

Species diversity of the green macroalgal genus *Caulerpa* (Caulerpaceae, Chlorophyta) in the area of Ko Kut, Ko Chang, and nearby islands, Trat Province

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Abstract

The preliminary survey of the Project "Species diversity and genetic resources assessment of the genus Caulerpa (Caulerpaceae, Chlorophyta) in Thailand for the principal conservation and sustainable utilization" was kicked off in Trat Province as representative of the upper part of the Gulf of Thailand. Caulerpa, some other seaweeds and seagrasses were sampled mainly by scuba diving in the area of Ko Kut, Ko Chang, and nearby islands, totaling ten sampling sites from 19-23 December 2022. Unfortunately, it seems like December is not the right season for Caulerpa sampling, hence, not many Caulerpa were found. Ko Kradad, Ko Khai Hua Ro, and Ko Mak were the only three sites that Caulerpa could be collected. Based on morphological characters solely, at least four Caulerpa species were identified. For better precision of species diversity in the genus Caulerpa in these areas, twenty *Caulerpa* voucher specimens were prepared, and twelve of them were sent for DNA extraction and molecular analysis. The molecular results are pending.

Keywords

species diversity, Caulerpa, Trat, preliminary, Thailand

Introduction

The genus *Caulerpa* (Caulerpaceae, Chlorophyta) is one of the most distinctive genera of seaweeds by being identifiable solely based on its habits (Wakey and Bucol, 2014). The thallus, a nonseptate siphonous structure is composed of horizontal stolon anchored by colorless rhizoids, bearing erect photosynthetic fronds (assimilators) of extremely diverse morphology including thread-like, blade-like, pinnate, spongy and vesicular structures. *Caulerpa* contains more than 100 accepted species and is found throughout tropical and subtropical seas around the world (Guiry and Guiry, 2024). Many of them exhibit polymorphism, showing different growth forms in different habitats which makes them difficult to identify.

Caulerpa is among the most prolific and widespread group of seaweed. Some *Caulerpa* species, *C. taxifolia* for example, have invaded some marine waters such as in the Mediterranean, Australia, USA, and Europe due to the aquarium trade and caused ecological

Citation. Sutti., S., S. Chungthanawong and R. Sumitrakij. 2024. Species diversity of the green macroalgal genus Caulerpa (Caulerpaceae, Chlorophyta) in the area of Ko Kut, Ko Chang, and nearby islands, Trat Province. In The 4th Thailand International Symposium on Natural History Museums: Era of Ecosystem Restoration on 21–22 December 2023 at Rama 9 Museum Thailand: 179–184 pp. and economic loss (Klein and Verlaque, 2008; Zubia *et al.*, 2020). On the other hand, some species of *Caulerpa* such as *C. lentillifera* and *C. racemosa* are farmed, consumed, and also exported to many countries, Philippines and Thailand, for instance. Regarding species diversity of the genus *Caulerpa* in Thailand, approximately 15 species were reported from both the Andaman Sea and Gulf of Thailand (Coppejans *et al.*, 2017).

The Trat site is located at the coastal zone of Trat Province in the Gulf of Thailand, bordering Cambodia. The province is the easternmost part of the country, covering 2,819 km² of land-area and 7,257 km² of sea-area with 165.5 km coastline. Trat is has been regarded as one of the richest coastal ecosystems in Thailand due to its high abundances of mangroves, coral reefs, as well as number of small and big islands (Munprasit *et al.*, 2020). Among those islands, Ko Kut and Ko Chang are the biggest two.

This is the preliminary survey of the "Species diversity and genetic resources assessment of the genus *Caulerpa* (Caulerpaceae, Chlorophyta) in Thailand for the principal conservation and sustainable utilization Project". Ko Kut, Ko Chang, and some nearby islands were selected as the representatives of the upper Gulf of Thailand sites.

