

## An Enigmatic *Caulerpa macrodisca* Decaisne (Chlorophyta) from the Mangrove Channels on the Andaman Sea Coast of Thailand

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### ABSTRACT

A phylogenetic analysis of DNA sequence data revealed that the morphological *Caulerpa* entities, previously reported as *C. corynephora* Montagne (or *C. racemosa* var. *corynephora* (Montagne) Weber-van Bosse) and *C. ashmeadii* Harvey from mangrove channels on the Thai Andaman Sea coast, are both representatives of *C. macrodisca* Decaisne. The analyzed Andaman Sea specimens formed one of four subclades in a monophyletic *C. macrodisca* clade that was sister to *C. megadisca* Belton & Gurgel. Ramuli arrangement of *C. macrodisca* from the Andaman Sea was mostly in opposite pairs and sometimes distichous. The shape of the ramuli varied from almost cylindrical to clavate or turbinate. No pyrenoid was observed. *Caulerpa macrodisca* with characteristic large peltate ramuli has only been reported in Thailand from the Gulf of Thailand. Its identity was confirmed with DNA sequence data from a herbarium specimen. It is recommended to refer to the three morphological *C. macrodisca* entities as ecads; i.e., *C. macrodisca* ecad *macrodisca*, ecad *corynephora*, and ecad *ashmeadii*.

**Keywords:** ITS rDNA sequence, Macroalgae, Morphological plasticity, *TufA* gene

### 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 *tufA* gene and RUBISCO large subunit (*rbcL*) gene sequence

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Received 21 March 2019 / Accepted 16 June 2019

data. They provided names, DNA barcodes (i.e., reference *tufA* 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 *tufA* 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 (Gury and Gury, 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 METHODS

### *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 1). 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.

Table 1. Collection data, Genbank accession numbers (those in bold are published here for the first time), and references for specimens used in the present study. n.a., not available.

<i>tufA</i> Genbank accession <sup>a)</sup>	ITS Genbank accession	Location	Figure	Reference	Voucher <sup>c)</sup>
<b><i>Caulerpa racemosa</i> (Forsskål) J.Agardh</b>					
-	AY206420	Green Island, Taiwan		Yeh and Chen (2004)	n.a.
-	AY206421	Nanwan, Taiwan		Yeh and Chen (2004)	n.a.
AJ417947	-	Galeta, Panama		Famà <i>et al.</i> (2002)	n.a.
AJ417956	-	Florida, USA		Famà <i>et al.</i> (2002)	n.a.
DQ652422	DQ652263	Aquarium trade (California, USA)		Stam <i>et al.</i> (2006)	CA060
DQ652425	-	US Virgin Islands		Stam <i>et al.</i> (2006)	FL045
-	DQ652265	Aquarium trade (California, USA)		Stam <i>et al.</i> (2006)	CA014
-	JF932267	Gujarat, India		Kazi <i>et al.</i> (2013)	CITS04
FM956045	<b>MK481938</b> <sup>b)</sup>	West Papua, Indonesia		Sauvage <i>et al.</i> (2013), This study	L SGAD0712247
FM956051	<b>MK481937</b> <sup>b)</sup>	Java Sea, Indonesia		Sauvage <i>et al.</i> (2013), This study	L SGAD0509638
JN645169	-	Grande Terre, New Caledonia		Sauvage <i>et al.</i> (2013)	IRD5641
JN817659	-	Kimberley, WA, Australia		Belton <i>et al.</i> (2014)	PERTH 08292736
JN817665*	-	Kimberley, WA, Australia		Belton <i>et al.</i> (2014)	PERTH 08292728
KF256097	-	West Papua, Indonesia		Belton <i>et al.</i> (2014)	L 078917
<b><i>Caulerpa megadisca</i> Belton &amp; Gurgel</b>					
JN645149	-	Grande Terre, New Caledonia		Sauvage <i>et al.</i> (2013)	IRD5639
JN645154	-	Île des Pins, New Caledonia		Sauvage <i>et al.</i> (2013)	IRD5636
JN817656	-	Lizard Island, Queensland, Australia		Belton <i>et al.</i> (2014)	AD A92609
JN817657*	-	Lizard Island, Queensland, Australia		Belton <i>et al.</i> (2014)	AD A90107
KF256091	-	Yaukuvelailai Island, Fiji		Belton <i>et al.</i> (2014)	US DML40342
<b><i>Caulerpa macrodisca</i> Decaisne</b>					
DQ652428	-	Aquarium trade (Florida, USA)		Stam <i>et al.</i> (2006)	FL158
DQ652429	-	Aquarium trade (Florida, USA)		Stam <i>et al.</i> (2006)	FL159
FM956053*	-	Java Sea, Indonesia		Sauvage <i>et al.</i> (2013)	L SGAD0509359
FM956054	-	East Kalimantan, Indonesia		Sauvage <i>et al.</i> (2013)	L 03-341
FM956055	<b>MK481944- MK481949</b> <sup>b)</sup>	West Papua, Indonesia		Sauvage <i>et al.</i> (2013), This study	L SGAD0712635
FM956075	-	Klong Yang, Krabi, Thailand	Figs. S1, S2	Sauvage <i>et al.</i> (2013)	GENT HEC16156 <sup>d)</sup>
JN645165	-	Grande Terre, New Caledonia		Sauvage <i>et al.</i> (2013)	IRD5635
JN817666	-	Lizard Island, Queensland, Australia		Belton <i>et al.</i> (2014)	AD A88056

