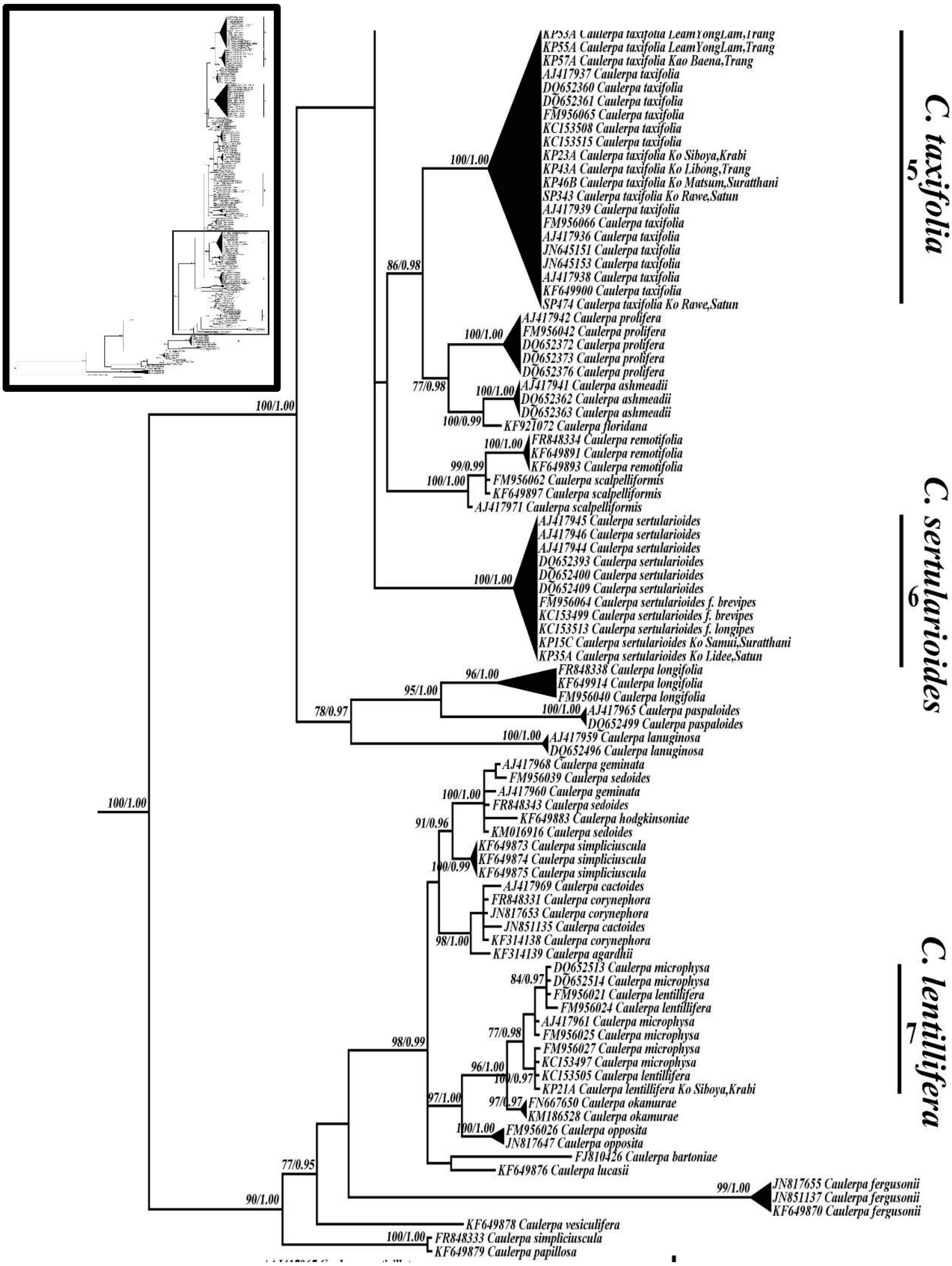

**Figure 4** C., Bayesian *tuf*A phylogenetic tree showed *Caulerpa* diversity from *C. taxifolia* clade to *C. papillosa*, of which there were 3 Thai *Caulerpa* clades, *i.e.*, *C. taxifolia*, *C. sertularioides* and *C. chemnitzia*. (continous)

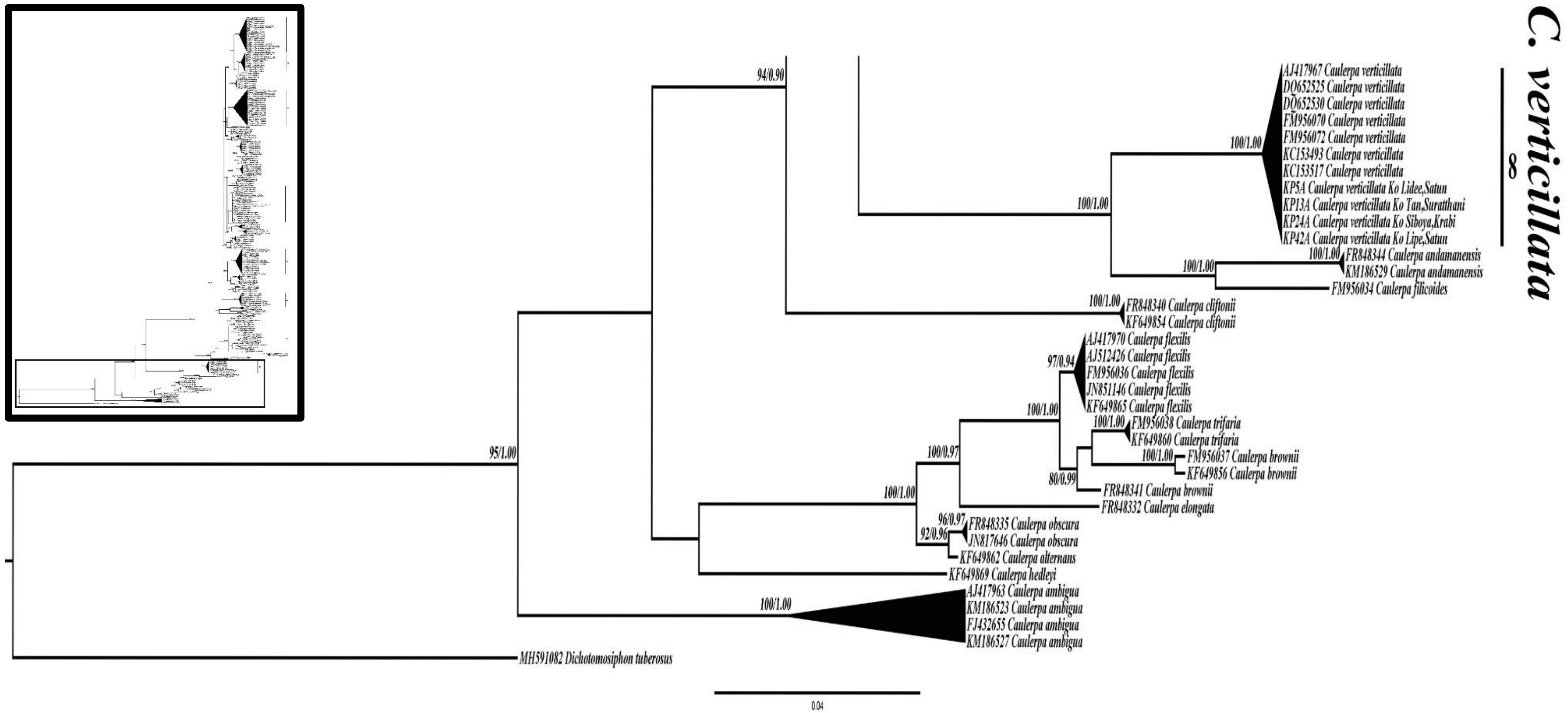


**Figure 4** D., Bayesian *tuf*A phylogenetic tree showed *Caulerpa* diversity from *C. verticillata* clade to *C. ambigua* clade with the outgroup (*D. tuberosus*), of which there was a Thai *Caulerpa* clades, *i.e.*, *C. verticillata*. (continous)

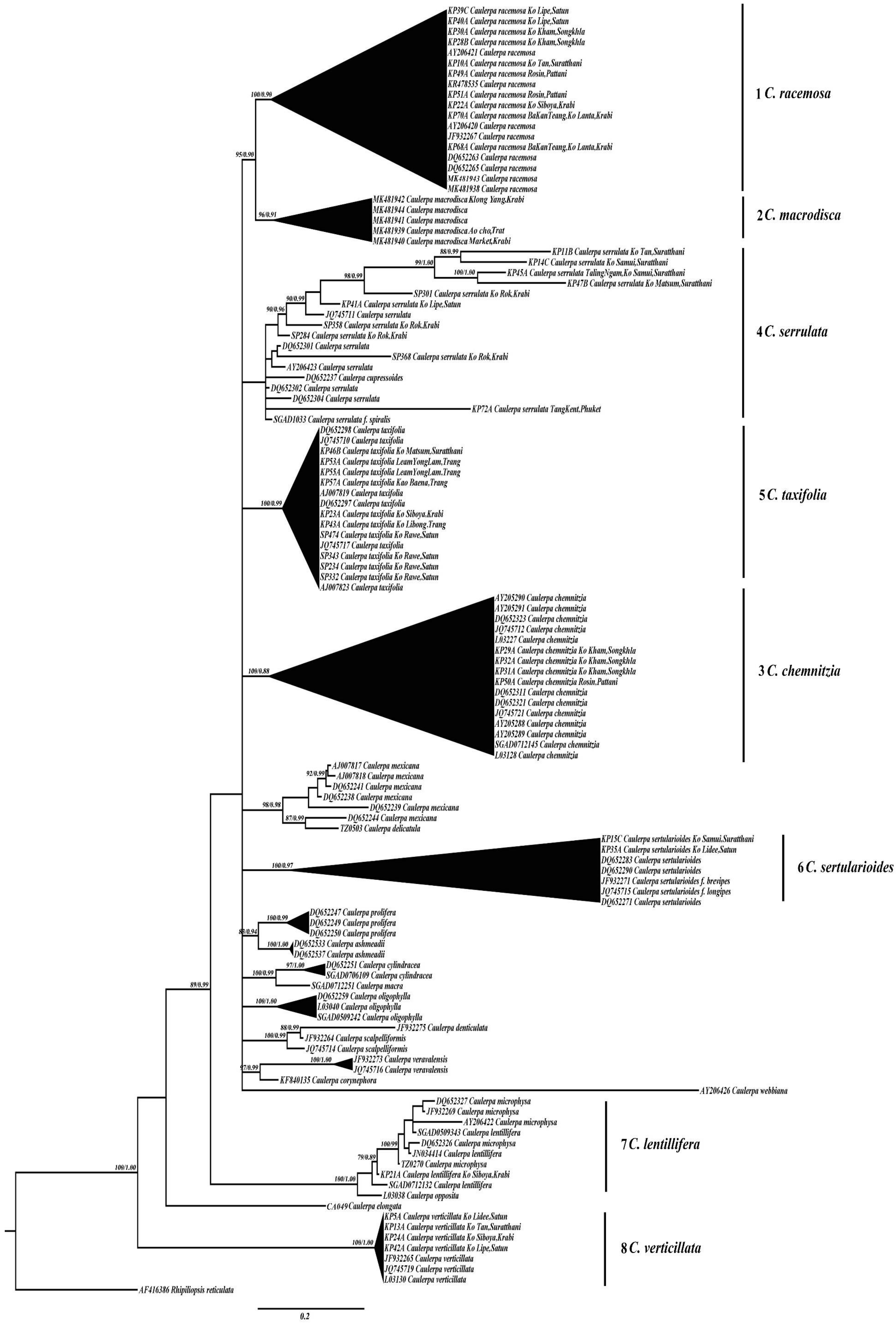


**Figure 5** Bayesian phylogenetic tree constructed from a total of 123 complete ITS1, 2 sequences (alignment = 1,114 bp in length). Numbers at nodes indicate BP and PP values. BP < 70% and PP < 0.7 are not shown. The scale is 0.2 expected changes


**Figure 6** Eight *Caulerpa* species found in Thailand (Scale bar = 1 cm) A., *C. chemnitzia*, B., *C. lentillifera*, C-E., *C. macrodisca* (C., *C. macrodisca* ecad *ashmeadii*, no scale bar, D., *C. macrodisca* ecad *corynephora* and E., *C. macrodisca* ecad *macrodisca*), F-G., *C. racemosa* (F., *C. racemosa* ecad *chemnitzia* and G., *C. racemosa* ecad *racemosa*), H., *C. serrulata*, I., *C. sertularioides*, J., *C. taxifolia* and K., *C. verticillata*.

**Table 4** Summary of morphological characteristics in the genus *Caulerpa* from Thailand (Min-Max ± SE, cm), Measurements from dried specimens in square brackets “[]” n.m., not measured.

| Features | *C. chemnitzia* | *C. lentillifera* | *C. macrodisca* | | | *C. racemosa* | | *C. serrulata* | *C. sertularioides* | *C. taxifolia* | *C. verticillata* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | ecad *macrodisca* | ecad *corynephora* | ecad *ashmeadii* | ecad *racemosa* | ecad *chemnitzia* | | | | |
| Diameter of stolon | 0.17–0.34±0.009 | 0.13–0.18±0.006 | 0.15–0.18±0.003 | 0.23–0.34±0.006 | n.m. | 0.13–0.39±0.004 | 0.15–0.25±0.003 | 0.11–0.36±0.005 | 0.07–0.29±0.006 | 0.11–0.32±0.007 | 1.8–3.9 x $10^{-2}$ ±0.001 |
| Rachis shape | Terete | Terete | Terete | Terete | Terete | Terete | Terete | Terete | Terete | Terete | Terete |
| Assimilator shape | Irregulary | Irregulary | Irregulary | Distichous | Distichous | Irregulary | Irregulary | Flat, dichotomous branching | Distichous | Distichous | Filiform, dichotomous branching |
| Assimilator margin | Entire | Entire | Entire | Entire | Entire | Entire | Entire | Serrate | Entire | Entire | Entire |
| Assimilator length | 0.87–6.75±0.280 | 1.8–5.7±0.441 | [4.13–6.05±0.285] | 2.89–10.48±0.369 | n.m. | 0.44–5.98±0.059 | 0.84–4.8±0.134 | 0.59–5.36±0.108 | 0.56–7.89±0.263 | 1.8–11.42±0.354 | 0.2–1.1±0.461 |
| Assimilator width | 0.47–1.2±0.035 | 0.46–0.7±0.013 | [0.54–1.23±0.112] | 0.65–0.94±0.027 | n.m. | 0.29–1.88±0.019 | 0.33–1.39±0.031 | 0.22–4.13±0.066 | 0.21–1.56±0.051 | 0.13–1.46±0.033 | 0.09–0.53±0.187 |
| Arrangement of ramuli | Irregularly | Opposite decussate | Alternate, Irregularly | Opposite | Opposite, or alternate at base | Alternate, Irregularly | Irregularly | - | Opposite | Opposite | Whorled |
| Length of ramuli | 0.01–0.43±0.013 | 0.19–0.23±0.005 | [≈0.01] | 0.51–1.22±0.034 | n.m. | 0.2–0.67±0.008 | 0.01–0.47±0.009 | 0.36–4.67±0.097 | 0.17–01.27±0.035 | 0.26–0.77±0.016 | 0.12–0.34 x $10^{-2}$ ±0.092 |
| Diameter of ramuli | 0.11–0.78±0.016 | 0.21–0.23±0.002 | [0.64–0.9±0.038] | 0.14–0.5±0.021 | n.m. | 0.22–0.71±0.008 | 0.11–1.01±0.013 | 0.21–0.59±0.007 | 0.02–0.05±0.001 | 0.07–0.13±0.002 | 3–7 x $10^{-3}$ ±0.002 |
| Shape of ramuli | Mushroom-like, peltate and turbinate shape | Spherical shape | Large peltate shape | Clavate, turbinate, or rarely peltate shape | Cylindrical shape | Subspherical to spherical shape | Mushroom-like, peltate and turbinate shape | - | Cylindrical shape | Upright falcate shape | Filiform |
| Shape of ramulus tip | - | - | - | - | Obtuse | Rounded | - | - | Acuminate | Acuminate | Three spines |
| Shape of ramulus base | - | - | - | - | - | Cuneate | - | - | - | - | - |
| Diameter of a constricted of ramuli | - | - | - | - | - | - | - | - | - | 0.05–0.12±0.002 | - |
| Length of stalks | 0.03–0.6±0.01 | 0.05–0.09±0.004 | [0.11–0.25±0.023] | - | n.m. | 0.06–0.47±0.006 | 0.03–0.43±0.006 | - | - | - | - |
| Diameter of stalks | 0.05–0.23±0.005 | 0.06–0.07±0.001 | [0.05–0.07±0.004] | - | n.m. | 0.11–0.31±0.003 | 0.05–0.25±0.007 | - | - | - | - |
| Diameter of a constricted of stalks | - | 0.04–0.05±0.001 | - | - | n.m. | - | - | - | - | - | - |
| Shape of tip | Acute | Rounded | Disc | Acute | Acute | Rounded | Acute | Teeth | Mucronate | Mucronate | Mucronate |

