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# *Caulerpa wysorii sp. nov.*, a denuded *Caulerpa* (Chlorophyta) resembling *C. sertularioides* when 'dressed'

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**ABSTRACT**
Routine DNA barcoding with the chloroplast gene *tuf*A identified novel records of an unknown and denuded (i.e. lacking branchlets) *Caulerpa* species previously reported from the Florida Middle Grounds. *tuf*A barcoding not only confirmed the matching molecular identity of this taxon with several newly collected denuded specimens from shallow habitats of the Yucatán shelf, but also with a set of plumose specimens previously collected from mesophotic depths of the West Florida shelf and recorded as *Caulerpa sertularioides*. Sequencing of two additional, faster-evolving DNA markers, i.e. nuclear ITS and the newly tested chloroplast-encoded *rpo*A, each confirmed conspecificity of the above morphotypes and the molecular separation of the new taxon from *C. sertularioides* and filiform species found in the region, namely *C. cupressoides* f. *denudata* and *C. fastigiata*. In light of our sequencing results and review of the early taxonomic history of filiform *Caulerpa*, we propose the description of *Caulerpa wysorii sp. nov.* for this new species that exhibits two distinct morphotypes in currently available collections, i.e. filiform or plumose assimilators. We also subsume *C. tongaensis* within the broadly distributed *C. fastigiata*, whose lectotype is also designated herein. In addition to *Caulerpa wysorii sp. nov.*, we discovered yet another unknown denuded species from the Campeche Banks represented by a single specimen thus far.

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**KEYWORDS**
*Caulerpa cupressoides*; *C. denudata*; *C. fastigiata*; *C. longiseta*; *C. nuda*; *C. tongaensis*; Cryptic species; Endemic; Mesophotic; *rpo*A

## INTRODUCTION

Recent DNA sequencing of mesophotic *Caulerpa* specimens found within 30–70 m depths in the Gulf of Mexico revealed their unsuspected molecular identity to an undescribed taxon previously collected by Dr. Brian Wysor (Roger Williams University, Bristol, Rhode Island) from the Florida Middle Grounds (LAF-8-12-00-1-52 in LAF, accessioned in GenBank as AJ417962 by Famà *et al.* 2002). These newly sequenced specimens, originally annotated as *C. sertularioides* (S.G. Gmelin) M. Howe, display large but delicate plumose assimilators that differ widely from the depiction of B. Wysor's specimen, which consists of a wiry thallus with terete assimilators devoid of any branchlets (see fig. 12 in Sauvage *et al.* 2014). Based on its chloroplast gene *tuf*A sequence, B. Wysor's novel taxon belongs to a clade of endemics from the Western Tropical Atlantic (WTA) that also includes *C. ashmeadii* Harvey and *C. floridana* W.R. Taylor and is phylogenetically distant from the clade of *C. sertularioides* (Famà *et al.* 2002; Sauvage *et al.* 2014) as well as filiform species of Indo-Pacific origin (see Supplementary File 1).

Since publication of a photographic illustration for B. Wysor's wiry *Caulerpa* (Sauvage *et al.* 2014, fig. 12), several collaborators have come forward with denuded specimens collected from shallow waters of the southern Gulf of Mexico (Campeche and Yucatán) and vicinity (Cuba). These specimens bear uncertain (provisional) annotations varying by collectors and location, such as '*C. denudata*', *C. fastigiata*, simply *Caulerpa* sp., or '*C. requienii*'. The former combination was never described, and *denudata* represents an infraspecific epithet of *C. cupressoides* (Vahl) C. Agardh (see further below). The combination '*C. requienii*' was also never published and represents a filiform *Caulerpa* from the Red Sea (after *Herpochaeta requienii* Montagne) that is of uncertain affiliation and unlikely related to the above WTA clade (see Wynne *et al.* 2009 and Supplementary File 1). Brian Wysor's wiry *Caulerpa* is annotated (informally) with yet another epithet as '*Caulerpa "nuda*"' (Sauvage *et al.* 2014, fig. 12). This epithet corresponds to the subspecific rank f. *nuda* created by Weber-van Bosse (1898; i.e. f. *nuda*) for some morphotypes of *C. cupressoides* var. *mamillosa* (Montagne) Weber

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Supplemental data for this article can be accessed on the publisher's website.

Bosse from Guadeloupe, French West Indies, whose fronds exhibit naked bases (i.e. devoid of branchlets). Weber-van Bosse (1908) also published *C. cupressoides* f. *denudata* Weber Bosse (see above) for ‘relaxed’ mesophotic specimens (> 50 m) from the Seychelles. The assimilators of *C. cupressoides* f. *denudata* are mostly bare but exhibit occasional diminutive pinnules. Among all of the above, *C. fastigiata* Montagne 1837 represents a diminutive taxon with stolons entangled as a mat that cannot be distinguished with the naked eye. This feature is in sharp contrast with the conspicuous external features (i.e. stolons and assimilators) of B. Wysor’s taxon, which is also much larger. Although *C. fastigiata* was sequenced from Indonesia by Draisma *et al.* (2014), its molecular identity still requires clarification in the WTA, where it was originally described from Cuba by Montagne (1837; see Fig. S1). Filiform specimens assigned to *C. cupressoides* f. *denudata* in the region have also not been sequenced prior.

In the Gulf of Mexico, early collections of wiry specimens with similar habit to B. Wysor’s denuded *Caulerpa*, are those of the German naturalist Arthur C.V. Schott from Celestún, Yucatán, Mexico. These consist of two separate herbarium sheets numbered 369 (in BM and F, Figs 1, 2), each annotated with several uncertain determinations. The first specimen (BM001139864, Fig. 1) is annotated as ‘*Caulerpa* sp? Celestun, Yuc. v.13.1865′ with more recent anonymous annotations suggesting ‘*C. ligulata Harv.*, *C. filiformis*?’ and ~~‘? *Caulerpa ?tongaensis?*’~~. The second specimen (C0532532F, Fig. 2) is labelled as ‘*Caulerpa simplex, mihi*’ (by A. Schott himself in 1865 or possibly W.H. Harvey who first reviewed his collections, thus prior to his death in 1866; Godínez-Ortega 2008), later annotated by W.G. Farlow (about 1898) as ‘*C. cupressoides* (Vahl) Ag.’ and finally as ‘*Caulerpa* sp.’ by W.R. Taylor. In reviewing these epithets (see Supplementary File 1), none appears applicable to the novel WTA taxon because they correspond to other well-accepted taxa [e.g. *C. filiformis* (Suhr) K. Hering 1841] or were subsequently described for a different taxon (*C. simplex* Levring 1938, a synonym of *C. brachypus* Harvey 1860). Nonetheless, the crossed-out annotation referring to *C. tongaensis* Papenfuss (1943), a taxon considered by some as a heterotypic synonym of the broadly recorded *C. fastigiata* (see N’Yeurt & Tsuda 2014, p. 4), requires further attention in the context of the present investigation. The later identification of A. Schott’s filiform specimen to *C. cupressoides* (Fig. 2) by W.G. Farlow also further underscores its potential confusion with B. Wysor’s novel taxon (i.e. as ‘*C. nuda*’ or ‘*C. denudata*’, two infraspecific ranks of *C. cupressoides*, see further above).

Here, we report on our molecular sequencing of wiry *Caulerpa* specimens from the Gulf of Mexico (including *C. fastigiata*) and plumose morphotypes identified by routine DNA barcoding with the chloroplast gene *tuf*A. We also introduce the chloroplast gene polymerase alpha subunit (*rpo*A) as a potential aid in molecular systematics of the genus *Caulerpa* and further verify our results by generating the nuclear ITS rDNA marker for a few specimens. Following our molecular investigation, we propose the description of *Caulerpa wysorii sp. nov*. We also re-evaluate the status of *C. tongaensis* and designate a lectotype for *C. fastigiata*.

