

Crossing the Atlantic: The tropical macroalga *Caulerpa ashmeadii* Harvey 1858 as a recent settler in Porto Santo Island (Madeira archipelago, North-eastern Atlantic)

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ARTICLE INFO

Keywords:

Caulerpa ashmeadii
Biogeography
Distribution
Webbnesia
Soft-bottom
Climate change
Mesophotic meadows

ABSTRACT

The green macroalga *Caulerpa ashmeadii* was recently found in the North-eastern Atlantic Ocean, on the southern coast of Porto Santo Island (Madeira archipelago). *Caulerpa ashmeadii* was detected in a subtidal soft-bottom forming scattered patches, mostly among the green calcareous macroalgae *Halimeda incrassata* and *Penicillus capitatus*, within a depth range of 18–26 m. Some isolated patches were also observed in bare sand. The species is currently distributed over an estimated area of approximately 0.22 km². *Caulerpa ashmeadii* may represent a recent settler (Nov. 2020), although the hypothesis of the species being present elsewhere in Porto Santo prior to our discovery cannot be discarded. Alternatively, the presence of *C. ashmeadii* in Porto Santo may indicate the influence of climate change on the composition and structure of benthic communities in the Webbnesia region, which further stresses the importance of conducting more intensive biological and environmental monitoring programmes. Overall, this new record constitutes an exceptional eastern expansion of this marine species by more than 4200 km, comprising its first register in the Eastern Atlantic Ocean.

1. Introduction

Members of the siphonous green algal order Bryopsidales are ecologically important species known to form large meadows in many tropical and subtropical regions, particularly in mesophotic soft-seabed where they significantly increase habitat complexity to support associated diversity (e.g. Spalding, 2012; Ribeiro et al., 2019; Sauvage et al., 2020). All bryopsidalean species share a siphonous (coenocytic) thallus construction made of a tubular filament devoid of cross walls (i.e. a giant multinucleate cell harbouring millions of nuclei and organelles moving via cytoplasmic streaming, Raven, 1981; Bold and Wynne, 1985; Graham et al., 2009) and some genera develop rhizoids allowing them to 'root' the thallus in unconsolidated sediments and uptake nutrients from it (e.g. *Caulerpa*, *Halimeda*, *Udotea*).

Among bryopsidalean species, the genus *Caulerpa* J.V. Lamouroux 1809 (Bryopsidales, Chlorophyta) currently comprises > 100 accepted

species in tropical and sub-tropical shallow marine waters worldwide (Zubia et al., 2020; Guiry and Guiry, 2022). *Caulerpa* species have horizontal, creeping green stolons from which rhizoids grow downward to anchor the thallus by penetrating soft-bottom substratum or tightly adhering to hard (reef or rocky) substratum. Uprights axes are called fronds (or assimilators) and bear branchlets (or pinnules), whose overall morphotypes are used to distinguish species. Unfortunately, many *Caulerpa* species are phenotypically very plastic as a response to environmental factors (Ohba et al., 1992; Littler and Littler, 2000; Belton et al., 2014), which complicates their taxonomic identification.

The propagation of *Caulerpa* species is mainly clonal, either by patch expansion or thallus fragmentation (Ruesink and Collado-Vides, 2006), which has favoured invasion where they have been introduced. This is the case of *Caulerpa taxifolia* (M. Vahl) C. Agardh 1817 whose strain is thought to have originated from southeastern Australian waters (Jousson et al., 2000; Meusnier et al., 2004) and *Caulerpa cylindracea* Sonder

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1845 (Belton et al., 2014, previously described as *C. racemosa* var. *cylindracea*, Verlaque et al., 2003), initially thought as endemic to southwestern Australia, although later published records of this species in New Caledonia and Indonesia challenge this hypothesis (Sauvage et al., 2013; Darmawan et al., 2021). Invasive *Caulerpa* species in the Mediterranean Sea have significantly altered benthic communities and reduced biodiversity on large spatial scales (i.e. Verlaque, 1994; Verlaque et al., 2000; Boudouresque et al., 2009).

Coastal marine habitats are among the most vulnerable and heavily invaded ecosystems (Kolar and Lodge, 2001; Hochberg and Gotelli, 2005), and islands are known to be particularly prone to ecological damage by introduced species (Simberloff, 2013). Yet, to undergo range expansion, a species must first enter a new region, and its arrival per se does not guarantee establishment and subsequent positive population growth (Burgess et al., 2012; Sadowski et al., 2018). Hence, early detection through monitoring programmes is critical. As part of regular monitoring in the island of Porto Santo, Madeira, the recent discovery of a large distichous *Caulerpa* species, forming dense patches in soft-bed habitats in the southeastern part of the island prompted the present investigation.