### b. *Caulerpa* morphological diversity in Thailand

For morphological study, five species including *C. lentillifera, C. serrulata, C. sertularioides, C. taxifolia,* and *C. verticillata* showed clear morphological criteria for specific identification based on ramuli arrangement and shapes. Other three species including *C. racemosa*, *C. macrodisca* and *C. chemnitzia* were in the *C. racemosa* - *peltata* complex presenting considerable levels of morphological plasticity. Thus, another tool, *i.e.*, DNA marker, was important key for their identifications. In this study, there were two morphotypes of *C. racemosa* named here *C. racemosa* ecad *racemosa* and *C. racemosa* ecad *chemnitzia,* while *C. macrodisca* was divided into three morphologies named *C. macrodisca* ecad *macrodisca, C. macrodisca* ecad *corynephora* and *C. macrodisca* ecad *ashmeadii*. Of which, *C. racemosa* ecad *chemnitzia* and *C. chemnitzia* were indistinguishable because of their overlapping morphology. So, molecular data was a necessary tool for their specific identification and classification. The summary of morphological characteristics is described in Table 4.

Lineage 1 (Figs 4A, 5) represents *Caulerpa racemosa* (Forsskål) J.Agardh and does not only include specimens with spherical ramuli identified as *C. racemosa* (KP10 from Surat Thani, the Gulf of Thailand, KP39, KP40 from Satun, KP22, KP68 and KP70 from Krabi, the Andaman Sea), but also peltate specimens from the lower Gulf of Thailand morphologically attributed to *C. chemnitzia* (KP28A, KP30A from Ko Kham, Songkhla and KP49A, KP51A from Rosin Pattani). There was obviously morphological difference. Peltate specimens (as *C. chemnitzia* morphology) normally showed considerable morphological varieties of ramuli from mostly turbinate to peltate or sometimes mushroom-like, while spherical specimens (as *C. racemosa* morphology) presented erect assimilators bearing densely globose to subspherical ramuli. The spherical specimens previous morphologically identified as *C. racemosa* var. *macrophysa* (see Lewmanomont, 2008 fig. 6). However, there were extremely morphological variable of *C. racemosa* (Coppejans and Beeckman, 1989) led to consider *C. racemosa* var. *macrophysa* as belonging in this species (Coppejans, 1992). For molecular data, our results confirmed a monophyletic group of *C. racemosa*. Thus, these spherical specimens were currently regarded as *C. racemosa* ecad *racemosa*, while peltate specimens were referred as *C. racemosa* ecad *chemnitzia*.

Lineage 2 (Figs 4A, 5) represents *Caulerpa macrodisca* Decaisne containing previous specimens morphologically identified as *C. peltata* var. *macrodisca* (KUMF04404, from intertidal shore of Trat, the Gulf of Thailand), *C. corynephora* (as *C. racemosa* var. *corynephora*, *sensu* Lewmanomont, 2008, figs 11, 12) (KP4 from fresh market in Krabi, KP73, KP74 from Khlong Yang, Krabi and KUMF06874 from mangrove channel in Satun, the Andaman Sea coast) and *C. ashmeadii* (KUMF06872, *sensu* Lewmanomont, 2008, from mangrove channel in Satun, the Andaman Sea) together. The congruent *tuf*A and ITS phylogenies strongly suggested that the three morphological entities in Thai waters represented a single species and will be onwards referred to as *C. macrodisca* ecad *ashmeadii*, *C. macrodisca* ecad *corynephora* and *C. macrodisca* ecad *macrodisca.* This clade will be discussed in more detail in the second part "An enigmatic *Caulerpa macrodisca* Decaisne (Chlorophyta) from the mangrove channels on the Andaman Sea coast of Thailand".

Lineage 3 (Figs 4A, 5) represents *Caulerpa chemnitzia* (Esper) J.V.Lamouroux and includes specimens previously identified as *C. peltata* (*sensu* Lewmanomont, 2008, fig. 10). *Caulerpa chemnitzia* specimens (KP29, KP31, KP32 from Ko Kham, Songkhla and KP50 from Losin, Pattani in the lower Gulf of Thailand) showing the morphology of peltate form. Erect assimilators bearing considerable morphological varieties of ramuli from mostly turbinate to peltate shape, that disc diameter was overlapping with *C. racemosa* ecad *chemnitzia*, but smaller than *C. macrodisca* ecad *macrodisca* (Belton *et al.*, 2014; Price, 2011). Both phylogenies illustrated monophyletic clade of *C. chemnitzia*, *C. macrodisca* and *C. racemosa* respectively with strong supported values.

Lineage 4 (Figs 4B, 5) represents *Caulerpa serrulata* (Forsskål) J.Agardh from both coastlines (sensu Lewmanomont, 2008, fig. 14). *Caulerpa serrulata* specimens (KP11, KP14, KP45, KP47 from Surat Thani, the Gulf of Thailand, KP41 from Satun, KP72 from Phuket, SP372 from Trang and SP358, SP284 and SP301 from Krabi, the Andaman Sea) presented morphology of flattened twisted branches with serrate margin and dichotomous branching. *Tuf*A and ITS topologies showed a mixed of *C. serrulata* and *C. cupressoides* together as previous studies (Famà *et al.*, 2002; Stam *et al.*, 2006; Draisma *et al.*, 2014). However, *C. cupressoides* was separated from *C. serrulata* by the *rbc*L gene (Kazi *et al.*, 2013), and presented different morphology of flattened serrate branches with upcurved pointed tip. Thus, an apparent morphological distinct was able to separate into two species (de Senerpont Domis *et al.*, 2003).

Lineage 5 (Figs 4C, 5) represents *Caulerpa taxifolia* (M.Vahl) C.Agardh growing on sandy bottom substrate from both seashores. *Caulerpa taxifolia* specimens (KP46 from Surat Thani, the Gulf of Thailand, SP234, SP332, SP343 and SP474 from Satun, KP23 from Krabi and KP43, KP53, KP55, and KP57 from Trang, the Andaman Sea coasts) (sensu Lewmanomont, 2008, fig. 16) showed a feather-like assimilator with flattened upright sickle-shaped (falcate) ramuli and opposite arrangement. Its morphological characters and molecular phylogeny result presented congruent results of a species *C. taxifolia*.

Lineage 6 (Figs 4C, 5) represents *Caulerpa sertularioides* (S.G.Gmelin) M.Howe from both seacoasts. *Caulerpa sertularioides* specimens (KP15 from Surat Thani, the Gulf of Thailand, KP35 from Satun, the Andaman Sea coasts) (sensu Lewmanomont, 2008, fig. 15) showed a feather-like assimilator bearing slender cylindrical pinnules oppositely arranged. This morphological distinct data was supported their molecular phylogenies suggested that referred as *C. sertularioides*.

Lineage 7 (Figs 4C, 5) represents *Caulerpa lentillifera* J.Agardh (KP21A) from Ko Siboya, Krabi (sensu Lewmanomont, 2008, fig.5). Four rows of spherical translucent ramuli constricted at the base appeared on each erect assimilator. For *tuf*A and ITS topology, this lineage was mixed with *C. microphysa* similar to a previous result of Kazi *et al.* (2013). Moreover, both species were not difference in *rbc*L gene and 18S rDNA sequence (Kazi *et. al*., 2013). So, this study might be considered that both taxa was belong to a species.

Lineage 8 (Figs 4D, 5) represents *Caulerpa verticillata* J.Agardh from both Thai coasts. Specimens (KP13 from Surat Thani, the Gulf of Thailand, KP24 from Krabi, KP5 and KP42 from Satun, the Andaman Sea coasts) (sensu Lewmanomont, 2008, fig. 17) presented filiform thallus with dichotomous branching of whorled branched. A strongly supported of a monophyletic clade of *C. verticillata* also indicated as a single species, *C. verticillata*.

**2. *Caulerpa* distribution in Thai waters**

Eight species were found on the Andaman coast, *i.e.*, *C. chemnitzia, C. lentillifera, C. macrodisca* ecad *ashmeadii, C. macrodisca* ecad *corynephora, C. racemosa* ecad *racemosa, C. serrulata, C. sertularioides, C. taxifolia* and *C. verticillata*. Seven species were found in the Gulf of Thailand, *i.e.*, *C. chemnitzia, C. macrodisca* ecad *macrodisca, C. racemosa* ecad *chemnitzia, C. racemosa* ecad *racemosa, C. serrulata, C. sertularioides, C. taxifolia* and *C. verticillata* (Table 5).

**Table 5** List of *Caulerpa* species were found from both Thai waters.

| ***Caulerpa* species** | **The Andaman Sea** | **The Gulf of Thailand** |
|---|---|---|
| 1. *C. chemnitzia* | ✔ | ✔ |
| 2. *C. lentillifera* | ✔ | |
| 3. *C. macrodisca* | | |
| *C. macrodisca* ecad *ashmeadii* | ✔ | |
| *C. macrodisca* ecad *corynephora* | ✔ | |
| *C. macrodisca* ecad *macrodisca* | | ✔ |
| 4. *C. racemosa* | | |
| *C. racemosa* ecad *chemnitzia* | | ✔ |
| *C. racemosa* ecad *racemosa* | ✔ | ✔ |
| 5. *C. serrulata* | ✔ | ✔ |
| 6. *C. sertularioides* | ✔ | ✔ |
| 7. *C. taxifolia* | ✔ | ✔ |
| 8. *C. verticillata* | ✔ | ✔ |

During the field surveys, *C. macrodisca* ecad *corynephora* and *C. macrodisca* ecad *ashmeadii* only were found in the Andaman mangrove channels. *Caulerpa lentillifera* was only found in Krabi province (the Andaman Sea coast), but there was previously reported of *C. lentillifera* from the upper Gulf of Thailand by Lewmanomont (2008). On the other hand, *C. macrodisca* ecad *macrodisca* was found in the upper Gulf of Thailand and *C. racemosa* ecad *chemnitzia* was found in the lower Gulf of Thailand (Ko Kham, Songkhla and Losin, Pattani) growing on the rock together with *C. chemnitzia*.

Sixteen *Caulerpa* species have been recorded from Thai waters (Lewmanomont, 2008; Coppejans *et al.*, 2017), but only 8 species were confirmed by molecular data in the present study. A summary of *Caulerpa* distribution in Thai waters is shown in Figure 7. This map shows the highest *Caulerpa* diversity in Krabi, Satun and Trang provinces in the upper Strait of Malacca (the Andaman Sea coast) followed by Trat and Surat Thani provinces (the upper and middle Gulf of Thailand). Seven previously recorded morphological species *C. ambigua*, *C. ashmeadii*, *C. cupressoides*, *C. fastigiata*, *C. manorensis*, *C. mexicana* and *C. microphysa* sensu Lewmanomont (2008) and Coppejans *et al*. (2017) were not found in this present study. Those recorded species were not amplified both sequences from their extracted herbarium specimens to confirm their identities. However, they have clearly their own morphological criteria leading to accept those identified species except *C. ashmeadii*, which will be discussed more in part 2 of this thesis.

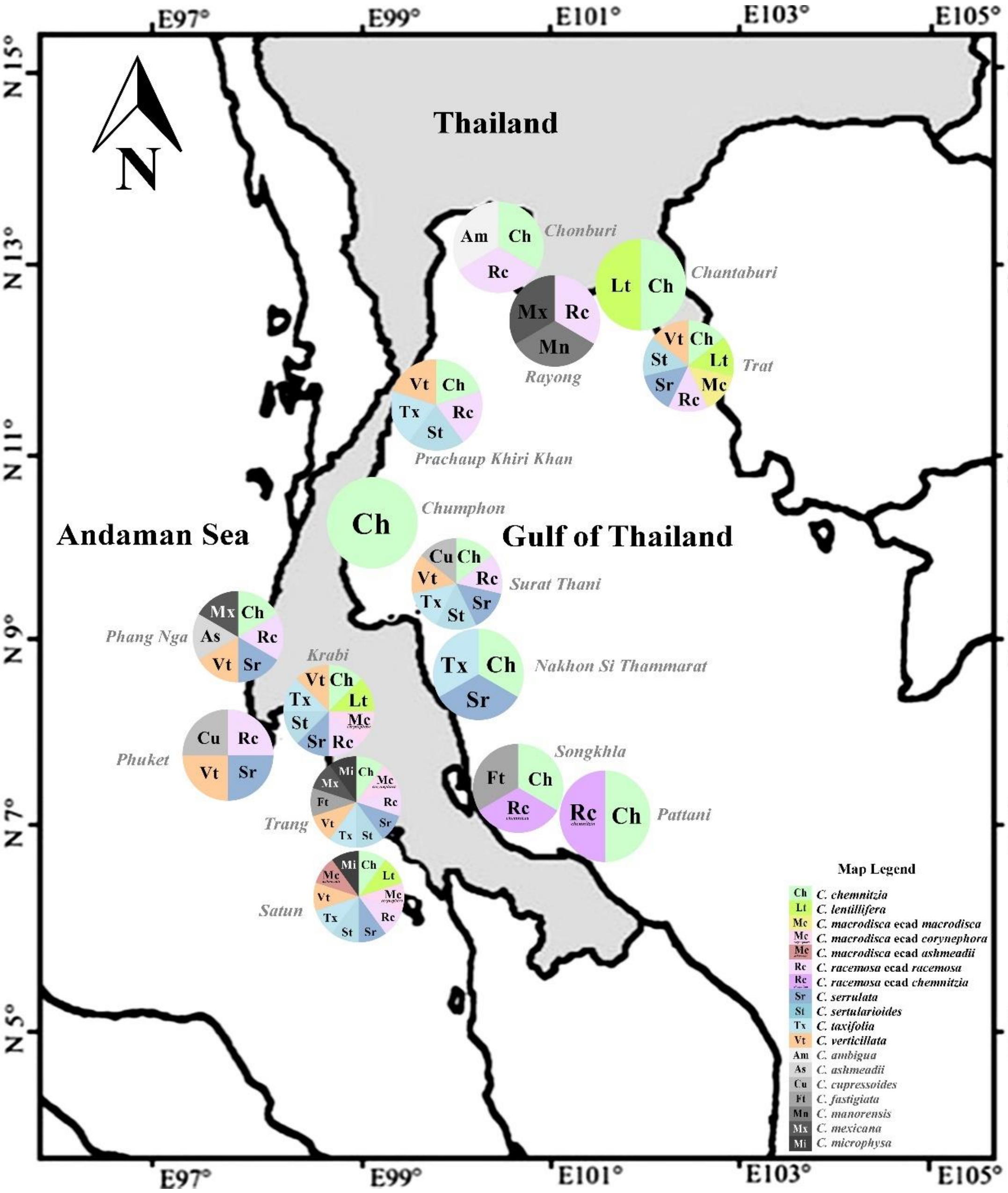


**Figure 7** Map showing the distribution of *Caulerpa* species in Thailand Data from herbarium specimens in NHM, Coppejans *et al.* (2017), Lewmanomont (2008), and the present. Color legend: Thai *Caulerpa* species collected and reported in the present study, Dark-gray legend: species not found in the present study but found in the NHM herbarium or reported in the literature

