A DNA-Assisted Diversity Assessment of the Genus *Caulerpa* (Chlorophyta) in Thailand

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ABSTRACT

The green algal genus *Caulerpa* is widely distributed in tropical and subtropical seas. However, its taxonomy is significantly complicated by high morphological plasticity and overlapping morphologies. Previous taxonomic studies of *Caulerpa* in Thailand were based on morphological observations. Meanwhile, molecular studies have challenged the taxonomic status of several *Caulerpa* species. This study aims to reassess *Caulerpa* diversity in Thailand using DNA barcodes –specifically, chloroplast-encoded *tuf*A gene and nuclear ITS rDNA sequences –derived from newly collected specimens and to re-evaluate previous records from Thailand. DNA barcode analysis identified nine *Caulerpa* species: *C. chemnitzia, C. cupressoides, C. lentillifera, C. macrodisca, C. racemosa, C. serrulata, C. sertularioides*, *C. taxifolia,* and *C. verticillata*. In addition, based on distinctive morphological characteristics, three additional species were recognized from Thailand, though they were not collected during recent field surveys: *C. ambigua, C. fastigiata,* and *C. manorensis*. Notably, *C. fastigiata* has not been recorded for the past 50 years. The presence of *C. mexicana* in Thailand remains uncertain due to the lack of DNA barcode confirmation. Other previously reported *Caulerpa* species from Thailand were determined to be misidentifications. Specimens with peltate ramuli from the same collection site, initially presumed to be conspecific, were revealed by DNA barcoding to belong to two distinct species (*C. racemosa* and *C. chemnitzia*), highlighting the importance of molecular tools in resolving *Caulerpa* taxonomy.

Keywords: Bryopsidales, DNA barcoding, Ecad, ITS rDNA, Reference sequence, *Tuf*A gene

INTRODUCTION

Caulerpa J.V.Lamouroux is a coenocytic green algal genus in the monogeneric family Caulerpaceae Kützing. It is characterized by numerous branching cell wall ingrowths that traverse the cell lumen, providing structural support to the thallus (Zubia *et al*., 2020). *Caulerpa* species show a complex external morphology, with thalli consisting of a creeping stolon from which rhizomes and fronds (assimilators) emerge in opposite directions. The morphology of the assimilators, which may bear branchlets (ramuli or pinnae) of various forms, is the primary characteristic used for identification within the genus. Currently, *Caulerpa* comprises 106 accepted species (Guiry and Guiry, 2024), including three fossil taxa. These species are classified into six subgenera, with the subgenus *Caulerpa* containing the majority, further divided into two sections (Draisma *et al*., 2014; Zubia *et al*., 2020).

Southern Australia, the Caribbean, and the Indo-Malay archipelago have been identified as regions with the highest *Caulerpa* diversity (Prud'homme van Reine *et al*., 1996) based on morphological observations. High morphological

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plasticity and overlapping characteristics have complicated the taxonomy of the genus, necessitating the use of DNA sequence data to resolve taxonomic uncertainties (Stam *et al*., 2006; Belton *et al*., 2014; 2019; Sauvage *et al*., 2021). Molecular studies have shown that some *Caulerpa* species cannot be reliably identified based on morphology alone, leading to the adoption of the *tuf*A gene as a DNA barcode for species identification. Several regional studies have assessed *Caulerpa* diversity using *tuf*A sequences (Fernández-García *et al*., 2016; Belton *et al*., 2019; Dumilag *et al*., 2019; Darmawan *et al*., 2021). Draisma and Sauvage (2024a) compiled a *tuf*A reference sequence library of 89 *Caulerpa* species, including cryptic species and dark taxa, though it does not yet cover all accepted species.

Thailand is located on the Southeast Asian peninsula. Its east coast borders the Gulf of Thailand, an ecoregion of the Sunda Shelf marine province in the Central Indo-Pacific biogeographic realm (Spalding *et al*., 2007). Its west coast borders the Andaman Sea and the northern Malacca Strait, which belong to the Andaman Sea Coral Sea ecoregion of the Andaman province of the Western Indo-Pacific realm (Figure 1). The earliest records of *Caulerpa* in Thailand were reported by Reinbold (1901), with subsequent records published by Egerod (1971; 1974; 1975), Lewmanomont and Ogawa (1995), Hodgson *et al*. (2004), and Lewmanomont *et al*. (2007). Lewmanomont (2008) published the most recent comprehensive taxonomic study based on specimens collected between 1969 and 2007 deposited at Kasetsart Museum of Fisheries (KUMF), reporting 16 morphological species.

The first DNA sequences of *Caulerpa* specimens collected in Thailand were published by Sauvage *et al*. (2013) in their study of the *C. racemosa-peltata* complex. These Thai specimens initially identified as *C. nummularia* Harvey ex J.Agardh and *C. racemosa* cf. var. *corynephora* (Montagne) Weber Bosse were later reassigned to, respectively, *C. chemnitzia* (Esper) J.V.Lamouroux and *C. macrodisca* Decaisne by Belton *et al*. (2014) who resolved the *C. racemosa-peltata* complex using *tuf*A DNA sequences. Pattarach *et al*. (2019) further concluded that *C. peltata* var. *macrodisca* (Decaisne) Weber Bosse, *C. ashmeadii* Harvey, and *C. racemosa* var. *corynephora* (Montagne) Weber Bosse reported by Lewmanomont (2008) represented three growth forms of a single species, *C. macrodisca* Decaisne. They proposed referring to these growth forms as ecads, a classification that avoids taxonomic recognition of infra-specific ranks such as varieties and forms. *Caulerpa ashmeadii* and *C. corynephora* Montagne remain valid species, but their distribution appears restricted to the Atlantic Ocean and Australia, respectively (Sauvage *et al*., 2014; 2021; Belton *et al*., 2019).

Recent seaweed diversity assessments in Thailand have expanded knowledge of *Caulerpa* occurrence. Coppejans *et al*. (2010) documented four species in Nakhon Si Thammarat Province (western Gulf of Thailand), while Coppejans *et al*. (2017) recorded ten species in the northern Malacca Strait (Krabi, Trang, and Satun Province). However, these studies relied solely on morphological identifications, lacking DNA sequence confirmation. Sutti *et al*. (2024) reported four *Caulerpa* species from Trat Province (northeastern Gulf of Thailand) using *tuf*A sequences, but the sequences have not been publicly released (Table 1). Phang *et al*. (2016) listed three additional *Caulerpa* species– *C. filiformis* (Suhr) Hering, *C. scalpelliformis* (R.Brown ex Turner) C.Agardh, and *C. urvilleana* Montagne –based on the report by Reinbold (1901) based on specimens collected during the Danish Siam expedition 1899–1900 (Bruun, 1961). Additionally, Reinbold (1901) reported *C. sedoides* f. *crassicaulis* (J.Agardh) Weber Bosse, which was not listed in Phang *et al*. (2016), but was listed in the checklist of Thai algae published in 1995 with reference to Reinbold (1901) (Lewmanomont *et al*., 1995). However, none of these aforementioned four species have been reported from Thailand since Reinbold's initial records. Reinbold, however, reported *C. filiformis* (Harvey) J.Agardh instead of *C. filiformis* (Suhr) Hering. The incorrect species author of Reinbold's record was introduced in Hodgson *et al*. (2004). The illegitimate name *Caulerpa filiformis* J.Agardh (1873) was later replaced by *C. tongaensis* Papenfuss (1943) and recently subsumed into *C. fastigiata* (Sauvage *et al*., 2021).

