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DNA Barcodes of *Caulerpa* Species (Caulerpaceae, Chlorophyta) from the Northern Philippines

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Species discrimination in *Caulerpa* – a seaweed genus of economic importance – can be difficult because its members are often morphologically variable, occasionally with some morphological overlap between species. One of the most common approaches to circumvent this challenge is the integration of phenotypic data with information gained from short reference DNA sequences or DNA barcodes. Previous investigations based on this approach have incorporated only a limited number of specimens of *Caulerpa* from the Philippines. The present study aimed to identify *Caulerpa* collections from the northern Philippines aided with DNA barcodes. *Tuf*A gene sequence data of recent intertidal and shallow subtidal collections confirmed the presence of seven genetically recognized *Caulerpa* species in the northern Philippines, namely *C. chemnitzia, C. cupressoides, C. elongata, C. oligophylla, C. racemosa*, *C. serrulata*, and *C. sertularioides. Caulerpa elongata* and *C. oligophylla* were confirmed occurring in the area for the first time. With the addition of species recorded previously but not found during the present study, this brings the total number of *Caulerpa* species in the region to 15 species.

Keywords: *Caulerpa elongata, Caulerpa oligophylla,* DNA barcoding, taxonomy, *tuf*A gene

INTRODUCTION

Caulerpa is a genus of marine green algae comprising 97 currently recognized species (Guiry and Guiry 2018). The genus is pantropical in distribution but also extends into temperate waters. Its members often form turf communities in the shallow intertidal (Belton *et al.* 2015) to depths of up to 100 m (Belsher and Meinesz 1995). *Caulerpa* is predominantly an Old World assemblage, having so far the highest species diversity in Australia (Price 2011, Belton *et al*. 2019). Despite the occurrence of several invasive pest taxa (Meinesz 2002, Klein and Verlaque 2008), species of *Caulerpa* can constitute important components within their native ranges (Alcala *et al.* 1972, Kuenen and Debrot 1995, Crockett and Keough 2014).

A significant number of *Caulerpa* species are characterized by an erect branch forming the central axis (rachis) with branchlets (ramuli), collectively forming the assimilator. The primary criteria for species discrimination include the branching of the assimilator and shape of the ramuli. These morphological traits, however, can exhibit a high degree of variation under different environmental conditions (Peterson 1972, Calvert 1976, Ohba and Enomoto 1987, Ohba *et al.* 1992, Gacia *et al.* 1996, Riosmena-Rodríguez *et al.* 2014) and their convergence in classical character-systems has led to considerable taxonomic confusion (*e.g.,* Calvert *et al.* 1976, Coppejans and Prud'homme van

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Reine 1992, Prud'homme van Reine *et al.* 1996). Analysis of short DNA sequences – referred to as DNA barcodes – has been widely adopted by phycologists, enabling them to identify unknown specimens in reference to the established classification (Saunders and Kucera 2010, Pasha 2016). Today, species discrimination in *Caulerpa* is generally based on a number of genomic loci, with the chloroplast gene *tuf*A encoding elongation factor TU being used almost universally (Famà *et al.* 2002; de Senerpont Domis *et al.* 2003; Stam *et al.* 2006; Kazi *et al.* 2013; Sauvage *et al.* 2013; Belton *et al.* 2014, 2015, 2019; Draisma *et al.* 2014; Wang *et al.* 2015; Fernández-García *et al.* 2016).

Accordingly, Belton *et al.* (2014, 2019) proposed *tuf*A DNA barcodes (*i.e.*, reference sequences) for 37 *Caulerpa* species and analyzed them using a single-locus species delimitation method (General Mixed Yule Coalescent or GMYC) together with other statistical criteria. When possible, they chose the *tuf*A sequences of specimens nearest to the type location as barcode to represent the species. They also advised to abandon the use of infraspecific ranks (variety or form) and use terminology without formal taxonomic assignment (ecads). However, other entities might still show consistent morphological and genetic distinctions – for example, *Caulerpa taxifolia* var. *distichophylla* from *Caulerpa taxifolia* var. *taxifolia* – in its invasive habitat (Jongma *et al.* 2013).