# Discussion

## 1. *Caulerpa* diversity in Thai waters

This study showed a taxonomic revision of the genus *Caulerpa* in Thailand at the species level. The congruent molecular identifications using *tuf*A gene and ITS sequence in combination with morphological data of recent collections in Thailand reported the occurrence of eight *Caulerpa* species in Thai waters; *C. chemnitzia, C. lentillifera, C. macrodisca* (ecad *ashmeadii*, ecad *corynephora* and ecad *macrodisca*), *C. racemosa* (ecad *chemnitzia* and ecad *racemosa*), *C. serrulata, C. sertularioides, C. taxifolia* and *C. verticillata*. Other previously reported species, *i.e.*, *C. ambigua, C. cupressoides, C. fastigiata, C. manorensis, C. mexicana* and *C. microphysa* sensu Lewmanomont (2008) were not found during this survey and unconfirmed their identities using molecular evidence. Thus, this study only found half of the *Caulerpa* diversity in Thailand reported in Lewmanomont (2008). That might be because of insufficient sampling, monitoring time and seasonal variations, which highly influenced on the diversity and distribution of any marine macroalgae (Prathep *et al*., 2005; Thongroy *et al*., 2007). Lewmanomont (2008) has been worked on her specimen collections for a decade spending more times to revisit all study sites in both monsoons, while this study ran from 2017–2019. Thus, it might be better for diversity research to extend more monitoring times and data collections.

In this study, there are eight *Caulerpa* species in Thailand. Of which, five taxa; *C. lentillifera*, *C. serrulata*, *C. sertularioides, C. taxifolia* and *C. verticillata* were clearly identified based on morphological criterion following Lewmanomont (2008) and Coppejans *et al*. (2010, 2017) supporting by both molecular data (Famà *et al.*, 2002; Belton *et al.*, 2014; Draisma *et al.*, 2014). However, others; *C. chemnitzia*, *C. racemosa* and *C. macrodisca* were later defined to the correct species based on molecular results.

Thai *Caulerpa* specimens previously identified as *C. corynephora* (as *C. racemosa* var. *corynephora*) sensu Lewmanomont *et al*. (2008) and Coppejans *et al*. (2017) are a variety of *C. macrodisca*. It illustrates a terete rachis with diverse ramuli from mainly clavate, turbinate to peltate formed without a constriction at the basis that not similar to the type description by Montagne (1845) and the lectotype illustration of *C. corynephora* in Price (2011). Price (2011) reported that the true *C. corynephora* was limited in Australia presenting an annulated rachis forming transverse articulations with mostly opposite clavate ramuli arrangement. Those obvious dissimilar morphologies were supported by a phylogeny of this study indicating in a monophyletic clade of *C. macrodisca* (section *Caulerpa* crown clade) with strong support values (Fig. 4). Thus, all previous *C. corynephora* specimens in Thai were consequently renamed as *C. macrodisca* ecad *corynephora*. In addition, a confused species, *C. macrodisca* ecad *ashmeadii* (morphologically identified as *C. ashmeadii* sensu Lewmanomont (2008)) showed terete rachis consisted with cylindrical ramuli (slightly upcurved at base) with swollen tip in opposite arrangement (sometimes alternate arrangement at base), which not matched with the type illustration of *C. ashmeadii* by Harvey (1858). The type morphology of *C. ashmeadii* presented erect opposite straight cylindrical ramuli arrangement with obtuse tip. Moreover, *C. ashmeadii* is a tropical native species distributed in the Atlantic Ocean (Zaleski and Murray, 2006). Thus, the newly collected specimens previously identified as *C. ashmeadii* demonstrated as *C. macrodisca* ecad *ashmeadii* using *tuf*A phylogeny in combination with morphological data. Other informations is explained in the second part.

Sympatric growing specimens (in Songkhla and Pattani) previously identified as *C. chemnitzia* based on original peltate ramuli morphology, represented 2 different species; *C. chemnitzia* and *C. racemosa*. In this study, the comparison demonstrates the biggest degrees of morphological plasticity in the complex particularly on assimilator length, ramuli length and diameter. Their qualitative characters are similar, while quantitative features are overlapping. Accordingly, PCA and ANOVA results (not shown) showed no significant difference on any character of their morphometric analysis. This corroborates the unreliability of morphological identification in the former *C. racemosa-peltata* complex (Belton *et al.*, 2014). Another study by de Senerpont Domis *el al*. (2003) showed congruous result that it was not morphological distinct exhibited on *C. racemosa* complex because its morphological plasticity responding environmental factors. Thus, the *Caulerpa racemosa-peltata* complex cannot be discriminated from a difference in morphology evidently.

Previously, there were many physiological studies about morphological plasticity of *Caulerpa*. The interrelated contribution of temperature and light intensity have important effect on external morphological constructs (Calvert, 1976; Ohba *et al.*, 1992; Komatsu *et al.*, 1997). Peterson (1972) found that *C. racemosa* (as *Caulerpa racemosa* var. *uvifera* (C.Agardh) J.Agardh) and *C. lamourouxii* (as *Caulerpa racemosa* var. *lamourouxii* (Turner) Weber-van Bosse) had diverse morphological growth under several illuminate situations that might help balancing their photosynthesis and respiration processes. In addition, Ohba and Enomoto (1987) found a high degree of morphological variation of *C. chemnitzia* (as *Caulerpa racemosa* var. *laetevirens*) that formed laetevirens-type (cylindrical ramuli) in the low temperatures-high light intensities conditions, and formed peltata-type (discoid shape ramuli) in various temperatures-low light intensities condition and intermediated type (trumpet shaped ramuli) in others. Hence, environmental variation can affect the external morphology that was causes of taxonomy problem in this genus.

Nevertheless, Yeh and Chen (2004) who studied various varieties of *C. racemosa* confirmed that nuclear rDNA and ITS sequences are helpful resolution of the inter- and infraspecific identification. The result is consistent with the study of Ohba and Enomoto (1987) showing that *C. racemosa* var. *laetevirens*, var. *peltata* and var. *turbinata* are varieties of a single species. Therefore, molecular tool is an important key to confirm specific identification of the complex. Hence, our coherent phylogenetic results apparently indicate sufficient authority for specific delimitation of the green algal genus *Caulerpa* using *tuf*A gene combination with ITS region. This present study suggested that both DNA markers are appropriate makers for molecular identifications of the genus, but the reference database for *tuf*A sequences is much larger. So, the *tuf*A gene also provided obviously interspecific identification, while ITS rDNA sequences were still needed for subspecies level as a supporting data (Stam *et al.*, 2006; Kazi *et al.*, 2013; Draisma *et al.*, 2014).

### *2. Caulerpa* distribution in Thai waters

This study showed that species compositions among sites were different. This might be a result of physical factors such as habitat types, environment factors, an indirect effect of sea surface currents (SSCs).

The biology of *Caulerpa* showed that a strong creeping stolon with numerous long rhizoids usefully attaches on various substrate in different habitat types, such as dead coral, rocky shore, muddy bottom and sandy bottom (Lee, 2008). For example, *C. taxifolia* and *C. sertularioides* normally grow on the sandy bottom in the inter- to subtidal zone. Four *Caulerpa* species; *C. racemosa, C. lentillifera, C. serrulata* and *C. chemnitzia* were generally found on hard substrates, *i.e*., dead coral, coral rubble or rock in tide pool of rocky shore or coral reef habitat. *Caulerpa verticillata* can grow on both hard substrate and sand in the intertidal zone. Only *C. macrodisca* was found in the mangrove channels growing on floating fish cages or muddy bottom, and some specimens could attach on rock in the intertidal zone (Coppejans *et al*., 2017; Lewmanomont, 2008). In Thailand, environment factors of both Thai coasts were different in each location (see in Table 1). Each organism requires a niche such as habitat type and physical environment for its suitable growth and reproduction (Begon *et al*., 2006), that might mainly influence on species composition in each study site.

Pongparadon *et al.* (2015) also found higher diversity of the green algal genus *Halimeda* from the Andaman Sea coast than the Gulf of Thailand similar to another studies, *i.e.*, Wichachucherd *et al*. (2014) and Pongparadon *et al*. (2017). The Gulf of Thailand was influenced by the mix of the Philippines Sea current and the East China Sea current in the Northeast monsoon providing the clockwise current direction in the Thai Gulf. The circulated currents directly affected connectivity reduction with open water that possible limited the species distribution within the Gulf of Thailand. For the Andaman Sea coast, not only the current form Indian Ocean water masses flow through the Thai Andaman Sea coast in both monsoons, but also a current from the South China in the Northeast monsoon possibly provide more opportunity of diverse species for settlement. Thus, the water mass might be driving forces of various marine organism appearances (Chen, 1999; Huang, 1999; Hu *et al*., 2011). Thus, the differences in SSCs could powerfully indirectly affect genetic diversity and distribution patterns of *Caulerpa* along the Thai coastline.

In the Southeast Asia, eight species, *C. chemnitzia*, *C. lentillifera*, *C. macrodisca*, *C. racemosa*, *C. serrulata*, *C. sertularioides*, *C. taxifolia* and *C. verticillata* were generally distributed as native species in the Coral Triangle (Prud'homme van Reine *et al*., 1996). Then, they were common dispersal along both coasts of Thailand connected to other countries in Southeast Asia region and Australia (Zaleski and Murray, 2006; Guiry and Guiry, 2019) except *C. lentillifera,* which was only found on the Andaman coast.

The present study is the first report of *C. lentillifera* from natural habitat in Krabi (the Andaman Sea coast). However, Lewmanomont (2008) recorded *C. lentillifera* in the Eastern Gulf of Thailand (Ko Chik, Chantaburi and Ko Kradat, Trat) for ten year ago. Ratana-arporn and Chirapart (2006) also reported the cultivate *C. lentillifera* from coastal shrimp aquacultures in Phetchaburi province (the Gulf of Thailand), and from Chachoengsao province by Chirapart *et al*. (2011). This species is becoming as a commercial algal species, which generally cultivated *C. lentillifera* from the Gulf of Thailand in each fishery agriculture along both sides of Thailand. *Caulerpa* was rapid growth under the culture conditions without any attentive maintenance. Thus, that might be some fragment of culture *C. lentillifera* was unexpectedly released to the Andaman Sea, and grow in the new locations here.

Although, *C. macrodisca* ecad *corynephora* and *C. macrodisca* ecad *ashmeadii* was limited in brackish water (27–30 ppt) of the Andaman Sea, while a larger peltate ramuli; *C. macrodisca* ecad *macrodisca* was restricted in the Gulf of Thailand. More information about this species has been discussed in part 2 confirmed by molecular and morphological data.

In addition, several *Caulerpa* species previously recorded in Thailand; *C. cupressoides*, *C. manorensis* and *C. mexicana* were not found during our field survey. It might be because of their rareness. *Caulerpa ambigua* and *C. fastigiata* were not found either and may have been overlooked due to their small size. Moreover, their identities could not be confirmed with DNA sequence data obtained from herbarium specimens. However, their morphological evidences seem obvious enough to confidently predict specific identification.

# Part 2

## An enigmatic *Caulerpa macrodisca* Decaisne (Chlorophyta) from the mangrove channels on the Andaman Sea coast of Thailand

# An enigmatic *Caulerpa macrodisca* Decaisne (Chlorophyta) from the mangrove channels on the Andaman Sea coast of Thailand

(A submitted manuscript of Journal of Fisheries and Environment, JFE)

Kattika Pattarach, Jaruwan Mayakun and Stefano G.A. Draisma

## Introduction

The common tropically to subtropically distributed genus *Caulerpa* J.V.Lamouroux is a coenocytic siphonous green alga. Plants consist of horizontally growing stolons with downward growing rhizoids and upright branched or unbranched fronds (assimilators). Lateral branchlets (ramuli) on the assimilators can be of various shapes e.g., cylindrical (terete), trumpet-shaped (turbinate), club-shaped (clavate), sickle-shaped (falcate), disc-shaped (peltate) or globular (vesiculate). The shape and arrangement of ramuli are important keys in species identification in this genus (Coppejans and Beeckman, 1989). However, morphological plasticity is known in the genus, induced by environmental factors like light intensity (Calvert, 1976) and temperature (Ohba *et al*., 1992). Moreover, some species have overlapping morphologies (Draisma *et al*., 2014; Belton *et al*., 2014, 2019). Correct species identification is therefore a challenge. The morphological plasticity within this genus has resulted in a long-standing source of uncertain and unstable taxonomy (Famà *et al*., 2002; Sauvage *et al*., 2013; Belton *et al*., 2014).

It was long debated whether several species with vesiculate and peltate ramuli represent different species or varieties of a single or a few species. These taxa have been referred to as the "*Caulerpa racemosa-peltata* complex". Belton *et al*. (2014) recognized 11 distinct species in the complex based on chloroplast-encoded *tuf*A gene and RUBISCO large subunit (*rbc*L) gene sequence data. They provided names, DNA barcodes (*i.e.*, reference *tuf*A sequences), and morphological descriptions for nine species, but stated that reliable morphological characterization remains not possible for several species due to high levels of phenotypic plasticity and morphological overlap.

They strongly suggested the use of molecular-based identifications and to refrain from recognizing any infra-specific ranks (*i.e.*, varieties and forms) within these species, but instead the use of morphological entities without formal taxonomic status (*e.g.*, ecad, ecotype) for highly plastic species.