This study aims to reassess *Caulerpa* diversity in Thailand through a new field inventory along both coastlines, incorporating *tuf*A barcodes for species identification. However, *tuf*A has limitations in resolving species in the subgenus *Caulerpa* (Stam *et al*., 2006; Kazi *et al*., 2013; Sauvage *et al*., 2013). Therefore, the nuclear-encoded Internal Transcribed Spacers of the ribosomal cistron (ITS1-5.8S-ITS2, hereafter referred to as ITS) was also analyzed for a subset of specimens and in cases where *tuf*A amplification was unsuccessful. ITS has been demonstrated to provide greater resolution than *tuf*A in *Caulerpa* species identification (Kazi *et al*., 2013). Additionally, we attempted to locate and re-evaluate voucher specimens from Reinbold's (1901) descriptions, which lacked illustrations, to re-assess their taxonomic identities.

MATERIALS AND METHODS

Caulerpa collections of the Danish Siam expedition 1899–1900

The Global Biodiversity Information Facility (GBIF, 2024) was searched for *Caulerpa* specimens collected in Thailand. Additionally, curators from the *Botanische Staatssammlung München* (M), the *Herbarium Hamburgense* at the University of Hamburg (HBG), and the Herbarium of the University of Copenhagen (C) were contacted and requested to search their collections for *Caulerpa* specimens collected by Schmidt during the Danish Siam Expedition (1899–1900).

Sampling and morphological identification

New *Caulerpa* collections were made between November 2015 and July 2018 through surveys spanning intertidal to subtidal zones (using snorkeling and SCUBA diving) in five of six west coast provinces and ten of seventeen east coast provinces (inset, Figure 1). Additionally, Supattra Pongparadon provided silica-preserved specimens collected in 2011 (Table 1) and an additional collection was obtained in April 2023.

Specimens were photographed before and after collection using a Canon 60D or Olympus Tough TG-4 digital camera. Each specimen was herbarium-dried after a small portion (1–2 cm) was quickly dried and preserved in silica gel for later DNA extraction. Initial morphological identifications were based on species descriptions in Lewmanomont (2008) and Coppejans *et al*. (2010; 2017).

DNA barcoding

DNA extractions, polymerase chain reaction (PCR) amplification, and sequencing followed Pattarach *et al*. (2019), except that amplicons were sequenced only with the forward primer. Two samples were amplified using the *tuf*A652R reverse primer (Draisma *et al*., 2014) and sequenced in both directions. Chromatograms were carefully inspected, and primer sites were removed. All newly generated *tuf*A sequences (687–933 nucleotides in length) were assembled in BioEdit v.7.0.5.3 (Hall, 1999) into a single 1,230-nucleotide alignment, incorporating previously published sequences of Thai specimens (Table 1) and the *Caulerpa tuf*A Reference Sequence alignment v.2 (Draisma and Sauvage, 2024b). This reference sequence alignment also includes nine *tuf*A sequences from the genus *Pseudochlorodesmis* Børgesen, used as an outgroup.

A Maximum Likelihood (ML) tree was generated on the IQ-TREE web server (http://iqtree.cibiv.univie.ac.at/) (Nguyen *et al*., 2015; Trifinopoulos *et al*., 2016; Minh *et al*., 2020). The ModelFinder function was used to determine the best-fitting substitution model (Kalyaanamoorthy *et al*., 2017), and branch support was assessed using ultrafast bootstrap approximation (UFBoot, 1,000 replicates) (Hoang *et al*., 2018) and the Shimodaira-Hasegawa-like approximate likelihood ratio test (SH-aLRT, 1,000 replications) (Guindon *et al*., 2010). Data were partitioned by codon position (Chernomor *et al*., 2016), with gaps (-) treated as missing data. A nonparametric bootstrap analysis (100 replications) (Felsenstein, 1985) was also conducted. UFBoot values ≥95% approximate a 95% probability that a clade is valid, while standard bootstrap and SH-aLRT, values ≥80% indicate strong support. The resulting phylogenetic tree was visualized in FigTree 1.4.4 (Rambaut, 2018) and rooted using *Pseudochlorodesmis* as an outgroup. A species was confirmed if it formed a strongly supported clade with its reference *tuf*A sequence.

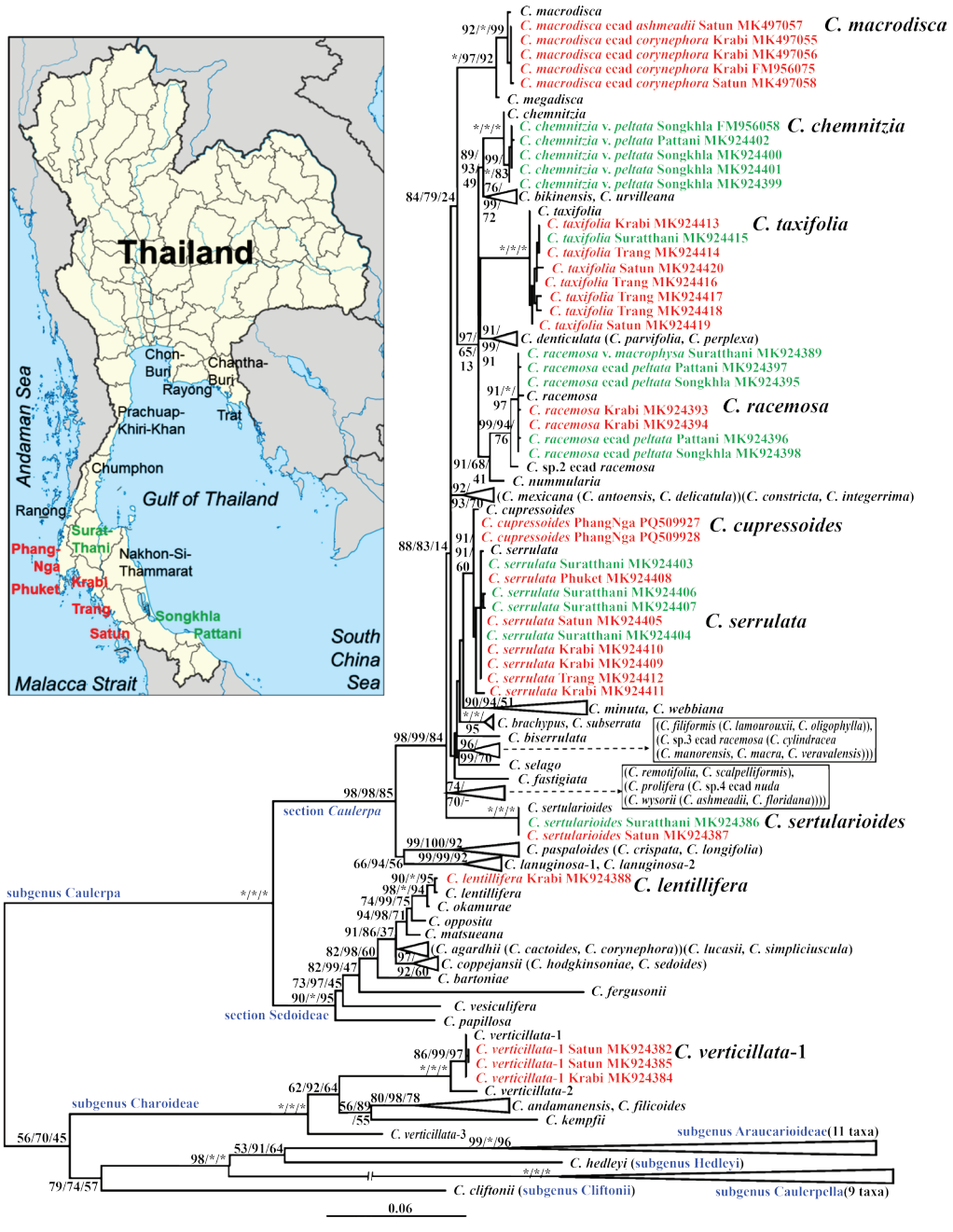

Figure 1. Maximum Likelihood tree based on 134 *tuf*A sequences of *Caulerpa* spp. and nine of *Pseudochlorodesmis* spp. (outgroup). The outgroup is not shown in this tree. Scale bar = substitutions per site. Taxon labels of *tuf*A reference sequences indicated in black. Taxon labels of Thai specimens indicated in bold red (from the west coast) and bold green (from the east coast). *Caulerpa* subgenera and sections indicated in bold blue. Clades not containing Thai specimens are collapsed and their internal phylogenies are indicated to their right. The long branch leading to the subgenus *Caulerpella* is shortened. Species clades with Thai representatives are named in a larger bold black font to the right. Clade support values (SH-aLRT / UFBoot / standard bootstrap) are given at nodes, where * indicates 100% and - that the node did not occur in the standard bootstrap tree. The inset in the upper left shows a map of Thailand with provincial borders. Names of provinces surveyed in the present study are given. *Caulerpa* was collected in red and green provinces.