## MATERIAL AND METHODS

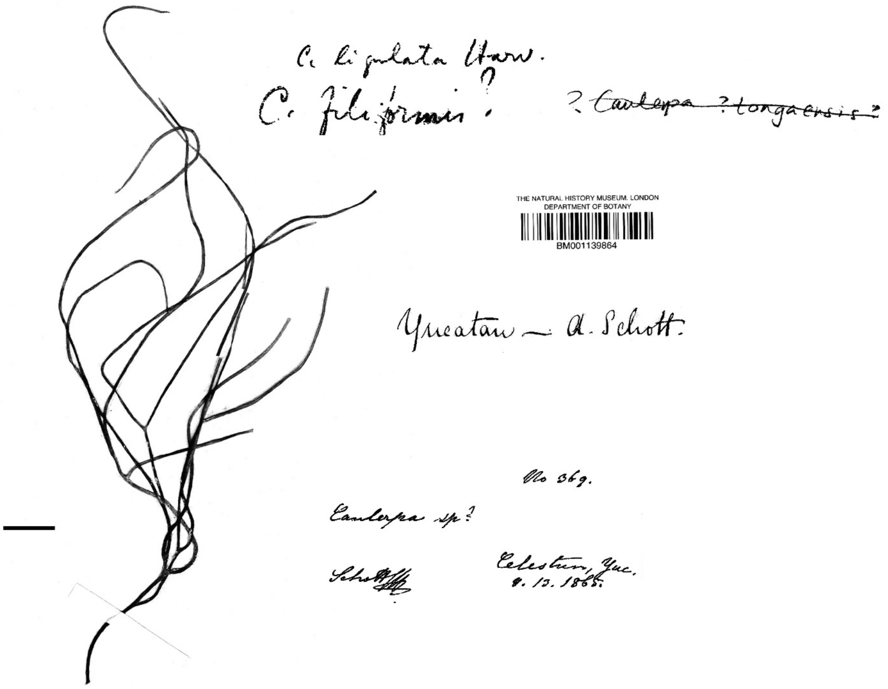
### Specimens

Mesophotic specimens with plumose habit identified as ‘*C. sertularioides*’ originated from the Gulf of Mexico using a Hourglass-design box dredge with minimum tows (see Fredericq *et al.* 2014) in rhodolith bed habitats found in the vicinity of the Florida Middle Grounds and the Dry Tortugas (e.g. NSF-III-7-5-06-8-3, Fig. 3, Table 1). By contrast, most of the newly collected wiry morphotypes from the northwestern Yucatán Peninsula of Mexico (e.g. typical specimens, Fig. 4) originated from snorkelling depths (< 3 m), except for one denuded mesophotic specimen housed in LAF that originated from dredges conducted in the Campeche Banks, Mexico (LAF6574, Table S1, no herbarium sheet available for illustration). Additional samples were represented by *C. fastigiata* from Florida (Fig. 5), Palau (South Pacific), and Indonesia (South East Asia). One filiform specimen from Cuba was received with a provisional annotation as ‘*C. requienii*’ (see Introduction), which after close examination revealed very few triangular papillae and was therefore corrected to *C. cupressoides* f. *denudata* (HANC0484, Fig. 6) following Weber-van Bosse (1908). Specimens of *C. sertularioides* from shallow waters of Belize (Fig. S2, Fisheries Department permit #0009-18), Caribbean Panama, Campeche, and the Dry Tortugas (U.S. National Park Service permits #DRTO-2013-SCI-0015 and #DRTO-2017-SCI-0004) were also processed to verify their molecular identity in relation to the above plumose morphotypes (Table S1). Two of these specimens (LAF-8-3-10-1-11 and TS2126, Fig. 7 and Fig. S2) with long, thin assimilators generally assigned to *C. sertularioides* f. *longiseta* (Bory) Svedelius (1906) (see taxonomic notes in Supplementary File 1), exhibited a striking resemblance to the delicate mesophotic ‘*C. sertularioides*’ from the Gulf of Mexico. Miscellaneous *Caulerpa* specimens were also sequenced from Yucatán and elsewhere (Table S1).

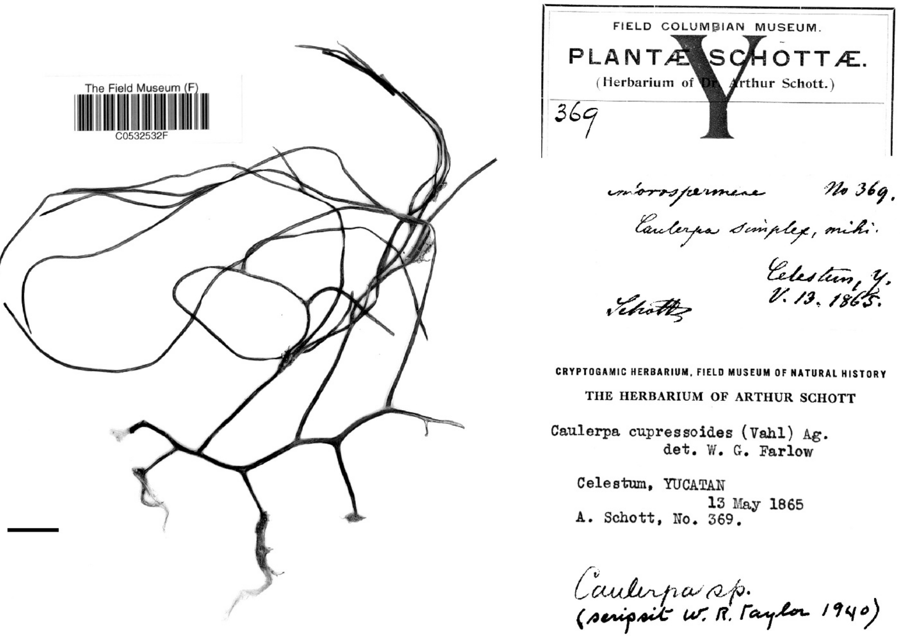
### DNA sequencing

DNA was extracted according to the modified protocol of Dellaporta *et al.* (1983) described in Hughey *et al.* (2001) for herbarium specimens, or with a DNeasy Plant MiniKit (Qiagen, Valencia, California, USA) for silica gel-dried specimens. Amplification of *tuf*A was conducted as previously indicated (e.g. Sauvage *et al.* 2013) and *rpo*A with newly designed primers targeting >600 base pairs, *rpo*A_F1 5′-TGAACATG AATATTCGAGTT-3′ (Tm = 46.7°C) and *rpo*A_R1 5′-TGCCTAAATCAAAATCAAAG-3′ (Tm = 46.4°C). A nested forward primer *rpo*A_F2 5'-TTTTCGAAATATGGACAAAGG-3′ (Tm = 48.8°C) was also designed to facilitate amplification of difficult templates, or resequencing the most informative section of the above fragment. We also generated nuclear ITS rDNA (spanning ITS1, 5.8S and ITS2) with primers published in Stam *et al.* (2006) or Kazi *et al.* (2013) in order to verify congruence with chloroplast markers for a mesophotic plumose ‘*C. sertularioides*’ (TS1923) and a denuded, filiform specimen from shallow waters of Campeche (CAMP2), as well as a delicate *C. sertularioides* (i.e. f. *longiseta*, TS2126) from shallow waters of Carrie Bow Cay,

**1**



**2**



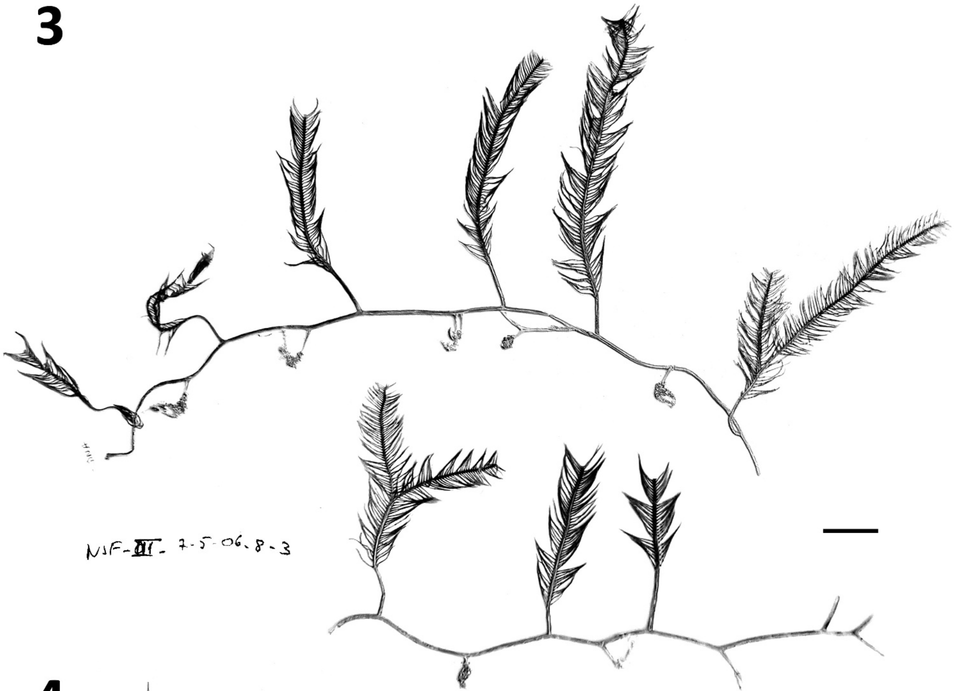
**Figs 1, 2.** Specimen and annotations found on A. Schott's herbarium sheets no. 369 from Celestún, Yucatán, Mexico. Note the uncertain determination within each sheet and incongruent determination between sheets. Scale bars = 2 cm.

**Fig. 1**. Specimen and annotations found on herbarium sheet BM001139864.

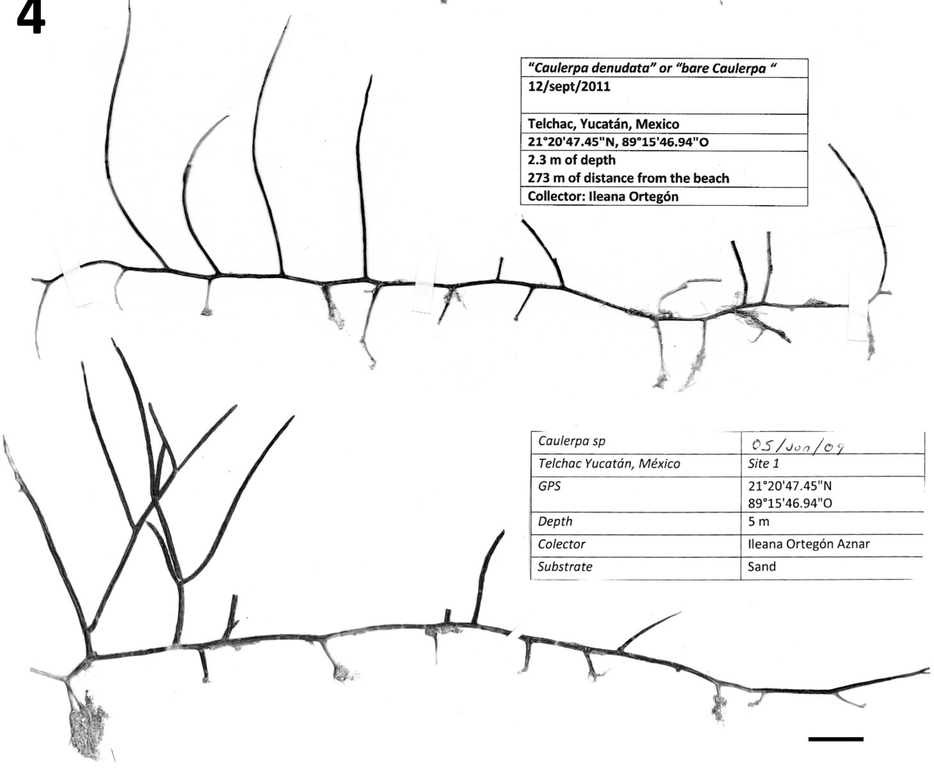
**Fig. 2**. Specimen and annotations found on herbarium sheet (C0532532F).