One of the Philippine hotspots of seaweed diversity is its northern territory (Cordero 1977a, Trono 1998). The area includes the provinces of Ilocos Norte and Cagayan, which respectively encompass the northeast and northwest areas of Luzon Island. Its extreme northern boundary extends across the volcanic island series of Calayan and Batanes (Fig. 1). Going under the most popular local name, "Ar-arusip," *Caulerpa* holds a significant place as a traditional food in the region (Blanco 1938, Moreland

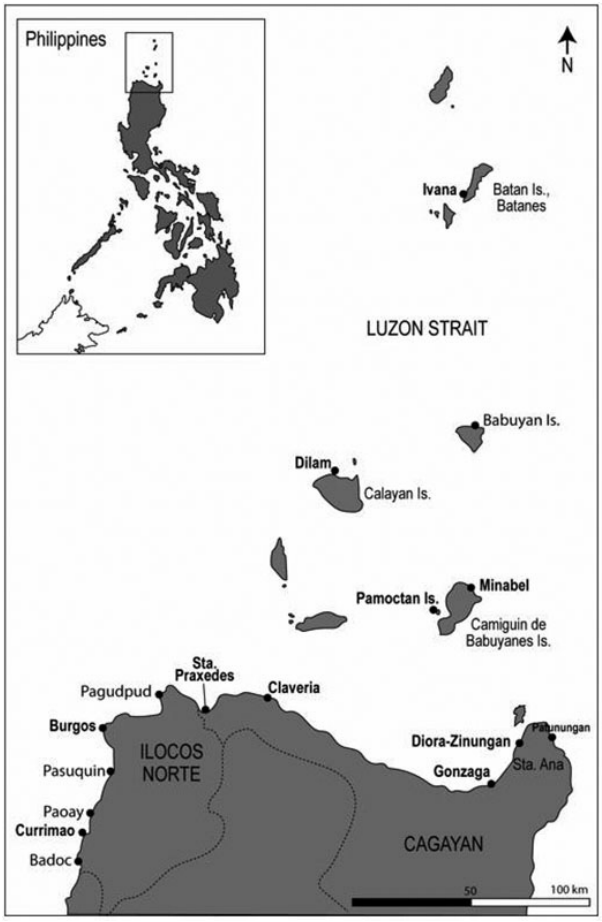

Figure 1. Map showing the geographic distribution of *Caulerpa* species reported in the northern Philippines. Collection sites are written in boldface.

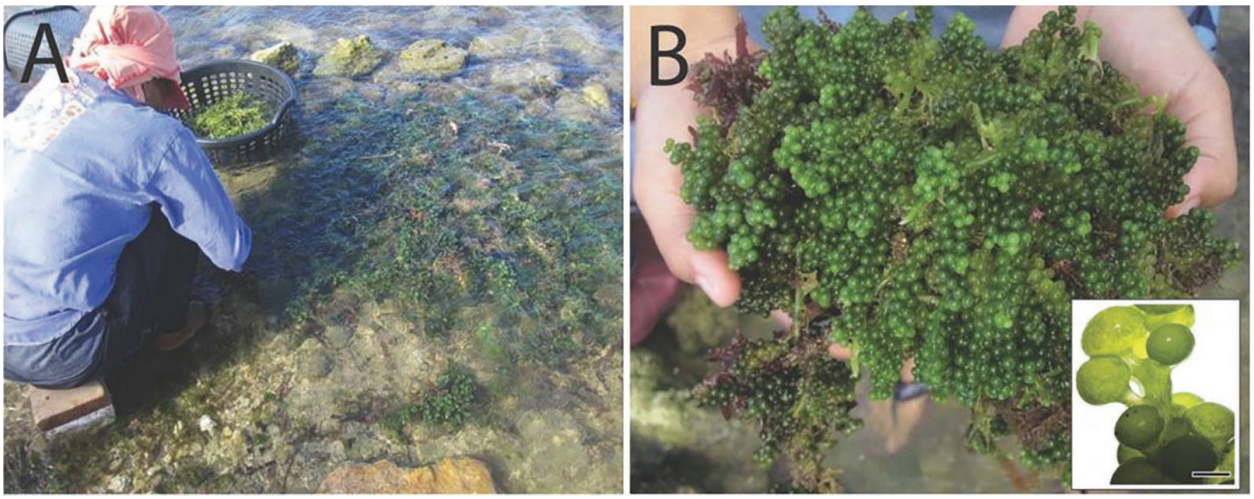

Figure 2. Gathering and collection of *Caulerpa racemosa* ("Ar-arusip") in Sitio, Daisy, Paayas, Burgos, Ilocos Norte, northern Philippines. (A) A local gatherer cleaning *Caulerpa* produce near shore on a low tide. (B) Habit of *C. racemosa* showing its typical spherical ramuli (inset), scale = 2 mm.

Table 1. List of species and distribution of *Caulerpa* reported from the northern Philippines. Species names are updated from the original publication. The currently accepted names are based on Algaebase (Guiry and Guiry 2018), an international standard database for algal taxonomy, nomenclature, and distribution information.