Belton *et al*. (2014) recognized *Caulerpa macrodisca* Decaisne (homotypic synonyms: *Caulerpa racemosa* var. *macrodisca* (Decaisne) Weber Bosse 1898 and *Caulerpa peltata* var. *macrodisca* (Decaisne) Weber Bosse 1898) as sister-species of a newly described *C. megadisca* Belton & Gurgel. They described *C. macrodisca* as having large peltate ramuli arranged around an upright axis, and noted morphological variations of ramuli from disc-like to slightly mushroom-like. The specimens molecularly identified as *C. macrodisca* in Belton *et al*. (2014) came from Indonesia (7 specimens), Thailand (1), Australia (2), New Caledonia (1), and the aquarium trade (1), of which some were previously published (Stam *et al.*, 2006; Sauvage *et al*., 2013). Three specimens from the Thousand Islands in the Java Sea were collected nearest to the type location of *C. macrodisca* (Anambas Islands, Indonesia) and from one of these three the *tuf*A sequence was selected as reference sequence for the species. The DNA sequence of the Thai specimen was originally published in Sauvage *et al.* (2013), but its morphology was not discussed and neither in Belton *et al.* (2014). However, it was submitted to Genbank as *C. racemosa* var. *corynephora* (Montagne) Weber-van Bosse, the currently accepted name of which is *C. corynephora* Montagne (Guiry and Guiry, 2019) and its morphology deviates from the description of *C. macrodisca sensu* Belton *et al.* (2014). Moreover, molecular studies showed that the two species are not closely related, *C. corynephora* belonging to the *Caulerpa* section *Sedoideae* J.Agardh *ex* De Toni and *C. macrodisca* to the section *Caulerpa* (Belton *et al.,* 2015, 2019).

In Thailand, *C. corynephora* has only been reported from the Andaman Sea coast growing in mangrove channels, often on floating fish cages (Lewmanomont 1978, 2008; Coppejans *et al.,* 2017). In Thailand, *Caulerpa macrodisca* with characteristic peltate ramuli has only been reported from the Gulf of Thailand (Lewmanomont, 2008, as *C. peltata* var. *macrodisca*). Its identity was not confirmed with DNA sequence data. The aims of the present study are to confirm the identity of *C. macrodisca* in the Gulf of Thailand and the morphological *Caulerpa* entity previously reported as *C. corynephora* (or *C. racemosa* var. *corynephora*) from mangrove channels on the Andaman Sea coast using DNA sequence data and to describe their morphological variation.

## Materials and methodology

### Specimen collection and morphological characterization

The intertidal and subtidal Andaman Sea coast and the Gulf of Thailand were explored by snorkeling and SCUBA diving from 2016–2018. Six *Caulerpa* specimens used in the present study were newly collected from mangrove channels on the Thai Andaman Sea coast and Langkawi, Malaysia, and from a market in Krabi province, Thailand (Table 3). Freshly collected specimens were stripped of epiphytes, photographed using a CANON 60D camera, and various morphological characters such as stolon diameter, assimilator length and ramulus diameter were measured using a caliper. Once dried, specimens were measured using NIH ImageJ software (Rasband, 1997). A small piece (1–2 cm) was preserved in silica gel for later DNA extraction. Specimens were herbarium pressed and labeled following Coppejans *et al*. (2010). Subsequently, specimens were identified following key references (Lewmanomont, 2008; Coppejans *et al*., 2017). Attempts to make new collections of *C. macrodisca* with peltate ramuli in Thailand were unsuccessful. Therefore, we attempted to determine DNA sequence data from a herbarium specimen (KUMF04404) with peltate ramuli from the Gulf of Thailand.

**Molecular study**

DNA was extracted using the ZR Plant/Seed DNA MiniPrep™ Kit (Zymo Research Corporation, New York, USA) following manufacturer's instructions. Two DNA markers were targeted, *i.e.*, the chloroplast-encoded *tuf*A gene and the nuclear internal transcribed spacers (ITS1 and ITS2) of the ribosomal cistron. *Tuf*A and ITS amplifications were done in a final reaction volume of 20 µl containing 0.2 mM dNTPs, 0.2 µM of each primer, 0.1× Titanium® Taq DNA polymerase and 10× buffer (Clontech Laboratories Inc., Takara Bio company, CA, USA), and 1 µl DNA template (3–20 ng/µl). Forward and reverse primers for *tuf*A amplifications were, respectively, *tuf*A (5´-TGAAACAGAAMAWCGTCATTATGC-3´) and *tuf*AR (5´-CCTTCN CGAATMGCRAAWCGC-3´) (Famà *et al*., 2002) or *tuf*AR1 (5'-CCATAGGAATT GGACTATCA-3') (Stam *et al.* 2006), annealing at, respectively, nucleotide (nt) positions 210, 1184, and 1096 in a complete *tuf*A gene (1230 nt) of *C. chemnitzia* (Esper) J.V.Lamouroux (Genbank NC032042, Lam and Lopez-Bautista, 2016, as *C. racemosa*). For ITS amplifications, the primers H1F (5´-CTCTGAACCTTCGCACG TAGA-3´) (Kooistra *et al*., 2002) and ITS4 (5´-TCCTCCGCTTATTGATATGC-3´) (White *et al*., 1990) were used. Double-stranded DNA amplifications were performed in a S1000™ thermal cycler (Bio-Rad Laboratories, California, USA). PCR amplification was started at 96 ºC for 4 minutes as a denaturation step, followed by 40 cycles of 30 s at 94 ºC, 30 s at 52 ºC (for *tuf*A) or 48 ºC (for ITS), and 60 s at 72 °C for denaturing, annealing and extension steps, and a final extension step at 72 °C for 6 minutes. PCR purification and sequencing were done by Macrogen Inc. (Seoul, Korea) using the amplification primers.

The DNA sequence data set was complemented with sequences from Genbank. In addition, previously unpublished (S.G.A. Draisma) *tuf*A and/or ITS sequences of *C. macrodisca* and *C. racemosa* specimens from Indonesia were available for this study (Table 3). These *tuf*A sequences were determined as described in Draisma *et al*. (2014). ITS was amplified as described in Stam *et al*. (2006) and subsequently sequenced from cloned amplicons as described in Draisma *et al*. (2012). The herbarium vouchers of these Indonesian specimens are housed in the Naturalis Biodiversity Center in Leiden, The Netherlands, which at the time of our study did not loan any specimens. Sequences were aligned using the MUSCLE software (Edgar, 2004). Molecular species identification was done by Maximum Likelihood (ML) and Bayesian Inference (BI) phylogenetic inference. ML was performed in MEGA v7 (Kumar *et al*., 2016) using the General Time Reversible model with Gamma distribution and invariable sites. Clade support was assessed by bootstrap analysis (Felsenstein, 1985) performed with 1,000 pseudoreplicates. BI was performed in MrBayes (Huelsenbeck and Ronquist, 2001) using Markov Chain Monte Carlo chains (MCMC) for 40,000,000 generations, sampled every 4,000$^{th}$ generation with 10% burnin period. The *tuf*A dataset included *C. macrodisca* and its sister-species *C. megadisca. C. racemosa* was used as an outgroup, because it was shown to be the sister-clade of these two species in Belton *et al*. (2014). A specimen was identified as *C. macrodisca* if in the ML and BI *tuf*A trees it was a member of a supported (ML bootstrap percentage (BP) ≥ 80%, BI posterior probability (PP) ≥ 0.95) clade that also included the *C. macrodisca* DNA barcode sequence (FM956053) and was sister to a clade including the *C. megadisca* DNA barcode sequence (JN817657). No previously published ITS sequences were available for *C. macrodisca* and *C. megadisca*. Newly generated ITS sequences were analyzed with previously published *C. racemosa* ITS sequences as an outgroup.

# Results

## Phylogenetic analyses and species confirmation

The *tuf*A alignment (40 taxa) was 843 nt positions (234–1076) in length after trimming. The ITS alignment (17 sequences from 12 taxa) was 652 nt positions long including 66 gapped positions. Figure 1 shows the BI *tuf*A phylogeny of the *Caulerpa* species under study. The ML tree (not shown) was congruent with the BI tree, revealing the same clades. ML BP and BI PP values are shown in Figure 1. A strongly supported *C. macrodisca* clade (ML BP = 80%, BI PP = 0.98) was sister to a strongly supported *C. megadisca* clade (ML BP = 94%, BI PP = 0.97). The sister-relationship had high to maximum support (ML BP = 96%, BI PP = 1.00). Within the *C. macrodisca* clade four subclades could be discerned, *i.e.*, an Australasian clade (Australia and New Caledonia), an Andaman Sea clade (Thailand and Malaysia), a Java Sea clade, and a Coral Triangle clade (East Kalimantan and West Papua) (Fig. 1). Specimens from the aquarium trade grouped with the Java Sea clade. Relationships among the four subclades remained unresolved. Only the Australasian subclade showed sequence variation (the New Caledonian specimen differed from the Australian specimens). All *Caulerpa* specimens from the Malaysian and Thai mangrove channels on the Andaman Sea coast grouped together in the same *C. macrodisca* subclade.

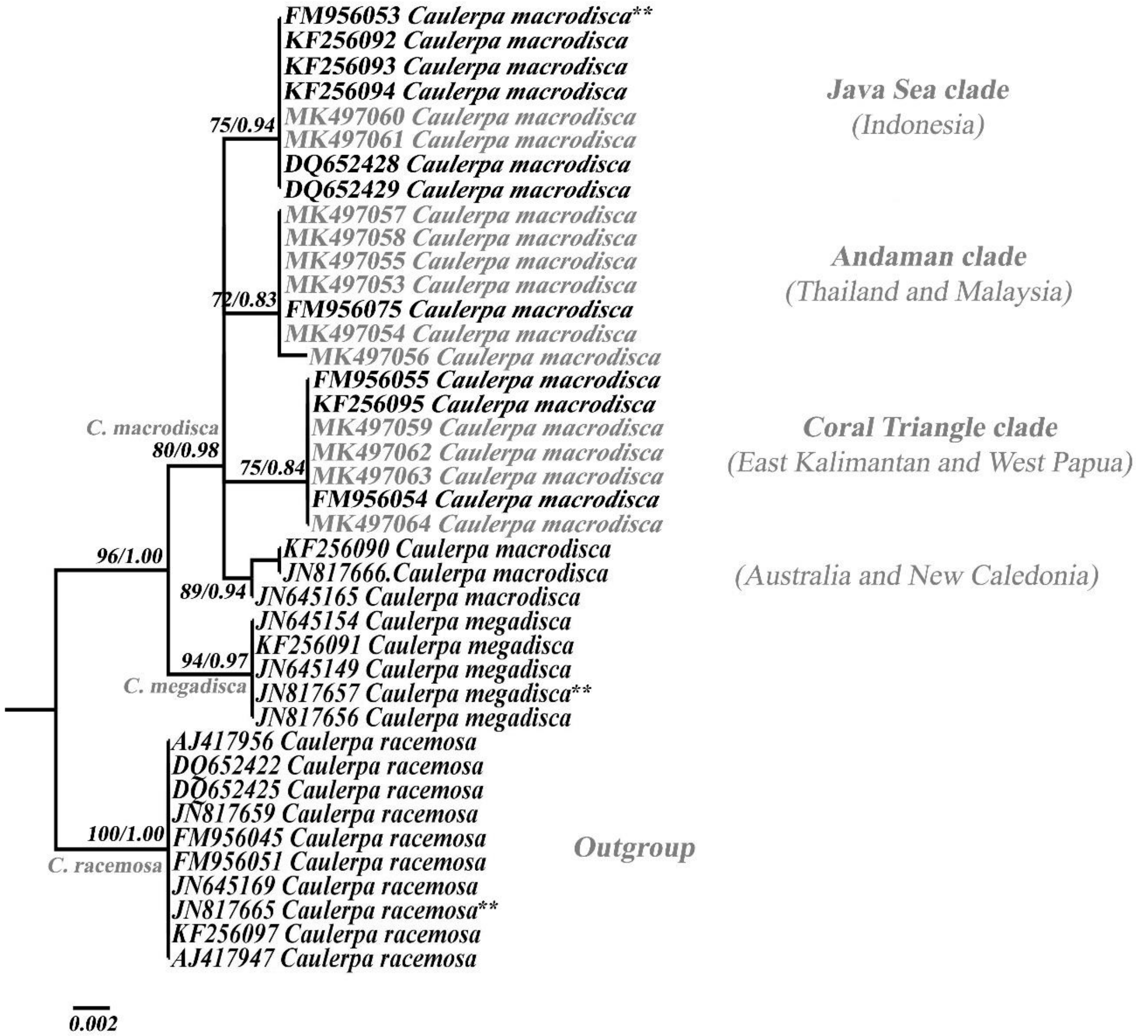


**Figure 8** Bayesian phylogenetic tree constructed from 40 partial *tuf*A DNA sequences of three *Caulerpa* species; *C. racemosa* (outgroup, n=10), *C. megadisca* (n=5), and *C. macrodisca* (n=25). (alignment = 843 bp in length). Taxon labels include GenBank accession numbers. Numbers at branch nodes correspond to Maximum Likelihood bootstrap percentages (BP) and Bayesian Inference posterior probabilities (PP). BP < 70% and PP < 0.7 are not shown. The scale is 0.002 expected changes per site. Taxon labels in gray are newly generated sequences.

** indicate proposed DNA barcode sequences for each species (Belton *et al.*, 2014).

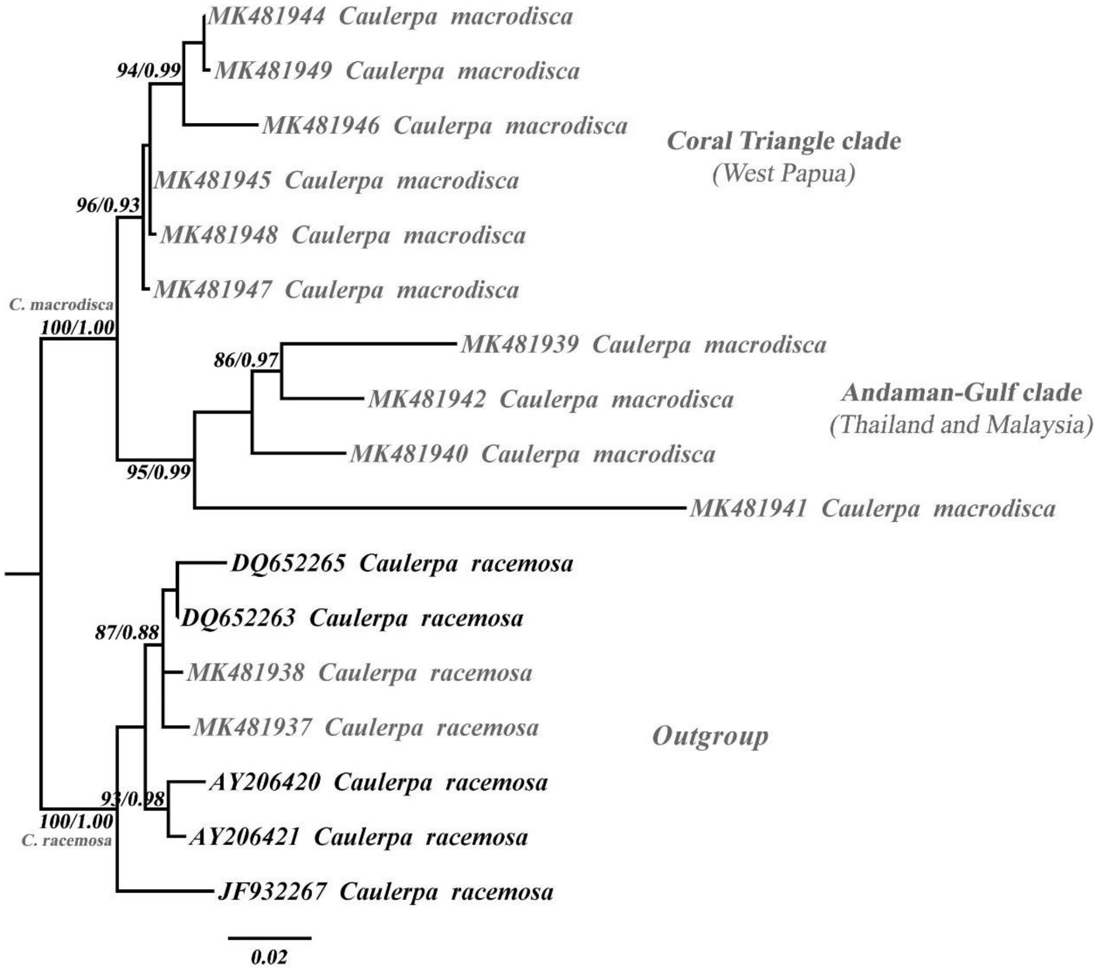


**Figure 9** Bayesian phylogenetic tree constructed from 10 *C. macrodisca* and 7 *C. racemosa* (outgroup) ITS rDNA sequences (alignment = 652 bp in length). Taxon labels include GenBank accession numbers. Numbers at branch nodes correspond to Maximum Likelihood bootstrap percentages (BP) and Bayesian Inference posterior probabilities (PP). BP < 70% and PP < 0.7 are not shown. The scale is 0.02 expected changes per site. Taxon labels in gray are newly generated sequences. The six sequences in the Coral Triangle clade were generated from a single individual.