Belize. ITS amplicons were cloned (e.g. as Draisma *et al.* 2012 or Sauvage *et al.* 2016) to resolve polymorphism. Amplification for both ITS rDNA and *rpo*A followed the same cycling profile as for *tuf*A but with adjusted annealing temperatures to 45°C. PCR products (i.e. *tuf*A and *rpo*A amplicons) or colony-PCR products (i.e. cloned ITS amplicons) of correct sizes were processed for Sanger sequencing on the UL Lafayette ABI Prism® 3130XL Genetic Analyser (Life Technologies Corporation, Grand Island, New York, USA) with the BDX64 enhancer (MCLAB, San Francisco, California, USA) for BigDye® 3.1 (Life Technologies Corporation, Grand Island, New York, USA) as described in Sauvage *et al.* (2014). Resulting chromatograms were assembled into contigs, cropped from incorporated primers and further edited using Sequencher v.5.4.6 (Gene Codes, Ann Arbour, Michigan, USA). All generated sequences were deposited into the GenBank database under accessions MT441785–MT441819 for *tuf*A, MT441761–MT441784 for *rpo*A and MT431674–MT431685 for ITS (see Table S1).

3



4



| "*Caulerpa denudata*" or "*bare Caulerpa* " |
|---|
| 12/sept/2011 |
| Telchac, Yucatán, Mexico |
| 21°20'47.45"N, 89°15'46.94"O |
| 2.3 m of depth |
| 273 m of distance from the beach |
| Collector: Ileana Ortegón |

| *Caulerpa sp* | 05/Jun/09 |
|---|---|
| *Telchac Yucatán, México* | *Site 1* |
| *GPS* | 21°20'47.45"N<br>89°15'46.94"O |
| *Depth* | 5 m |
| *Colector* | Ileana Ortegón Aznar |
| *Substrate* | Sand |

**Figs 3, 4.** Morphological variability exhibited by *Caulerpa wysorii sp. nov*. Note that while plumose morphotypes have been collected so far only in mesophotic depths, wiry specimens are known from both shallow (Yucatán Peninsula) and mesophotic depths (see Table 1 and Fig. 10). Scale bars = 2 cm.

**Fig. 3**. Delicate plumose morphotype (paratype specimen NSF-III-7-5-06-8-3).

**Fig. 4**. Wiry morphotypes (top and bottom specimens correspond to TS1667 and TS1666 respectively).

## Phylogenetics

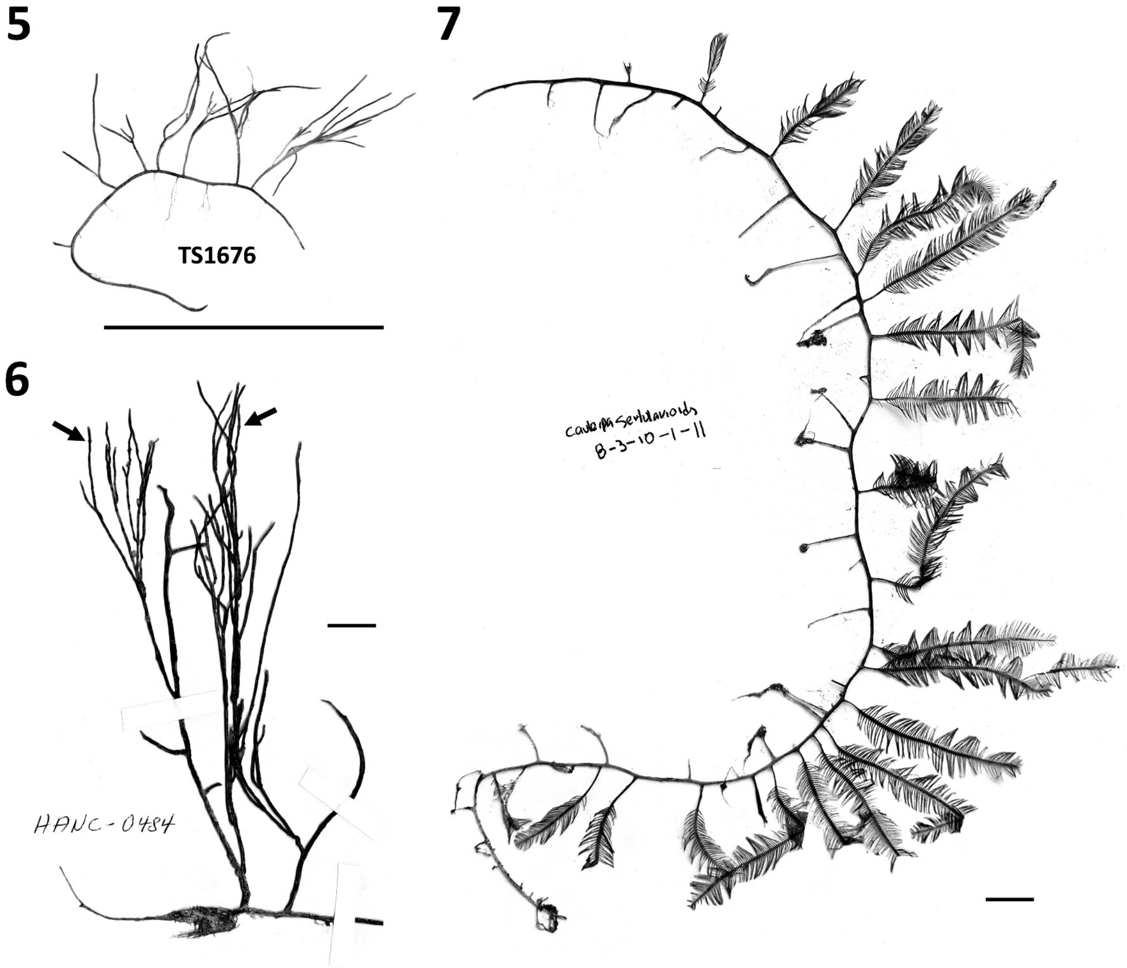
Newly generated chloroplast *tuf*A sequences were added to a multiple alignment built for a previous study on the molecular confirmation of the WTA endemic *Caulerpa floridana* (Sauvage *et al.* 2014). For figure clarity (i.e. tree size), the multiple alignment was first reduced to taxa/clades of interest and subsequently complemented with sequences from recent studies (e.g. Kazi *et al.* 2013; Fernández-García *et al.* 2016), mainly for *C. cupressoides* and *C. sertularioides*. Following communications with Wytze T. Stam, we also obtained chromatograms of

**Table 1.** Sequenced specimens of *Caulerpa wysorii sp. nov*., including the holotype and paratypes. Note that mesophotic specimens were either plumose or wiry, while all identified shallow specimens sequenced in the present study were wiry. Voucher ID corresponds to the provisional identifications as annotated on their respective voucher.

| Date | Specimen | Depth | Latitude/Longitude | Site | Voucher ID | Morphology |
|---|---|---|---|---|---|---|
| 12 Aug. 2000 | LAF-8-12-00-1-52[a] | 33 m | 28°33.06′N 84°16.47′W | Florida Middle Grounds | *C. nuda* | Wiry |
| 3 Jun. 2004 | NSF-I-41-3-6-04[b] | 62 m | 24°43.19′N 83°13.88′W | Dry Tortugas | *C. sertularioides* | Plumose |
| 4 Jul. 2006 | NSF-III-7-4-06-4-3[b] | 45 m | 28°34.24′N 84°28.77′W | Florida Middle Grounds | *C. sertularioides* | Plumose |
| 5 Jul. 2006 | NSF-III-7-5-06-8-3[b] | 38 m | 28°05.20′N 83°46.16′W | Florida Middle Grounds | *C. sertularioides* | Plumose |
| 5 Jun. 2009 | TS1666 | <5 m | 21°20.79′N 89°15.78′W | Yucatán | *Caulerpa* sp. | Wiry |
| 12 Sep. 2011 | TS1667 | <3 m | 21°20.79′N 89°15.78′W | Yucatán | *C. denudata* | Wiry |
| 13 Jun. 2013 | TS1881 | <3 m | 21°20.79′N 89°15.78′W | Yucatán | *Caulerpa* sp. | Wiry |
| 27 Jun. 2013 | TS1882 | <3 m | 21°18.22′N 89°39.70′W | Yucatán | *Caulerpa* sp. | Wiry |
| 27 Jun. 2013 | TS1883 | <3 m | 21°18.22′N 89°39.70′W | Yucatán | *Caulerpa* sp. | Wiry |
| 10 Sep. 2014 | TS1923 | 65 m | 24°42.05′N 83°16.84′W | Dry Tortugas | *C. sertularioides* | Plumose |
| 3 Apr. 2017 | CAMP2 | <2 m | 19°49.63′N 90°34.19′W | Campeche | *C. fastigiata*? | Wiry |

[a] Holotype deposited at LAF. Original collection from B. Wysor previously published as *Caulerpa* sp. in GenBank (AJ417962) and illustrated in Sauvage *et al*. (2014).

[b] Plumose paratypes deposited at LAF; specimen NSF-III-7-5-06-8-3 is depicted in Fig. 3.



**Figs 5–7.** Habit of species found in the Western Tropical Atlantic (WTA) that may be confused with wiry or plumose morphotypes of *Caulerpa wysorii sp. nov*. Scale bars = 2 cm.

**Fig. 5**. Habit of *C. fastigiata* (TS1676) following mat separation to reveal stolons and assimilators. Note the very diminutive size of this species.

**Fig. 6**. Habit of a filiform specimen of *C. cupressoides* (HANC-0484), whose morphotype is generally assigned to f. *denudata*. The presence of diminutive spines (branchlets) that were visible upon close examination of the specimen are highlighted with arrows.