Species	Northern Philippine distribution[a]	Reference
Caulerpa chemnitzia (Esper) J.V.Lamouroux	Cagayan – Babuyan Is.; Ilocos Norte – Burgos, Pasuquin; Cagayan – Camiguin de Babuyanes Is., Calayan Is.	Gilbert 1942[bc], Cordero 1983[b], Marcos-Agngarayngay 1983b[b], This study
Caulerpa cupressoides (Vahl) C.Agardh	Batanes – Batan Is.; Cagayan –Camiguin de Babuyanes, Claveria, Sta. Ana; Ilocos Norte – Burgos	Cordero 1983, Marcos-Agngarayngay 1983a; This study
var. *lycopodium* Weber-van Bosse	Ilocos Norte – Currimao	Hurtado-Ponce 1983a, Hurtado-Ponce and Modelo 1983
var. *elegans* (P.Crouan & H.Crouan) Børgesen	Batanes – Batan Is.	Cordero 1977b
var. *turbinata* (J.Agardh) Fernández-García & Riosmena-Rodríguez	Ilocos Norte – Burgos	Belleza and Liao 2007[c]
Caulerpa elongata Weber-van Bosse	Batanes – Batan Is.; Cagayan –Camiguin de Babuyanes Is.; Ilocos Norte – Burgos	This study
Caulerpa fergusonii Murray	Ilocos Norte – Currimao	Hurtado-Ponce 1983a,b; Hurtado-Ponce and Modelo 1983; Tungpalan 1983
Caulerpa lentillifera J.Agardh	Ilocos Norte – unstated locality	Moreland 1979, Agngarayngay *et al.* 2005
Caulerpa macrodisca Decaisne	Cagayan – unstated locality; Ilocos region – probably in an unstated locality in Ilocos Norte	Zaneveld 1956[e]
Caulerpa nummularia Harvey ex J.Agardh	Batanes – Batan Is.	Cordero 1976[f]
Caulerpa oligophylla Montagne	Cagayan – Sta. Ana	This study
Caulerpa parvifolia Harvey	Cagayan – Babuyan Is.	Gilbert 1942[g]
Caulerpa racemosa (Forsskål) J.Agardh	Batanes – Batan Is.; Cagayan –Camiguin de Babuyanes Is., Sta. Ana, Sta. Praxedes; Ilocos Norte –Badoc, Burgos, Currimao, Pagudpud, Pasuquin, Paoay	Blanco 1938, Zaneveld 1956, Moreland 1979, Marcos-Agngarayngay 1983a,b; Tungpalan, 1983; Agngarayngay *et al.* 2005; This study
var. *clavifera* (Turner) Weber-van Bosse	Cagayan – Babuyan Is.	Gilbert 1942
Caulerpa serrulata (Forsskål) J.Agardh	Batanes – Batan Is.; Cagayan –Camiguin de Babuyanes, Claveria, Gonzaga, Sta. Ana; Ilocos Norte –Burgos, Currimao	Blanco 1938; Gilbert 1942; Cordero 1983; Hurtado-Ponce 1983a: Hurtado-Ponce & Modelo 1983; Marcos-Agngarayngay 1983a,b; This study
var. *boryana* (J.Agardh) Gilbert f. *longifolia* Gilbert	Ilocos Norte – Currimao	Gilbert 1942; Hurtado-Ponce and Modelo 1983
f. *lata* (Weber-van Bosse) Tseng	Ilocos Norte – unstated locality	Gilbert 1942
var. *serrulata*	Ilocos Norte – Burgos	Belleza and Liao 2007
Caulerpa sertularioides (S.G.Gmelin) Howe	Cagayan – Gonzaga; Ilocos Norte –Burgos, Currimao	Blanco 1938; Zaneveld 1956; Cordero 1983; Hurtado-Ponce 1983a; Hurtado-Ponce and Modelo 1983; Marcos-Agngarayngay 1983a,b; This study
f. *brevipes* (J.Agardh) Svedelius	Batanes – Batan Is.	Cordero 1977b
f. *longiseta* (Bory de Saint-Vincent) Svedelius	Ilocos Norte – unstated locality	Gilbert 1942
Caulerpa subserrata Okamura	Ilocos Norte – Burgos, Currimao	Cordero 1980; Hurtado-Ponce 1983a: Hurtado-Ponce and Modelo 1983; Marcos-Agngarayngay 1983a
Caulerpa taxifolia (Vahl) C. Agardh	Batanes – Batan Is.; Ilocos Norte –Burgos	Cordero 1977b; Cordero 1983
Caulerpa webbiana Montagne	Ilocos Norte – Burgos	Cordero 1983
var. *pickeringii* (Harvey & Bailey) Eubank	Ilocos Norte – Burgos	Trono 2004