We only succeeded in amplifying the ITS sequence from the peltate *C. macrodisca* herbarium specimen (KUMF04404) from the Gulf of Thailand. It was nested inside the *C. macrodisca* clade in the BI ITS tree (Fig. 9). The ML ITS tree (not shown) was congruent with the BI tree and ML BP and BI PP are shown in Figure 2. *C. macrodisca* from the Gulf of Thailand was nested inside a strongly supported (ML BP = 95%, BI PP = 0.99) clade comprised of *Caulerpa* from the Andaman Sea. This clade was sister to *C. macrodisca* from West Papua (represented by six ITS sequences from a single individual) with maximum support.

**Morphological characterization**

*Caulerpa* specimens from the Andaman Sea resembled *C.* (*racemosa* var.) *corynephora sensu* Lewmanomont (2008, figs 12, 13) and *sensu* Coppejans *et al.* (2017, fig. 32) with the exception of KUMF06872, which resembled *Caulerpa ashmeadii* Harvey *sensu* Lewmanomont (2008, figs 1, 2). However, the DNA sequence analysis described above clearly identified them as *C. macrodisca*. We will onwards refer to the three morphological *C. macrodisca* entities in Thailand as ecad: *C. macrodisca* ecad *macrodisca* (typical form bearing peltate ramuli, Figs 10A, B), ecad *corynephora* (with clavate and/or turbinate ramuli, Figs 10C–H, J–M), and ecad *ashmeadii* (with cylindrical ramuli, Figs 10I, N). Table 2 summarizes the morphological features of *C. macrodisca sensu* Belton *et al*. (2014) (*i.e.*, ecad *macrodisca*) and the three ecads in Thailand. The measurements for *C. macrodisca* ecad *corynephora* specimens (KP73 and KP74) were from both fresh and herbarium specimens (indicated in Table 2). The recently collected specimens KUMF06872 and KUMF06874 were not available for measurement..

**Table 6** Features of specimens assigned to *C. macrodisca* in the present study and the *C. macrodisca* description provided in Belton *et al.* (2014). Measurements from fresh specimens in square brackets "[]". n.a., not available. n.m., not measured.

| **Characters** | ***C. macrodisca* Decaisne**<br><br>***sensu* Belton *et al.* (2014)** | ***C. macrodisca* ecad *macrodisca***<br><br>**from the Gulf of Thailand** | ***C. macrodisca* ecad *corynephora***<br><br>**from the Andaman Sea** | ***C. macrodisca* ecad *ashmeadii***<br><br>**from the Andaman Sea** |
|---|---|---|---|---|
| Specimens examined | see Belton *et al*. (2014) | KUMF04404 | PSU KP73A, PSU KP74A, GENT HEC16156, KUMF06874 | KUMF06872 |
| Figures | fig. 9C in Belton *et al.* (2014) | Fig. 10A | Figs 10C─D, F─H, Figs 11A–B | Fig. 10I |
| Habitat | n.a. | Intertidal | Mangrove channel, subtidal | Mangrove channel, subtidal |
| Stolon diameter (mm) | (1.0─)1.5─3.0 | 1.5─1.8 | 1.4─2.3 [2.3─3.4] | n.m. |
| Assimilator height (cm) | 1.0─5.0 | 4.1─6.0 | 2.7─9.2 [2.9─10.5] | n.m. |
| Ramuli | | | | |
| Arrangement | Semi-crowded, radially arranged around an axis | Alternate or irregular | In opposite pairs along the axis, but sometimes alternately opposite (distichous) near the base. KUMF06874 distichous throughout | Mostly in opposite pairs along the axis |
| Shape | Distinctly peltate | Distinctly peltate | Clavate to mostly turbinate (rarely peltate) | Cylindrical with a swollen tip |
| Length (mm) | 5.0─8.0 | *ca*. 1.0 | 4.0─10.9 [5.1─12.2] | n.m. |
| Diameter (mm) | 5.0─10.0 | 6.4─9.0 | 1.2─4.0 [1.4─5.0] | n.m. |

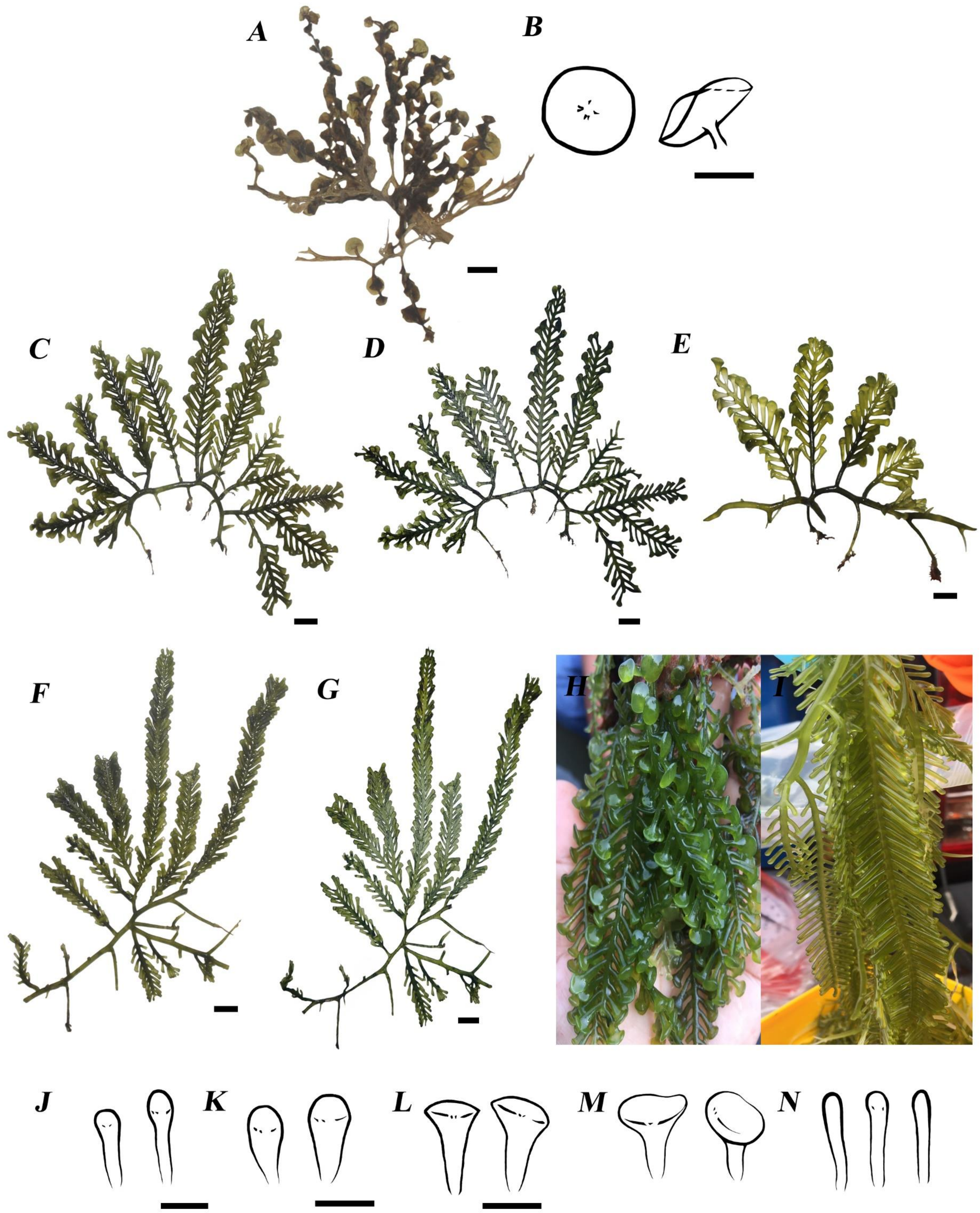


**Figure 10** *Caulerpa macrodisca* from Thailand and Malaysia (A, C–G, scale bar = 1 cm; B, J–L, scale bar = 0.5 cm; H–I, M–N, no scale bar): (A, B) *C. macrodisca* ecad *macrodisca* (KUMF04404) from Ao Cho, Trat; (A) Habit (herbarium); (B) Drawings of peltate ramuli in apical (left) and lateral (right) views; (C, D) *C. macrodisca* ecad *corynephora* (KP73) from Klong Yang, Krabi; (C) Fresh specimen; (D) Herbarium; (E) *C. macrodisca* ecad *corynephora* (KP65) from Kilim river, Langkawi, Malaysia (fresh); (F, G) *C. macrodisca* ecad *corynephora* (KP74) from Klong Yang, Krabi; (F) Fresh specimen; (G) Herbarium; (H) *C. macrodisca* ecad *corynephora* (KUMF06874) from Che Bilang, Satun; (I) *C. macrodisca* ecad *ashmeadii* (KUMF06872) from Tung Wa, Satun; (J–L) Drawings of ramuli of *C. macrodisca* ecad *corynephora*; (J, K) club-shaped, clavate and (L, M) trumpet-shaped, turbinate; (N) Drawings of cylindrical ramuli of *C. macrodisca* ecad *ashmeadii* (KUMF06872).

## Discussions

The *tuf*A and ITS phylogenies (Figs 8, 9) showed that *Caulerpa* taxa from Thailand that were previously assigned to *Caulerpa* (*racemosa* var.) *corynephora* and *Caulerpa ashmeadii* (Lewmanomont, 2008; Phang *et al*., 2008) actually belong to *Caulerpa macrodisca sensu* Belton *et al*. (2014) in the *Caulerpa* section *Caulerpa*. The ITS phylogeny supported that *Caulerpa macrodisca* ecad *macrodisca* from the Gulf of Thailand is conspecific with the other two ecads from the Andaman Sea coast.

The general habit of the *C. macrodisca* ecad *macrodisca* specimen (KUMF04404) (Fig. 10) matched the lectotype illustration of *C. macrodisca* by Decaisne (1846 (1846–1864), pl. 1, fig. 1), but Decaisne's illustration has no scale and dimensions are therefore unknown. However, Decaisne (1842) reported that the peltate disc was 1 cm in his original description. The habit and dimensions of KUMF04404 were in agreement with those of *C. macrodisca* given in Belton *et al.* (2014, table 1, fig. 9C) (Table 6). However, KUMF04404 (Fig. 10A) showed a branched assimilator, which was not reported by Belton *et al*. (2014). The assimilator length of the dried specimen KUMF04404 was 4–6 cm and its ramulus discs 6–9 mm in diameter (Fig. 10B). Lewmanomont (2008, fig. 11) reported an (unbranched) assimilator length of 2.5–7 cm and a disc diameter of 4.5–17.5 mm in intertidal *C. macrodisca* (as *C. peltata* var. *macrodisca*) from Trat province (no voucher numbers given). Specimens from the mangrove channels on the Andaman Sea coast molecularly identified as *C. macrodisca* (Fig. 10) also showed branched assimilators (Figs 10C–N), but deviated in ramuli shape and arrangement from ecad *macrodisca.* The ramuli in *C. macrodisca* ecad *corynephora* (KP73 and KP74) can be up to 12 mm long and are gradually widening from 1.4 mm towards a 5 mm wide rounded (club-shaped, clavate, Figs 10J–K) or blunt (trumpet-shaped, turbinate, Figs 10L–M) terminus. The ramuli in *C. macrodisca* ecad *ashmeadii* (KUMF06872) are cylindrical throughout except for a swollen terminus (Fig. 10N). Ramuli in *C. macrodisca* ecad *corynephora* and ecad *ashmeadii* were arranged in opposite pairs or distichously (alternating) along the rachis.

Earlier molecular phylogenies unambiguously demonstrated that true *C. corynephora* (type location Torres Strait, Australia) belongs to the *Caulerpa* section *Sedoideae* which is characterized by species with pyrenoid-associated chloroplasts and ramuli on a constricted pedicel (Draisma *et al.*, 2014; Belton *et al.*, 2015, 2019). Several species in the section *Sedoideae*, including *C. corynephora*, have an annulated rachis (Draisma *et al.*, 2014), a character not found outside the section *Sedoideae*. Pyrenoids and an annulated rachis are present in *Caulerpa corynephora* (Price, 2011; Belton *et al*., 2015, 2019) and this species has only been confirmed from northern and western Australia (Belton *et al*., 2019). Lewmanomont's (1978, 2008) and Phang *et al*.'s (2008, no description given) identification of *C. racemosa* var. *corynephora* from mangrove channels on the Andaman Sea coast of Thailand and Malaysia may have been based on illustrations in Weber-van Bosse (1898, pl. xxxiii, figs 10–14, 1913, figs 27, 28). However, these illustrations are not an accurate representation of either the holotype specimen or Montagne's (1845) own illustration of *C. corynephora* (Price, 2011). Pyrenoids, constricted pedicels, and annulations were not observed in *C. macrodisca* ecad *corynephora* specimens from Thailand and Malaysia, although Coppejans *et al*. (2017, p. 41) stated in the description of *C. corynephora* from Thailand "*The plasts possess pyrenoids, separating C. corynephora from the C. racemosa-peltata complex; this is confirmed by molecular data (Prud'homme van Reine in litteris 2014)*". However, this statement was based on a miscommunication between Coppejans and Prud'homme van Reine (E. Coppejans and W.F. Prud'homme van Reine, pers. comm. to S.G.A. Draisma 2018). Coppejans *et al*. (2017, fig. 32C) also showed a specimen with cylindrical ramuli, yet they assigned it to *C. corynephora*, not to *C. ashmeadii*. Collection no. HEC16156 A–D (in GENT, as *C. racemosa* var. *corynephora*) consists of multiple sheets, *i.e.*, HEC16156-A (Fig. 11A) and HEC16156-A´ (Fig. 11B). The latter contains four separate stolon fragments with assimilators with various ramuli shapes including cylindrical with swollen tips.