Table 1. (cont.) Collection data, Genbank accession numbers (those in bold are published here for the first time), and references for specimens used in the present study. n.a., not available.

<i>tufA</i> Genbank accession <sup>a)</sup>	<i>ITS</i> Genbank accession	Location	Note	Reference	Voucher <sup>c)</sup>
KF256090	-	Kimberley, WA, Australia		Belton <i>et al.</i> (2014)	PERTH 08292663
KF256092	-	Java Sea, Indonesia		Belton <i>et al.</i> (2014)	L SGAD0509415
KF256093	-	Java Sea, Indonesia		Belton <i>et al.</i> (2014)	L SGAD0509390
KF256094	-	Java Sea, Indonesia		Belton <i>et al.</i> (2014)	L SGAD0509510
KF256095	-	West Papua, Indonesia		Belton <i>et al.</i> (2014)	L SGAD0712405
<b>MK497059</b>	-	East Kalimantan, Indonesia		This study	L 03-344
<b>MK497060</b>	-	Java Sea, Indonesia		This study	L SGAD0509332
<b>MK497061</b>	-	Java Sea, Indonesia	Fig. 9C in Belton <i>et al.</i> (2014) <sup>g)</sup>	This study	L SGAD0509539 <sup>e)</sup>
<b>MK497062</b>	-	West Papua, Indonesia		This study	L SGAD0712194
<b>MK497063</b>	-	West Papua, Indonesia		This study	L SGAD0712198
<b>MK497064</b>	-	West Papua, Indonesia		This study	L SGAD0712199
<b>MK497053</b>	<b>MK481940</b>	Fresh market, Krabi, Thailand		This study	PSU KP4D <sup>d)</sup>
<b>MK497054</b>	<b>MK481941</b>	Kilim river, Langkawi, Malaysia	Fig. 3E	This study	PSU KP65A <sup>d)</sup>
<b>MK497055</b>	<b>MK481942</b>	Klong Yang, Krabi, Thailand	Figs. 3C, D	This study	PSU KP73A <sup>d)</sup>
<b>MK497056</b>	-	Klong Yang, Krabi, Thailand	Figs. 3F, G	This study	PSU KP74A <sup>d)</sup>
<b>MK497057</b>	-	Tung Wa, Satun, Thailand	Fig. 3I	This study	KUMF06872 <sup>f)</sup>
<b>MK497058</b>	-	Che Bilang, Satun, Thailand	Fig. 3H	This study	KUMF06874 <sup>d)</sup>
-	<b>MK481939</b>	Ao Cho, Trat, Thailand	Fig. 3A	This study	KUMF04404 <sup>e)</sup>

<sup>a)</sup> Proposed *tufA* DNA barcode sequences (Belton *et al.*, 2014) are indicated with \*.

<sup>b)</sup> Sequenced from cloned PCR product

<sup>c)</sup> Abbreviations before the space indicate herbarium codes (following Thiers, 2019). Stam *et al.*'s (2006) vouchers are housed in the private herbarium of L.J. Walters, Kazi *et al.*'s (2013) voucher is housed at Taxonomic Reference Centre for seaweeds at the Council of Scientific and Industrial Research-Central Salt and Marine Chemicals Research Institute (CSIR-CSMCRI), Bhavnagar, Gujarat, India, Sauvage *et al.*'s (2013) vouchers are housed at Institut de Recherche pour le Développement (IRD), Nouméa, New Caledonia, KUMF vouchers are housed at Faculty of Fisheries, Kasetsart University, Bangkok, Thailand.