[a]refer to Fig. 2; [b]named as *C. peltata;* [c]named as *C. peltata* var. *peltata;* [d]named as *C. racemosa* var. *turbinata;* [e]named as *C. peltata* var. *macrodisca;* [f]named as *C. peltata* var. *nummularia;* [g]named as *C. brachypus* f. *parvifolia*

1979, Hurtado-Ponce 1983b, Marcos-Agngarayngay 1983b, Tungpalan 1983, Agngarayngay *et al.* 2005) and its collection for market is a common subsistence activity in the area (Fig. 2).

The nomenclatural and taxonomic differences between earlier records of *Caulerpa* species for the area (see Table 1 for complete references) and those from more recent studies (Kazi *et al.* 2013; Sauvage *et al.* 2013; Draisma *et al.* 2014; Belton *et al.* 2014, 2015, 2019; Fernández-García *et al.* 2016) made updating previous records and distributions a challenge, and the utility of DNA barcodes to reliably assign species names cannot be overemphasized. Here, we fulfill this knowledge gap by generating *tuf*A gene barcodes and updating the list and distributions of *Caulerpa* species found in the northern Philippines.

MATERIALS AND METHODS

Collection

Specimens of *Caulerpa* were obtained from 11 sites in the northern Philippines (Table 2, see also Fig. 2). Intertidal and shallow subtidal collections were made by wading or snorkeling at low tide. A total of 47 specimens (Table 3) were collected and analyzed in this study. Initial identification based on morphology was chiefly based on Trono (1997, 2004). Voucher specimens were prepared following Trono and Ganzon-Fortes (1988) and were lodged in the Far Eastern University Herbarium (FEUH) (abbreviation follows Thiers 2019).

DNA Extraction and tufA Gene Amplification

Sampled fragments (if ramuli are present, 2–5 pieces) of each *Caulerpa* specimen preserved in 95% ethanol or

Table 3. Collection information of *Caulerpa* specimens used in this study.

	Species	Isolate Number	Voucher	Site No.[a]	Genbank Accession
1	*C. chemnitzia*	RVD20010	FEUH002081	4	KT861498
2		RVDJ12	FEUH003099	8	KT861508
3		RVDL12	FEUH003100	8	KT861513
4		RVDL13	FEUH003101	8	KT861514
5		RVDL15	FEUH003102	8	KT861516
6		RVDL19	FEUH003087	2	KT861519
7	*C. cupressoides*	RVD20025	FEUH002073	4	KT861486
8		RVD20043	FEUH002074	6	KT861487
9		RVD20056	FEUH002075	10	KT861488
10		RVD20058	FEUH002076	10	KT861489
11		RVDL2	FEUH003096	7	KT861490
12		RVDL7	FEUH003086	1	KT861491
13	*C. elongata*	RVD20026	FEUH002078	4	KT861492
14		RVDJ1[b]	FEUH003093	7	KT861493
15		RVDJ10[b]	FEUH003088	1	KT861494
16		RVDL10[b]	FEUH003089	1	KT861495
17	*C. oligophylla*	RVD20057[c]	FEUH002250	10	KT861506
18		RVD20064[c]	FEUH002252	11	KT861507
19		RVDL18[c]	FEUH003098	10	KT861518
20	*C. racemosa*	RVD20001	FEUH002079	3	KT861496
21		RVD20005	FEUH002080	3	KT861497
22		RVD20011	FEUH002082	4	KT861499
23		RVD20012	FEUH002083	4	KT861500
24		RVD20013	FEUH002084	4	KT861501
25		RVD20020	FEUH002085	4	KT861502
26		RVD20023	FEUH002087	4	KT861503
27		RVD20024	FEUH002088	4	KT861504

Table 2. List of 11 collecting sites located in the northern Philippines, their respective coordinates, and date of collection.