Materials and Methods

Field work

Collecting was carried out in Ao Salat, Ao Klong Chao, northwest of Ko Kut, Ko Rad and east of Ko Kut from 19–20 December 2022 and in Ko Klum, Ko Lao Ya, Ko Kradad, Ko Khai Hua Ro, and Ko Mak from 22–23 December 2022, totaling ten sites (Figure 1). The collection was done by SCUBA-diving in 3–7 m depth. *Caulerpa* and some other seaweeds and seagrasses specimens were collected, rinsed with seawater and stored in plastic bags by adding some salt to protect the plants from fouling. Small pieces of selected *Caulerpa* were kept in silica gel for DNA extraction and molecular analysis. Underwater photographs were taken if needed.



Figure 1. Research area.

DNA extraction and molecular analysis

Silica gel specimens were sent to the Faculty of Fisheries, Kasetsart University for DNA extraction and amplification. PCR products were purified and subjected to commercial sequencing. The *tuf*A gene (a partial section, ~ 950 bp) was chosen as it has been shown to have sufficient species-level resolution in the Bryopsidales, including *Caulerpa* (Kazi *et al.*, 2013; Vieira *et al.*, 2016; Darmawan *et al.*, 2021).

Individual sequences obtained in this study were compared to additional sequences retrieved from GenBank in order to produce the phylogeny of *Caulerpa*. *Caulerpella ambigua* was used as an outgroup as it was found to be the most basal taxon to all the *Caulerpa* species. A maximum likelihood (ML) tree was constructed by the bootstrap resampling method.

Reference materials

After arrival in the laboratory, specimens were sorted and prepared as reference herbarium specimens. Seaweed and seagrass vouchers were deposited in the collection of the Thailand Natural History Museum (THNHM).

Results and Discussion

Regarding ten sampling sites (Figure 1), *Caulerpa* were found only in three sites: Ko Kradad, Ko Khai Hua Ro, and Ko Mak at the 3–5 m depth. Ko Kradad, Ko Khai and Ko Mak share common characteristics of the sites such as sandy seabeds with rocks and dead corals mainly occupied by brown seaweeds. *Caulerpa* species are known to occupy a range of environmental niches. Most of them are intertidal species. They attach to a hard substrate but can also grow in unconsolidated sand using their rhizoids (Zubia *et al.*, 2020; Darmawan *et al.*, 2021).



Figure 2. Underwater photographs of some *Caulerpa* species. **a)** *Caulerpa lentillifera* at Ko Kradad; **b)** *Caulerpa racemosa* at Ko Kradad; **c)** *Caulerpa serrulata* at Ko Khai Hua Ro; **d.** *Caulerpa sertularioides* at Ko Khai Hua Ro.

