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Research Article

Further expansion of the alien seaweed *Caulerpa taxifolia* var. *distichophylla* (Sonder) Verlaque, Huisman & Procacini (Ulvophyceae, Bryopsidales) in the Eastern Mediterranean Sea

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Abstract

Caulerpa taxifolia var. *distichophylla* (Sonder) Verlaque, Huisman and Procacini is a green alga of Australian origin recently reported as an alien species in the Mediterranean Sea, where it is known from SE Turkey, Sicily, Cyprus and Malta. In the current study we present additional records of this taxon, expanding its known distribution into the Eastern Mediterranean Sea, and provide additional records from Cyprus and the first records from Rhodes Island (Greece). Our specimens were identified through a combination of morphological and molecular methods involving sequencing of ITS and tufA. Locally, *C. taxifolia* var. *distichophylla* occurred in high abundances and dominated the benthic community, suggesting that has the potential to become a major pest in the Mediterranean. It was also observed over a very wide depth range, from the sea surface to at least 100 m depth, on a variety of natural soft and hard substrates as well as abandoned fishing nets, suggesting a broad environmental plasticity. One of the findings reported here constitutes the deepest record of an alien *Caulerpa* in the Mediterranean Sea, even though it remains to be demonstrated that it actually grows at this site and depth rather than being merely a drift specimen.

Key words: alien, *Caulerpa taxifolia* var. *distichophylla*, green algae, Eastern Mediterranean, Cyprus, Rhodes

Introduction

The genus *Caulerpa* is represented by at least 7 taxa in the Mediterranean Sea, namely: *C. prolifera* (Forsskål) J.V. Lamouroux; *C. chemnitzia* (Esper) J.V. Lamouroux [as *Caulerpa racemosa* var. *occidentalis* (J.Agardh) Børgesen]; *C. mexicana* Sonder *ex* Kützing; *C. scalpelliformis* (R. Brown *ex* Turner) C. Agardh; *C. taxifolia* (M. Vahl) C. Agardh; *C. cylindracea* Sonder; and *C. racemosa* var. *lamourouxii* f. *requienii* (Montagne) Weber-van Bosse (Verlaque et al. 2000; Verlaque et al. 2003; Cormaci et al. 2014; Guiry and Guiry 2014). With the sole exception of *C. prolifera*, all *Caulerpa* taxa are considered to be alien introductions to the Mediterranean Sea (Zenetos et al. 2010). Among them, *C. cylindracea* and *C. taxifolia* are included in the list of worst alien invaders for the Mediterranean Sea (Streftaris and Zenetos 2006), occasionally having severe effects in the marine environment (Meinesz et al. 2001; Klein and Verlaque 2008).

Recently, an additional *Caulerpa* taxon has been reported from the Mediterranean Sea, namely *Caulerpa taxifolia* var. *distichophylla* (Sonder) Verlaque, Huisman and Procaccini. It was first reported as *C. taxifolia* from the coasts of South Turkey (Cevik et al. 2007), and later reported from Sicily (Cormaci and Furnari 2009; Meinesz et al. 2010; Jongma et al. 2013; Musco et al. 2014), Cyprus

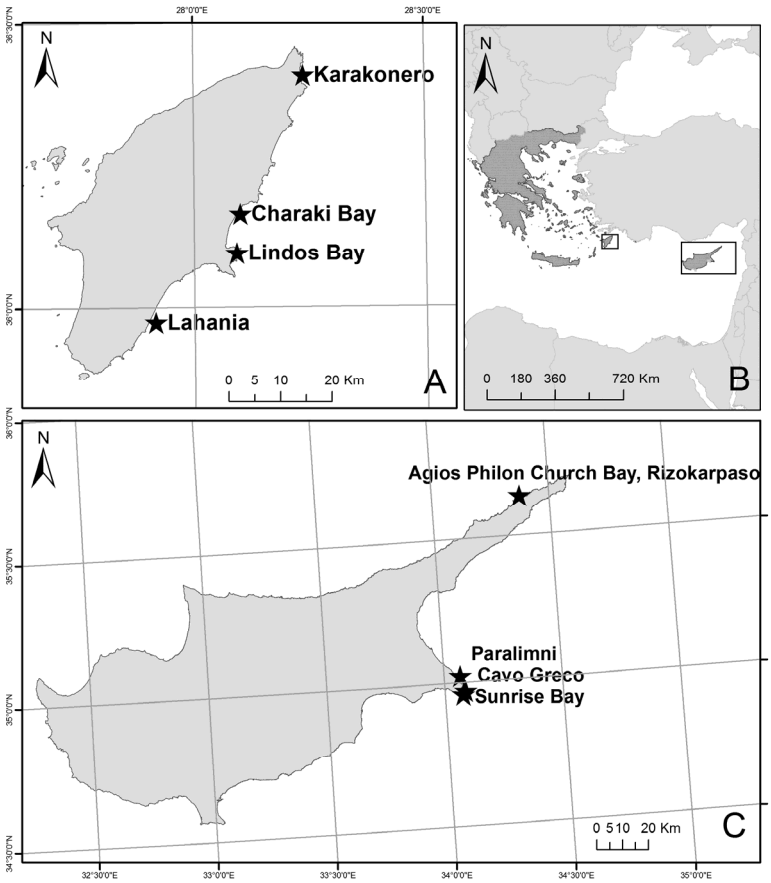

Figure 1. Map of sites where *Caulerpa* specimens were found. A: Rhodes; B: Overview of the region; C: Cyprus. Rhodes sites: 1–3/11/2010 – Lindos Bay, 17/06/2014 – Charaki Bay, 12/09/2014 – Karakonero Bay, 14/10/2014 – Lahania. Cyprus sites: June 2009 – Agios Philon Church Bay, Rizokarpaso (Dipkarpaz), 27/06/2012 – Sunrise Bay, 5/7/2012 & 30/10/2014 – Cavo Greco.

(Çicek et al. 2013; Tsiamis et al. 2014) and Malta (Schembri et al. 2015). Based on molecular work, it was shown that *C. taxifolia* var. *distichophylla* is also an alien species in the Mediterranean Sea, originating from Australia (Jongma et al. 2013).