Table 1. Collection details and GenBank accessions of all *Caulerpa* specimens from Thailand with DNA sequence data. Optimal BLAST matches of ITS sequences are also given.

Caulerpa species [a)]	Voucher [b)]	Location	Province	Coordinates [c)]	Collection date	Collector	GenBank accession (*tuf*A)	GenBank accession (ITS)	Reference	Optimal BLAST match ITS (% identity), reference [d)]
C. chemnitzia	n.a.	Ko Kham	Songkhla	(6°58'19"N 100°51'27"E)	11/12/2006	E. Coppejans?	FM956058	n.d.	Sauvage *et al.* (2013)	
C. chemnitzia	PSU KP29A	Ko Kham	Songkhla	6°58'19"N 100°51'27"E	21/10/2016	K. Pattarach	MK924399.2	n.d.	This study	
C. chemnitzia	PSU KP31A	Ko Kham	Songkhla	6°58'19"N 100°51'27"E	21/10/2016	K. Pattarach	MK924400.2	n.d.	This study	
C. chemnitzia	PSU KP32A	Ko Kham	Songkhla	6°58'19"N 100°51'27"E	21/10/2016	K. Pattarach	MK924401.2	n.d.	This study	
C. chemnitzia	PSU KP50A	Ko Losin	Pattani	7°18'59"N 101°55'59"E	30/08/2017	K. Pattarach	MK924402.2	MN413520	This study	DQ652320 (90.49), Stam *et al.* (2006)
C. cupressoides	PSU SGAD 2304255	Lam Kaen	Phang-Nga	8°36'02"N 98°14'20"E	28/04/2023	S. Draisma	PQ509927	n.d.	This study	
C. cupressoides	PSU SGAD 2304256	Lam Kaen	Phang-Nga	8°36'02"N 98°14'20"E	28/04/2023	S. Draisma	PQ509928	n.d.	This study	
C. lentillifera	THNHM-P-20220073	Ko Kradad	Trat	(11°50'36"N, 102°31'15"E)	23/12/2022	S. Sutti *et al.*	n.a.	n.d.	Sutti *et al.* (2024)	
C. lentillifera	PSU KP21A	Ko Siboya	Krabi	7°53'00"N, 98°58'31"E	23/03/2016	K. Pattarach	MK924388.2	MN413511	This study	JF932269 (99.61), Kazi *et al.* (2013)
C. macrodisca	BR HEC16156	Khlong Yang	Krabi	7°49'49"N, 99°05'48"E	12/04/2007	E. Coppejans *et al.*	FM956075	n.d.	Sauvage *et al.* (2013)	
C. macrodisca	PSU KP73A	Khlong Yang	Krabi	7°49'46"N 99°05'49"E	13/07/2018	K. Pattarach	MK497055.2	MK481942	Pattarach *et al.* (2019)	
C. macrodisca	PSU KP74A	Khlong Yang	Krabi	7°49'46"N 99°05'49"E	13/07/2018	K. Pattarach	MK497056.2	n.d.	Pattarach *et al.* (2019)	
C. macrodisca	KUMF06872	Tung Wa	Satun	7°06'23"N 99°44'45"E	03/09/2018	?	MK497057.2	n.d.	Pattarach *et al.* (2019)	
C. macrodisca	KUMF06874	Che Bilang	Satun	6°39'26"N 99°58'38"E	03/09/2018	?	MK497058.2	n.d.	Pattarach *et al.* (2019)	

Table 1. Cont.

Caulerpa species [a)]	Voucher [b)]	Location	Province	Coordinates [c)]	Collection date	Collector	GenBank accession (*tuf*A)	GenBank accession (ITS)	Reference	Optimal BLAST match ITS (% identity), reference [d)]
C. macrodisca	KUMF04404	Ao Cho	Trat	12°03'31"N 102°32'52"E	26/01/1991	K. Lewmanomont	n.d.	MK481939	Pattarach *et al.* (2019)	
C. racemosa	THNHM-P-20220014	Ko Khai Hua Ro	Trat	(11°52'04"N, 102°31'46"E)	23/12/2022	S. Sutti *et al.*	n.a.	n.d.	Sutti *et al.* (2024)	
C. racemosa	PSU KP10A	Ko Tan	Surat Thani	9°22'23"N 99°57'24"E	16/02/2016	K. Pattarach	MK924389.2	n.d.	This study	
C. racemosa	PSU KP22A	Ko Siboya	Krabi	7°53'00"N, 98°58'31"E	23/03/2016	K. Pattarach	n.a.[e)]	MN413512	This study	AY206420 (98.15), Yeh and Chen (2004)
C. racemosa	PSU KP39C	Ko Lipe	Satun	6°29'10"N 99°17'57"E	28/01/2017	K. Pattarach	n.a.[e)]	MN413513	This study	JF932272 (88.05), Kazi *et al.* (2013)
C. racemosa	PSU KP40A	Ko Lipe	Satun	6°29'10"N 99°17'57"E	30/01/2017	K. Pattarach	n.a.[e)]	MN413514	This study	DQ652264 (92.43), Stam *et al.* (2006)
C. racemosa	PSU KP68A	Bakan Teang, Ko Lanta	Krabi	7°29'29"N 99°04'32"E	02/03/2018	K. Pattarach	MK924393.2	n.d.	This study	
C. racemosa	PSU KP70A	Bakan Teang, Ko Lanta	Krabi	7°29'29"N 99°04'32"E	02/03/2018	K. Pattarach	MK924394.2	MN413515	This study	AY206420 (98.74), Yeh and Chen (2004)
C. racemosa	PSU KP28B	Ko Kham	Songkhla	6°58'19"N 100°51'27"E	21/10/2016	K. Pattarach	MK924395.2	MN413516	This study	AY206421 (99.16), Yeh and Chen (2004)
C. racemosa	PSU KP49A	Ko Losin	Pattani	7°18'59"N 101°55'59"E	29/08/2017	K. Pattarach	MK924396.2	MN413517	This study	MT991597 (91.04), Chen *et al.* (unpublished)
C. racemosa	PSU KP51A	Ko Losin	Pattani	7°18'59"N 101°55'59"E	30/08/2017	K. Pattarach	MK924397.2	MN413518	This study	AJ297651 (96.19), Durand *et al.* (2002)
C. racemosa	PSU KP30A	Ko Kham	Songkhla	6°58'19"N 100°51'27"E	21/10/2016	K. Pattarach	MK924398.2	MN413519	This study	AY206421 (95.24), Yeh and Chen (2004)
C. serrulata	THNHM-P-20220115	Ko Mak	Trat	(11°49'49"N, 102°30'11"E)	23/12/2022	S. Sutti *et al.*	n.a.	n.d.	Sutti *et al.* (2024)	
C. serrulata	PSU KP11B	Ko Tan	Surat Thani	9°22'23"N 99°57'24"E	16/02/2016	K. Pattarach	MK924403.2	n.d.	This study	

Table 1. Cont.