**Fig. 7**. Habit of a delicate specimen of *C. sertularioides* (LAF-8-3-10-1-11), whose morphotype is generally assigned to f. *longiseta*. Note the striking resemblance of this specimen with plumose morphotypes of *Caulerpa wysorii sp. nov*. displayed in Fig. 3.

*C. ashmeadii* DQ652368 (FL171 from Stam *et al.* 2006), whose *tuf*A haplotype differed erroneously from all other specimens sequenced in the region (sequence corrected online as DQ652368.2 by GenBank staff on 26 March 2020). The Maximum Likelihood analysis for *tuf*A was run in RAxML v8.2.12 (Stamatakis 2014) with a GTR+I+G model of evolution applied per codon position, 200 restarts and 1000 bootstrap replicates (Fig. 8). For faster-evolving markers *rpo*A and ITS, which both produce multiple alignments with numerous insertions-deletions (i.e. indels or gaps), we computed identity, gap and substitution with BLASTn (Altschul *et al.* 1990) for comparison with *tuf*A (Table 2). For *rpo*A, we also report a multiple alignment based on translated amino-acids to highlight sequence 'signature' variation with a focus on the clade of WTA endemics including the closely related species *C. prolifera* (Forsskål) J.V. Lamouroux, *C. sertularioides*, and *C. taxifolia* (Vahl) C. Agardh (see Fig. 9, produced with BoxShade 3.21 by K. Hofmann and M. Baron, EMBnet). For nuclear ITS, we also report BLASTn similarity levels against a curated collection of sequences downloaded from GenBank (Figs S3, S4). We also provide *rpo*A and ITS guide trees in Supplementary Figs S5, S6.

## Vertical distribution

Bathymetric data for the Gulf of Mexico were downloaded from the Coastal Ocean Observing System website (https://geo.gcoos.org/; original ASCII XYZ data 'gom_srtm30_plus.

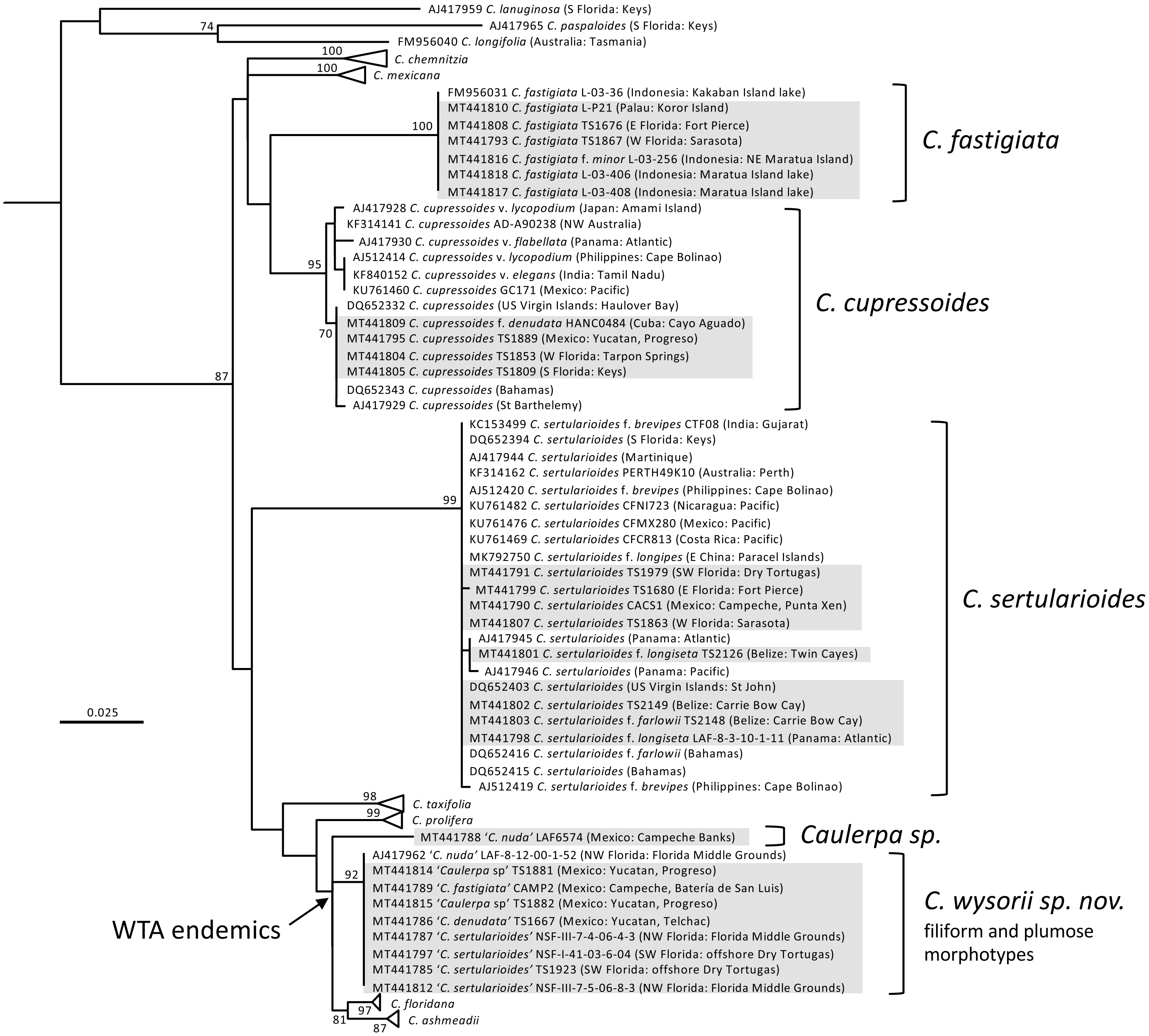


Fig. 8. Phylogenetic position of *Caulerpa wysorii sp. nov.* based on the chloroplast gene *tuf*A. The topology and bootstrap support values (< 70% not reported) were obtained with RAxML. Labels shaded in grey correspond to newly generated sequences. Note that plumose and filiform specimens confirmed as *Caulerpa wysorii sp. nov.* are reported on the tree according to annotations on their respective voucher (i.e. provisional identifications).

Table 2. Comparison of BLASTn pairwise alignment between *Caulerpa wysorii sp. nov*. and two closely related species *C. ashmeadii* and *C. prolifera* for the three markers sequenced in the present study. Number in parenthesis represent the length of the alignment, number of common base pairs (identity), inserted indels (gap), or variable nucleotides (substitution). Note the lack of indels (i.e. gaps) in *tuf*A as compared to fast evolving nuclear ITS and chloroplast *rpo*A.

| *C. wysorii vs. C. ashmeadii* | % Identity | % Gap | % Substitution |
|---|---|---|---|
| *tuf*A[a] (908 bp) | 98.8% (897 bp) | 0% (0 bp) | 0.2% (11 bp) |
| *rpo*A[a] (639 bp) | 92.5% (591 bp) | 4.7% (30 bp) | 2.8% (18 bp) |
| ITS[b] (612 bp) | 91.8% (562 bp) | 3.6% (22 bp) | 4.6% (28 bp) |
| *C. wysorii vs. C. prolifera* | % Identity | % Gap | % Substitution |
| *tuf*A[c] (908 bp) | 98.4% (897 bp) | 0% (0 bp) | 1.6% (14 bp) |
| *rpo*A[c] (639 bp) | 93.3% (585 bp) | 1.9% (12 bp) | 4.8% (30 bp) |
| ITS[d] (612 bp) | 90.1% (602 bp) | 4.2% (28 bp) | 5.7% (38 bp) |

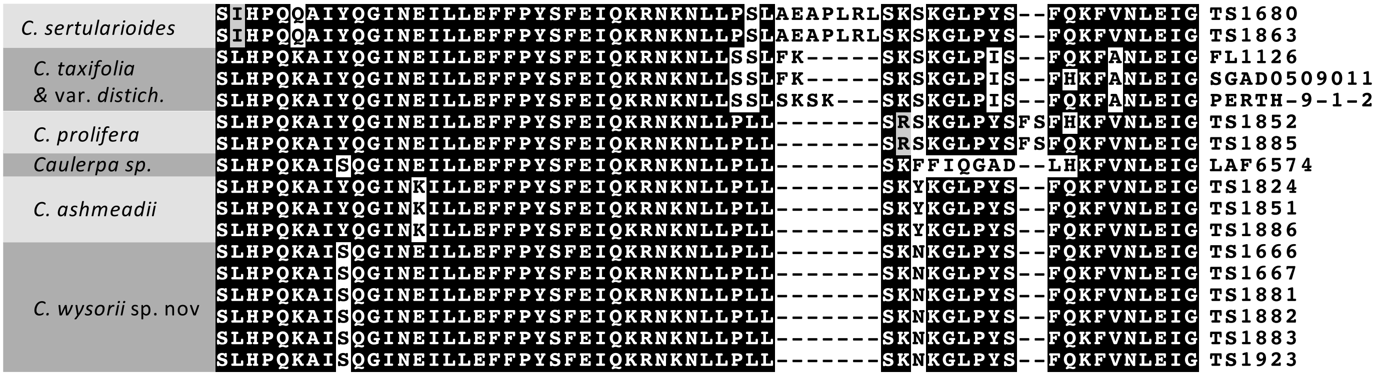
[a] TS1923 vs TS1824.
[b] TS1923 vs DQ652534.
[c] TS1923 ribotype 1 vs TS1852.
[d] TS1923 ribotype 1 vs AJ228989.

txt' derived from the STRM30 PLUS V6 global bathymetry grid; Becker *et al.* 2009). Within this dataset, we selected narrow transects from the main locations of collection in order to examine the overall shelf profile and depth of collection for the new taxon investigated (Fig. 10). Transects were drawn from Progreso and Campeche city for samples from the Yucatán shelf, Mexico, and from Clearwater and Key West for samples from the West Florida shelf, USA. Plots were produced in R with package ggplot2 (Wickham 2016) and the profiles smoothed with the 'gam' option (general additive model).