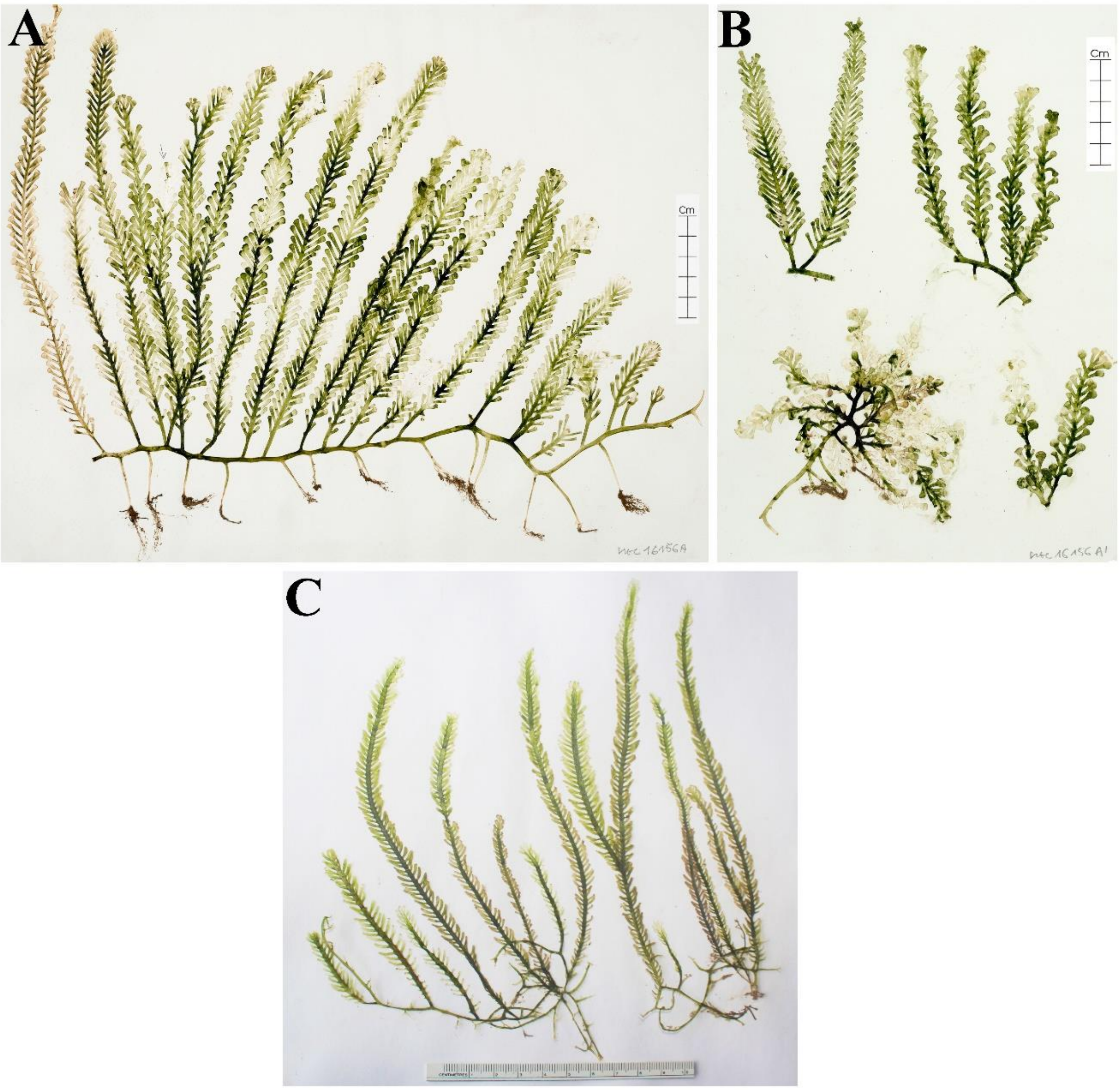

**Figure 11** Herbarium specimens of A., *C. macrodisca* ecad *corynephora* HEC16156A-D in GENT, as *C. racemosa* var. *corynephora* (Herbarium sheet HEC16156A), B., *C. macrodisca* ecad *corynephora* HEC16156A-D in GENT, as *C. racemosa* var. *corynephora* (Herbarium sheet HEC16156A´) and C., *C. ashmeadii* from Phang Nga province (KL8301 in KUMF)

KUMF06872 (Fig. 10I) from Satun province, originally identified as *C. ashmeadii*, also represents an ecad of *C. macrodisca*. PCR amplification was unsuccessful for KL8301 (in KUMF, Fig. 11C) from Phang Nga province (also on the Andaman Sea coast), which was also morphologically identified as *C. ashmeadii*. It appears not to be the same specimen as the *C. ashmeadii* from Phang Nga depicted in Lewmanomont (2008, figs 1, 2, no voucher number given). However, specimens identified as *C. ashmeadii* from Phang Nga differ from the type specimen of *C. ashmeadii* (type locality Florida) illustrated in Harvey (1858, pl. XXXVIII.A), which shows straight cylindrical ramuli with obtuse tips in an opposite arrangement, whereas ramuli in KL8301 (Fig. 11C) and the specimen depicted in Lewmanomont (2008, figs 1, 2) are slightly curved up. *Caulerpa ashmeadii* has only been confirmed with molecular data from the Caribbean and the Gulf of Mexico and is thought to be confined to the Atlantic (Famà *et al*., 2002; Stam *et al*., 2006; Sauvage *et al*., 2014). However, *C. ashmeadii* has been reported from the Indo-Pacific. Besides the above mentioned Thai records, it has been reported from India (Umamaheswara Rao 1969) and Vietnam (Nguyen *et al*., 1993). Subsequent Indo-Pacific *C. ashmeadii* reports (Silva *et al*., 1996; Sahoo *et al*., 2001; Nguyen, 2007; Nguyen *et al*., 2013; Phang *et al*., 2016) can all be traced back to the original reports which could not be accessed by the present authors. *Caulerpa ashmeadii* was not found by Kazi *et al*. (2013) who molecularly identified Indian *Caulerpa* species. Kazi *et al*. (2013) found and molecularly identified *Caulerpa veravalensis* Thivy & V.D.Chauhan from India (Thivy and Chauhan, 1963), a species morphologically similar to *C. ashmeadii*, but with compressed ramuli. *Caulerpa veravalensis, C. ashmeadii,* and *C. macrodisca* all belong to the *Caulerpa* section *Caulerpa*, but are not closely related to each other. Each has another sister-species. Herbarium specimens from Thailand identified as *C. ashmeadii* (voucher KL8301 in KUMF (Fig. 11C) and figs 1–2 in Lewmanomont, 2008) are morphologically similar to *C. veravalensis* (see fig. S9 in Kazi *et al*., 2013). However, whether ramuli originally were compressed or cylindrical cannot be discerned from herbarium specimens. In her description of *C. ashmeadii* from Phang Nga mangroves, Lewmanomont (2008) mentioned that ramuli are cylindrical, whereas ramuli in Indian intertidal *C. veravalensis* are compressed (Kazi *et al*., 2013).

Another brackish water *Caulerpa* species with terete ramuli, reminiscent of a lanky *C. macrodisca* ecad *ashmeadii*, was described from Swan River, Western Australia, *i.e. Caulerpa lagara* Carruthers, Walker & Huisman (Carruthers *et al*., 1993). It has not been reported since its original description and was therefore not included in Belton *et al*. (2019), a re-assessment of southern Australian *Caulerpa* using DNA sequence data. Conspecificity of *C. lagara* and *C. macrodisca* is not supported by Carruthers *et al*.'s study (1993). They cultured estuarine *C. lagara* in 20, 30, and 40 ‰ salinity, where it did not develop peltate ramuli. In Draisma *et al*. (2014, table S2) it was suggested that *C. lagara* might be a synonym of *C. pinnata* C.Agardh (type location Sri Lanka), but DNA sequence data is lacking for both taxa. Carruthers *et al*. (1993) and Silva *et al*. (1996) mention a resemblance of *C. racemosa* var. *corynephora* (Montagne) Weber-van Bosse to, respectively, *C. lagara* and *C. pinnata*.

*Caulerpa macrodisca* ecad *corynephora* and ecad *ashmeadii* are morphologically distinct from *C. macrodisca* ecad *macrodisca* and form a distinct clade within *C. macrodisca* in the *tuf*A phylogeny (Fig. 8) and therefore could merit the official taxonomic status variety or form. However, this is not supported by the ITS phylogeny (Fig. 9) where *C. macrodisca* ecad *macrodisca* from the Gulf of Thailand (not included in the *tuf*A tree) is nested inside the Andaman Sea clade. The phylogenetic pattern may rather be a reflection of a biogeographic pattern. Any infra-specific classification is currently not warranted without *tuf*A sequence data of *C. macrodisca* ecad *macrodisca* from the Gulf of Thailand and a study of the morphology of the members of the other *C. macrodisca* subclades (Fig. 8). The differences in morphology are more likely environmentally induced. *C. macrodisca* var. *macrodisca* grew intertidally in the Gulf of Thailand and *C. macrodisca* on the west coast of the Thai-Malay peninsula always grew subtidal in mangrove channels hundreds of meters from the sea. Morphological variation in *Caulerpa* can be caused by several environmental factors such as light intensity, temperature and salinity (Peterson, 1972; Calvert, 1976; Ohba *et al.*, 1992). Ohba and Enomoto (1987) reported that *Caulerpa racemosa* var. *laetevirens* (Montagne) Weber Bosse (probably *C. chemnitzia*) exhibited various ramulus shapes (cylindrical, turbinate and peltate) at different temperatures and light intensities. The estuarine environment of the Andaman mangrove channels may induce the *C. macrodisca* ecad *corynephora* and ecad *ashmeadii* morphologies. Culture experiments should provide more insight into the morphological plasticity of *C. macrodisca*.

# CONCLUSION

In conclusion, this study was found eight *Caulerpa* species, *i.e.*, *C. chemnitzia, C. lentillifera, C. macrodisca, C. racemosa, C. serrulata, C. sertularioides, C. taxifolia* and *C. verticillata.* There were three distinct morphotypes of *C. macrodisca* in Thailand. *C. macrodisca* ecad *macrodisca* was only found in the Gulf of Thailand. *C. macrodisca* ecad *corynephora* and *C. macrodisca* ecad *ashmeadii* were only found in mangrove channels on the Andaman Sea coast. In addition, there were two distinct morphotypes of *C. racemosa* in Thai water including *C. racemosa* ecad *chemnitzia* and *C. racemosa* ecad *racemosa*.

The present study highlights the importance of DNA sequence data for reliable species identification in the *Caulerpa racemosa-peltata* complex and to determine the true extent of morphological variation within each species. It is recommended to apply the use of morphological entities like ‘ecads’ which do not have formal taxonomic status for highly plastic species such as *C. macrodisca* and *C. racemosa*. Moreover, morphological variations especially on thallus size and ramuli shape of *Caulerpa* in Thailand might affected by environmental factors because of it’s morphological plasticity.

For seven previous species, this study not found any specimens during this field work, and their identities were not confirmed by molecular study. Then, this result lead to decrease macroalgal diversity in Thailand. Thus, further studies may focus a modeling approach to estimate any environmental conditions predicting the further situation of any marine macroalgae in Thai water.

**Taxonomic conclusions**

1. *Caulerpa chemnitzia* (Esper) J.V.Lamouroux (Fig. 12A)

Synonyms : *Fucus chemnitzia* Esper, *Ahnfeldtia chemnitzia* (Esper) Trevisan, *Chauvinia chemnitzia* (Esper) Kützing and *Caulerpa racemosa* var. *chemnitzia* (Esper) Weber-Van Bosse

Description : Green thallus; rhizoid 0.23–1.56 cm long; large widely horizontal stolon 0.17–0.34 cm in diameter, abundant growth on rock at intertidal zone; erect assimilator, 0.47–1.2 cm in width, 0.87–6.75 cm in length; terete axis 0.1–0.22 cm in diameter; bearing various ramulus shapes from mostly turbinate to peltate into mushroom-like, 0.11–0.78 cm in diameter, supported by short stalks 0.03–0.6 cm long; acute tips.

Vouchers, collection month and location : KP29, KP31 (Ko Kham, Songkhla in October, 2016), KP32 and KP50 (Losin, Pattani in August, 2017)

2. *Caulerpa lentillifera* J.Agardh (Figs 12B–C)

Synonyms : *Ahnfeldtia lentillifera* (J.Agardh) Trevisan and *Chauvinia lentillifera* (J.Agardh) Kützing

Description : Light green thallus; rhizoid 0.11–0.78 cm long; horizontal stolon 0.13–0.18 cm in diameter, abundant growth on rock and dead coral at intertidal zone; Erect branch with dense and short ramuli on axis; terete axis, 0.1–0.15 cm in diameter; spherical translucent ramulus shape, 0.21–0.23 cm in diameter, supported by short stalks 0.05–0.09 cm long, prominently constricted at the base, imbricately arranged or in rows of four; rounded tips; chloroplast with pyrenoid.

Vouchers, collection month and location : KP6 and KP21 (Ko Siboya, Krabi in February and April, 2016)

3. *Caulerpa macrodisca* Decaisne (Figs 12D–G)

Synonyms : *Ahnfeldtia macrodisca* (Decaisne) Trevisan, *Chauvinia macrodisca* (Decaisne) Kützing, *Caulerpa peltata* var. *macrodisca* (Decaisne) Weber-van Bosse and *Caulerpa racemosa* var. *macrodisca* (Decaisne) Weber-van Bosse

*Caulerpa macrodisca* ecad *macrodisca* (Figs 12D–E)

Description : Green thallus; rhizoid 0.61–2.35 cm long; terete stolon, 0.15–0.18 cm diameter, abundant growth on sandy or muddy bottoms of turbid water; erect assimilators, 0.54–1.23 cm in width, 4.13–6.05 cm in length; terete axis 0.06–0.1 cm diameter, bearing large thin disc, 0.64–0.9 cm diameter; with short stalks, sometimes, a single peltate branch (genearally 1 cm. or more diameter) was consisted along terete stolon; disk tips.

Vouchers, collection month and location : KUMF04404 (Ao Cho, Trat in January, 1991)

*Caulerpa macrodisca* ecad *corynephora* (Figs 12F–G)

Description : Light green thallus; rhizoid 0.84–5.05 cm long; horizontal stolon, 0.23–0.34 mm in diameter, abundant growth on fish's cage in the brackish water of the Andaman mangrove channels; erect assimilators, 0.65–0.94 cm in width, 2.89–10.48 cm in length; terete axis, 0.14–0.21 cm in diameter, constriction at the basis; bearing generally 2 to 4 longitudinal rows with opposite various shapes of ramulus from mostly clavate (1 mm in diameter) to turbinate into rarely peltate (disc 5 mm in diameter, nearly the top), 0.14–0.5 cm in diameter; without constriction at the base; acute tips; chloroplasts without associated pyrenoids.

Vouchers, collection month and location : KP65 (Kilim river, Malaysia in December, 2017), KP73, KP74 (Khlong Yang, Krabi in July, 2018) and KUMF06874 (mangrove channel, Satun in September, 2018)

*Caulerpa macrodisca* ecad *ashmeadii*

Description : light green thallus, growth in the Andaman mangrove channel; erect assimilators bearing generally opposite ramuli arrangement (sometime alternate at base); cylindrical ramulus shape with swollen tips

Vouchers, collection month and location : KUMF06872 (mangrove channel, Satun in September, 2018)

4. *Caulerpa racemosa* (Forsskål) J.Agardh (Figs 12H–L)

Synonyms : *Fucus racemosus* Forsskål

*Caulerpa racemosa* ecad *racemosa* (Figs 12H–I)

Description : Dark green thallus; rhizoid 0.28–4.64 cm long; large widely horizontal stolon 0.13–0.39 cm in diameter, abundant growth on rock and dead coral at intertidal zone; strong short erect branch, 0.29–1.88 cm in width, 0.44–5.98 cm in length; with dense ramuli; terete axis 0.09–0.47 cm in diameter, with constriction at the basis; subspherical to spherical (vesiculate) ramulus shape with rounded tip, 0.22–0.71 cm in diameter; supported by short stalk 0.06–0.47 mm long, 0.11–0.31 cm in diameter; with constriction at the base, 0.07–0.26 cm in diameter; rounded tip.