Caulerpa species [a)]	Voucher [b)]	Location	Province	Coordinates [c)]	Collection date	Collector	GenBank accession (*tuf*A)	GenBank accession (ITS)	Reference	Optimal BLAST match ITS (% identity), reference [d)]
C. serrulata	PSU KP14C	Bo Put, Ko Samui	Surat Thani	9°34'23"N 100°03'36"E	17/02/2016	K. Pattarach	MK924404.2	n.d.	This study	
C. serrulata	PSU KP41A	Ko Lipe	Satun	6°29'10"N 99°17'57"E	30/01/2017	K. Pattarach	MK924405	n.d.	This study	
C. serrulata	PSU KP45A	Taling Ngam, Ko Samui	Surat Thani	9°27'19"N 99°56'03"E	25/08/2017	K. Pattarach	MK924406.2	n.d.	This study	
C. serrulata	PSU KP47B	Ko Matsum	Surat Thani	9°22'15"N 99°58'29"E	26/08/2017	K. Pattarach	MK924407.2	n.d.	This study	
C. serrulata	PSU KP72A	Tangkhen Bay, Ko Phuket	Phuket	7°48'41"N 98°24'21"E	04/03/2018	K. Pattarach	MK924408.2	n.d.	This study	
C. serrulata	PSU SP284	Ko Rok	Krabi	(7°13'N, 99°03'E)	02/12/2011	S. Pongparadon	MK924409	MN413521	This study	DQ652306 (96.95), Stam *et al*. (2006)
C. serrulata	PSU SP301	Ko Rok	Krabi	(7°13'N, 99°03'E)	13/02/2011	S. Pongparadon	MK924410.2	MN413522	This study	DQ652306 (85.93) Stam *et al*. (2006)
C. serrulata	PSU SP358	Ko Rok	Krabi	(7°13'N, 99°03'E)	16/02/2011	S. Pongparadon	MK924411.2	MN413523	This study	DQ652303 (95.73), Stam *et al*. (2006)
C. serrulata	PSU SP372	Ko Rok	Krabi	(7°13'N, 99°03'E)	17/02/2011	S. Pongparadon	MK924412.2	n.d.	This study	
C. sertularioides	THNHM-P-20220014	Ko Khai Hua Ro	Trat	(11°52'04"N, 102°31'46"E)	23/12/2022	S. Sutti *et al*.	n.a.	n.d.	Sutti *et al*. (2024)	
C. sertularioides	PSU KP15C	Bo Put, Ko Samui	Surat Thani	9°34'23"N 100°03'36"E	17/02/2016	K. Pattarach	MK924386	n.d.	This study	
C. sertularioides	PSU KP35A	Ko Lidee	Satun	6°47'12"N 99°45'48"E	04/12/2016	K. Pattarach	MK924387	MN700269	This study	DQ652283 (98.91), Stam *et al*. (2006)
C. taxifolia	PSU KP23A	Ko Siboya	Krabi	7°53'N, 98°58'31"E	23/03/2016	K. Pattarach	MK924413	MN413524	This study	DQ652292 (100), Stam *et al*. (2006)
C. taxifolia	PSU KP43A	Ko Libong	Trang	7°15'19"N 99°27'12"E	23/05/2017	K. Pattarach	MK924414	MN413525	This study	DQ652292 (99.83), Stam *et al*. (2006)

Table 1. Cont.

Caulerpa species [a]	Voucher [b]	Location	Province	Coordinates [c]	Collection date	Collector	GenBank accession (*tuf*A)	GenBank accession (ITS)	Reference	Optimal BLAST match ITS (% identity), reference [d]
C. taxifolia	PSU KP46B	Ko Matsum	Surat Thani	9°22'16"N 99°58'30"E	26/08/2017	K. Pattarach	MK924415.2	MN413526	This study	AJ228978 (99.82), Jousson *et al*. (1998)
C. taxifolia	PSU KP53A	Leam Yong Lam	Trang	7°23'15"N 99°20'12"E	01/10/2017	K. Pattarach	MK924416.2	MN413527	This study	AJ228978 (99.64), Jousson *et al*. (1998)
C. taxifolia	PSU KP55A	Leam Yong Lam	Trang	7°22'54"N 99°20'12"E	01/10/2017	K. Pattarach	MK924417.2	MN413528	This study	AJ228978 (99.82), Jousson *et al*. (1998)
C. taxifolia	PSU KP57A	Khao Bae Na	Trang	7°24'26"N 99°20'22"E	01/10/2017	K. Pattarach	MK924418.2	MN413529	This study	AJ228978 (99.64), Jousson *et al*. (1998)
C. taxifolia	PSU SP343	Ko Rok	Krabi	(7°13'N, 99°03'E)	15/02/2011	S. Pongparadon	MK924419.2	MN413530	This study	AJ228978 (99.64), Jousson *et al*. (1998)
C. taxifolia	PSU SP474	Ko Rawi	Satun	6°33'19"N 99°15'26"E	18/12/2011	S. Pongparadon	MK924420.2	MN413531	This study	AJ228978 (99.13), Jousson *et al*. (1998)
C. taxifolia	PSU SP332	Ko Rawi	Satun	6°33'19"N 99°15'26"E	15/02/2011	S. Pongparadon	n.d.	MN413533	This study	AJ228978 (98.40), Jousson *et al*. (1998)
C. taxifolia	PSU SP234	Ko Rawi	Satun	9°22'16"N 99°58'30"E	26/08/2017	S. Pongparadon	n.d.	MN413532	This study	AJ228978 (98.27), Jousson *et al*. (1998)
C. verticillata-1	PSU KP5A	Ko Lidee	Satun	6°47'08"N 99°45'42"E	01/10/2017	K. Pattarach	MK924382	MN413508	This study	JF932265 (99.34), Kazi *et al*. (2013)
C. verticillata-1	PSU KP13A	Ko Tan	Surat Thani	9°22'23"N 99°57'24"E	16/02/2016	K. Pattarach	n.a.[e]	MN413509	This study	JF932265 (99.50), Kazi *et al*. (2013)
C. verticillata-1	PSU KP24A	Ko Siboya	Krabi	7°53'00"N 98°58'31"E	23/03/2016	K. Pattarach	MK924384	MN413510	This study	JF932265 (99.50), Kazi *et al*. (2013)
C. verticillata-1	PSU KP42A	Ko Lipe	Satun	6°29'10"N 99°17'57"E	30/01/2017	K. Pattarach	MK924385	n.d.	This study	

Note: n.a. = not available; n.d. = not determined; 'ko' = island in Thai;

[a] Species authors given in Table 2;

[b] BR, Meise Botanical Garden, Belgium; KUMF, Kasetsart University Museum of Fisheries, Bangkok; PSU, Prince of Songkla University Herbarium, Hat Yai, Thailand; THNHM, Thailand Natural History Museum, Pathum Thani;

[c] Coordinates within brackets are estimates based on location descriptions;

[d] Only matches to sequences covering both ITS1 and ITS2;

[e] *Tuf*A of this specimen was amplified and sequenced, but the quality of the chromatogram was insufficient for an unambiguous read and the sequence is therefore not published here

There is no curated reference sequence database available for the ITS rDNA marker in *Caulerpa*, and ITS alignments are often ambiguous. Therefore, ITS sequences were compared against accessions in the GenBank sequence database (https://www.ncbi.nlm.nih.gov/genbank/) (Benson *et al*., 2013) using the Basic Local Alignment Search Tool (BLAST), https://blast.ncbi.nlm.nih.gov/ (Johnson *et al*., 2008). The highest-scoring BLAST hits covering both spacer regions were critically evaluated. The ITS results were then compared with *tuf*A data to assess congruency.