## RESULTS

As mentioned earlier, a motivation for this study was the discovery through routine DNA barcoding of mesophotic specimens identified as '*C. sertularioides*' matching the sequence of B. Wysor's denuded *Caulerpa* from the Florida Middle Grounds (also mesophotic, GenBank accession AJ417962; Famà *et al.* 2002). Here, we report four such mesophotic plumose specimens (Table 1, Fig. 8) from two distant locations of the West Florida shelf in the Gulf of Mexico, including the Florida Middle Ground, where both morphotypes (plumose and denuded) are therefore found (Fig. 10, Fig. S8). The DNA sequences of the above plumose specimens were generated from herbarium sheets as well as from a recently collected specimen from the vicinity of the Dry Tortugas (TS1923) grown in a mesocosm at UL Lafayette for several months before vanishing (Fig. 11). By contrast, all newly sequenced filiform specimens matching the above taxon were from shallow nearshore habitats (< 5 m) of Yucatán and Campeche states (Figs 8, 10, S8). However, the denuded specimen LAF6574 collected from a mesophotic depth of the Campeche Banks represented yet another unknown *Caulerpa* species (Fig. 8, Table 1). This specimen's *tuf*A sequence has no direct match in GenBank and branches within the cluster of WTA endemics comprising *C. ashmeadii, C. floridana* and B. Wysor's taxon (Fig. 8). In comparison to *tuf*A, which aligns unambiguously across *Caulerpa* species, nuclear ITS and the newly tested chloroplast marker *rpo*A displayed a large amount of nucleotide insertions/deletions between sister species and higher substitution rates (as measured via BLASTn pairwise alignment, Table 2).

The marker *rpo*A showed clear sequence signature across species at the nucleotide (not shown) and amino acid level (Fig. 9), displaying multiple codon substitutions and insertions/deletions (for figure clarity, the sequence signatures of *Caulerpa fastigiata* and *C. cupressoides* are not shown because of their large divergence, see guide tree instead, Fig. S5). *Rpo*A further confirmed the absence of molecular variation within the two morphotypes of B. Wysor's taxon (i.e. filiform and plumose) and distinctness of the denuded specimen LAF6574 from Campeche Banks (Figs 9, S5). Nuclear ITS rDNA exhibited some polymorphism in the plumose specimen TS1923 as compared to the filiform specimen CAMP2 but this polymorphism was not consistent with one morphotype or the other (Figs S3–S4, S6); the sequences also displayed overall BLASTn similarities >97% (Fig. S3) that are within the range of intraspecific divergence observed for other well-sampled *Caulerpa* species such as *C. sertularioides* (about 96%, Fig. S4). ITS signatures for representatives of B. Wysor's taxon exhibited 89%–92% identity with close relatives such as *C. ashmeadii* and *C. prolifera* (Table 2, Fig. S3). *Tuf*A sequencing of a shallow plumose



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SIHPQQAIYQGINEILLEFFPYSFEIQKRNKNLLPSLAEAPLRLSKSKGLPYS--FQKFVNLEIG TS1863
SLHPQKAIYQGINEILLEFFPYSFEIQKRNKNLLSSLFK-----SKSKGLPIS--FQKFANLEIG FL1126
SLHPQKAIYQGINEILLEFFPYSFEIQKRNKNLLSSLFK-----SKSKGLPIS--FHKFANLEIG SGAD0509011
SLHPQKAIYQGINEILLEFFPYSFEIQKRNKNLLSSLSKSK---SKSKGLPIS--FQKFANLEIG PERTH-9-1-2
SLHPQKAIYQGINEILLEFFPYSFEIQKRNKNLLPLL-------SRSKGLPYSFSFHKFVNLEIG TS1852
SLHPQKAIYQGINEILLEFFPYSFEIQKRNKNLLPLL-------SRSKGLPYSFSFQKFVNLEIG TS1885
SLHPQKAISQGINEILLEFFPYSFEIQKRNKNLLPLL-------SKFFIQGAD--LHKFVNLEIG LAF6574
SLHPQKAIYQGINKILLEFFPYSFEIQKRNKNLLPLL-------SKYKGLPYS--FQKFVNLEIG TS1824
SLHPQKAIYQGINKILLEFFPYSFEIQKRNKNLLPLL-------SKYKGLPYS--FQKFVNLEIG TS1851
SLHPQKAIYQGINKILLEFFPYSFEIQKRNKNLLPLL-------SKYKGLPYS--FQKFVNLEIG TS1886
SLHPQKAISQGINEILLEFFPYSFEIQKRNKNLLPLL-------SKNKGLPYS--FQKFVNLEIG TS1666
SLHPQKAISQGINEILLEFFPYSFEIQKRNKNLLPLL-------SKNKGLPYS--FQKFVNLEIG TS1667
SLHPQKAISQGINEILLEFFPYSFEIQKRNKNLLPLL-------SKNKGLPYS--FQKFVNLEIG TS1881
SLHPQKAISQGINEILLEFFPYSFEIQKRNKNLLPLL-------SKNKGLPYS--FQKFVNLEIG TS1882
SLHPQKAISQGINEILLEFFPYSFEIQKRNKNLLPLL-------SKNKGLPYS--FQKFVNLEIG TS1883
SLHPQKAISQGINEILLEFFPYSFEIQKRNKNLLPLL-------SKNKGLPYS--FQKFVNLEIG TS1923
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Fig. 9. Amino-acid variation among *Caulerpa wysorii sp. nov*. and closely related taxa for the chloroplast gene *rpo*A. Note the lack of intraspecific variation among specimens confirmed as *Caulerpa wysorii sp. nov*. as compared to e.g. variation found between *C. taxifolia* and its variety *distichophylla.*

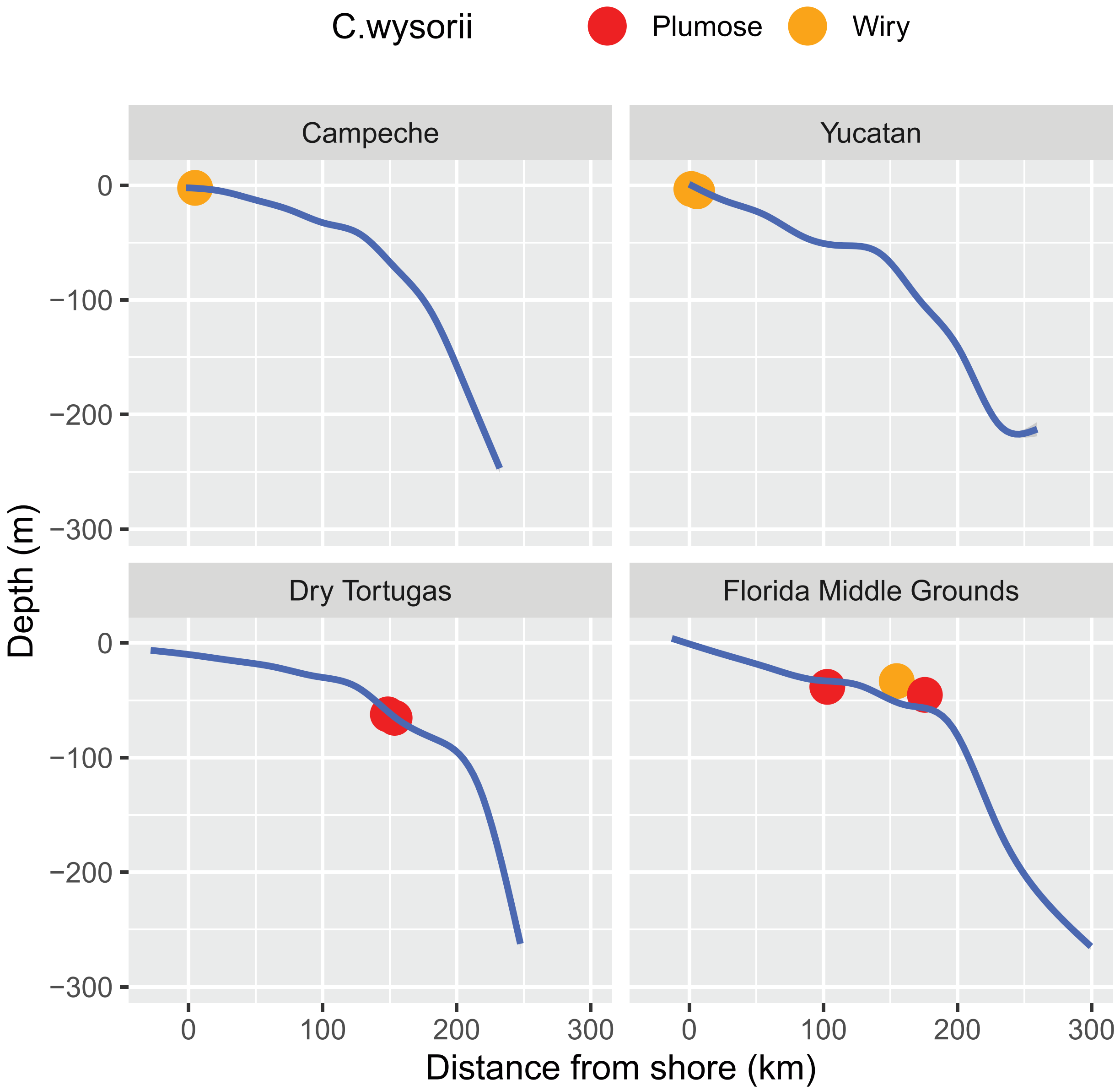
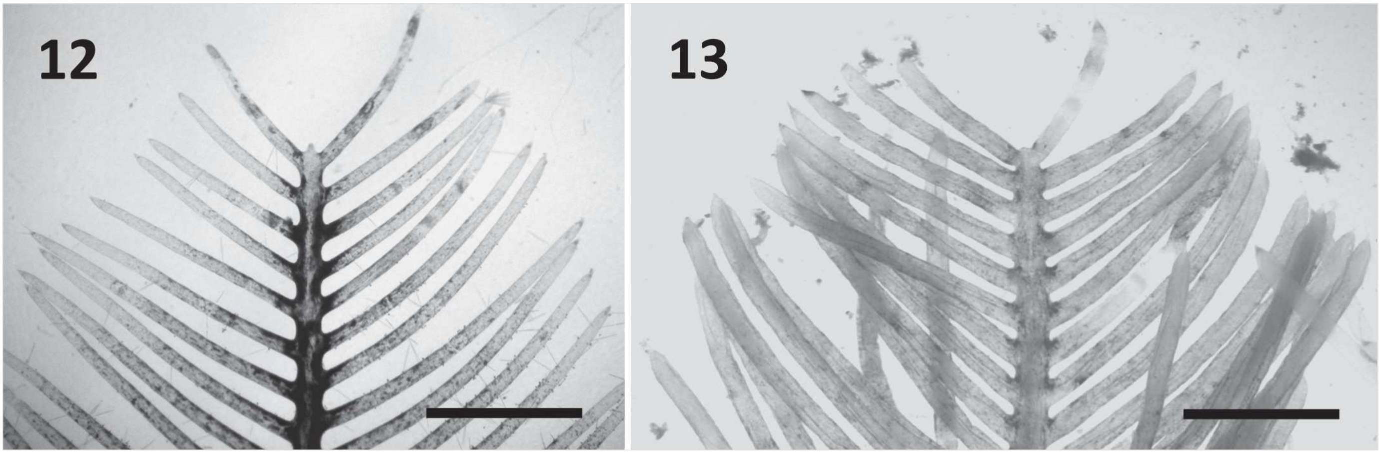