<sup>d)</sup> *C. macrodisca* ecad *corynephora*

<sup>e)</sup> *C. macrodisca* ecad *macrodisca*

<sup>f)</sup> *C. macrodisca* ecad *ashmeadii*

<sup>g)</sup> Belton *et al.* (2014) erroneously referred to the illustrated specimen as L 0509359 (W.F. Prud'homme van Reine, pers. comm.). Their proposed *tufA* reference sequence for *C. macrodisca* is indeed from L SGAD0509359.

### 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 *tufA* gene and the nuclear internal transcribed spacers (ITS1 and ITS2) of the ribosomal cistron. *TufA* 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<sup>-1</sup>). Forward and reverse primers for *tufA* amplifications were, respectively, *tufA* (5'-TGAAACAGAAMAWCGTCATTATG C-3') and *tufAR* (5'-CCTTCNCGAATMGCRAA WCGC-3') (Famà *et al.*, 2002) or *tufAR1* (5'-CC ATAGGAATTGGACTATCA-3') (Stam *et al.* 2006), annealing at, respectively, nucleotide (nt) positions 210, 1184, and 1096 in a complete *tufA* 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'-CTCTGAACCTTCGCACGT AGA-3') (Kooistra *et al.*, 2002) and ITS4 (5'-TCC TCCGCTTATTGATATGC-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 °C for 4 minutes as a denaturation step, followed by 40 cycles of 30 s at 94 °C, 30 s at 52 °C (for *tufA*) or 48 °C (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) *tufA* and/or ITS sequences of *C. macrodisca* and *C. racemosa* specimens from Indonesia were available for this study (Table 1). These *tufA* 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<sup>th</sup> generation with 10% burn-in period. The *tufA* 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 *tufA* 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 AND DISCUSSION

### *Phylogenetic analyses and species confirmation*

The *tufA* 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 *tufA* 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.

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. 2). 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

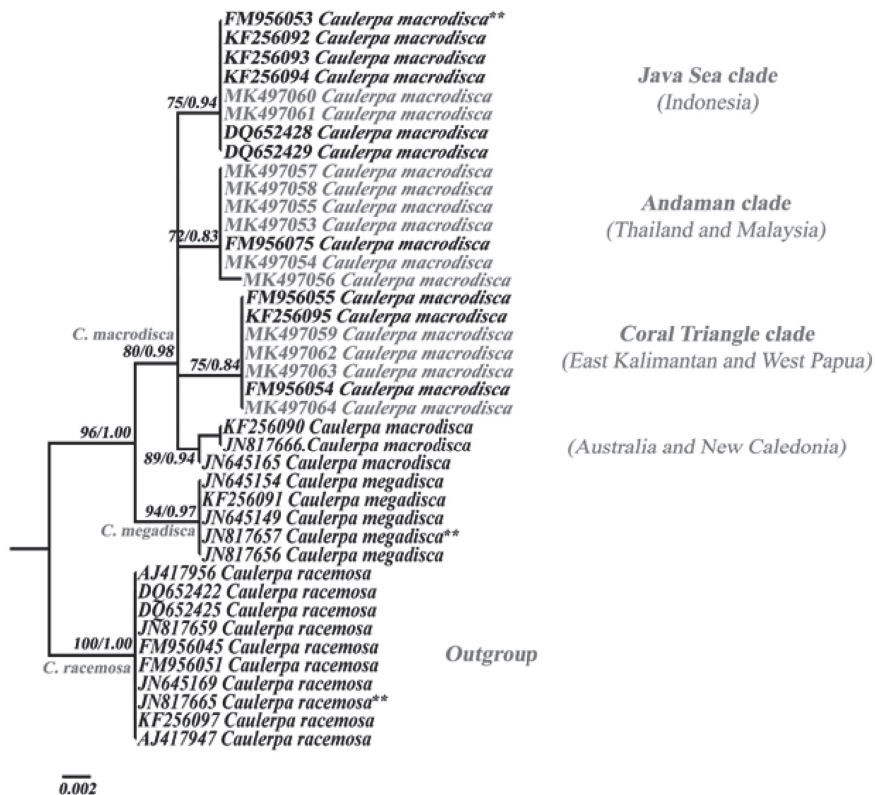


Figure 1. Bayesian phylogenetic tree constructed from 40 partial *tufA* DNA sequences of three *Caulerpa* species: *C. racemosa* (outgroup, n=10), *C. megadisca* (n=5), and *C. macrodisca* (n=25) (alignment = 843 bp in length). Taxon labels include Genbank accession numbers. Numbers at branch nodes correspond 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).