RESULTS AND DISCUSSION

Caulerpa collections of the Danish Siam expedition (1899–1900)

Seven *Caulerpa* specimens collected by Schmidt during the Danish Siam expedition were located in the *Botanische Staatssammlung München* (M). All but one were identified by Reinbold and listed in Reinbold (1901) under the following names: *C. fastigiata* var. *minor, C. filiformis* Harv. (now recognized as *C. fastigiata*), *C. freycinetti* (now *C. serrulata*), *C. lentillifera* var. *longistipitata* (identified by Anna Weber-van Bosse), *C. peltata* (now *C. chemnitzia*), *C. plumaris* (now *C. sertularioides*), and *C. racemosa* var. *clavifera*.

Additionally, seven specimens representing four species were located in the Herbarium of the University of Copenhagen (C): *C. fastigiata* var. *minor* (voucher code C-A-99749), *C. racemosa* var. *uvifera* (C-A-99750 and C-A-99751), *C. sedoides* (C-A-99745), and *C. freycinetti* (C-A-99746, C-A-99747, and C-A-99748). The specimen C-A-99748, originally identified as *C. freycinetti* var. *pectinata* (now *C. serrulata*), may actually be *C. cupressoides* f. *disticha*, as its rachis does not appear markedly compressed (Coppejans and Prud'homme van Reine, 1992). However, this is difficult to confirm from a pressed herbarium specimen. Reinbold (1901) did not provide a description. The identification of *C. sedoides* (C-A-99745) was later corrected by Hideo Ohba on 30 June 1998 to *C. lentillifera*, with which we agree. *Caulerpa sedoides* is restricted to southern Australia (Belton *et al*., 2019). No *Caulerpa* collections by Schmidt/Reinbold were located in Hamburg (HBG).

None of the above records appear in the GBIF database. However, six additional *Caulerpa* records from the Danish Siam Expedition were located via the GBIF website in the Smithsonian Institution (US) (*C. serrulata* and *C. racemosa* var. *uvifera*) (Orrell and Informatics and Data Science Center-Digital Stewardship, 2024a; 2024b), Naturalis Biodiversity Center (L) (*C. filiformis* Harv. and *C. serrulata*) (Bijmoer *et al*., 2024a; 2024b), and the New York Botanical Garden (NY) (*C. serrulata* and *C. racemosa*) (Ramírez *et al*., 2024a; 2024b).

The specimens identified by Reinbold as *C. scalpelliformis, C. urvilleana* f. *tristicha* (J.Agardh) Weber Bosse, and *C. verticillata* J.Agardh could not be located for verification. Of these, only *C. verticillata* has been reported from Thailand after Reinbold's initial records. The other two records remain uncertain and may represent misidentifications. *Caulerpa scalpelliformis* is restricted to temperate waters in southern Australia (Belton *et al*., 2019). Reinbold described the Thai specimens as "fragment only which perhaps is to be referred to as var. *intermedia* Weber Bosse", a name now synonymous with *C. denticulata* Decaisne (Draisma *et al*., 2014). *Caulerpa denticulata* (var. *intermedia*) is widespread in the tropical Atlantic and Indian Oceans, with the only Pacific records from Japan (Guiry and Guiry, 2024).

Caulerpa urvilleana was historically considered a synonym of *C. cupressoides*, but Draisma *et al*. (2014) demonstrated that they are distinct using *tuf*A DNA sequences. Reinbold (1901) stated "*C. urvilleana* is by forms narrowly connected to *C. freycinetti* (=*C. serrulata*); I think our only fragmentary specimen is to be referred to *C. urvilleana* f. *tristicha* (J.Agardh) Weber Bosse, because there are three rows of teeth on one part of the frond". However, *C. cupressoides* is highly variable and includes forms with three rows of teeth (Coppejans and Prud'homme van Reine, 1992; Price, 2011). Hodgson *et al*. (2004) noted the difficulty in distinguishing herbarium specimens of *C.* (*cupressoides* var.) *urvilleana* from twisted *C. serrulata* herbarium specimens. Since Reinbold's

identifications were based on herbarium specimens and the voucher of his *C. urvilleana* record could not be found, uncertainty remains regarding its true identity.

GBIF (2024) lists 352 *Caulerpa* records from Thailand, representing all species reported in Lewmanomont (2008) except *C. ashmeadii* and *C. manorensis*. However, an unidentified specimen in the University of Michigan Herbarium (UM Herbarium Data Group and IPT Admin L, 2024a) was identified as *C. manorensis* in this study. Specimens examined by Lewmanomont (2008) are housed in KUMF and are not included in GBIF.

The ramuli of Atlantic *C. ashmeadii* are cylindrical (Sauvage *et al*., 2014), whereas the Thai specimen attributed to *C. ashmeadii* by Lewmanomont (2008) has clavate ramuli, suggesting it is most likely a growth form of *C. macrodisca* (Pattarach *et al*., 2019). The majority of the 352 GBIF records are housed in the PSU Herbarium (210 records), but are not scanned in GBIF (Saelao and Tuntiprapas, 2024). One PSU herbarium record (PSU-SWC-0172), initially identified as *C. scalpelliformis* (not reported in Lewmanomont, 2008), was re-identified as *C. taxifolia* in this study.

Records from BR (Meise Botanic Garden, 2024a) included *C. nummularia* (BR-16182A-B). However, based on morphological features, this specimen is more likely *C. chemnitzia*. The key distinguishing characteristic of *C. nummularia* is the presence of secondary peltate ramuli arising from an underlying peltate ramulus (Belton *et al*., 2014), which was not observed in this specimen. The Thailand Biodiversity Information Facility (TH-BIF, 2024) also lists *C. prolifera* (Forsskål) J.V.Lamouroux as housed in the Marine Science Institute of Burapha University, though no further details were provided and it was not examined in this study. This species is considered to be restricted to the Atlantic (Sauvage *et al*., 2021).

New Caulerpa collections

New collections were made in five out of six visited provinces on the west coast and in three out of ten visited provinces on the east coast (Figure 1, Table 1). A total of 43 new *Caulerpa* specimens were collected, for which the *tuf*A and/or ITS DNA sequences were determined. Collection details and GenBank accessions are shown in Table 1. The *tuf*A alignment included 143 sequences, incorporating nine outgroup taxa and 43 *Caulerpa* sequences from Thailand. ModelFinder selected the following models for codon positions: first (GTR+F+G4), second (HKY+F+G4), and third (GTR+F+I+G4). A Maximum Likelihood tree (Log-likelihood -11923.730) was generated and is shown in Figure 1 with the outgroup pruned. Monophyly of all *Caulerpa* subgenera and sections was strongly supported (SH-aLRT and standard bootstrap ≥80%, UFBoot ≥95%).

Specimens from Thailand were distributed across two subgenera and three sections: subgenus *Charoideae* section *Charoideae* and subgenus *Caulerpa* sections *Caulerpa* and *Sedoideae* (Figure 1). The subgenus *Charoideae* is represented in Thailand by a single species, *C. verticillata*-1. Draisma and Sauvage (2024a) recognized three cryptic *C. verticillata* clades, included in the *tuf*A reference library as *C. verticilata*-1, -2, and -3. All sequenced Thai specimens were assigned to *C. verticillata*-1 (Figure 1). Subgenus *Caulerpa* section *Sedoideae* is also represented by a single species in Thailand, *C. lentillifera*. The remaining Thai specimens were assigned to seven species in subgenus *Caulerpa* section *Caulerpa* (Figure 1). Representatives of each of the nine species identified in the present study are shown in Figures 2 and 3.