Vouchers, collection month and location : KP10 (Ko Tan, Surat Thani in February, 2016), KP7, KP22 (Ko Siboya, Krabi in February and April, 2016), KP39, KP40 (Ko Lipe, Satun in January, 2017), KP68, KP69 and KP70 (Ko Lanta, Krabi in March, 2018)

*Caulerpa racemosa* ecad *chemnitzia* (Figs 12J–L)

Description : Green thallus; rhizoid 0.12–2.76 cm long; horizontal stolon 0.15–0.25 cm in diameter, abundant growth on rock at intertidal zone; erect assimilators, 0.33–1.39 cm in width, 0.84–4.81 cm in length; terete axis 0.1–0.19 cm in diameter, with constriction at the basis; bearing various ramulus shapes from mostly turbinate to peltate or sometimes mushroom-like, 0.11–1.01 cm in diameter, supported by short stalks 0.03–0.43 cm long; acute tips.

Vouchers, collection month and location : KP28, KP30 (Ko Kham, Songkhla in October, 2016), KP49 and KP51 (Losin, Pattani in August, 2017)

5. *Caulerpa serrulata* (Forsskål) J.Agardh (Fig. 12M)

Synonyms : *Fucus serrulatus* Forsskål and *Caulerpa freycinetii* f. *serrulata* (Forsskål) Weber-van Bosse

Description : Heavy green thallus with yellow tip; descending branches having branched rhizoid at the ends 0.1–5.74 cm long; large widely horizontal stolon 0.11–0.36 cm in diameter, widespread and dense colonies on rock and dead coral at intertidal zone; dichotomous branching, twist once or twice; upright flattened branches with serrate margin; branch 0.21–0.59 cm in width, 0.36–4.67 cm in length; supported by cylindrical stalks 4 mm long or less; teeth tips.

Vouchers, collection month and location : KP11 (Ko Tan, Surat Thani in February, 2016), KP14, KP45 (Ko Samui, Surat Thani in February, 2016 and August, 2017), KP41 (Ko Lipe, Surat Thani in January, 2017), KP47 (Ko Matsum, Surat Thani in August, 2017), KP72 (Tang Khen Bay, Phuket in March, 2018), SP358, SP284, SP301, SP358, SP368 (Ko Rok, Krabi in February, 2011) and SP372 (Ko Kradan, Trang in February, 2011)

6. *Caulerpa sertularioides* (S.G.Gmelin) M.Howe (Figs 12N–O)

Synonyms : *Fucus sertularioides* S.G.Gmelin

Description : Green widespread thallus, feather like erect branches; rhizoid 0.06–3.78 cm long; large strong stolon 0.07–0.29 cm in diameter, abundant growth on sand, rock and dead coral at intertidal zone; distichously arranged assimilators along axis, slender, cylindrical, oppositely arranged, pinnules ramulus; branch, 0.21–1.56 cm in width, 0.56–7.89 cm in length, rarely dichotomous, branching 1–2 times; yellow mucronate tips.

Vouchers, collection month and location : KP15 (Ko Samui, Surat Thani in February, 2016), KP34, KP35 and KP61 (Ko Lidee, Satun in November-December, 2016 and December, 2017)

7. *Caulerpa taxifolia* (M.Vahl) C.Agardh (Figs 12P–R)

Synonyms : *Fucus taxifolius* M.Vahl

Description : Green thallus; rhizoid 0.58–6.6 cm long; horizontal stolon 0.11–0.32 cm in diameter, abundant growth on rock at intertidal zone; upright branch with opposite ramuli arrangement, usually branched; branches, 0.133–1.46 cm in width, 1.8–11.42 cm in length; clearly flattened and upright falcate ramulus shape 0.07–0.13 cm wide, 0.26–0.77 cm long, with obviously constricted at base; mucronate tips.

Vouchers, collection month and location : KP8, KP23 (Ko Siboya, Krabi in February and April, 2016), KP43 (Ko Libong, Trang in May, 2017), KP46 (Ko Matsum, Surat Thani in August, 2017), KP53, KP54, KP55, KP56 (Leam Yong Lam, Trang in September, 2017), KP57 (Khao Bae Na, Trang in September, 2017), SP332, SP343, SP234 (Ko Rawi, Satun in February, 2011) and SP474 (Ko Adang-Rawi, Satun in December, 2011)

8. *Caulerpa verticillata* J.Agardh (Figs 12S–U)

Synonyms : *Stephanocoelium verticillatum* (J.Agardh) Kützing

Description : Green filiform thallus; rhizoid 0.2–2.81 mm long; stolon 0.18–0.39 mm diameter, abundant tuft on rock and dead coral; 4–7 times dichotomously branched, whorled branching 0.94–5.28 mm wide, 2–10.97 mm high, dense overlap layers (more than 5 layers); branchlets cylindrical 0.03–0.07 mm width, 0.19–3.36 mm long; tree spine tips.

Vouchers, collection month and location : KP1, KP5, KP13, KP62 (Ko Lidee, Satun in January, 2015–2016 and December, 2017), KP9, KP24 (Ko Siboya, Krabi in February and April, 2016) and KP42 (Ko Lipe, Satun in January, 2017)

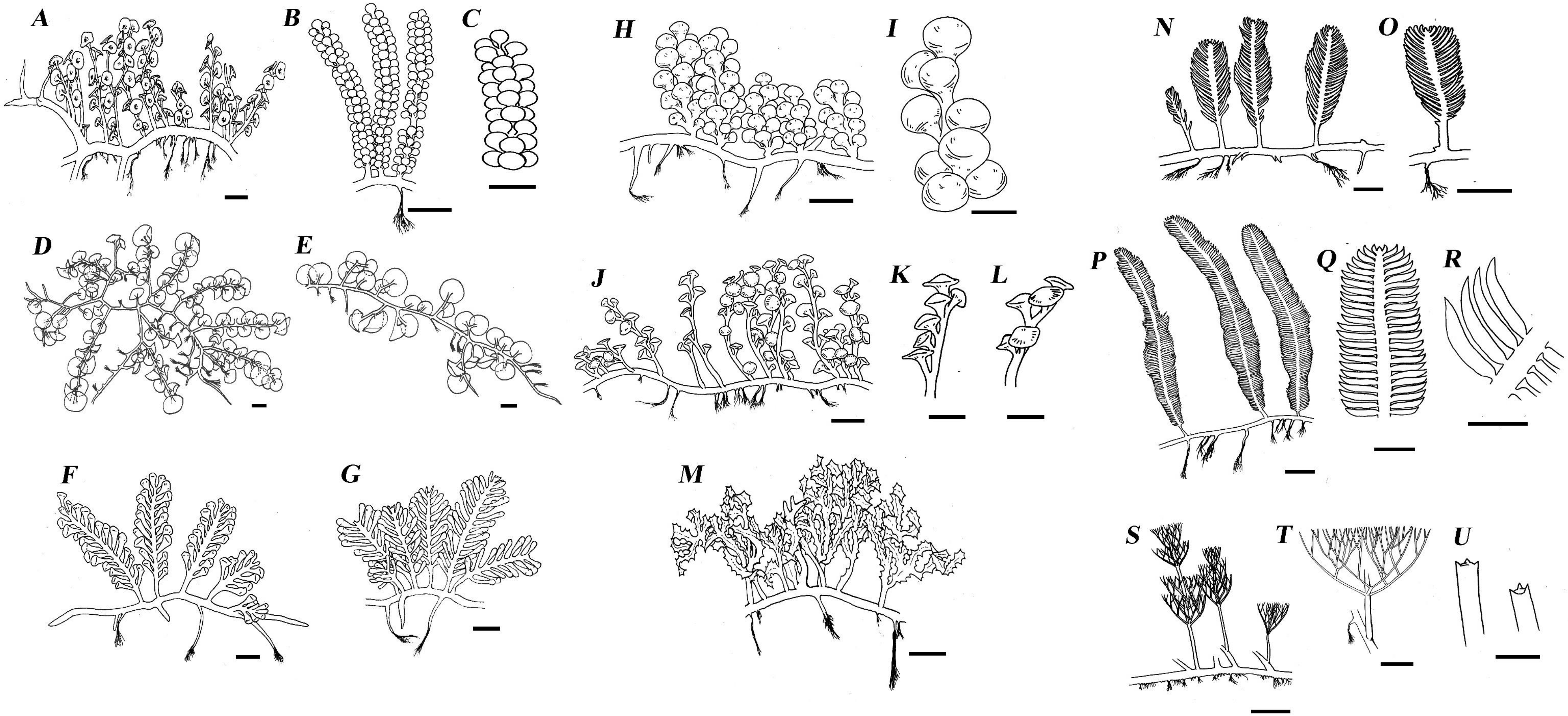


**Figure 12** Eight *Caulerpa* species were found in Thailand (A–G., H., J., M., P., scale bar = 1 cm; I., K–L., N–O., Q–R., T., scale bar = 0.5 cm; S., scale bar = 1 mm; U., scale bar = 50 µm) including A., *C. chemnitzia*, B–C., *C. lentillifera*, D–G., *C. macrodisca* (D–E., *C. macrodisca* ecad *macrodisca* and F–G., *C. macrodisca* ecad *corynephora*), H–L., *C. racemosa* (H–I., *C. racemosa* ecad *racemosa* and J–L., *C. racemosa* ecad *chemnitzia*), M., *Caulerpa serrulata*, N–O., *C. sertularioides*, P–R., *C. taxifolia* and S–U., *C. verticillata*.

# REFERENCES


Bakker, F. T., Olsen, J. L. and Stam, W. T. 1995. Evolution of nuclear rDNA ITS sequences in the *Cladophora albida/sericea* clade (Chlorophyta). Journal of Molecular Evolution 40: 640–651.

Belleza, D. F.C. and Liao, L. M. 2007. Taxonomic inventory of the marine green algal genus *Caulerpa* (Chlorophyta, Bryopsidales) at the University of San Carlos (CEBU) herbarium. The Philippines Scientist 44: 71–104.

Belton, G. S., Prud'homme van Reine, W. F., Huisman, J. M., Draisma, S. G.A. and Gurgel, C. F.D. 2014. Resolving phenotypic placticity and species designation in the morphologically challenging *Caulerpa racemosa-peltata* complex (Caulerpaceae, Chlorophyta). Journal of Phycology 50: 32–54.

Belton, G. S., Draisma, S. G.A., Prud'homme van Reine, W. F., Huisman, J. M. and Gurgel, C. F.D. 2019. A molecular based taxonomic revision of *Caulerpa* (Chlorophyta, Caulerpaceae) from southern Australian based on *tuf*A and *rbc*L gene sequence data. Phycologia DOI: 10.1080/00318884.2018.1542851.

Begon, M., Townsend, C. R. and Harper, J. L. 2006. Ecology. 4th ed. Blackwell Publishing Ltd, Oxford. 3–633.

Bold, H. C. and Wynne, M. J. 1985. Introduction to the Algae. Structure and Reproduction. 2nd ed. Prentice Hall. 1706pp.

Calvert, H. E. 1976. Culture studies on some Florida species of *Caulerpa*: Morphological responses to reduced illumination. British Phycological Journal 11: 203–214.

Carruthers, T. J.B., Walker, D. I. and Huisman, J. M. 1993. Culture studies on two morphological types of *Caulerpa* (Chlorophyta) from Perth, Western Australia, with a description of a new species. Botanica Marina 36: 589–596.

Chen, C. A. 1999. Analysis of scleractinian distribution in Taiwan indicating a pattern congruent with sea surface temperatures and currents: examples from Acropora and Faviidae corals. Zoological Studies 38(2): 119–129.

Chirapart, A., Pimla, S., Praiboon, J., Boonprab, K., Akakabe, Y., Matsui K. and Kajiwara, T. 2011. Contribution to the knowledge of morphometry and sulfated polysaccharide from Thai species of *Caulerpa*. Algal Resources 3: 177-184.

Coppejans, E. and Beeckman, T. 1989. *Caulerpa* section Sedoideae (Chlorophyta, Caulerpales) form the Kenyan coast. Nova Hedwigia 49: 381–393.

Coppejans, E., Prathep, A., Leliaert, F., Lewmanomont, K. and De Clerck, O. 2010. Seaweeds of Mu Ko Tha Lae Tai. 1st ed. Biodiversity research and training program. Bangkok, Thailand. 77–92.

Coppejans, E., Prathep, A., Lewmanomont, K., Hayashizaki, K. I., De Clerck, O., Leliaert, F. and Terada, R. 2017. Seaweeds and Seagrasses of the Southern Andaman Sea coast of Thailand. The Kagoshima University Museum. Asahi Printing Co., Ltd., Kagoshima, Japan. 39–53.

Coppejans, E. 1992. Marine algae of Papua New Guinea (Madang Prov.) 2. A revised and completed list of *Caulerpa* (Chlorophyta-Caulerpales). Blumea 36: 383–410.

Dawson, E. Y. 1954. Note on tropical Pacific marine algae. Bulletin of the Southern California Academy of Sciences 53: 1–7.

de Clerck, O., Bolton. J. J., Anderson, R. J. and Copperjans, E. 2005. Guide to the seaweeds of Kwazulu-Natal. Volume 33. Flanders marine institute, Flemish community, National botanic garden of Belgium. Meise, Belgium. 62–75.

de Clerck, O., Guiry, M. D., Leliaert, F., Samyn, Y. and Verbruggen, H. 2013. Algal taxonomy: a road to nowhere? Journal of Phycology 49: 215–25.

de Senerpont Domis, L. N., Famà, P., Bartlett, A. J., Prud'homme van Reine, W. F., Espinosa, C. A. and Trono, G. C. 2003. Defining taxon boundaries in members of the morphologically and genetically plastic genus *Caulerpa* (Caulerpales, Chlorophyta). Journal of Phycology 39: 1019–1037.

Decaisne, J. 1842. Essais sur une classification des algues et des polypiers calcifères de Lamouroux. Annales des Sciences Naturelles, Botanique, Seconde série. 17: 297–380. pls 14–17.

Decaisne, J. 1846–1864. Botanique. In: Voyage autour du monde sur la frégate la Vénus, Théodore Morgand, (ed. A. Du Petit Thouars). Gide et Cie, Paris. 297–381.

Draisma, S. G.A., Prud'homme van Reine, W. F., Sauvage, T., Belton, G. S., Gurgel, C. F.D., Lim, P. E. and Phang, S. M. 2014. A re-assessment of the infrageneric classification of the genus *Caulerpa* (Caulerpaceae, Chlorophyta) inferred from a time-calibrated molecular phylogeny. Journal of Phycology 50: 1020–1034.

Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput Nucleic Acids Res. 32(5): 1792–1797. https://www.drive5.com/muscle/; searched on 19 March 2019.

Egerod, L. 1971. Some marine algae form Thailand. Phycologia 10(1): 121–142.

Egerod, L. 1974. Report of the marine algae collected on the fifth Thai-Denish Expedition of 1966: Chlorophyceae and Phaeophyceae. Botanica Marina 17(3): 130–157.

Egerod, L. 1975. Marine algae of Andaman Sea coast of Thailand: Chlorophyceae. Botanica Marina 18(1): 41–66.

Famà, P., Wysor, B., Kooistra, W. H.C.F. and Zuccarello, G. C. 2002. Molecular phylogeny of the genus *Caulerpa* (Caulerpales, Chlorophyta) inferred from chloroplast *tuf*A gene. Journal of Phycology 38: 1040–1050.