The discovered species differs from the four *Caulerpa* species known from Porto Santo and the Madeira archipelago listed in Table 1. These species include *Caulerpa chemnitzia* (Esper) J.V.Lamouroux 1809 (previously recorded in the area as *Caulerpa racemosa* (Forsskål) J.Agarth 1873 as well as *C. racemosa* var. *peltata* (J.V.Lamouroux) Eubank 1944 see Belton et al., 2014:48 for taxonomy information on the reinstatement of *C. chemnitzia*), *Caulerpa prolifera* (Forsskål) J.V.Lamouroux 1809, *Caulerpa mexicana* Sonder ex Kützing 1849, and *Caulerpa webbiana* Montagne 1837 (including *C. webbiana* f. *disticha* Vickers 1896). Among the above, *C. mexicana* represents another distichous species reported for Porto Santo (Cruz-Reyes et al., 2001), but its pinnules are flat and end in an apiculus, while the newly discovered specimens presented cylindrical and obtuse pinnules. Hitherto, no occurrence of this kind of specimens has been reported within the Madeira archipelago. More broadly, within Webbsnesia archipelagos (i.e. the biogeographic area encompassing the Canary Islands, Madeira and Salvage archipelagos, Freitas et al., 2019), namely in the Canary Islands, and besides *C. mexicana*, only two other distichous species have been recorded, *Caulerpa sertularioides* (S.G. Gmelin) M. Howe 1905 and *C. taxifolia*. Yet, their pinnules also differ from those of the newly discovered specimens, and both bear apiculi (Afonso-Carrillo et al., 1984; Gil-Rodríguez and Afonso-Carrillo, 1980).

Here, combining morphological and molecular data, we report the discovery of *C. ashmeadii* in the subtidal soft-bottom habitats of the island of Porto Santo (Madeira archipelago), which represents the first register of the species in the Eastern Atlantic Ocean. This report is

Table 1
Distribution (presence/absence) of the genus *Caulerpa* in the different islands of the Madeira archipelago.

	Madeira	Porto Santo	Selvagens	Desertas
<i>C. chemnitzia</i> (Esper) J.V. Lamouroux 1809	+ ^{1,2}	+ ¹	+ ^{1,2}	+ ^{1,2}
<i>C. mexicana</i> Sonder ex Kützing 1849		+ ³	+ ³	
<i>C. prolifera</i> (Forsskål) J.V. Lamouroux 1809	+ ⁴	+ ⁴		
<i>C. webbiana</i> Montagne 1837	+ ⁵	+ ⁵	+ ⁵	+ ⁶

¹as *C. racemosa*, Audiffred and Prud'homme van Reine, 1985; Cruz-Reyes et al. (2001); Ferreira et al. (2012, 2018).

²as *C. racemosa* var. *peltata*, Audiffred and Weisscher (1984); Audiffred and Prud'homme van Reine (1985); Verlaque et al. (2003); Ferreira et al. (2018).

³Cruz-Reyes et al. (2001) and Lizán (2019).

⁴Levring (1974) and Neves et al. (2018).

⁵Levring (1974) and Ribeiro and Neves (pers. obs. 2017).

⁶as *C. webbiana* f. *disticha* Vickers 1896; Parente et al. (2000).

complemented by a map of the currently confirmed *C. ashmeadii* occurrence/spreading area and the characterization of its habitat.

2. Material and methods

2.1. Study area

The island of Porto Santo is the second largest island of the Madeira archipelago (area: 42 km², coastline: 38 km) located 43 km northeast of the island of Madeira, at ca. 700 km off NW-Africa (Fig. 1). Porto Santo coastline differs strongly from North to South; the northern coast is mostly crenulated with abrupt cliffs and rare pocket beaches, while the southern coast is mainly bordered by a sandy beach lined by a fore dune ridge (i.e., an arcuate embayment, Silva et al., 2019). The southern coast has a gentle slope from the shoreline down to the 50 m isobath, especially in its eastern part. The distance from shore down to the 50 m isobath varies between 700 (at the West, at Ilhéu da Cal) and ~3000 m in the East. The seafloor is composed primarily by sediments (fine to medium sands, φ (phi = 1–3) with an average thickness between 10 and 20 m (on the W sector of the bay down to depths of 30 m, and S of Ilhéu de Cima at depths between 40 and 100 m) and less than 5 m on the remainder of the bay (Instituto Hidrográfico, 2008). Overall, the shallower subtidal habitats of southern Porto Santo are mainly sedimentary and directly linked to the intertidal environment formed by the extensive sandy beach described above. These sedimentary habitats are subject to wave exposure mostly from the South and to tidal currents.