No.	Field Num-	Catalog Number	Genus	Specific Epithet	Date Collected	Locality	District	Re- marks
1	100	TUNUN D 2022 0072	0.1	1.1	22 D 1	V V 1 1	V Cl	
1	\$408	1HNHM-P-2022-00/2	Caulerpa	serrulata	23 December 2022	Ko Kradad	Ko Chang	
2	s409	THNHM-P-2022-0073	Caulerpa	lentillifera	23 December 2022	Ko Kradad	Ko Chang	tufA
3	s409	THNHM-P-2022-0074	Caulerpa	lentillifera	23 December 2022	Ko Kradad	Ko Chang	
4	s410	THNHM-P-2022-0075	Caulerpa	sp.	23 December 2022	Ko Kradad	Ko Chang	
5	s421	THNHM-P-2022-0087	Caulerpa	racemosa	23 December 2022	Ko Khai Hua Ro	Ko Chang	tufA
6	s422	THNHM-P-2022-0088	Caulerpa	racemosa	23 December 2022	Ko Khai Hua Ro	Ko Chang	tufA
7	s423	THNHM-P-2022-0089	Caulerpa	racemosa	23 December	Ko Khai	Ko Chang	
0	- 40.4	TINUNA D 2022 0000	C1		2022 22 December	Hua Ko	V. Chana	
ð	\$424	IHNHM-P-2022-0090	Cauterpa	serrulala	25 December	Ko Khai	Ko Chang	
0	a125	TUNIUM D 2022 0001	Caularna	sorrulata	2022 23 December	Tiua Ko Ko Khai	Ko Chana	
,	5 4 23	11111111111-1-2022-0071	Cauterpa	serruiuiu	25 December	KU Klidi Hua Ro	K0 Chang	
10	e∕126	THNHM_P_2022_0002	Caularna	sartularioidas	2022 23 December	Ko Khai	Ko Chang	
10	3720	1111111111-1-2022-0072	Cunerpu	ser innar ionies	2022	Hua Ro	Ko Chang	
11	s426	THNHM-P-2022-0093	Caulerna	sertularioides	2022 23 December	Ko Khai	Ko Chang	
	5120		Cumerpu	Ser futur fordes	2022	Hua Ro	ne chung	
12	s426	THNHM-P-2022-0094	Caulerpa	sertularioides	23 December	Ko Khai	Ko Chang	
			1		2022	Hua Ro	0	
13	s439	THNHM-P-2022-0105	Caulerpa	serrulata	23 December	Ko Khai	Ko Chang	
					2022	Hua Ro	·	
14	s440	THNHM-P-2022-0106	Caulerpa	sertularioides	23 December	Ko Khai	Ko Chang	
					2022	Hua Ro		
15	s441	THNHM-P-2022-0107	Caulerpa	sertularioides	23 December 2022	Ko Khai Hua Ro	Ko Chang	
16	s448	THNHM-P-2022-0114	Caulerpa	sertularioides	23 December	Ko Mak	Ko Chang	tufA
17	s449	THNHM-P-2022-0115	Caulerpa	serrulata	23 December 2022	Ko Mak	Ko Chang	tufA
18	s450	THNHM-P-2022-0116	Caulerpa	sertularioides	23 December	Ko Mak	Ko Chang	
10	450	TINUN (D 2022 0117	C 1	. 1 1	2022 22 D 1	V. M.1.	V Cl	
19	s430	1HNHM-P-2022-0117	Cauterpa	sertularioides	23 December 2022	Ko Mak	Ko Chang	
20	s450	THNHM-P-2022-0118	Caulerpa	sertularioides	23 December 2022	Ko Mak	Ko Chang	

Table 1. Selected silica gel samples handling to DNA extraction and molecular analysis.



Figure 3. ML tree based on *tuf*A sequence data.

Morphological characters and molecular analysis (Figures 2–3, Table 1) support four *Caulerpa* species including *Caulerpa lentillifera*, *Caulerpa racemosa*, *Caulerpa serrulate*, and *Caulerpa sertularioides* from Trat. Basically, most *Caulerpa* were identified by the differences in gross morphology of fronds, stolons, and rhizoids with the form and size of ramuli. Within the *Caulerpa*, the most taxonomically troublesome taxa are those currently associated with *C. racemosa* (Belton *et al.*, 2014). However, only four specimens initially identified as *C. racemosa* were collected with solely *tufA* (plastid elongation factor) sequence data support. *tufA* has been selected as the DNA barcode marker for the genus *Caulerpa* (Belton *et al.*, 2014; Darmawan *et al.*, 2021). However, *tufA* alone is not variable enough to resolve all *Caulerpa* species relationships. Rubisco large subunit (*rbcL*) is the other marker purposed for *Caulerpa* species identification. The *rbcL* result of this preliminary survey is pending.

According to personal interviews from local people in Ko Kut and Ko Chang, *Caulerpa* can be found nearby at all sites selected especially in the hot season or between March to April. Hence, December is not the right season for *Caulerpa* sampling. For better understanding regarding species diversity of the genus *Caulerpa* in Thailand, more sampling sites covering both the Gulf of Thailand and Andaman Sea are necessary. Moreover, molecular analysis using appropriate *Caulerpa* DNA markers is needed.

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