In the current paper we present data showing further expansion of *C. taxifolia* var. *distichophylla* in the Eastern Mediterranean Sea (identified on the basis of both morphological and molecular data) and a survey of different benthic habitats affected by this new arrival. Besides reporting additional records from Cyprus, we report the taxon for the first time from Greece (Rhodes Island).

Materials and methods

Sampling

During 1–3 November 2010, benthic sampling was conducted along the coasts of Rhodes Island and a few individuals of *Caulerpa taxifolia* var. *distichophylla* were collected at a depth of 20 m with a Smith McIntire grab (0.1 m^2 surface) on crushed shells and sand substrate in Lindos Bay (east of Rhodes Island, SE Greece, Figure 1). These samples were preserved in ethanol. Additional surveys and sampling of the species were conducted on 17 June 2014 by scuba diving on hard substrate at Charaki Bay on the east coast of Rhodes down to 18 m depth. Samples from Charaki Bay were preserved in CTAB (Cetyltrimethylammonium-bromide) buffer (Gachon et al. 2009) for DNA extraction.

On 27 June 2012, while surveying the coastal waters of Cyprus, a few specimens of *Caulerpa taxifolia* var. *distichophylla* were collected with a Van Veen grab (0.1 m^2 surface) from 34 m depth, off Sunrise Bay on the east coast of Cyprus. On 5 July 2012, specimens of the same taxon were collected from two depths (22 and 42 m) along a single line transect in Cavo Greco. Additional specimens were collected from the 42 m station on 28 February 2014.

Finally, diving-based surveys and sampling of *C. taxifolia* var. *distichophylla* were conducted in Cyprus at Cavo Greco, on the east coast of Cyprus at depths between 36 – 40 m, on July 16, 2014.

Table 1. Herbarium specimens of *Caulerpa taxifolia* var. *distichophylla* originating from this study. Herbarium abbreviations follow Thiers (2014).

Collection date and site	Herbarium / Accession number
June 27, 2012 – Sunrise Bay, Cyprus (34 m)	HDFMR89 (Dept. of Fisheries and Marine Research, Cyprus)
June 5, 2012 – Cavo Greco, Cyprus (22 m)	HDFMR90 (Dept. of Fisheries and Marine Research, Cyprus)
July 5, 2012 – Cavo Greco, Cyprus (42 m)	HDFMR91 (Dept. of Fisheries and Marine Research, Cyprus)
June 17, 2014 – Charaki, Rhodes	BM001180495 (London, UK)
June 17, 2014 – Charaki, Rhodes	PC0167419 (Paris, France)
June 17, 2014 – Charaki, Rhodes	ABDUH:1/42666 (University of Aberdeen, Scotland, UK)
June 17, 2014 – Charaki, Rhodes	UC2037100 (Berkeley CA, USA)
June 17, 2014 – Charaki, Rhodes	ATHU (East Mediterranean Seaweed Herbarium at the National and Kapodistrian University of Athens, Greece)
February 28, 2014 – Cavo Greco, Cyprus	BM001180496 (London, UK)
February 28, 2014 – Cavo Greco, Cyprus	PC0167420 (Paris, France)
February 28, 2014 – Cavo Greco, Cyprus	ATHU (East Mediterranean Seaweed Herbarium at the National and Kapodistrian University of Athens, Greece)

Table 2. Specimens used for DNA sequencing in the present study.

Species name	Date of collection	Locality	% identity to closest relative with publicly available sequences (%)	Query cover (%)	e value	EBI accession numbers for new sequences (chloroplast partial tufA gene for elongation factor TU)	EBI accession numbers for new sequences (each containing 3'-18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2 and 5'-28S rRNA gene)
Caulerpa taxifolia var. *distichophylla*	17/06/ 2014	Rhodes Island, Greece	100	100	0	LN559617	-
Caulerpa taxifolia var. *distichophylla*	28/02/ 2014	Cavo Greco, Cyprus	100	100	0	LN559618	-
Caulerpa taxifolia var. *distichophylla*	17/06/ 2014	Rhodes Island, Greece	99	99	0	-	LN559619
Caulerpa taxifolia var. *distichophylla*	28/02/ 2014	Cavo Greco, Cyprus	99	99	0	-	LN559620

In addition, the taxon was found in the Paralimni area by snorkeling, at 0.5 to 0.8 m depth, on July 18, 2014. The most recent findings of *C. taxifolia* var. *distichophylla* were from Karakonero Bay, Rhodes (12 September 2014 at 35 m depth and Lahania, Rhodes (14 October 2014 at 20, 50, and 100 m depths); collected at both stations with a Smith McIntyre grab. One final sample was collected with a Van Veen grab at a depth of 48 m off Cavo Greco, Cyprus, on 30 October 2014.

Herbarium specimens of *Caulerpa taxifolia* var. *distichophylla* were deposited in herbaria of the University of Aberdeen, UK Scotland (ABD); the Natural History Museum, London (BM); the Muséum National d'Histoire Naturelle, Paris (PC); the University of California, Berkeley (UC); and the East Mediterranean Seaweed Herbarium at the National and Kapodistrian University of Athens (ATHU) (Table 1). The 2012 specimens from Cyprus were preserved in 4% formaldehyde-seawater and deposited in the collection of the Department of Fisheries and Marine Research, Cyprus (HDFMR; Table 1). The 2012 specimens were compared to a few small unclassified *Caulerpa* specimens deposited in the HDFMR collection since 2008: HDFMR44, *Caulerpa* sp. Ammochostos district area – SE coast of Cyprus, 2008.

Identification based on morphological features

The following characters were assessed for the morphological identification of specimens: stolon width, the shape, width and length of fronds and pinnules, the number of rhizoidal pillars and pinnules, and the morphology and width of the midrib.

DNA extraction, PCR amplification and sequencing

DNA extractions were performed on material from specimens collected from both Cyprus and Rhodes in 2014 (Table 2) preserved in CTAB extraction buffer (Gachon et al. 2009) using DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). A mortar and pestle were used in order to grind the algal tissue and then 700µL CTAB (preheated to 65ºC) were added to the ground material, followed by disruption with TissueLyser II (Qiagen). The subsequent protocol followed the procedure as in DNeasy Plant Mini Kit.