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. 3A, B), ecad *corynephora* (with clavate and/or turbinate ramuli, Figs. 3C–H, J–M), and ecad *ashmeadii* (with cylindrical ramuli, Figs. 3I, 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.

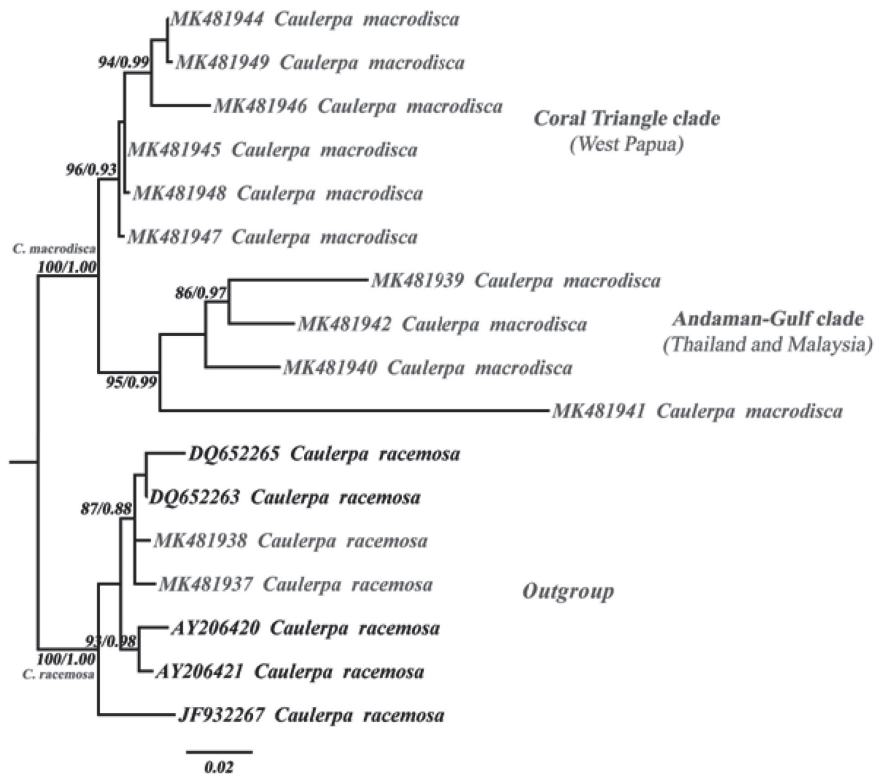


Figure 2. Bayesian phylogenetic tree constructed from 10 *C. macrodisca* and 7 *C. racemosa* (outgroup) ITS rDNA sequences (alignment = 652 bp in length). Taxon labels include Genbank accession numbers. Numbers at branch nodes correspond to Maximum Likelihood bootstrap percentages (BP) and Bayesian inference 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.