Guiry, M. D., and Guiry, G. M. 2019. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org; searched on 17 March 2019.

Händeler, K., Wägele, H., Wahrmund, U., Rüdinger, M. and Knoop, V. 2010. Slugs' last meals: molecular identification of sequestered chloroplasts from different algal origins in Sacoglossa (Opisthobranchia, Gastropoda). Molecular Ecology Resources 10: 968–978.

Harvey, W. H. 1858. Nereis Boreali-Americana: or contributions to a history of the marine Algae of North America. Part III. Chlorospermeae. Smithsonian Contributions to Knowledge 10: 18–20. pl. XXXVIII.A.

Hu, Z. M., Uwai, S., Yu, S. H., Komatsu, T., Ajisaka, T. and Duan, D. L. 2011. Phylogeographic heterogeneity of the brown macroalga *Sargassum horneri* (Fucaceae) in the northwestern Pacific in relation to late Pleistocene glaciation and tectonic configurations. Molecular Ecology 20: 3894–3909.

Huang, S. F. 1999. Floristic studies on the benthic marine algae of Northeastern Taiwan. Taiwania. 44(2): 271–298.

Huelsenbeck, J. P. and Ronquist, F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754–755. http://nbisweden.github.io/MrBayes/download.html; searched on 19 March 2019.

Kazi, M. A., Reddy, C. R.K., and Jha, B. 2013. Molecular phylogeny and barcoding of *Caulerpa* (Bryopsidales) based on the *tuf*A, *rbc*L, 18S rDNA and ITS rDNA genes. PLOS ONE 8(12): 1–13.

Kazi, M. A., Reddy, C. R.K. and Jha, B. unpublished. Characterization of *Caulerpa cylindracea/corynephora/veravalensis* complex from India. Marine Biotechnology and Ecology, CSIR-Central Salt and Marine Chemicals Research Institute, Giju Bhai Bhadeka Marg, Bhavnagar, Gujarat, India.

Komatsu, T., Meinesz, A. and Buckles, D. 1997. Temperature and light responses of alga *Caulerpa taxifolia* introduced into the Mediterranean sea. Marine Ecology Progress Series 146: 145–153.

Kooistra, W. H.C.F., Coppejans, E. G.G. and Payri, C. 2002. Molecular systematics, historical ecology, and phylogeography of *Halimeda* (Bryopsidales). Molecular Phylogenetics and Evolution 24: 121–138.

Kumar, S., Stecher, G. and Tamura, K. 2016. Molecular Biology and Evolution. 33: 1870–1874. https://www.megasoftware.net/; searhed on 19 March 2019.

Kützing, F. T. 1843. Phycologia generalis oder anatomie, physiologie und systemkunde der tange. Mit 80 farbig gedruckten Tafeln, gezeichnet und gravirt vom Verfasser. 307–308.

Lamouroux, J. V.F. 1809. Observations sur la physiologie des algues marines, et description de cinq nouveaux genres de cette famille. Nouveau Bulletin des Sciences, par la Société Philomathique de Paris. 1: 330–333.

Lee, H. W. and Kim, M. S. 2015. Species delimitation in the green algal genus *Codium* (Bryopsidales) from Korea using DNA barcoding. Acta Oceanologica Sinica 34(4): 114–124.

Lee, R. E. 1999. Phycology. 3rd ed. Cambridge University Press, New York. 105–155.

Lee, R. E. 2008. Phycology. 4th ed. Cambridge University Press, New York. 178–188.

Lewmanomont, K. 1978. Some edible algae of Thailand. The Kasetsart Journal 12(2): 119–129.

Lewmanomont, K., Wongrat, L. and Supanwanid, C. 1995. Algae in Thailand. Office of environmental policy and planning. 1st ed. Integrated promotion technology Co., Ltd, Bangkok, Thailand. 45–50.

Lewmanomont, K. 2008. Some *Caulerpa* and *Caulerpella* (Bryopsidales, Chlorophyta) from Thailand. In: Taxonomy of Southeast Asian Seaweeds, Institute of Ocean and Earth Sciences, University of Malaya, Monograph Series 2 (eds S. M. Phang, K. Lewmanomont and P. E. Lim). UM Press, Kuala Lumpur. 1–14.

Mahakham, W. 2011. DNA barcodes of plants: basic concept, application and limitation. Thai Journal of Botany 3(1): 1–30.

Meinesz, A., Benichoua, L., Blachier, J., Komatsu, T., Lemée, R., Molenaar, H. and Mari, X. 1995. Variations in the Structure, Morphology and Biomass of *Caulerpa taxifolia* in the Mediterranean Sea. Botanica Marina 38: 499–508.

Menez, E. G. and Calumpong, H. P. 1982. The Genus *Caulerpa* from Central Visayas, Philippines. Smithsonian contributions to the marine science 17: 1–21.

Montagne, C. 1845. Plantes cellulaires. In: Voyage au Pôle Sud et dans l'Océanie sur les corvettes l'Astrolabe et la Zelée exécuté pendant les années 1837–1838–1839–1840, sous le commandement de M. J. Dumont d'Urville, Botanique (eds. J.B. Hombron and H. Jacquinot). Paris. XIV+319.

Nguyen, H. D., Huynh, Q. N., Tran, N. B. and Nguyen, V. T. 1993. Marine algea of north Vietnam (Rong biê n phí a bac Viê t Nam). Science and Technic Publishing House. Hanoi. 344pp.

Nguyen, T. V. 2007. Chlorophyta (Ng à nh rong luc). In: Flora of Vietnam (Thuc V â t Chi Vi ê t Nam) 10 (eds. B. T. Nguyen, L. D. Tran, L. K. Phan, T. V. Nguyen, T. Nguyen, P. X. Vu and K. K. Nguyen). Science and Technic Publishing House, Hanoi. 279pp.

Nguyen, T. V., Le, N. H., Lin, S. M., Steen, F. and De Clerck, O. 2013. Checklist of the marine macroalgae of Vietnam. Botanica Marina 56: 207–227.

Ohba, H. and Enomoto, S. 1987. Culture studies on *Caulerpa* (Caulerpales, Chlorophyceae) II. Morphological variation of *C. racernosa* var. *laetevirens* under various culture condltions. Japanese Society of Phycology 35: 178–188.

Ohba, H., Nashima, H. and Enomoto, S. 1992. Culture studies on *Caulerpa* (caulerpales, chlorophyceae) III. Reproduction, development and morphological variation of laboratory-cultured *C. racemosa* var. *peltata*. The botanical magazine 105: 589–600.

Olsen, J. L., Stam, W. T., Berger, S. and Menzel, D. 1994. 18S rDNA and evolution in the Dasycladales (Chlorophyta): Modern living fossils. Journal of Phycology 30: 729–744.

Olsen, J. L., Valero, M., Meusnier, I., Boele-Bos, S. and Stam, W. T. 1998. Mediterranean *Caulerpa taxifolia* and *C. maxicana* (Chlorophyta) are not conspecific. Journal of Phycology 34: 850–856.

Pattarach, K., Mayakun, J. and Draisma, S. G.A. An Enigmatic *Caulerpa macrodisca* Decaisne (Chlorophyta) from the Mangrove Channels on the Andaman Sea Coast of Thailand. Journal of Fisheries and Environment, submitted.

Pattarach, K. 2016. The study of genetic variation of green algae: genus *Caulerpa* in Thailand. BSc thesis Project Biology (331–491), Department of Biology, Faculty of Science, Prince of Songkla University 2015. 65pp.

Pečnikar, Ž. F. and Buzan, E. V. 2014. 20 years since the introduction of DNA barcoding: from theory to application. Journal of Applied Genetics 55: 43–52.

Peterson, R. D. 1972. Effects of light intensity on the morphology and productivity of *Caulerpa racemosa* (Forsskal) J Agardh. Microneslca 8(1–2): 63–86.

Phang, S. M., Lewmanomont, K., Prathep, A., Largo, D., Abdullah, S. A., Han, N. A., Lim, W. L., Nam, H. S., Andriana, R., Pongparadon, S., Lim, P. E. and Yeoung, H. Y. 2008. Some Chlorophyta of Southeast Asia, with one new record of *Caulerpa* for Malaysia and Indonesia. In: Taxonomy of Southeast Asian Seaweeds, Institute of Ocean and Earth Sciences, University of Malaya, Monograph Series 2 (eds S. M. Phang, K. Lewmanomont and P. E. Lim). UM Press, Kuala Lumpur. 27–32.

Phang, S. M., Yeong, H. Y., Ganzon-Fortes, E. T., Lewmanomont, K., Prathep, A., Hau, L. N., Gerung, G. S. and 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.

Pongparadon, S., Zuccarello, G. C., Phang, S. M., Kawai, H., Hanyuda, T. and Prathep, A. 2015. Diversity of *Halimeda* (Chlorophyta) from the Thai-Malay Peninsula. Phycologia 54: 349–66.

Prathep, A., Pongparadon, S., Darakrai, A., Wichachucherd, B. and Sinutok, S. 2011. Diversity and distribution of seaweed at Khanom-Mu Ko Thale Tai National Park, Nakhon Si Thammarat Province, Thailand. Songklanakarin Journal of Science and Technology (SJST) 33(6): 633–640.

Price, I. R. 2011. A taxonomic revision of the marine green algal genera *Caulerpa* and *Caulerpella* (Chlorophyta, Caulerpaceae) in northern (tropical and subtropical) Australia. Australian Systematic Botany 24(3): 137–213.

Prud'homme van Reine, W. F., Verheij, E. and Coppejans, E. 1996. Species and ecads of *Caulerpa* (Ulvophyceae, Chlorophyta) in Malesia (South-East Asia): taxonomy, biogeography, and biodiversity. Netherlands Journal of Aquatic Ecology 30: 83.

Ratana-arporn, P. and Chirapart, A. 2006. Nutritional evaluation of tropical green seaweeds *Caulerpa lentillifera* and *Ulva reticulata*. Kasetsart Journal (Natural Science) 40: 75-83.

Rathbun, M. J. 1919. The Danish Expedition to Siam (1899–1900). K. danske Vidensk. Selak. Skr. 5(7): 301–367.

Rasband, W. S. 1997. ImageJ. U.S. National Institutes of Health (NIH), Bethesda, Maryland, USA. http://imagej.nih.gov/ij/; searched on 19 March 2019.

Reinbold, T. 1901. Marine algae (Chlorophyceae, Phaeophyceae, Rhodophyceae). Flora of Koh Chang: contributions to the knowledge of the vegetation in the Gulf of Siam 187–201.

Robledo, D. and Freile-Pelegr´ın, Y. 2005. Seasonal variation in photosynthesis and biochemical composition of *Caulerpa* spp. (Bryopsidales, Chlorophyta) from the Gulf of Mexico. Phycologia 44(3): 312–319.

Sahoo, D., Nivedita and Debasish. 2001. Seaweeds of Indian coast: A.P.H. Publishing. New Delhi. 283pp.

Sauvage, T., Payri, C., Draisma, S. G.A., Prud'homme van Reine, W. F., Verbruggen, H., Belton, G. S., Gurgel, C. F.D., Gabriel, D., Sherwood, A. R., and Fredericq, S. 2013. Molecular diversity of the *Caulerpa racemosa-Caulerpa peltata* complex (Caulerpaceae, Bryopsidales) in New Caledonia, with new Australasian records for *C. racemosa* var. *cylindracea*. Phycologia 52(1): 6–13.

Seidenfaden, G., Smitinand, T. and Thorson, G. 1968. Report on the Fifth Thai Danish Expedition (1966). Natural History Bulletin of the Siam Society 22: 245–261.

Silva, P.C., Basson, P. W. and Moe, R. L. 1996. Catalogue of the benthic marine algae of the Indian Ocean. University of California Publications in Botany 79: 1–1259.

Stam, W. T., Olsen, J. L., Zaleski, S. F., Murray, S. N., Brown, K. R. and Walters, L. J. 2006. A forensic and phylogenetic survey of *Caulerpa* species (Caulerpales, Chlorophyta) from the Florida coast, local aquarium shops, and e-commerce: establishing a proactive baseline for early detection. Journal of Phycology 42: 1113–1124.

Thivy, F. and Chauhan, V. D. 1963. *Caulerpa veravalensis*, a new species from Gujarat, India. Botanica Marina 5: 97–100.

Thongroy, P., Liao, L. M. and Prathep, A. 2007. Diversity, abundance and distribution of macroalgae at Sirinart Marine National Park, Phuket Province, Thailand. Botanica Marina 50: 88–96.

Trevisan, V. B.A. 1849. *Caulerpearum sciagraphia* Linnaea 22: 129–144.

Trono, G. C. 1997. Field guide and atlas of the seaweed resources of the Philipines. 1st ed. Bookmark Co., Ltd, Makati city, Philipines. 29–45.

Verlaque, M., Boudouresque, C. F., Meinesz, A. and Gravez, V. 2000. The *Caulerpa racemosa* complex (Caulerpales, Ulvophyceae) in the Mediterranean Sea. Botanica Marina 43: 49–68.

Weber-van Bosse, A. 1898. Monographie des Caulerpes. Annales du Jardin Botanique de Buitenzorg. 15: 243–401. pls XX [20] –XXXIV [34].

White, T. J., Bruns, T., Lee S. and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols (eds. M. A. Innis, D. H. Gelfand, J. J. Sninsky and T. J. White). Academic Press, New York. 315–322.

Wichachucherd, B., Prathep, A. and Zuccarello, G. C. 2014. Phylogeography of *Padina boryana* (Dictyotales, Phaeophyceae) around the Thai-Malay Peninsula. European Journal of Phycology 49: 313–323.

Wynne, M., Verbruggen, H. and Angel, D. 2009. The recognition of *Caulerpa integerrima* (Zanardini) comb. et stat. nov. (Bryopsidales, Chlorophyta) from the Red Sea. Phycologia 48(4): 291–301.

Yeh, W. J. and Chen, G. Y. 2014. Nuclear rDNA and internal transcribed spacer sequences clarify *Caulerpa racemosa* vars. from other *Caulerpa* species. Aquatic Botany 80: 193–207.

Zaleski, S. F. and Murray, S. N. 2006. Taxonomic diversity and geographic distributions of aquarium-traded species of *Caulerpa* (Chlorophyta:

Caulerpaceae) in southern California, USA. Marine Ecology Progress Series 314: 97–108.

# VITAE

**Name** Miss Kattika Pattarach

**Student ID** 5910220009

**Educational Attainment**

| Degree | Name of Institution | Year of Graduation |
|---|---|---|
| Bachelor of science (Biology) (First Class Honors) | Prince of Songkla University | 2016 |

**Scholarship Awards during Enrolment**

Science achievement scholarship of Thailand (SAST)

**List of Publication and Proceeding**

Pattarach, K., Titioatchasai, J., Darakrai, A. and Mayakun, J. 2018. Effects of wave exposure and shore level on seagrass abundance and distribution in the intertidal community. Songklanakarin Journal of Science and Technology. 40(6): 1446–1450.

Pattarach, K., Phetcharat, S. and Mayakun, J. 2019. Some species of *Gracilaria* (Gracilariaceae, Rhodophyta) from Southern Thailand. In: Taxonomy of Southeast Asian Seaweeds, Institute of Ocean and Earth Sciences, University of Malaya, Monograph Series 17 (eds S. M. Phang, S. L. Song and P. E. Lim). UM Press, Kuala Lumpur. 47–57.