Site No.	Collection Site	Coordinates	Date of collection
1	Port Radiwan, Ivana, Batan Is., Batanes	20° 36' N, 121° 91' E	20 January 2015
2	Dilam, Calayan Is., Cagayan	19° 38' N, 121° 46' E	16 February 2015
3	Pangil, Currimao, Ilocos Norte	17° 59' 46" N, 120° 29' 50" E	08 June 2014
4	Sitio Daisy, Paayas, Burgos, Ilocos Norte	18° 36' 04" N, 120° 53' 15" E	09 June 2014
5	Mingay, Brgy. San Juan, Sta. Praxedes, Cagayan	18° 59' 86" N, 120° 99' 09" E	10 June 2014
6	Blue Lagoon, Taggat Norte, Claveria, Cagayan	18° 62' N, 120°86' E	11 June 2014
7	Pamoctan Is., Camiguin de Babuyanes Is., Cagayan	18° 89' 98" N, 121° 83' 82" E	11 February 2015
8	Sitio Minabel, Camiguin de Babuyanes Is., Cagayan	19° 00' 23" N, 121° 94' 48" E	12 February 2015
9	Brgy. Tapel, Gonzaga, Cagayan	18° 30' 10" N, 122° 8' 48" E	12 June 2014
10	Diora-Zinungan, Sta. Ana, Cagayan	18° 42' 62" N, 122° 12' 00" E	13 June 2015
11	Sitio Malolog, Patunungan, Sta. Ana, Cagayan	18° 44' 41" N, 122° 25' 92" E	14 June 2014

Table 3 continuation. . . . . .

28		RVD20046	FEUH002089	5	KT861505
29		RVDJ14	FEUH002334	8	KT861509
30		RVDL4	FEUH003094	7	KT861510
31		RVDL5	FEUH003095	7	KT861511
32		RVDL9	FEUH003092	1	KT861512
33		RVDL14	FEUH002090	8	KT861515
34		RVDL17	FEUH002335	11	KT861517
35	*C. serrulata*	RVD20002	FEUH002092	3	KT861520
36		RVD20008	FEUH002094	3	KT861521
37		RVD20051	FEUH002095	9	KT861522
38		RVD20053	FEUH002096	9	KT861523
39		RVD20059	FEUH002097	10	KT861524
40		RVD20062	FEUH002077	11	KT861525
41		RVDJ8	FEUH003091	1	KT861526
42		RVDL3	FEUH003097	7	KT861527
43		RVDL8	FEUH003090	1	KT861528
44	*C. sertularioides*	RVD20003	FEUH002098	3	KT861529
45		RVD20006	FEUH002099	3	KT861530
46		RVD20007	FEUH002100	3	KT861531
47		RVD20052	FEUH002101	9	KT861532

[a]refer to Table 2
[b]morphologically identified as *C. webbiana*
[c]morphologically identified as *C. racemosa*

silica gel were ground in liquid nitrogen. Genomic DNA was extracted using either the CTAB protocol described in Zuccarello *et al.* (2006) with no enzyme treatment or the Qiagen Plant MiniKit (Valencia, CA, USA) following the manufacturer's protocol.

PCR amplification was carried out using the protocols of Kazi *et al.* (2013) or Famà *et al.* (2002). The PCR primer combinations *tuf*AF-*tuf*AR (Famà *et al.* 2002), *tuf*AF1-*tuf*AR1/ *tuf*AR2/ *tuf*AR3 (Stam *et al.* 2006), and the combinations thereof were used. A total volume of 30 µL PCR cocktail containing 1× Buffer A (Vivantis™, Subang Jaya, Selangor, Malaysia); 2.5 mM $MgCl_2$, 0.75 mM of each primer; 0.2 mM dNTP; 0.5 × Taq polymerase; and 2.0 µL (ca. 30-100 $\mu g/\mu L^{-1}$) genomic DNA template were used. The thermocycler profile had an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, and an extension at 72 °C for 2 min. The final extension held at 72 °C for 5 min. The amplicons were checked through 1% agarose gel electrophoresis and were sent to FirstBase (Seri Kembangan, Selangor, Malaysia) for purification and sequencing. Contiguous sequences were assembled using DNA Baser ver. 3.5 (Heracle Biosoft, Argeș, Romania).