Polymerase chain reaction (PCR) was performed using specific primer pairs for the *tuf*A gene and ITS region, that had previously been used for *Caulerpa* spp. (Famà et al. 2002; Jongma et al. 2013; Stam et al. 2006). The chloroplast *tuf*A gene amplified with the primer pair tufAF-tufAR and using PCR conditions slightly modified from those reported by Famà et al. (2002). PCR amplification was performed in a total volume of 25 µL, containing 1.25 units/µL of Taq DNA Polymerase (Promega), 1 × GoTaq™ buffer, 5 mM $MgCl_2$, 1.25 mM dNTPs, 1.87 mM of each primer and 1 µL of template DNA (5–50 ng/µl). PCR amplification was carried out with the following profile: one cycle at 94° C for 4 min; 10 cycles at 94° C for 15 s, 1 min at 50° C and 2 min at 72° C; 35 cycles of 15 s at 94° C, 20 s at 50° C and 2 min at 72° C and one cycle at 72° C for 10 min.

For the nuclear ribosomal ITS1+2 region the primer pair JO4–JO6 developed by Stam et al. (2006) was used. PCR reactions were performed in a total volume of 25 µL and used 0.25 units/µL of Taq DNA Polymerase (Promega), 1 × GoTaq™ Buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 mM of each primer and 1 mL template DNA (5-50 ng/µl). The PCR profile included one cycle of 2 min at 94° C; 10 cycles of 15 s at 94° C, 1 min at 50° C and 2 min at 72° C; 30 cycles of 15 s at 94° C, 20 s at 50° C and 2 min at 72° C; and one cycle of 10 min at 72° C.

PCR products were run on a GelRed™ (Biotium) TBE agarose (1.2 %) gel to check for amplification and correct length. A single reaction was purified using the QIAquick PCR Purification Kit (Qiagen) and sent for sequencing using the Source Bioscience sequencing service. Approximately 50 ng of purified PCR products were sequenced using the original PCR primers.

The alignment of each gene sequence was created with BioEdit Sequence Alignment Editor (Hall 1999) and then the sequences were compared to published data by means of NCBI BLAST searches (Altschul et al. 1997).

Phylogenetic analysis

Publicly available sequences (Jongma et al. 2013) were merged with sequences newly generated in this study and aligned in MEGA6.0 (Tamura et al. 2013) using the ClustalW algorithm. Alignments were refined by eye. Finally Maximum Likelihood (ML) phylogenies were inferred under the Tamura–Nei model of evolution with gaps being treated as pairwise deletions and 1000 bootstrap pseudo-replicates to ensure statistical support of the nodes.

Results

Identification

Based on morphology, the *Caulerpa* specimens from Cyprus and Rhodes were identified as *Caulerpa taxifolia* var. *distichophylla*. They were characterized by a light green thallus, feather-like, delicate, with narrow postrate stolons and erect fronds bearing pinnules (Figure 2A); stolons slender, 0.4–1.0 mm thick, with short rhizoidal pillars 1–4 mm long (Figure 2B); about 13 pillars per 10 cm of stolon; erect fronds simple to 3 times branched, 2.5–10 cm high and 1.5–4.2 mm wide; erect fronds terete below, compressed towards the apex, bearing distichously and closely arranged (but never overlapping) pinnules in one plane; pinnules compressed, 0.8–2.3 mm long and 0.3–0.5 mm wide, up-curved, slightly constricted at their base and gradually tapering into a pointed tip (Figure 2C). No fertile specimens were found.

Field observations

In Cyprus, *C. taxifolia* var. *distichophylla* was found both in very shallow waters (Figure 3) and at 42 m depth (Figure 3); however, it was less dominant at the two Cypriot sites than at Charaki in Rhodes. The Cypriot specimens from the Cavo Greco area were collected from sediment comprised of fine biogenic sand: shell fragments/foraminiferans (at 34 m depth), dominated by the non-native seagrass *Halophila stipulacea* (Forsskål) Ascherson (at 42 m depth) and on muddy substratum dominated by *C. racemosa* var. *cylindracea* with scattered specimens of *Codium bursa* (Olivi) C. Agardh (48 m depth), respectively. They were also found growing on biogenic hard substrate (serpulid tubes

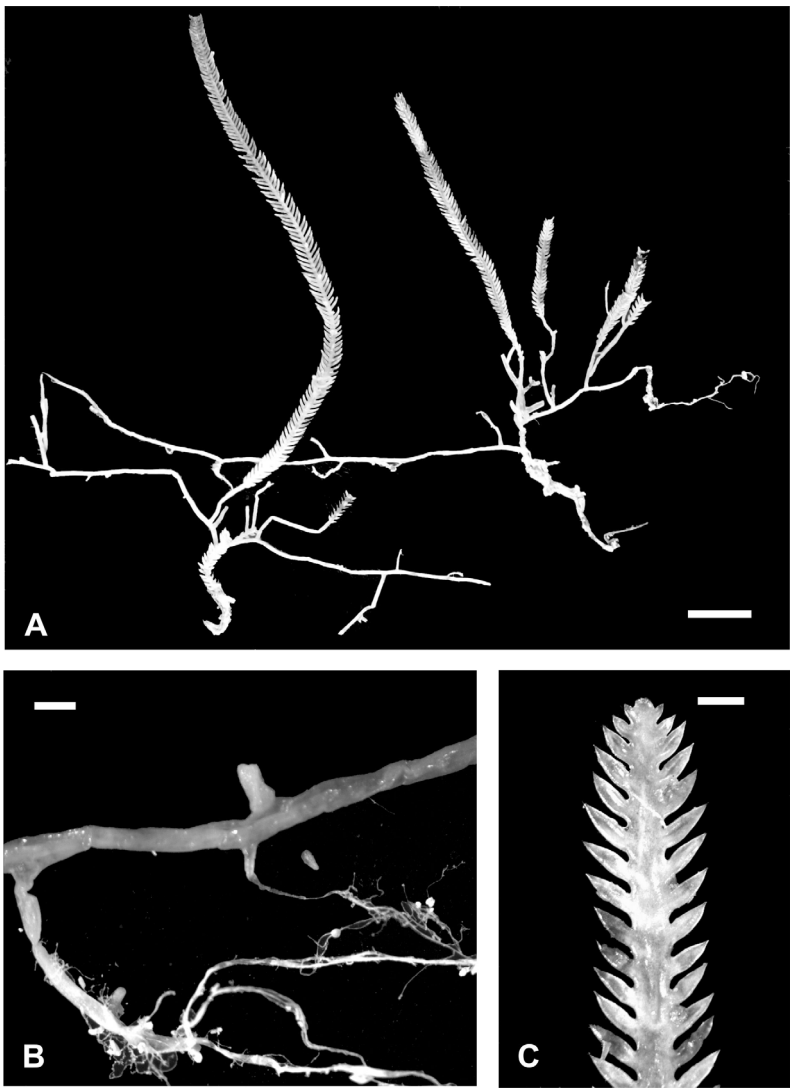

Figure 2. Morphological features of the *Caulerpa* specimens (HDFMR91). (A) Part of the thallus: scale bar 1 cm; (B) Details of stolon and rhizoidal pillars: scale bar 1 mm; (C) Details of the upper part of the frond and pinnules: scale bar 1 mm. Photographs by M. Aplikioti (DFMR).