**Fig. 10.** Onshore-offshore bathymetric profiles (0–300 m) and distribution of *Caulerpa wysorii sp. nov*. morphotypes (filiform and plumose). Note the presence of both morphotypes in collections from the West Florida Shelf (Dry Tortugas, and Florida Middle Grounds), while only filiform specimens have been collected from the Yucatan shelf so far (in Campeche and Yucatán states).

specimen strongly resembling the habit of mesophotic specimens (see illustration of LAF-8-3-10-1-11 vs NSF-III-7-5-06-8-3, Fig. 7 vs Fig. 3) confirmed its molecular identity to genuine *C. sertularioides* (Fig. 8). Likewise, sequencing of plumose specimens from shallow habitats of Campeche (CACS1) and Dry Tortugas (TS1979) also confirmed their identity as genuine *C. sertularioides* (Fig. 8). Morphological comparison between the assimilator of delicate (genuine) *C. sertularioides* with those of B. Wysor's taxon (Figs 12, 13) revealed narrower and cylindrical branchlets and rachis (i.e. the primary axis of the assimilator) in the former (Fig. 12). Those of the plumose morphotype of B. Wysor's taxon appeared laterally compressed in formalin preserved material available (Fig. 13). Unfortunately, no careful observations could be made on live material to confirm such characters, which also become obscure for both taxa in dried herbarium material.

Finally, sequencing *Caulerpa fastigiata* from coastal Florida (WTA) with *tuf*A confirmed the molecular identity of the species previously reported by Draisma *et al.* (2014) and its clear separation from B. Wysor's taxon (Figs 8, S5, S6). *Rpo*A sequence signatures also showed clear separation from other species (not shown on Fig. 9 for alignment clarity), likewise for ITS (e.g. Figs S3, S4). The *tuf*A sequences of *C. fastigiata* from the WTA showed no base pair variation with those from the Indo-Pacific (Indonesia and Palau). The filiform specimen of *C. cupressoides* f. *denudata* from Cuba (HANC-0484) was identical to previously published and newly generated *tuf*A sequences of typical *C. cupressoides* from the region (e.g. Florida and Yucatán) and likewise for *rpo*A (not shown in alignment for clarity but see Fig. S5). The ITS sequence for HANC-0484 was not determined.

Fig. 11. *Caulerpa wysorii sp. nov*. grown in the Seaweeds' lab mesocosm at the University of Louisiana at Lafayette (specimen TS1923). Note the striking resemblance of this new species' assimilators with those of *C. sertularioides*. The initially diminutive specimen (< 2 cm) with few distichous branchlets collected by dredging offshore the Dry Tortugas grew to a fully plumose thalli in the laboratory before suddenly vanishing. Assimilators on the picture are about 3–5 cm in length.



Figs 12, 13. Comparative habit of rachis and branchlets. Scale bars = 0.5 cm.

**Fig. 12**. *Caulerpa sertularioides* f. *longiseta* (live specimen).

**Fig. 13**. *Caulerpa wysorii sp. nov*. (formalin-preserved specimen NSF-III-7-5-06-8-3). Note the slightly broader rachis and branchlets in *Caulerpa wysorii sp. nov*., which also appear laterally compressed. In contrast, those of *C. sertularioides* appear cylindrical.

## DISCUSSION

Thanks to routine DNA barcoding of mesophotic *Caulerpa* from the Gulf of Mexico, we made the unexpected identification of specimens matching the *tuf*A sequence of B. Wysor's enigmatic denuded *Caulerpa* (LAF-8-12-00-1-52, accessioned on GenBank as AJ417962; Famà *et al*. 2002). These delicate, plumose specimens had never been considered for DNA barcoding before because of the well-accepted status of *C. sertularioides*, which we thought would lead to predictable sequencing results. The present study thus further highlights the importance of performing routine DNA barcoding without any *a priori* assumptions in order to reveal unsuspected cryptic diversity, i.e. to discover remote clades of look-alikes. Here, neither of the chloroplast markers *tuf*A nor *rpo*A revealed differences between denuded and plumose morphotypes (Fig 8, 9). This was also true with the nuclear ITS marker, which in spite of revealing some intraindividual polymorphism (common in *Caulerpa* species, e.g. Famà *et al.* 2000; Durand *et al.* 2002) was well within the range of nucleotide identity reported for other well-sampled species (approximately 96%, Figs S3–S4). ITS sequence signatures also clearly differed from other *Caulerpa* species (in congruence with the chloroplast markers), thus showing no evidence of introgressive hybridization between B. Wysor's taxon and e.g. *C. sertularioides*.

The newly tested chloroplast marker *rpo*A appeared more useful in supporting *tuf*A results and revealing additional variation than *tuf*A in some taxa (e.g. codon variation within *C. taxifolia* and its var. *distichophylla*, Fig. 9), and unlike ITS does not require cloning. As for ITS, we refrain from conducting state-of-the-art phylogenetics on such markers considering that individual positions of the multiple alignments cannot be ascertained with confidence. Indeed, *rpo*A or ITS rapidly accumulate insertions/deletions (see Fig. 9) with increasingly distant species, which can result in biased tree topologies and branch lengths that may not be representative of true evolutionary processes. In this context and in our opinion, reporting sequence signatures (Fig. 9) or BLASTn metrics (Table 2, Fig. S3) represent a much safer practice to explore haplotype variation underlying a given morphology with such markers. We expect that in future sequencing, *rpo*A may reveal interesting patterns of variation in some populations (infraspecific variation) , *rpoA* and ITS broadly distributed *Caulerpa* species. Here, while we did not test for universality of our *rpo*A primers, experimental amplification in other genera of siphonous green algae were successful. Overall, based on the molecular separation of B. Wysor's taxon from other *Caulerpa* species, including species developing filiform assimilators such as *C. fastigiata* (Fig. 5) and some specimens of *C. cupressoides* (Fig. 6), or the plumose species *C. sertularioides* (and more delicate morphotypes assigned to f. *longiseta*, Figs 7, S2), we propose the description of *Caulerpa wysorii* T. Sauvage & M.J. Wynne *sp. nov.* (see further below).

Relying on collaborative collection efforts with researchers located in the southern Gulf of Mexico, we confirm several new records of *Caulerpa wysorii sp. nov.* and thus extend its distribution from the mesophotic habitats of the Florida Middle Grounds to those of the Dry Tortugas and the shallow waters of the Mexican States of Campeche and Yucatán (Figs 10, S8). To our knowledge, the Yucatán Peninsula represents the only location in the Gulf of Mexico where *C. wysorii* occurs in shallow waters (< 5 m). Based on our limited sampling along this coastline, we do not know whether plumose morphotypes of *C. wysorii* also occur in the shallows of Campeche and Yucatán. However, records of *C. sertularioides* f. *longiseta* (resembling plumose morphotypes of *C. wysorii*) are known from the States of Campeche, Quintana Roo, and Yucatán (Espinoza-Avalos *et al.* 2015). Such records should be further investigated at the molecular level in future studies. We also do not know whether *C. wysorii* occurs at mesophotic depths of the Yucatán shelf where it might have been overlooked. Indeed, the bathymetric profile of the region may provide ample suitable habitat similar to those of the West Florida shelf (Figs 10, S8). Yet, shallow habitats of the Dry Tortugas, the Florida Keys and coastal Florida appear similar to those of nearshore Yucatán and Campeche but yielded no records of *C. wysorii* (personal observations and sequencing results herein). Therefore, the current vertical distribution (shallow vs deep, Fig. 10) of *C. wysorii* is intriguing and calls for further exploration. For instance, it is unknown if these shallow vs deep populations of the Yucatán vs West Florida shelves are connected in a possible source-sink manner, where deeper populations perhaps seed shallower ones as in the 'deep reef refugia' hypothesis (Bongaerts *et al.* 2010). Here, population genomics could possibly provide some answers.