DNA Barcode Analysis

An initial phylogenetic tree was constructed using a matrix containing representative *Caulerpa* species with available *tuf*A accessions. The final aligned matrix was 626 nt in length and contained only the 47 newly generated *tuf*A sequences and 33 published ones. The newly generated partial *tuf*A sequences covered the nt positions 295–920 in the complete *tuf*A gene (1230 nt) of *Caulerpa chemnitzia* (as *C. racemosa* in Genbank NC032042; Lam and Lopez-Bautista 2016). Reference *tuf*A sequences as proposed by Belton *et al*. (2014) were particularly included for the *Caulerpa racemosa-Caulerpa peltata* complex: *C. chemnitzia* (AJ512415, lineage 6); *Caulerpa lamourouxii* (KF256083, lineage 1); *Caulerpa oligophylla* (KF256085, lineage 2); and *C. racemosa* (KU761464, lineage 11). For those species with yet to have proposed barcode reference, we chose representative *tuf*A sequences generated from those identified specimens collected closest to their respective type locations: for *Caulerpa cupressoides* (type from St. Croix, Virgin Island, Vahl (1802)]; *Caulerpa elongata* [syntypes from Makassar (Ujung Pandang), Celebes, Indonesia and Tongatapu, Tonga, Weber-van Bosse 1898]; *Caulerpa sertularioides* (type from *Coraliis americanis*, Gmelin 1768 or tropical America, Lipkin and Silva 2002); and *Caulerpa webbiana* [type from Arrecife, Isla Lanzarote, Islas Canarias (Canary Islands), Montagne 1837] – the conspecific specimens from Florida USA (DQ652345, Stam *et al.* 2006); Palau (FR848332, Draisma *et al.* 2014); Martinique, Lesser Antilles (AJ417944, Famà *et al.* 2002); and Portugal (FM956073, Draisma *et al.* 2014) were used as reference barcodes, respectively. Since *Tuf*A sequence generated from *C. serrulata* collected from or near to its type locality in Yemen was unavailable, a confirmed conspecific specimen of *C. serrulata* (AJ512411) identified by de Senerpont Domis *et al.* (2003) – collected from Cape Bolinao, Pangasinan, Philippines – was used. For this study, species membership was determined on the basis of nucleotide divergence with only 0–4 nt differences as compared against their respective reference sequences. The phylogram was inferred under the Neighbor-joining (NJ) algorithm based on Tamura-3 parameter (T92+G) corrected distances as implemented in MEGA ver. 7.0 (Kumar *et al.* 2016). Branch support was assessed using 1000 bootstrap pseudoreplications.

RESULTS

Morphology-based Identification

We morphologically discriminated our entire collection to six taxa, namely *C. chemnitzia, C. cupressoides, C. racemosa*, *C. serrulata*, *C. sertularioides*, and *C.*

Table 4. Initial species assignment based on morphological features.

Species name	Observed diagnostic features	Remark[a]
C. chemnitzia	fronds with clavate, convex, to discoid ramuli	matched with *tuf*A conspecific barcodes
C. cupressoides	fronds with spiniform and upcurved ramuli	matched with *tuf*A conspecific barcodes
C. racemosa	fronds with clavate to spherical ramuli	matched with tufA conspecific barcodes, except isolates RVD20057, RVD20064, and RVDL18 whose genetic identification belongs to *C. oligophylla*
C. serrulata	fronds with twisted and canaliculated branches	matched with *tuf*A conspecific barcodes
C. sertularioides	fronds with slender, elongate, cylindrical, and distichous ramuli	matched with *tuf*A conspecific barcodes
C. webbiana	fronds with whorled and distichous ramuli running along the stolons, some stolon tips are deciduous; the whorled frond ramuli are longer than the those on the stolons	molecularly identified as *C. elongata*

[a]For this study, a matched conspecific barcode indicates 0 to 4 nt differences as compared to its respective reference sequences