and calcareous algae; at 22 m depth). During the survey at Charaki/Rhodes in June 2014, *C. taxifolia* var. *distichophylla* was found to occur in dense, monospecific mats between 9 and 18 m depth, on a variety of substrates (rocky, pebbles/shells, sand) in the entire area surveyed. Similar observations were made in Cyprus, where *C. taxifolia* var. *distichophylla* was found to occur in both deep (36 – 40 m) and shallow waters (<1 m), but it was never the dominant species. It sparsely covered the sandy substratum of Cavo Greco, along with *C. prolifera,* and it was present in small numbers on the rocky substratum of Paralimni. *C. taxifolia* var. *distichophylla* was found at 35 m depth at Karakonero bay and at 20, 50 and 100 m depth off Lahania. At Karakonero the substrate consisted of sand with *Halophila stipulacea*, while at Lahania it was very fine sand /almost mud with *Cymodocea nodosa* (Ucria) Ascherson at -20 m, and mud with *Halophila stipulacea* (-50 m) and mud (-100 m), respectively.

Molecular phylogeny

The cpDNA *tuf*A sequences from specimens collected from Rhodes and Cyprus (Cavo Greco) were 745 bp and 788 bp long, respectively. These sequences provide strong support that the specimens from Rhodes and Cyprus belong to *Caulerpa taxifolia* var. *distichophylla* (98/100).

The rDNA ITS1-5.8S-ITS2 sequences from Rhodes and Cyprus (Cavo Greco) were 587 bp long