Monophyly of each species with their respective *tuf*A reference sequences was strongly supported, except for the sister species *C. cupressoides* and *C. serrulata*. The nesting of *Caulerpa serrulata* inside a paraphyletic *C. cupressoides*, is consistent with the phylogeny presented by Lagourgue *et al*. (2024), which included *tuf*A sequences from 15 *C. cupressoides* and 14 *C. serrulata* specimens. BLAST analysis of the ITS rDNA sequences was congruent with the *tuf*A dataset (Table 1), however, ITS sequences were not determined for *C. cupressoides*. ITS showed considerable infra-specific variation in some species. Optimal BLAST matches showed identity ranges of 86–97% in *C. serrulata* (n = 3) and 88–99% in *C. racemosa* (n = 8), whereas identity values ranged from 98%–100% in *C. verticillata* (n = 3) and *C. taxifolia* (n = 10).

A priori morphological identifications of the newly collected *Caulerpa* specimens were largely congruent with DNA sequence analyses, with one exception. Some specimens from the southern Gulf of Thailand (Pattani and Songkhla Provinces) were initially identified as *C. chemnitzia* based on the presence of peltate ramuli (Figure 2b). However, DNA barcoding assigned them to the *C. racemosa* clade. These specimens are indicated as *C. racemosa* ecad *peltata* in Figure 1. Morphologically, they could not be distinguished from *C. chemnitzia* var. *peltata* collected at the same site (Ko Kham, Songkhla Province) (Figure 2a). *Caulerpa racemosa* with globular ramuli (Figure 2c), was not found at this site.

The nine *Caulerpa* species (Figures 2 and 3) confirmed with DNA sequences, were found on both coasts, except *C. cupressoides*, which was only confirmed from the west coast (Table 1). During field surveys, no specimens were collected that were identified morphologically as *C. ambigua, C. fastigiata, C. manorensis, C. microphysa,* and *C. mexicana*. The first three species have distinct morphologies (Figure 4a–4c) and do not resemble any of the species confirmed from Thailand with DNA sequences in the present study.

Caulerpa microphysa is considered a synonym of *C. lentillifera* (Draisma *et al*., 2014; Draisma and Sauvage, 2024a), and the morphological differences between *C. mexicana* (Figure 4d) and *C. taxifolia* remain ambiguous (Coppejans and Prud'homme van Reine, 1992). *Caulerpa ambigua* represents a cryptic species complex comprising at least nine molecular species (Draisma and Sauvage, 2024a). It has recently been observed in Phuket Province (S.G.A. Draisma, pers. obs., 1 Nov. 2021, Figure 4a) and Satun Province (Sutti, 2023). Draisma and Sauvage (2024a) questioned the distinct species status of *C. manorensis* due to minimal *tuf*A sequence differences compared to the morphologically similar *C. veravalensis* Thivy et V.D.Chauhan and the highly variable *C. macra* (Weber Bosse) Draisma

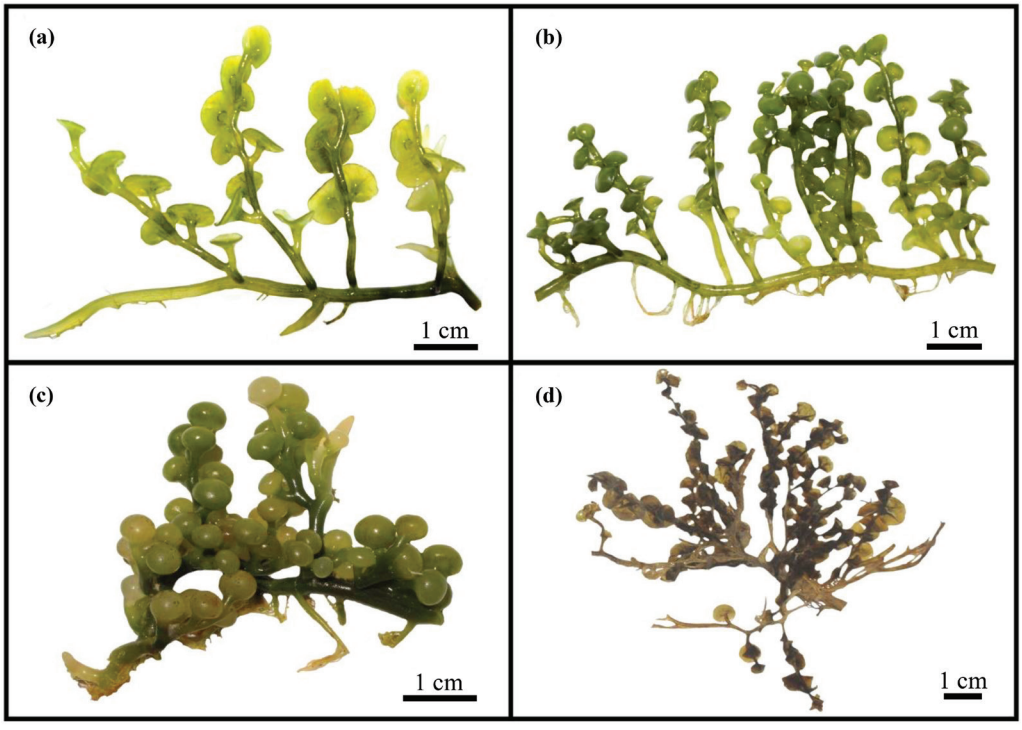

Figure 2. *Caulerpa* specimens from Thailand: (a) *C. chemnitzia* ecad peltata (PSU KP29A); (b) *C. racemosa* ecad *peltata* (PSU KP30A); (c) *C. racemosa* (PSU KP39C); (d) *C. macrodisca* (KUMF04404). Note: (a)–(c) fresh specimens (*ex situ*), (d) herbarium-pressed; Voucher codes in brackets (see Table 1).

et Prud'homme. They suggested these three taxa might represent a single species exhibiting high phenotypic plasticity, with *C. veravalensis* having nomenclatural priority. In Thailand, only the *C. manorensis* morphology has been observed, most recently in 2024 in Chonburi Province (Thanaphon Mana, pers. comm. via Anirut Klomjit to S.G.A. Draisma with *in situ* photo, voucher THNHM-P-2024-0001).

The last record of *C. fastigiata* from Thailand dates back to 1974 (Hodgson *et al*., 2004, Figure 4a). The collection site of the only Thai *C. fastigiata* records with detailed collection site information, was devoid of seaweeds during a revisit in 2021 in the same month as the 1963 collection (Orrell and Informatics and Data Science Center - Digital Stewardship, 2024c; UM Herbarium Data Group and IPT Admin L, 2024b).