Interestingly, in reviewing collections of Sylvia Earle in US, we encountered a series of mesophotic filiform specimens collected from the Florida Middle Grounds, which very possibly are of *Caulerpa wysorii sp. nov*. This includes specimen SAEH826 collected at about 25 m depth in 1975 (Fig. S7). Thus, the taxon may be common at this location where B. Wysor's original wiry specimen was also collected (see LAF-8-12-00-1-52, Fig. S8). Unfortunately, these specimens were formalin-fixed prior to mounting, and thus verifying their molecular identity may not be possible. Even more intriguing than its vertical distribution, is the absence of morphological gradation in our limited collection. Indeed, if the above morphotypes represent plastic responses induced by environmental conditions, one would expect to observe intermediate morphotypes, i.e. filiform specimens with some branchlets or plumose specimens with part of the frond denuded, as demonstrated in several *Caulerpa* species grown under low light conditions (see figs 1–16 in Calvert 1976). Unfortunately, our sample size for *C. wysorii* is currently quite limited due to its rarity and/or the logistics and costs associated with its sampling at mesophotic depths. Future observations may provide a revised account of morphological variation in this previously overlooked species. Nonetheless, aside from plasticity, a genetic factor related to its life cycle cannot be excluded. For instance, variable ploidy could very well affect morphology (see Sauvage *et al.* 2019 for a succinct discussion on ploidy in *Caulerpa*).

With hindsight from the present study, it appears that Taylor (1928) encountered *Caulerpa wysorii sp. nov.* in deep dredges conducted in the Dry Tortugas and was confused by it. He repeatedly refers to a slender and delicate, lax or degenerate 'deep-water form of *C. sertularioides*', which he discusses under *C. ashmeadii, C. fastigiata* or *C. sertularioides*, but ultimately labelled with the latter epithet (Taylor 1928, p. 96, p. 98, and p. 103). Our plumose morphotype of *C. wysorii* dredged from the vicinity of the Dry Tortugas, which was initially diminutive (< 2 cm) with few distichous branchlets but developed more fully in the UL Lafayette mesocosm (Fig. 11), is reminiscent of W.R. Taylor's observations. This specimen would also confirm the occurrence of the taxon within the (now) Dry Tortugas National Park where W.R Taylor conducted his dredges in the mid-1920s. Interestingly, recent offshore exploration (Fredericq *et al.* 2019) of mesophotic habitats of the Flower Garden Banks National Marine Sanctuary (Texas, USA) did not reveal any filiform or plumose specimens. This suggests that *C. wysorii* is perhaps restricted to the eastern half of the Gulf of Mexico. The currently known populations of *C. wysorii* seem to occur in expansive, gently sloping carbonate platforms of the Yucatán (i.e. Campeche Bank) and West Florida shelves (Figs 10, S8) on the path of the 'loop' current (Weisberg & Liu 2017). It is possible that the steep slopes found westward of Campeche and the Florida Middle Grounds (i.e. offshore southeastern Louisiana) as well as the south of the Dry Tortugas therefore limit its dispersal and distribution (see depth contour in Fig. S8). Further exploration is needed in these habitats as well as those southward in understudied, expansive carbonate platforms such as the Nicaraguan shelf. Here, considering that large denuded specimens may represent multiple species (i.e. *C. wysorii* or the unknown species LAF6574), any putative identification should probably be disambiguated with DNA barcodes (at least for a few specimens if locally abundant). Likewise, while a few characters may allow separation of plumose *C. wysorii* from delicate forms of *C. sertularioides* (Figs 12, 13 and diagnosis further below), additional observations on broader collections are needed for verification. Therefore, as for denuded specimens, identification of plumose ones, may remain DNA-assisted. Regarding the unknown species LAF6574, it is possible that future mesophotic collections will bring to light more developed thalli (i.e. assimilators with branchlets) in order to facilitate its description.

Finally, DNA sequencing of *Caulerpa fastigiata* clarified the molecular identity of this species in the WTA (type locality Cuba) and its widespread distribution in the Indo-Pacific Ocean reaching the Western Pacific based on our specimen from Palau. We also provided a photographic illustration (Fig. 5) to demonstrate the diminutiveness of the species as compared to other filiform *Caulerpa* in the region. Considering our results and observations in comparison to the photographic illustration of Harvey's *Herpochaeta filiformis* nom. ined. from Tonga (= *C. tongaensis* Papenfuss; see Fig. S9 and notes in Supplementary File 1), whose tuft-like habit strongly resembles that of *H. fastigiata* (Montagne) Montagne (1843) from the same locality and collections (see Fig. S10; *H. fastigiata* = *C. fastigiata* since Montagne had transferred *Caulerpa* to *Herpochaeta* leading to the combination), we propose that *C. tongaensis* Papenfuss (1943) should be regarded as a heterotypic synonym of *C. fastigiata* Montagne (1837). Such synonymy follows the opinion of others, such as N'Yeurt & Tsuda (2014; implied p. 4), and annotations by W.F. Prud'homme van Reine on the sheet of *H. filiformis* in LD (see Fig. S9). In searching type material, three specimens from the Berkeley and Hooker herbarium (both close collaborators of Montagne) can be found in BM (BM000701574–576, Fig. S11); however, Montagne's types are usually located in PC. Following a request to PC, multiple syntypes of *C. fastigiata* collected in 1836 by R. de la Sagra from Cuba (as listed in Montagne 1837) could be located (MA10296–MA10306, cross-referenced as PC0762816, PC0762819–27, PC0762830). Among these specimens, we designate specimen MA10305/PC07762821 (Fig. S1) as lectotype, and additional type specimens in PC and BM (BM000701575–576) as isolectotypes (on the fair assumption that all specimens were collected at the same time by R. de la Sagra). Specimen BM000701574 annotated from 'Brasilia' (Brazil?) is ambiguous, and therefore is not considered here as type material since the only listed location in the protologue of *C. fastigiata* is Cuba (Montagne 1837).

## ***Caulerpa wysorii* T. Sauvage & M.J. Wynne *sp. nov.***

Figs 3, 4, 13

DIAGNOSIS: Thallus olive green, darker when dried. Assimilators filiform or delicately plumose. Assimilators occasionally dichotomously or subdichotomously branching up to 20 cm when filiform and 12 cm when plumose. Rachis 0.8–1.1 mm in diameter. Plumose assimilators exhibit a smooth stipe, up to 2 cm long. Branchlets opposite, each up to 1.5 cm long, <1 mm diameter and with a sharp mucron. The branchlet length uniform throughout the length of the assimilator, decreasing close to assimilator tip. Branchlets slightly compressed in formalin-preserved material (Fig. 13), possibly cylindrical when fresh. Branchlets straight or slightly curved upward (Figs 3, 13) as in some delicate specimens of *C. sertularioides* generally assigned to f. *longiseta* (see TS2126 in Fig. S2) but much less than in typical *C. sertularioides* (see TS2149 in Fig. S2). Filiform assimilators cylindrical. Stolon smooth, 1-2 mm in diameter. Rhizoids forming a compact anchor in specimens examined.

HOLOTYPE: Filiform specimen LAF-8-12-00-1-52 (*tuf*A barcode AJ417962) originally collected by B. Wysor, 12 August 2000 from 33 m depth in the vicinity of the Florida Middle Grounds in the northeastern Gulf of Mexico (28° 33.06′N 84°16.47′W). Specimen deposited in the University of Louisiana Herbarium (LAF) and depicted in Sauvage *et al.* (2014, fig. 12).

PARATYPES: Mesophotic plumose specimens collected by S. Fredericq and deposited at LAF as NSF-I-41-3-6-04 (*tuf*A MT441797), 3 June 2004 from 62 m depth in the vicinity of the Dry Tortugas (24°43.19′N 83°13.88′W), NSF-III-7-4-06-4-3 (*tuf*A MT441787), 4 July 2006 from 45 m depth (28° 34.24′N 84°28.77′W) and NSF-III-7-5-06-8-3 (*tuf*A MT441812), 5 July 2006 from 38 m depth (28°05.20′N 83°46.16′W) in the vicinity of the Florida Middle Grounds.

ADDITIONAL DNA SEQUENCES: Barcodes for specimen TS1923 are MT441785 (*tuf*A gene), MT441774 (*rpo*A gene) and MT431677–679 (ITS1-5.8S-ITS2 rRNA gene, polymorphic ribotype) in GenBank.

TYPE LOCALITY: Florida Middle Grounds in the northeastern Gulf of Mexico.

ETYMOLOGY: This species is named after Dr. Brian Wysor of Roger Williams University, Bristol, Rhode Island, who published the first molecular data on a representative specimen of this newly recognized species (specimen LAF-8-12-00-1-52, Genbank AJ417962 in Famà *et al.* 2002).

DISTRIBUTION: Eastern Gulf of Mexico, in mesophotic habitats of the West Florida shelf and shallow habitats of the Yucatán shelf, Mexico.

HABITAT: Coral reef slopes and rhodolith beds, mixed substratum (sand and rubble).

SPECIMENS EXAMINED: Specimens listed in Table 1.

## Comments

*Caulerpa wysorii* is endemic to the Western Tropical Atlantic (WTA). Its plumose morphotypes exhibit a striking resemblance to some delicate thalli of *Caulerpa sertularioides* generally assigned to f. *longiseta*. However, the plumose morphotypes of *C. wysorii* are found thus far only in mesophotic habitats, whereas genuine *C. sertularioides* is generally recognised as a shallow tropical species. In the Gulf of Mexico, filiform specimens may be confused with relaxed growths of *C. cupressoides*, which can be differentiated by the presence of occasional triangular papillae. Filiform specimens can be easily differentiated from *C. fastigiata*, which is extremely diminutive, tuft-like, and does not exhibit clear external features (stolons) without magnification. While both filiform and plumose specimens are presently documented from mesophotic habitats of the Florida Middle Grounds, only filiform specimens are documented from shallow habitat of the Yucatán peninsula. The presence of *C. wysorii* in mesophotic habitats of the Yucatán shelf is possible but undetermined. It is seemingly absent in shallow habitats of the Dry Tortugas, the Florida Keys and coastal Florida. Future investigations should further explore western habitats of the Gulf of Mexico (e.g. Flower Garden Banks) and southern locations in the WTA. The morphological features of the species and their variability should be reassessed on fresh material accessible via snorkelling or scuba. No specimens with intermediate morphology (e.g. filiform with occasional branchlets) were encountered in our modest collections. DNA-assisted identification is recommended.