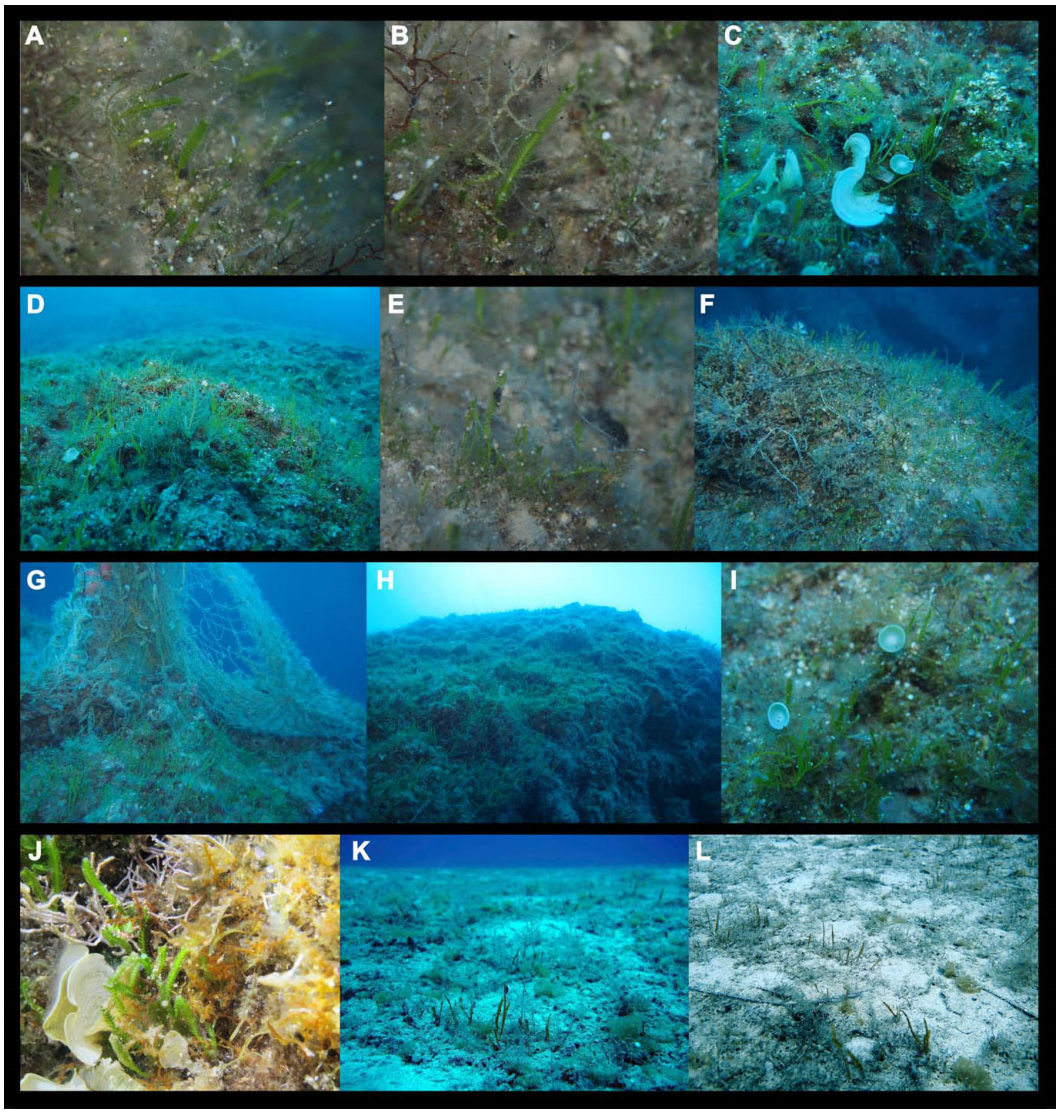

Figure 3. *Caulerpa taxifolia* var. *distichophylla* in situ off the coast of Rhodes (Charaki, A-I) and Cyprus (Paralimni, J; Cavo Greco, K-L), respectively. A-B: Macrophotographs of *C. taxifolia* var. *distichophylla*, showing both individual phylloids and stolons on macrophytobenthos turf on a steeply inclined underwater cliff. C: *C. taxifolia* var. *distichophylla* in community with *Padina* sp. and *Acetabularia* sp.. D-F: *C. taxifolia* var. *distichophylla* on undulating, otherwise flat soft-bottom benthos. E: In several instances, the snail *Bittium reticulatum* was observed on *C. taxifolia* var. *distichophylla*, possibly grazing on it. F: *C. taxifolia* var. *distichophylla* invading and overgrowing a *Cystoseira* community. G: *C. taxifolia* var. *distichophylla* colonizing an abandoned fishing net. H: Upward view of a dense community of *C. taxifolia* var. *distichophylla* covering a steeply inclined underwater cliff from around 15 m depth. I: At the same site, *C. taxifolia* var. *distichophylla* in a community with *Acetabularia* sp. J: *C. taxifolia* var. *distichophylla* in shallow inshore waters in Cyprus, in a community with *Padina* sp. and *Laurencia* sp.. K-L: Deep-water community (around 38 m depth) of *C. taxifolia* var. *distichophylla* at Cavo Greco, in a community with the native *Caulerpa prolifera*. Photographs by F.C. Küpper (Aberdeen; A-I), M. Marcou (DFMR; J-L).

and 586 bp, respectively. Likewise, the sequences obtained show strong support that the specimens from Rhodes and Cyprus fall within the *Caulerpa taxifolia* var. *distichophylla* (85/100).

All sequences of *tuf*A and ITS1-5.8S-ITS2 from both areas have been submitted to EMBL (European Bioinformatics Institute) and accession numbers obtained (Table 1). In Maximum Likelihood trees of both *tuf*A and ITS sequences, *C. taxifolia* var. *distichophylla* from both Rhodes and Cyprus clustered with specimens from Italy, Turkey, and Australia and were distinctly different from *C. taxifolia* from southern France and other localities (Figures 4 and 5).

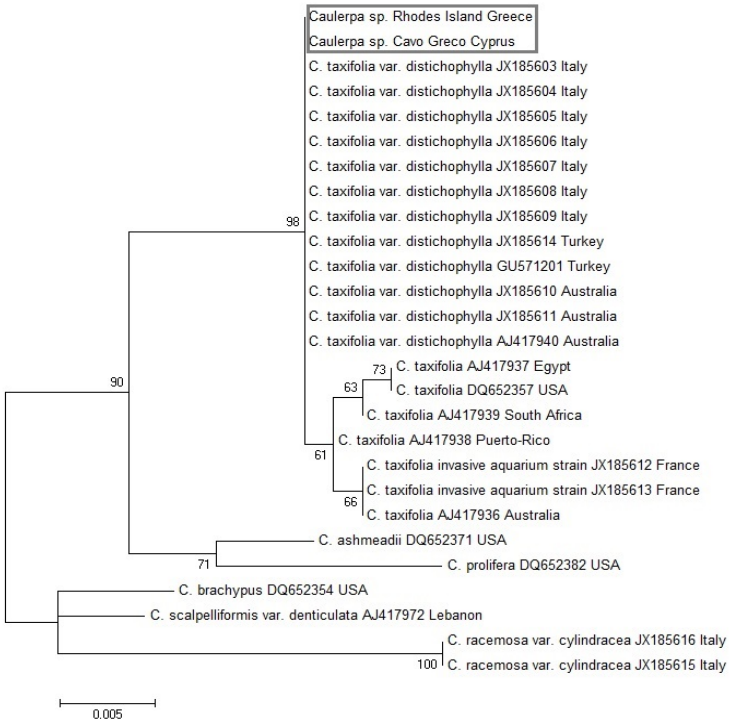

Figure 4. Maximum likelihood test of phylogeny of the *tuf*A cpDNA gene sequences obtained from the *Caulerpa taxifolia* var. *distichophylla* under investigation in this study. The grey box indicates sequences of *Caulerpa taxifolia* var. *distichophylla* in this study from Rhodes Island in Greece and Cavo Greco in Cyprus.

Discussion

Based on morphological features and molecular analyses, the green alga from Cyprus and Rhodes was confirmed to be *Caulerpa taxifolia* var. *distichophylla*. The sequences of *tuf*A and ITS were identical to previously published sequences from specimens of *C. taxifolia* var. *distichophylla* from Sicily (Jongma et al. 2013). In general, due to the morphological plasticity that characterizes the genus, identification of *Caulerpa* species based solely on morphological features can result in high misidentification rates (>12%), and is considered unreliable without supplementary use of molecular methods (Olsen et al. 1998; Stam et al. 2006). Indeed, the morphology of *C. taxifolia* var. *distichophylla* is somewhat similar to *C. cupressoides* (M. Vahl) C. Agardh; however, the latter is currently not known from the Mediterranean. Our specimens do not exhibit the usual morphologies of *C. mexicana* or *C. taxifolia*, or the typically robust morphology of the invasive aquarium strain of the latter, but they seem to agree with the description of a *C. taxifolia* var. *distichophylla* population found in south eastern Turkey by Cevik et al. (2007, as *C. taxifolia*).

The tropical green alga *C. taxifolia* was accidentally introduced to the Mediterranean Sea in 1984 from the Aquarium in Monaco (Meinesz and Hesse 1991; Jousson et al. 1998) and since then it has spread to at least seven Mediterranean countries: Croatia, France, Italy, Monaco, Spain and Tunisia (Meinesz et al. 2001). Its negative effect on benthic systems is significant and has led to concerns, e.g. for the decline of seagrass meadows in southern France and elsewhere in the Mediterranean, even though a causal link could not always be established (Glasby 2013). On the other hand, *C. mexicana* has been found in the Mediterranean since at least the 1940s (Olsen et al. 1998) and is mostly common in the Eastern Basin, where it has been reported from Israel, Lebanon, Syria and Egypt (Ukabi et al. 2012; Ukabi et al. 2014), with apparently little knowledge about its ecological impact.