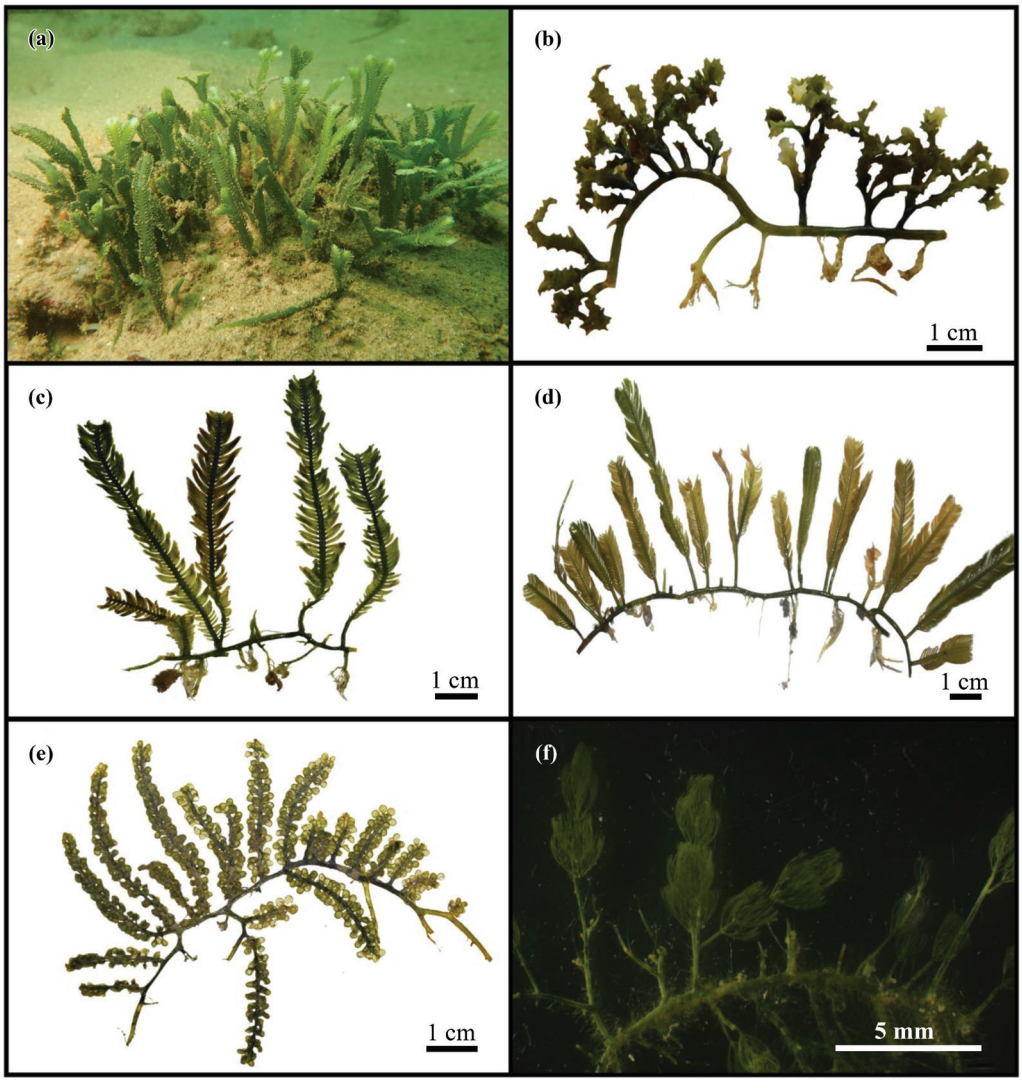

Figure 3. *Caulerpa* specimens from Thailand: (a) *C. cupressoides* (SGAD2304255); (b) *C. serrulata* (PSU KP11B); (c) *C. taxifolia* (PSU KP57A); (d) *C. sertularioides* (PSU KP61A); (e) *C. lentillifera* (PSU KP6A); (f) *C. verticillata* (PSU KP5A). Note: (a) *in situ*, (b)–(f) *ex situ*; Voucher codes in brackets (see Table 1).

Coppejans (1992) and Coppejans and Prud'homme van Reine (1992) found *Caulerpa* specimens with intermediate forms between *C. mexicana* and *C. taxifolia* in the Central Indo-Pacific and, therefore, they treated *C. mexicana* as an ecad of *C. taxifolia*. However, Famà *et al*. (2002) used *tuf*A DNA sequence to demonstrate that these taxa, both with type localities in the Atlantic, are distinct entities. Characteristics attributed to *C. mexicana* include ramuli that overlap in their central part, are not constricted at the base, and a flat rachis. In contrast, *C. taxifolia* features non-overlapping ramuli that are constricted at the base and a rachis that, while sometimes compressed, is never flat (Coppejans, 1992; Coppejans and Prud'homme van Reine, 1992; Littler and Littler, 2000; 2003; Lewmanomont, 2008; Coppejans *et al*., 2009). *Caulerpa mexicana* has never been confirmed from the Central Indo-Pacific via DNA sequencing. Karthick *et al*. (2020) found a specimen identified as *C. mexicana* from the Andaman Islands to be nested within the *C. taxifolia* clade based on *tuf*A sequences. A Thai specimen listed as *C. mexicana* in the US herbarium (Orrell and Informatics and Data Science Center - Digital Stewardship, 2024d) is likely *C. taxifolia* and a Thai specimen from BR exhibits traits of both *C. mexicana* and *C. taxifolia* (Meise Botanic Garden, 2024b). The distinction between these species, as well as certain growth forms of *C. chemnitzia* and *C. racemosa*, can only be reliably determined through DNA barcoding.

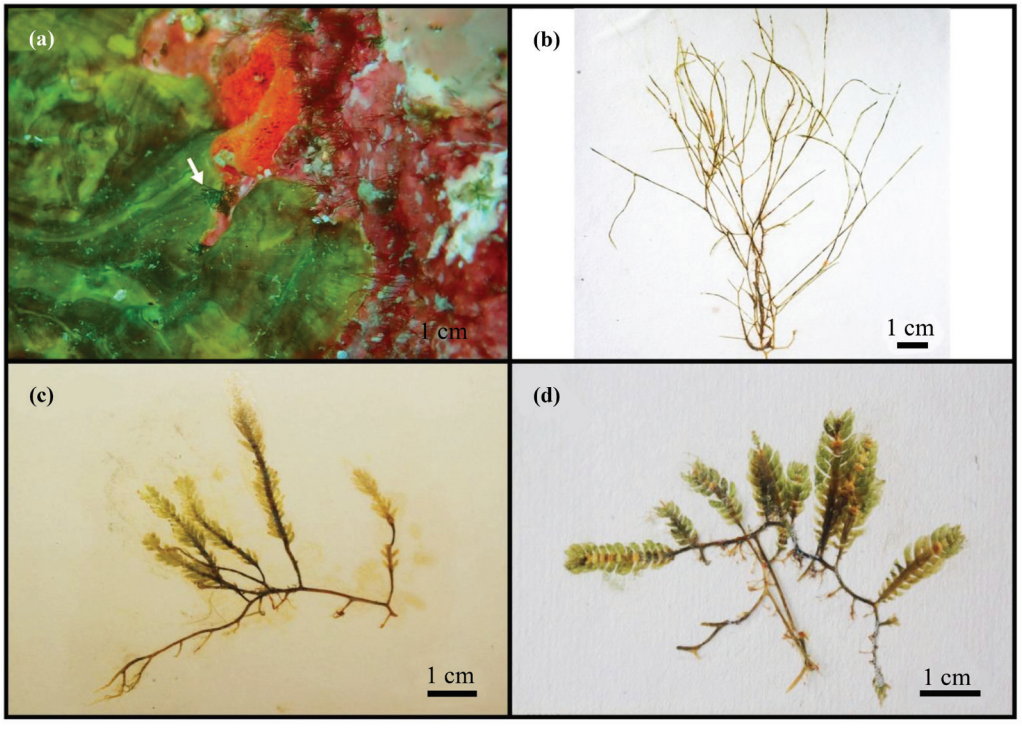

Figure 4. *Caulerpa* records from Thailand not found in the present study: (a) *C. ambigua* (arrow), estimated at 5 mm in size, growing from behind a *Lobophora* J.Agardh (Phaeophyceae) at Ko Waeo (Phuket, 1 Nov 2021, not collected); (b) The most recent collection of *C. fastigiata* in Thailand from Suan Son (Rayong, 29 Apr 1974, KUMF KL1750); (c) *C. manorensis* from Suan Son (Rayong, 10 May 1969, KUMF00250; (d) *C.* cf. *mexicana* from Kao Pilai (Phang-Nga, KUMF KL2131), discussed in Hodgson *et al*. (2004).