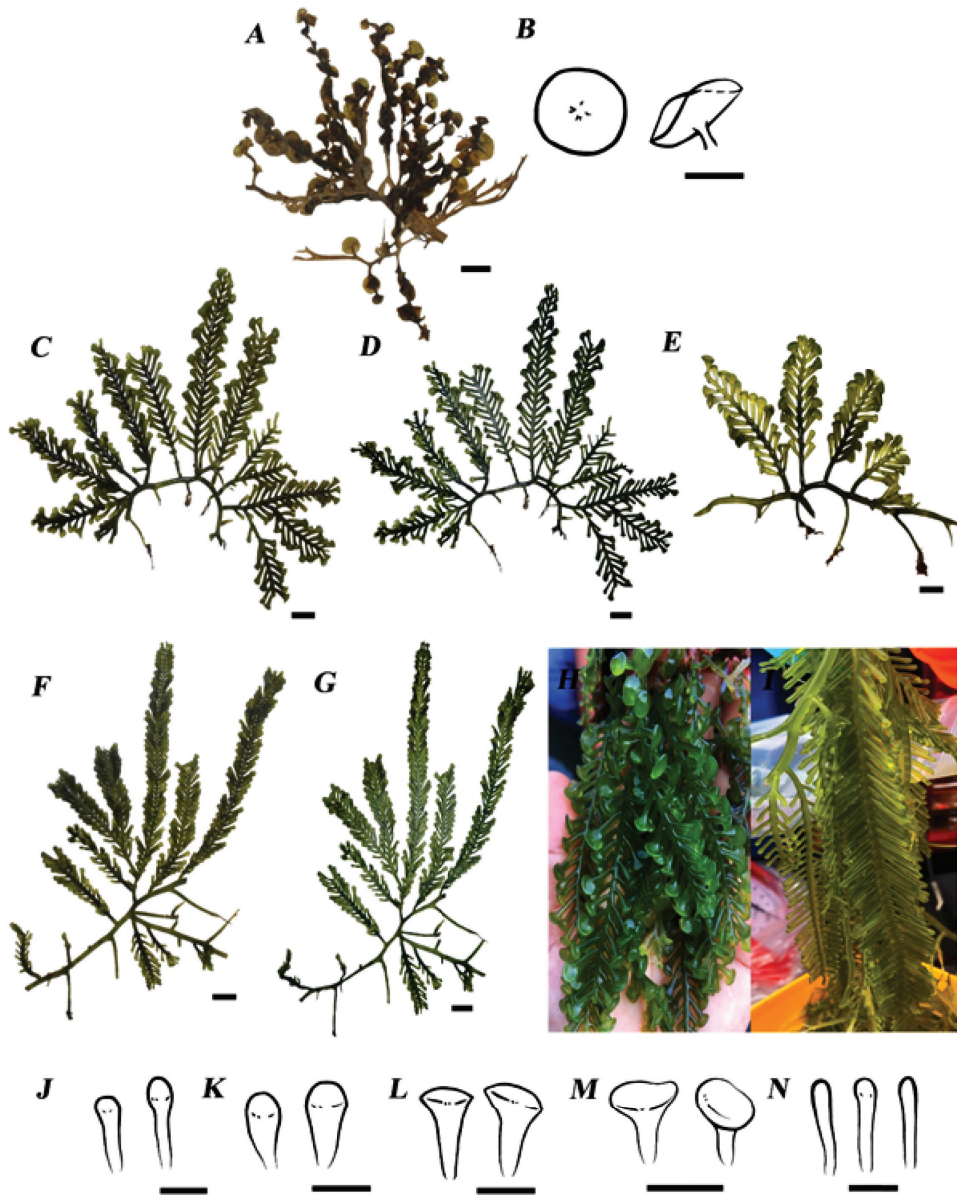


Figure 3. *C. macrodisca* from Thailand and Malaysia (A, C–G, scale bar = 1 cm; B, J–L, scale bar = 0.5 cm; H–I, no scale bar; M, scale bar  $\approx$  0.5 cm, measured from dried specimen KUMF06874; N, scale bar  $\approx$  0.5 cm, measured from dried specimen KUMF06872): (A, B) *C. macrodisca* ead *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* ead *corynephora* (KP73) from Klong Yang, Krabi; (C) Fresh specimen; (D) Herbarium; (E) *C. macrodisca* ead *corynephora* (KP65) from Kilim river, Langkawi, Malaysia (fresh); (F, G) *C. macrodisca* ead *corynephora* (KP74) from Klong Yang, Krabi; (F) Fresh specimen; (G) Herbarium; (H) *C. macrodisca* ead *corynephora* (KUMF06874) from Che Bilang, Satun; (I) *C. macrodisca* ead *ashmeadii* (KUMF06872) from Tung Wa, Satun; (J–M) Drawings of ramuli of *C. macrodisca* ead *corynephora*; (J, K) club-shaped, clavate and (L, M) trumpet-shaped, turbinate; (N) Drawings of cylindrical ramuli of *C. macrodisca* ead *ashmeadii* (KUMF06872).