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## REFERENCES

Altschul S.F., Gish W., Miller W., Myers E.W. & Lipman D.J. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215: 403–410. DOI: 10.1016/S0022-2836(05)80360-2.

Becker J.J., Sandwell D.T., Smith W.H.F., Braud J., Binder B., Depner J., Fabre D., Factor J., Ingalls S., Kim S.-H., *et al.* 2009. Global bathymetry and elevation data at 30 arc seconds resolution: SRTM30_PLUS. *Marine Geodesy* 32: 355–371. DOI: 10.1080/01490410903297766.

Bongaerts P., Ridgway T., Sampayo E.M. & Hoegh-Guldberg O. 2010. Assessing the 'deep reef refugia' hypothesis: focus on Caribbean reefs. *Coral Reefs* 29: 309–327. DOI: 10.1007/s00338-009-0581-x.

Calvert H.E. 1976. Culture studies on some Florida species of *Caulerpa*: morphological response to reduced illumination. *British Phycological Journal* 11: 203–214. DOI: 10.1080/00071617600650481.

Dellaporta S.L., Wood J. & Hicks J.B. 1983. A plant DNA mini preparation: version II. *Plant Molecular Biology Reporter* 1: 19–21. DOI: 10.1007/BF02712670.

Draisma S.G.A., Eurlings M.C.M. & Lim P.E. 2012. High intra-individual sequence variation in the nuclear rDNA LSU-5S intergenic spacer in the Sargassaceae (Fucales, Phaeophyceae). *Journal of Applied Phycology* 24: 1373–1379. DOI: 10.1007/s10811-012-9789-1.

Draisma S.G.A., Prudhomme van Reine W.F., Sauvage T., Belton G.S., Gurgel C.F.D., Lim P.E. & Phang S.M. 2014. A re-assessment of the infra-generic classification of the genus *Caulerpa* (Caulerpaceae, Chlorophyta) inferred from a time-calibrated molecular phylogeny. *Journal of Phycology* 50: 1020–1034. DOI: 10.1111/jpy.12231.

Durand C., Manuel M., Boudouresque C.F., 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. DOI: 10.0000/014461999371141.

Espinoza-Avalos J., Aguilar-Rosas L.E., Aguilar-Rosas R., Gómez-Poot J. M. & Raigoza-Figueras R. 2015. Presencia de Caulerpaceae (Chlorophyta) en la península de Yucatán, México. *Botanical Sciences* 93: 845–854. DOI: 10.17129/botsci.160.

Famà P., Olsen J.L., Stam W.T. & Procaccini G. 2000. High levels of intra- and inter-individual polymorphism in the rDNA ITS1 of *Caulerpa racemosa* (Chlorophyta). *European Journal of Phycology* 35: 349–356. DOI: 10.1080/09670260010001735951.

Famà P., Wysor B., Kooistra W.H.C.F. & Zuccarello G.C. 2002. Molecular phylogeny of the genus *Caulerpa* (Caulerpales, Chlorophyta) inferred from chloroplast *tuf*A gene. *Journal of Phycology* 38: 1040–1050. DOI: 10.1046/j.1529-8817.2002.t01-1-01237.x.

Fernández-García C., Wysor B., Riosmena-Rodríguez R., Peña-Salamanca E. & Verbruggen H. 2016. DNA-assisted identification of *Caulerpa* (Caulerpaceae, Chlorophyta) reduces species richness estimates for the Eastern Tropical Pacific. *Phytotaxa* 252: 252–304. DOI: 10.11646/phytotaxa.252.3.2.

Fredericq S., Arakaki N., Camacho O., Gabriel D., Krayesky D., Self-Krayesky S., Rees G., Richards J., Sauvage T., Venera-Ponton D. *et al.* 2014. A dynamic approach to the study of rhodoliths: A case study for the northwestern Gulf of Mexico. *Cryptogamie, Algologie* 35: 77–98. DOI: 10.7872/crya.v35.iss1.2014.77.

Fredericq S., Krayesky-Self S., Sauvage T., Richards J., Kittle R., Arakaki N., Hickerson E. & Schmidt W.E. 2019. The critical importance of rhodoliths in the life cycle completion of both macro- and microalgae, and as holobionts for the establishment and maintenance of marine biodiversity. *Frontiers in Marine Science* 5: 502. DOI: 10.3389/fmars.2018.00502.

Godínez-Ortega J.L. 2008. Colectores de algas de México (1787–1954). *Acta Botánica Mexicana* 85: 75–97. 2448-7589.

Harvey W.H. 1860. Characters of new algae, chiefly from Japan and adjacent regions, collected by Charles Wright in the North Pacific Exploring Expedition under Captain John Rodgers. *Proceedings of the American Academy of Arts and Sciences* 4: 327–335.

Hering K. 1841. Diagnoses algarum novarum a cl. Dre. Ferdinand Krauss in Africa Australi lectarum. *Annals and Magazine of Natural History [Series 1]* 8: 90–92.

Hughey J.R., Silva P.C. & Hommersand M.H. 2001. Solving taxonomic and nomenclatural problems in Pacific Gigartinaceae (Rhodophyta) using DNA from type material. *Journal of Phycology* 37: 1091–1109. DOI: 10.1046/j.1529-8817.2001.01048.x.

Kazi M.A., Reddy C.R.K. & Jha B. 2013. Molecular phylogeny and barcoding of *Caulerpa* (Bryopsidales) based on the *tuf*A, *rbc*L, 18S rDNA and ITS rDNA genes. *Plos One* 8: e82438. DOI: 10.1371/journal.pone.0082438.

Levring T. 1938. Verzeichnis einiger Chlorophyceen und Phaeophyceen von Südafrika. *Lunds Universitets Årsskrift, Ny Följd, Andra Afdelningen* 34: 1–25, pls I–IV.

Montagne C. 1837. Centurie de plantes cellulaires exotiques nouvelles. *Annales des Sciences Naturelles, Botanique, Série 2* 8: 345–370.

Montagne C. 1843. Quatrième centurie de plantes cellulaires exotiques nouvelles. Décades VII. *Annales des Sciences Naturelles, Botanique, Série 2* 20: 294–306, pl. 12.

N'Yeurt A.R. & Tsuda R.T. 2014. New records of marine algae from Tonga, Central Polynesia. *Marine Biodiversity Records*. 8. DOI:10.1017/S1755267214001110.

Papenfuss G.F. 1943. Notes on South African marine algae. II. *Journal of South African Botany* 9: 79–92.

Sauvage T., Payri C., Draisma S.G.A., Prud'homme van Reine W.F., Verbruggen H., Belton G.S., Gurgel F.D., Gabriel D., Sherwood A.R. & Fredericq S. 2013. Molecular diversity of the *Caulerpa racemosa-Caulerpa peltata* complex (Caulerpaceae, Bryopsidales) in New Caledonia, with new Australasian records for *C. racemosa* var. *cylindracea*. *Phycologia* 52: 6–13. DOI: 10.2216/11-116.1.

Sauvage T., Wynne M.J., Paul V.J. & Fredericq S. 2014. Morphological and molecular clarification of the enigmatic *Caulerpa floridana* W.R. Taylor (Chlorophyta, Bryopsidales) from the Dry Tortugas, Florida. *European Journal of Phycology* 49: 370–383. DOI: 10.1080/09670262.2014.947330.

Sauvage T., Schmidt W.E., Suda S. & Fredericq S. 2016. A metabarcoding framework for facilitated survey of endolithic phototrophs with *tuf*A. *BMC Ecology* 16: 8. DOI: 10.1186/s12898-016-0068-x.

Sauvage T., Schmidt W.E., Yoon H. & Fredericq S. 2019. Promising prospects of nanopore sequencing for algal hologenomics and structural variation discovery. *BMC Genomics* 20: 850. DOI: 10.1186/s12864-019-6248-2.

Stam W.T., Olsen J.L., Zaleski S.F., Murray S.N., Brown K.R. & Walters L.J. 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. DOI: 10.1111/j.1529-8817.2006.00271.x.

Stamatakis A. 2014. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313. DOI: 10.1093/bioinformatics/btu033.

Svedelius N. 1906. Reports on the marine algae of Ceylon. No. 1. Ecological and systematic studies of the Ceylon species of *Caulerpa*. *Ceylon Marine Biological Laboratory Reports* 2: 81–144.

Taylor W.R. 1928. The marine algae of Florida with special reference to the Dry Tortugas. *Publications of the Carnegie Institution of Washington* 379: [i-] v, [1]–219, 7 tables, 37 plates.

Weber-van Bosse A. 1898. Monographie des Caulerpes. *Annales du Jardin Botanique de Buitenzorg* 15: 243–401, pls XX [20]-XXXIV [34].

Weber-van Bosse A. 1908. Caulerpaceae. *Transactions of the Linnean Society of London, Second Series, Botany* 7: 171–173, pls 22, 23.

Weisberg R.H. & Liu Y. 2017. On the Loop Current penetration into the Gulf of Mexico. *Journal of Geophysical Research: Oceans* 122: 9679–9694. DOI: 10.1002/2017JC013330.

Wickham H. 2016. *ggplot2: elegant graphics for data analysis*, 2nd ed. Springer-Verlag, New York.

Wynne M.J., Verbruggen H. & Angel D.J. 2009. The recognition of *Caulerpa integerrima* (Zanardini) comb. et stat. nov. (Bryopsidales, Chlorophyta) from the Red Sea. *Phycologia* 48: 291–301. DOI: 10.2216/08-78.1.