This study suggests that *C. taxifolia* var. *distichophylla* has recently spread in the Eastern Mediterranean. Originating from South Western

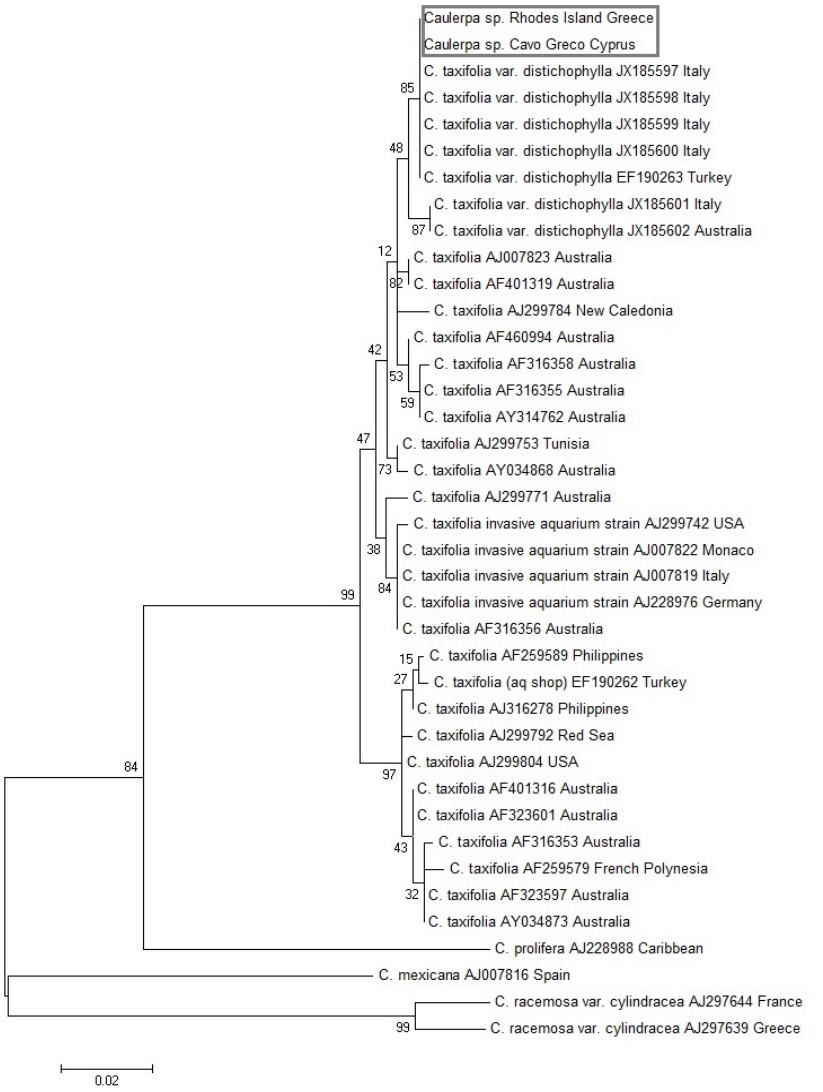

Figure 5. Maximum likelihood test of phylogeny of the ITS1-5.8S-ITS2 rDNA sequences obtained from the *Caulerpa taxifolia* var. *distichophylla* under investigation in this study. The grey box indicates sequences of *Caulerpa taxifolia* var. *distichophylla* in this study from Rhodes Island in Greece and Cavo Greco in Cyprus.

Australia where it is endemic (Jongma et al. 2013), *C. taxifolia* var. *distichophylla* was originally discovered in Iskenderun Bay, Turkey (approx. 200 km from Cyprus and 700 km from Rhodes, respectively) by Cevik et al. (2007), followed by records in South Eastern Sicily (Jongma et al. 2013; Musco et al. 2014), Cyprus (Çicek et al. 2013; Tsiamis et al. 2014) and, most recently, Malta (Schembri et al. 2015). Our records reveal further expansion of the taxon in the Eastern Mediterranean Sea. However, it is impossible to say at present whether this is occurring through natural dispersal or shipping or a combination of both.

Throughout this study, *C. taxifolia* var. *distichophylla* was found over a wide depth range, from the surface to at least 100 m depth. To the best of our knowledge, the findings off Lahania (Rhodes) constitute the deepest records not only of this taxon, but of any invasive *Caulerpa* species in general in the Mediterranean. However, while it seems possible that the species could survive at such a depth at that site, it is important to note that we cannot be sure whether

the plants were attached and developing. It would seem essential to confirm this record with *in situ* photography or a grab sample with substrate still attached. Moreover, a better understanding of its impact on circalittoral habitats in general would also be desirable. Klein and Verlaque (2008) reported *C. racemosa* from 0 to 70 m depths whereas the other invasive member of this genus, *C. taxifolia*, has been usually observed at lesser depths, even though the deepest record was made at 99 m at Cap d'Ail, France (Belsher and Meinesz 1995). Indeed, *C. racemosa* grows better at 30 than at 10 m depth (Cebrian and Ballesteros 2009), but it has never been recorded as deep as the *C. taxifolia* var. *distichophylla* reported here. Locally, like at the Charaki site (Rhodes Island, Greece), the taxon can be dominant, and it may be displacing native zoo- and macrophytobenthos, covering both hard and soft natural, as well as artificial (Figure substrates, thus suggesting a potentially invasive behavior. In contrast, at 38 m depth off Cavo Greco (Cyprus), the stands were much sparser than at Charaki in Rhodes. From a conservation perspective, it should be pointed out that in Rhodes, *C. taxifolia* var. *distichophylla* invades and covers *Cystoseira* communities, which are endangered in much of the Mediterranean due to a variety of anthropogenic causes (Thibaut et al. 2005). Moreover, it is noteworthy that following a two-year monitoring program covering 30 sites along the coast of Sicily, the taxon has been labelled an "invader" because of its impact on benthic system and ongoing spread in the Mediterranean Sea (Musco et al. 2014).

The genus *Caulerpa* contains several invasive taxa in the Mediterranean and other parts of the world, which correlates with the presence of an efficient wound-healing mechanism in this group (Welling et al. 2009), facilitating vegetative dispersal and establishment of new populations. At present, there is no knowledge about potential grazers of *C. taxifolia* var. *distichophylla* in the Mediterranean Sea. In several instances, the snail *Bittium reticulatum* (da Costa, 1778) was observed on *C. taxifolia* var. *distichophylla*, possibly feeding on it. This should be further investigated. In line with Musco et al. (2014), we hypothesize that *C. taxifolia* var. *distichophylla* has to be considered potentially invasive, and that the *Caulerpa taxifolia* species cluster in general seems to possess ecological features (Andreakis and Schaffelke 2012) rendering it particularly adapted to rapidly colonize the shallow-water Mediterranean ecosystem.