Table 2. 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	<i>C. macrodisca</i> <i>Decaisne</i> <i>sensu</i> Belton <i>et al.</i> (2014)	<i>C. macrodisca</i> <i>ecad macrodisca</i> from the Andaman Sea	<i>C. macrodisca</i> <i>ecad corynephora</i> from the Andaman Sea	<i>C. macrodisca</i> <i>ecad ashmeadii</i> from the Andaman Sea
Specimens examined	see Belton <i>et al.</i> (2014)	KUMF04404	PSU KP73A, PSU KP74A, GENT HEC16156 <sup>a)</sup> , KUMF06874 <sup>a)</sup>	KUMF06872 <sup>a)</sup>
Figures	Fig. 9C in Belton <i>et al.</i> (2014)	Fig. 3A	Figs. 3C–D, F–H, S1, S2	Fig. 3I
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 <sup>b)</sup> (mm)	5.0–10.0	6.4–9.0	1.2–4.0 [1.4–5.0]	n.m.

<sup>a)</sup> No measurements from these specimens, but observations from photographs.

<sup>b)</sup> Narrowest and widest part of clavate and turbinate ramuli. Only the disc diameter for peltate ramuli.

## Discussion

The *tufA* and ITS phylogenies (Figs. 1, 2) showed that *Caulerpa* taxa from Thailand that were previously assigned to *Caulerpa* (*racemosa* var.) *corynephora* and *Caulerpa ashmeadii* (Lewmanomont, 2008 and 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. 3) 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 2). However, KUMF04404 (Fig. 3A) 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. 3B). 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. 1) also showed branched assimilators (Figs. 3C–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. 3J–K) or blunt (trumpet-shaped, turbinate, Figs. 3L–M) terminus. The ramuli in *C. macrodisca* ecad *ashmeadii* (KUMF06872) are cylindrical throughout except for a swollen terminus (Fig. 3N). 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 and 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. S1) and HEC16156-A' (Suppl. Fig. S2). The latter contains four separate stolon fragments with assimilators with various ramuli shapes, including cylindrical with swollen tips.

KUMF06872 (Fig. 3I) from Satun Province, originally identified as *C. ashmeadii*, also represents an ecad of *C. macrodisca*. PCR amplification was unsuccessful for KL8301 (in KUMF, Fig. S3) 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. S3) 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. S3) 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 *tufA* phylogeny (Fig. 1) and therefore could merit the official taxonomic status variety or form. However, this is not supported by the ITS phylogeny (Fig. 2) where *C. macrodisca* ecad *macrodisca* from the Gulf of Thailand (not included in the *tufA* 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 *tufA* 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. 1). 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 subtidally 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, there occur 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. *C. macrodisca* ecad *corynephora* is the most

common morphotype. The present study highlights the importance of DNA sequence data for reliable species identification in the *Caulerpa racemosa-peltata* complex and to determine the 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*.

### ACKNOWLEDGEMENTS

We would like to express our sincere gratitude to Prof. Khanjanapaj Lewmanomont (Department of Fishery Biology, Kasetsart University, Thailand) for making available the KUMF herbarium specimens. Dr. Willem F. Prud'homme van Reine (Naturalis Biodiversity Center, Leiden, Netherlands) is thanked for useful discussions. We are appreciative to Assoc. Prof. Dr. Shao-Lun Liu (Department of Life Science, Tunghai University, Taiwan) for molecular research methodologies. The GENT herbarium staff is thanked for providing photographs of HEC16156. In addition, we are grateful to the team members of the Prince of Songkla University (PSU) Seaweed and Seagrass Research Unit (SSRU) for their field help. Financial support from the Science Achievement Scholarship of Thailand (SAST) to KP is acknowledged. This work was supported by Prince of PSU grant no. SCI610575S and partly supported by Faculty of Science, PSU and Graduate School, PSU.

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## SUPPLEMENTARY FIGURES



Figure S1. *Caulerpa macrodisca* ead *corynephora* from Krabi Province (HEC16156 A-D pro parte in GENT, as *C. racemosa* var. *corynephora*). Herbarium sheet HEC16156A.



Figure S2. *Caulerpa macrodisca* ead *corynephora* from Krabi Province (HEC16156 A-D pro parte in GENT, as *C. racemosa* var. *corynephora*). Herbarium sheet HEC16156A'.



Figure S3. *Caulerpa ashmeadii* from Phannga Province (KL8301 in KUMF), possibly representing *C. macrodisca* according to the present authors. Identification unconfirmed by DNA sequence data.