CONCLUSIONS

Nine *Caulerpa* species from Thailand have been confirmed using DNA barcoding and three additional species have been recognized based on morphology alone (Table 2). However, the presence of *C. fastigiata* in Thailand remains uncertain, as it has not been recorded in the past 50 years. Following Draisma and Sauvage (2024a), *C. microphysa* is considered a synonym of *C. lentillifera*. The status of *C. mexicana* in Thailand remains unresolved until DNA barcoding can confirm its occurrence.

It is recommended that *C. filiformis, C. sedoides, C. scalpelliformis*, and *C. urvilleana* be removed from the checklist of seaweeds of Thailand, as these species were only reported by Reinbold (1901). *C. filiformis sensu* Reinbold corresponds to *C. fastigiata*, while *C. sedoides sensu* Reinbold is actually *C. lentillifera*. Reinbold's vouchers of *C. scalpelliformis* and *C. urvilleana* could not be located, and they were likely misidentified. His *C. scalpelliformis* may have been *C. manorensis*, which had not been described at the time, while his *C. urvilleana* could represent *C. cupressoides*.

It is also proposed to remove *C. ashmeadii* and *C. corynephora* from the checklist and to classify all Thai records of these species under *C. macrodisca* (Pattarach *et al*., 2019). *Caulerpa corynephora* possesses an annulated rachis and chloroplasts with pyrenoids (Price, 2011; Belton *et al*., 2019), characteristics that have never been observed in Thai specimens identified under this name. The present study demonstrates the importance of DNA barcoding for accurately identifying members of the *C. racemosa-peltata* complex. The *tuf*A barcode alone is sufficient for identifying *Caulerpa* species in Thailand, while complementary ITS sequences are unnecessary except for distinguishing *C. cupressoides* and *C. serrulata*. For these two taxa, the *rbc*L gene is recommended (Draisma and Sauvage, 2024a).

Table 2. Updated list of *Caulerpa* species confirmed from Thailand, along with previous synonyms used in Thai records and references to misapplied names. Synonyms follow AlgaeBase (Guiry and Guiry, 2024) unless otherwise stated.

***Caulerpa* species**	**Synonyms reported from Thailand**	**Misapplied names with reference**	**Remark**
***Caulerpa* species confirmed from Thailand with DNA sequence data**			
C. chemnitzia (Esper) J.V. Lamouroux	*Caulerpa peltata* J.V. Lamouroux, *Caulerpa racemosa* var. *peltata* (J.V.Lamouroux) Eubank	*C. nummularia* (Harvey) Reinke (European Bioinformatics Institute (EMBL-EBI) and GBIF Helpdesk, 2024a; Meise Botanic Garden, 2024a)	*C. nummularia* voucher in BR re-identified (This study)
C. cupressoides (Vahl) C.Agardh			
C. lentillifera J.Agardh	*C. microphysa* (Weber Bosse) Feldmann	*C. sedoides* C.Agardh (Reinbold, 1901; Velasquez and Lewmanomont, 1975; Lewmanomont *et al*., 1995; Hodgson *et al*., 2004; TH-BIF, 2024)	All reports of *C. sedoides* (a temperate Australia species, Belton *et al*. (2019)) can be traced back to a single record by Reinbold (1901), which was re-identified as *C. lentillifera* (This study). For the synonymization of *C. microphysa* we follow Draisma *et al*. (2014) and Draisma and Sauvage (2024a).

Table 2. Cont.

***Caulerpa* species**	**Synonyms reported from Thailand**	**Misapplied names with reference**	**Remark**
C. macrodisca Decaisne	*C. peltata* var. *macrodisca* (Decaisne) Weber Bosse	*C. ashmeadii* Harvey (Lewmanomont, 2008), *C.* (*racemosa* var.) *corynephora* (Montagne) Weber Bosse (Hodgson *et al*., 2004; Lewmanomont *et al*., 1995; Lewmanomont, 1978; 2008; Longloy and Mangyu, 2007; Nuancharoen and Laohabanjong, 2011; Phang *et al*., 2016; Coppejans *et al,*. 2017; Lewmanomont and Chirapart, 2022; European Bioinformatics Institute (EMBL-EBI) and GBIF Helpdesk, 2024b)	Misapplication of the names *C. ashmeadii* and *C. corynephora* to *C. macrodisca* was reported by Pattarach *et al*. (2019)
C. racemosa (Forsskål) J.Agardh	*C. macrophysa* (Kützing) G.Murray	*C. racemosa* J.Agardh var. *microphysa* (Sonder ex Kützing) Taylor (Nuancharoen and Laohabanjong, 2011)	*C. racemosa* var. *microphysa* is a synonym of *C. microphysa* (= *C. lentillifera*), but the specimen depicted in Nuancharoen and Laohabanjong (2011) was re-identified as *C. racemosa* by the present authors
C. serrulata (Forsskål) J.Agardh *C. sertularioides* (S.G.Gmelin) M.Howe	*C. freycinetti* C.Agardh *C. plumaris* var. *longipes* J.Agardh		
C. taxifolia (M.Vahl) C.Agardh		*C. scalpelliformis* (R.Brown ex Turner) C.Agardh (Saelao and Tuntiprapas, 2024)	*C. scalpelliformis* specimen in PSU re-identified (This study)
C. verticillata-1 J.Agardh *sensu* Draisma and Sauvage (2024a)			*C. verticillata* currently represents three cryptic species (Draisma and Sauvage, 2024a). All sequenced Thai specimens belong to *C. verticillata*-1.
***Caulerpa* species recorded from Thailand not confirmed with DNA sequence data**			
C. ambigua Okamura	*Caulerpella ambigua* (Okamura) Prud'homme et Lokhorst		*C. ambigua* currently represents nine cryptic species (Draisma and Sauvage, 2024a). Most recent Thai record by Sutti (2023).
C. fastigiata Montagne	*C. filiformis* (Harvey) J.Agardh nom illeg.	*C. filiformis* (Suhr) Hering (Hodgson *et al*., 2004; Phang *et al*., 2016; TH-BIF, 2024)	All reports of *C. filiformis* (Suhr) Hering can be traced back to a single *C. filiformis* (Harvey) J.Agardh (illegitimate name) record by Reinbold (1901), which was renamed *C. tongaensis* Papenfuss (1943) and considered a synonym of *C. fastigiata* by Sauvage *et al*. (2021). Most recent Thai record from 1974 (This study)

Table 2. Cont.

***Caulerpa* species**	**Synonyms reported from Thailand**	**Misapplied names with reference**	**Remark**
C. manorensis Nizamuddin		*C. crassifolia* (C.Agardh) J.Agardh (Srimanobhas, 1980; Lewmanomont and Ogawa, 1995; Lewmanomont *et al*., 1995; 2007; TH-BIF, 2024), *C.* indet. (UM Herbarium Data Group and IPT Admin L, 2024a)	Misapplication of the name *C. crassifolia* (= *C. mexicana*) reported by Lewmanomont (2008). Unidentified *Caulerpa* in UM re-identified (This study).
Doubtful Thai *Caulerpa* records			
C. mexicana Sonder ex Kützing	*C. crassifolia* (C.Agardh) J.Agardh		The name *C. crassifolia* has been found on herbarium labels of *C. mexicana* specimens according to Hodgson *et al*. (2004). Morphological distinction between *C. mexicana* and *C. taxifolia* is ambiguous. *C. mexicana* has never been confirmed with a DNA sequence from the Central Indo-Pacific and Andaman Sea.
C. scalpelliformis (R.Brown ex Turner) C.Agardh			All reports can be traced back to Reinbold (1901), but the voucher was not found. *C. scalpelliformis* is considered a temperate Australian species (Belton *et al*., 2019).
C. urvilleana Montagne			All reports can be traced back to Reinbold (1901), but the voucher was not found.

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