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References

Altschul SF, Madden TL, Schaffer AA, Zhang JH, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* 25: 3389–3402, http://dx.doi.org/10.1093/nar/25.17.3389

Andreakis N, Schaffelke B (2012) Invasive marine seaweeds: Pest or prize. In: Wiencke C, Bischof K (eds), Seaweed Biology. Springer-Verlag, Berlin, Heidelberg, Germany, pp 235–262, http://dx.doi.org/10.1007/978-3-642-28451-9_12

Belsher T, Meinesz A (1995) Deep-water dispersal of the tropical alga *Caulerpa taxifolia* introduced into the Mediterranean. *Aquatic Botany* 51: 163–169, http://dx.doi.org/10.1016/0304-3770(95)00469-G

Cebrian E, Ballesteros E (2009) Temporal and spatial variability in shallow- and deep-water populations of the invasive *Caulerpa racemosa* var. *cylindracea* in the Western Mediterranean. *Estuarine Coastal and Shelf Science* 83: 469–474, http://dx.doi.org/10.1016/j.ecss.2009.04.026

Cevik C, Yokes MB, Cavas L, Erkol LI, Derici OB, Verlaque M (2007) First report of *Caulerpa taxifolia* (Bryopsidales, Chlorophyta) on the Levantine coast (Turkey, eastern Mediterranean). *Estuarine Coastal and Shelf Science* 74: 549–556, http://dx.doi.org/10.1016/j.ecss.2007.05.031

Çicek BA, Kurt O, Taskin E, Öztürk M (2013) First report of *Caulerpa taxifolia* var. *distichophylla* (Sonder) Verlaque, Huisman and Procaccini (Caulerpaceae, Chlorophyta) from Northern Cyprus. *Rapport Commission International Mer Mediterranee* 40: 600

Cormaci M, Furnari G (2009) Progetto ISPRA. Realizzazione ed aggiornamento delle schede tassonomiche delle specie non indigene di taxa vegetali: aggi ornamento ed implementazione dei records e delle segnalazioni delle stesse specie, ove presenti, nel mar Mediterraneo e nei mari Italiani, aggiornamento e studio critico della letteratura. Aggiornamento dei dati già in possesso dell'ISPRA (ex ICRAM): acrofitobenthos. Catania, Italy

Cormaci M, Furnari G, Alongi G (2014) Flora marina bentonica del Mediterraneo: Chlorophyta. *Bollettino delle sedute della Accademia Gioenia di Scienze Naturali in Catania* 47: 11–436

Durand C, Manuel M, Boudouresque CF, Meinesz A, Verlaque M, Le Parco Y (2002) Molecular data suggest a hybrid origin for the invasive *Caulerpa racemosa* (Caulerpales, Chlorophyta) in the Mediterranean Sea. *Journal of Evolutionary Biology* 15: 122–133, http://dx.doi.org/10.1046/j.1420-9101.2002.00370.x

Famà P, Wysor B, Kooistra W, Zuccarello GC (2002) Molecular phylogeny of the genus *Caulerpa* (Caulerpales, Chlorophyta) inferred from chloroplast tufA gene. *Journal of Phycology* 38: 1040–1050, http://dx.doi.org/10.1046/j.1529-8817.2002.t01-1-01237.x

Gachon CMM, Strittmatter M, Müller DG, Kleinteich J, Küpper FC (2009) Differential host susceptibility to the marine oomycete pathogen *Eurychasma dicksonii* detected by real time PCR: not all algae are equal. *Applied and Environmental Microbiology* 75: 322–328, http://dx.doi.org/10.1128/AEM.01885-08

Glasby TM (2013) *Caulerpa taxifolia* in seagrass meadows: killer or opportunistic weed? *Biological Invasions* 15: 1017–1035, http://dx.doi.org/10.1007/s10530-012-0347-1

Guiry MD, Guiry GM (2015) AlgaeBase. World-wide electronic publication. http://www.algaebase.org. National University of Ireland, Galway.[Accessed April 24, 2015]

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98

Jongma DN, Campo D, Dattolo E, D'Esposito D, Duchi A, Grewe P, Huisman J, Verlaque M, Yokes MB, Procaccini G (2013) Identity and origin of a slender *Caulerpa taxifolia* strain introduced into the Mediterranean Sea. *Botanica Marina* 56: 27–39, http://dx.doi.org/10.1515/bot-2012-0175

Jousson O, Pawlowski J, Zaninetti L, Meinesz A, Boudouresque CF (1998) Molecular evidence for the aquarium origin of the green alga *Caulerpa taxifolia* introduced to the Mediterranean Sea. *Marine Ecology Progress Series* 172: 275–280, http://dx.doi.org/10.3354/meps172275

Jousson O, Pawlowski J, Zaninetti L, Zechman FW, Dini F, Di Guiseppe G, Woodfield R, Millar A, Meinesz A (2000) Invasive alga reaches California - The alga has been identified that threatens to smother Californian coastal ecosystems. *Nature* 408: 157–158, http://dx.doi.org/10.1038/35041623

Klein J, Verlaque M (2008) The *Caulerpa racemosa* invasion: A critical review. *Marine Pollution Bulletin* 56: 205–225, http://dx.doi.org/10.1016/j.marpolbul.2007.09.043

Meinesz A, Belsher T, Thibaut T, Antolic B, Mustapha KB, Boudouresque C-F, Chiaverini D, Cinelli F, Cottalorda J-M, Djellouli A, El Abed A, Orestano C, Grau AM, Ivesa L, Jaklin A, Langar H, Massuti-Pascual E, Peirano A, Tunesi L, de Vaugelas J, Zavodnik N, Zuljevic A (2001) The introduced green alga *Caulerpa taxifolia* continues to spread in the Mediterranean. *Biological Invasions* 3: 201–210, http://dx.doi.org/10.1023/A:1014549500678