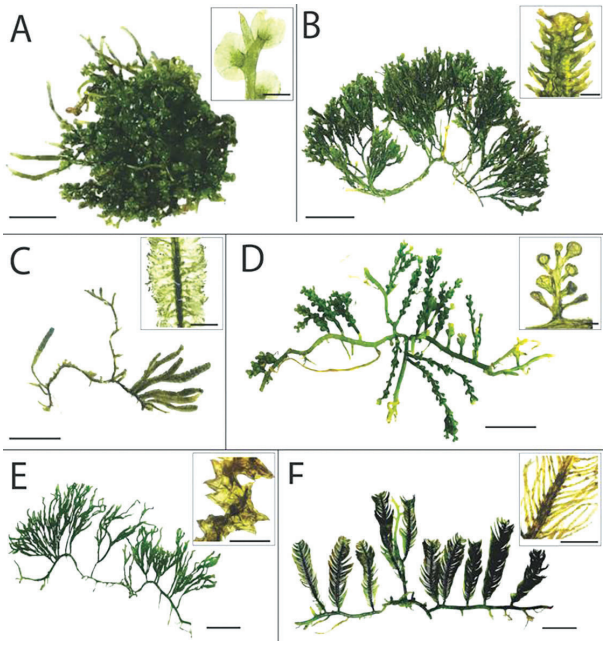

Figure 3. Habit of *Caulerpa* species from the northern Philippines with respective inset showing detailed part of a frond. (A) *C. chemnitzia*, FEUH002081, scale = 1 cm. Apical portion of a frond bearing peltate ramuli, scale = 2 mm. (B) *C. cupressoides*, FEUH002073, scale = 2 cm. Apical portion of frond bearing distichous elongate ramuli, scale = 1 mm. (C) *C. elongata,* FEUH003088, scale = 2 cm. Middle portion of a frond showing whorled and distichous ramuli, scale = 1 mm. (D) *C. oligophylla,* FEUH002078, scale = 2 cm. Apical portion of a frond bearing spherical to clavate ramuli, scale = 3 mm (E) *C. serrulata*, FEUH002096, scale = 1 cm. Middle portion of a frond with twisted and canaliculated branch, scale = 2 mm (F) *C. sertularioides,* FEUH002101, scale = 2 cm. Middle portion of a frond issuing oppositely-arranged cylindrical ramuli, scale = 2 mm.

webbiana. The diagnostic features for each of these species are summarized in Table 4. Habit representatives of *Caulerpa* species used in this study are shown in Fig. 3.

DNA Barcode-based Identification

When we integrated our analysis of *tuf*A barcodes to our data derived from morphology, all but "*C. webbiana"* and three specimens identified as *C. racemosa* from Sta. Ana, Cagayan (FEUH002250, FEUH002252, and FEUH003098) matched unambiguously with those reference *tuf*A barcodes – each represented by a distinct conspecific group of sequences in the NJ tree (Fig. 4). It appeared that those three morphologically defined *C. racemosa* specimens from Cagayan belonged genetically to *C. oligophylla*. Our morphologically identified specimens "*C. webbiana"* were found to be genetically conspecific to *C. elongata*.

DISCUSSION

The core objective of the study was to make use of DNA barcodes to assess the diversity of *Caulerpa* in the northern Philippines. Our *tuf*A gene dataset confirmed the occurrence of five previously recorded species from the area, namely *C. chemnitzia, C. cupressoides*, *C. racemosa*, *C. serrulata*, and *C. sertularioides*. For the first time, *C. elongata* and *C. oligophylla* were recorded for the region; the nearest sites being the southeastern (Taylor 1977) and western Luzon Island (de Senerpont Domis *et al.* 2003), respectively. Specimens GENT FL1174 and 1175 (supplementary data, table S1 in Draisma *et al.* 2014) collected from Apo Island, Negros Oriental, Philippines were identified as *C. elongata* but previously published in Genbank as *Caulerpa flexilis* (FM986357). The total