Meinesz A, Chancollon O, Cottalorda JM (2010) Observatoire sur l'expansion de *Caulerpa taxifolia* et *Caulerpa racemosa* en Méditerranée: campagne janvier 2008 - juin 2010. Université Nice Sophia Antipolis E.A. 4228 ECOMERS Publ

Meinesz A, Hesse B (1991) Introduction of the tropical alga Caulerpa taxifolia and its invasion of the northwestern Mediterranean. *Oceanologica Acta* 14: 415–426

Meusnier I, Valero M, Olsen JL, Stam WT (2004) Analysis of rDNA ITS1 indels in *Caulerpa taxifolia* (Chlorophyta) supports a derived, incipient species status for the invasive strain. *European Journal of Phycology* 39: 83–92, http://dx.doi.org/10.1080/0967026031000164 6531

Musco L, Andaloro F, Mikac B, Mirto B, Mirto S, Vega Fernandez T, Badalamenti F (2014) Concern about the spread of the invader seaweed *Caulerpa taxifolia* var. *distichophylla* (Chlorophyta: Caulerpales) to the Western Mediterranean. *Mediterranean Marine Science* 15/3: 532–538

Olsen JL, Valero M, Meusnier I, Boele-Bos S, Stam WT (1998) Mediterranean *Caulerpa taxifolia* and *C. mexicana* (Chlorophyta) are not conspecific. *Journal of Phycology* 34: 850–856, http://dx.doi.org/10.1046/j.1529-8817.1998.340850.x

Schaffelke B, Murphy N, Uthicke S (2002) Using genetic techniques to investigate the sources of the invasive alga *Caulerpa taxifolia* in three new locations in Australia. *Marine Pollution Bulletin* 44: 204–210, http://dx.doi.org/10.1016/S0025-326X(01)00202-8

Schembri PJ, Barbara J, Deidun A, Lanfranco E, Lanfranco S (2015) It was only a matter of time: occurrence of *Caulerpa taxifolia* (Vahl) C. Agardh var. *distichophylla* (Sonder) Verlaque, Huisman and Procaccini in the Maltese Islands (Chlorophyta, Ulvophyceae, Caulerpaceae). *BioInvasions Records* 4: 9–16, http://dx.doi.org/10.3391/bir.2015.4.1.02

Stam WT, Olsen JL, Zaleski SF, Murray SN, Brown KR, Walters LJ (2006) A forensic and phylogenetic survey of *Caulerpa* species (Caulerpales, Chlorophyta) from the Florida coast, local aquarium shops, and e-commerce: Establishing a proactive baseline for early detection. *Journal of Phycology* 42: 1113–1124, http://dx.doi.org/10.1111/j.1529-8817.2006.00271.x

Streftaris N, Zenetos A (2006) Alien Marine Species in the Mediterranean - the 100 'Worst Invasives' and their Impact. *Mediterranean Marine Science* 7: 87–117, http://dx.doi.org/10.1 2681/mms.180

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution* 30: 2725–2729, http://dx.doi.org/10.1093/molbev/mst197

Thibaut T, Pinedo S, Torras X, Ballesteros E (2005) Long-term decline of the populations of Fucales (*Cystoseira* spp. and *Sargassum* spp.) in the Albères coast (France, northwestern Mediterranean). *Marine Pollution Bulletin* 50: 1472–1489, http://dx.doi.org/10.1016/j.marpolbul.2005.06.014

Thiers B (2014) Index Herbariorum: A Global Directory of Public Herbaria and Associated Staff. New York Botanical Garden's Virtual Herbarium. New York, NY

Tsiamis K, Taskin E, Orfanidis S, Stavrou P, Argyrou M, Panayotidis P, Tsioli T, Cicek BA, Marcou M, Küpper FC (2014) Checklist of seaweeds of Cyprus (Mediterranean Sea). *Botanica Marina* 57: 153–166, http://dx.doi.org/10.1515/bot-2014-0006

Ukabi S, Dubinsky Z, Steinberger Y, Israel A (2012) Surveying *Caulerpa* (Chlorophyta) species along the shores of the eastern Mediterranean. *Mediterranean Marine Science* 13: 5–11, http://dx.doi.org/10.12681/mms.18

Ukabi S, Shemesh E, Dubinsky Z, Hershkovits G, Israel A (2014) Molecular authentication of *Caulerpa* (Chlorophyta) species along the eastern (Israeli) Mediterranean shores. *Botanica Marina* 57: 67–71, http://dx.doi.org/10.1515/bot-2013-0093

Verlaque M, Boudouresque CF, Meinesz A, Gravez V (2000) The *Caulerpa racemosa* complex (Caulerpales, Ulvophyceae) in the Mediterranean Sea. *Botanica Marina* 43: 49–68, http://dx.doi.org/10.1515/BOT.2000.005

Verlaque M, Durand C, Huisman JM, Boudouresque CF, Le Parco Y (2003) On the identity and origin of the Mediterranean invasive *Caulerpla racemosa* (Caulerpales, Chlorophyta). *European Journal of Phycology* 38: 325–339, http://dx.doi.org/10.1080/0967026031000161 2592

Welling M, Pohnert G, Küpper FC, Ross C (2009) Rapid biopolymerisation during wound plug formation in green algae. *Journal of Adhesion* 85: 825–838, http://dx.doi.org/10.1080/00218460903291452

Zenetos A, Gofas S, Verlaque M, Cinar ME, Raso JEG, Bianchi CN, Morri C, Azzurro E, Bilecenoglu M, Froglia C, Siokou I, Violanti D, Sfriso A, San Martin G, Giangrande A, Katagan T, Ballesteros E, Ramos-Espla A, Mastrototaro F, Ocana O, Zingone A, Gambi MC, Streftaris N (2010) Alien species in the Mediterranean Sea by 2010. A contribution to the application of European Union's Marine Strategy Framework Directive (MSFD). Part I. Spatial distribution. *Mediterranean Marine Science* 11: 381–493, http://dx.doi.org/10.12681/mms.87

The following supplementary material is available for this article:

Table S1. Primary geo-referenced species record data for *Caulerpa taxifolia* var. *distichophylla*.

Table S2. List of species used in this study for phylogenetic analysis of *Caulerpa taxifolia* var. *distichophylla* and GenBank accession numbers of the cpDNA tufA gene and the ITS-rDNA region, respectively.

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