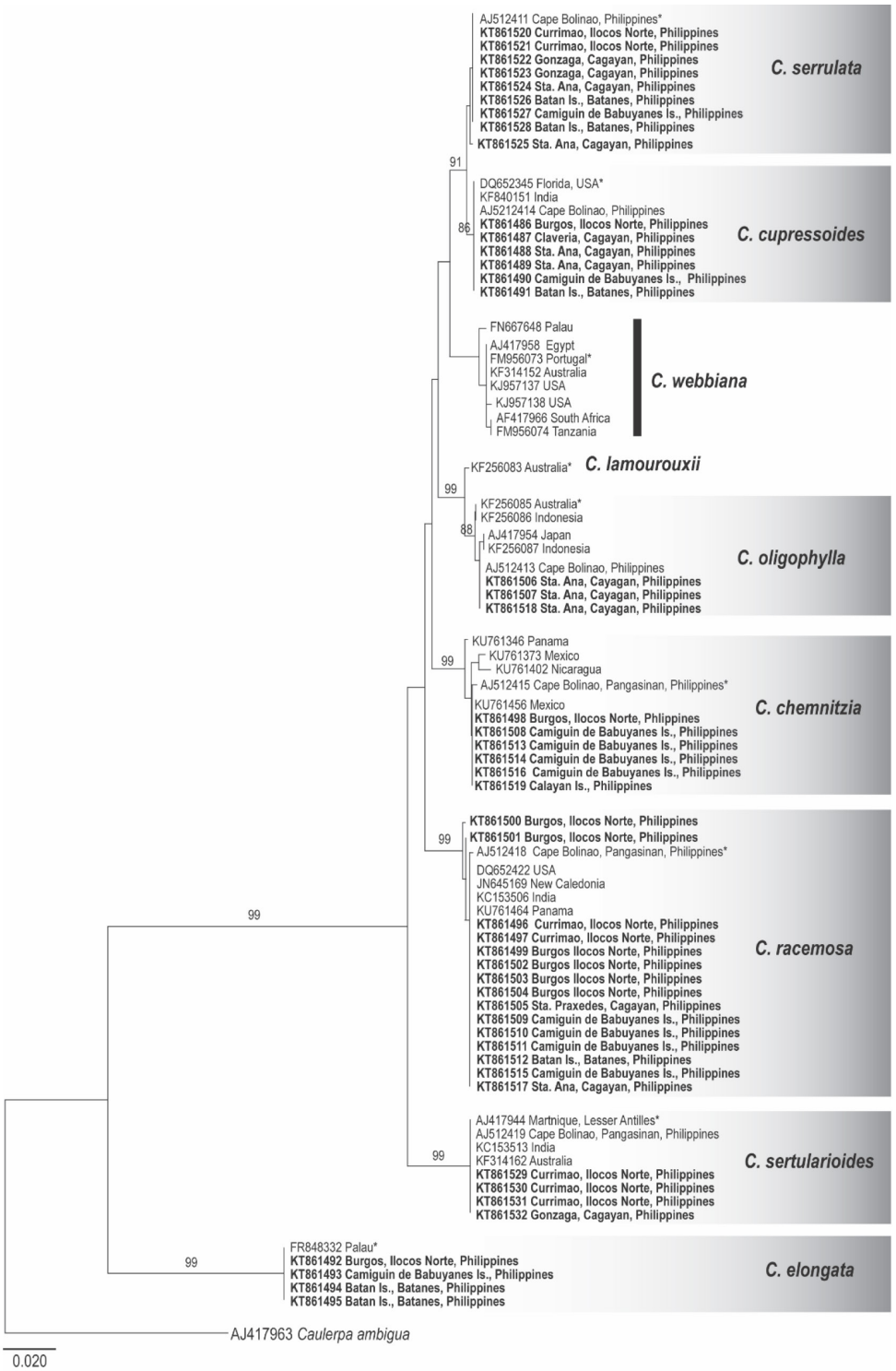

Figure 4. Neighbor-joining (NJ) tree based on *tuf*A gene sequences. Numbers above nodes indicate bootstrap support. Support values <85 are not shown. An asterisk indicates DNA barcode/reference sequence.

number of described *Caulerpa* from the northernmost record in the Philippines now stands at 15 with 10 subspecific entities (Table 1). We expect that additional surveys and molecular investigations for *Caulerpa* in other parts of their range in the northern Philippines will help to re-evaluate the occurrence of other species unconfirmed here or will render new distributional records for the area.

Confirmed barcodes of *C. elongata* from the northern

Philippines cast doubt on previous records of *C. webbiana* for the region. *C. webbiana* was recorded from Burgos, Ilocos Norte (Cordero 1983, Trono 2004) – the same site where we confirmed the presence of *C. elongata.* The illustration provided by Trono (2004) for his *C. webbiana* var. *pickeringii* resembles our *C. elongata* samples in habit. *C. elongata* and *C. webbiana* have been historically uncertain as to how they can be discriminated unambiguously (Weber-van Bosse 1898, Eubank 1946, Coppejans 1992, Price 2011, N'Yeurt and Payri 2007). In *C. elongata,* the whorled frond ramuli are longer than those on the stolons (Price 2011). The ramuli might also cover the stolon extending to their tips (Weber-van Bosse 1898). These characters were also observed among our samples, albeit the same traits also seen under the phenotypic continuum of *C. webbiana* (Weber-van Bosse 1898, Price 2011). Recent molecular evidences indicated that *C. elongata* and *C. webbiana* uphold species distinction and are indeed genetically distant from each other (Draisma *et al.* 2014). Molecular characterization of the specimens going under the name *C. webbiana* collected from the region, or elsewhere, is recommended.

The northern Philippines *C. racemosa* represented at least two morphologically cryptic species in need of genetic evaluation prior to reliable species placement. We identified *C. oligophylla* from our Sta. Ana, Cagayan collection, from which *C. racemosa* was also confirmed morphologically and genetically. The original *C. oligophylla* is characterized by its creeping stolon devoid of ramuli (Montagne 1842, Belton *et al.* 2014). Le Hau *et al.* (2015), however, alluded that – together with *C. lamourouxii – C. oligophylla* closely resembles *C. racemosa*, having a few to numerous spherical ramuli that are oppositely arranged along the assimilator. Our recent collection of *C. oligophylla* conformed to that description. Testing whether the species should be treated as *C. oligophylla*, *C. lamourouxii*, or *C. racemosa* needs confirmation by DNA sequence data (Belton *et al.* 2014).

In conclusion, we expanded the available DNA barcodes to include *tuf*A gene sequences of *Caulerpa* from the northern Philippines and revised the records of *Caulerpa* from this region in an updated list of species and distributions. It is hoped that this study will serve as a reference for future elucidation of the complete species diversity of this economically important genus in the Philippines.

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