2.2. Habitat characterization and specimen

Since 2016, twice a year (summer and autumn), SCUBA-diving surveys have been used to monitor the benthic subtidal habitats at Porto Santo island at ten locations (Fig. 1). The surveys provided detailed and quantitative observation of seabed habitats at each location. Following the unexpected detection of the *Caulerpa* species in November 2020 in Porto Santo at 33.05° N, 16.30° W, more detailed surveys were conducted at this location using the methodology described in Neves et al. (2021). The processed data was then imported into SIGMA (a GIS platform developed with QGIS Development Team, QGIS org, 2021) and the discovered patches plotted (Fig. 1). A total of four surveys were performed in November 2020, July and November 2021, and March 2022. During the first three surveys several specimens were collected and deposited in the Funchal Natural History Museum herbarium (as MADM4080, MADM4083, MADM4084 and MADM4085) and consist of morphological vouchers preserved as herbarium sheet, in 4% formalin, and in 96% ethanol. During preparation of the present manuscript, a fourth survey was carried out in March 2022, with several specimens collected and silica-dried. These specimens were then used for DNA barcoding with *tufA* according to previously published amplification and sequencing protocols (e.g. Stam et al., 2006). Two specimens were sequenced from “Controlo Areia” (CoAr1 and CoAr2; black dot 1 in Fig. 1) and two others from “Pedra do Jota” (PeJo1 and PeJo2, black dot 10 in Fig. 1) (Fig. 2). *tufA* barcodes were analyzed via BLASTn searches for species molecular identity confirmation and to retrieve closely related species for phylogenetic reconstruction with RAXML (1000 tree search restarts and 1000 bootstrap repeats with a GTR+G+I model of evolution; Stamatakis, 2014).

3. Results

3.1. Species identification and description

The *Caulerpa* specimens are light to dark green, with upright, distichously branched (feather-like) fronds (9–12 cm high, 1–3 cm wide) rising from a creeping stolon (1–2 mm diameter), anchored by colourless rhizoids (1–2 mm diameter) to the substrate at intervals between 2.5 and 4 cm (Fig. 3b and d). The fronds' pinnules are cylindrical (1–2 cm

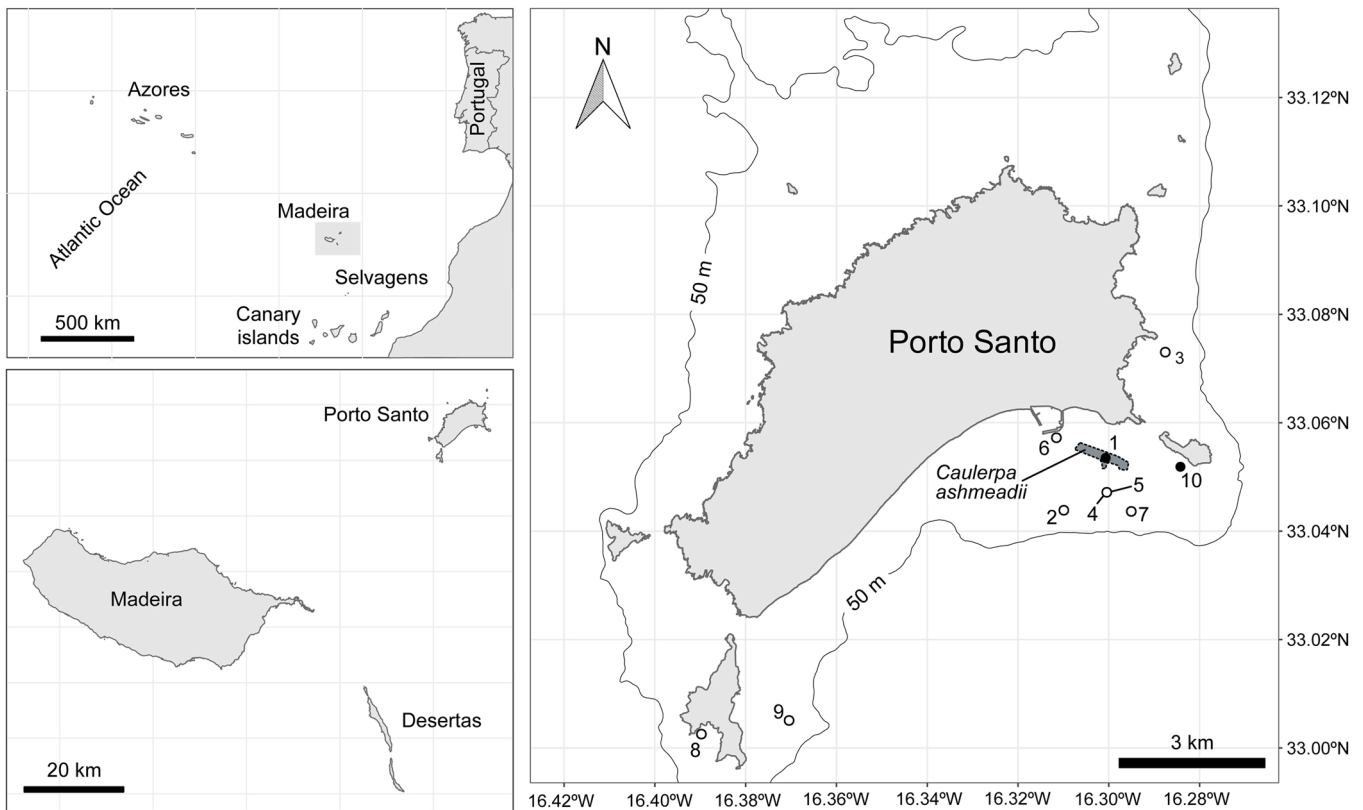


Fig. 1. (counterclockwise from the upper left corner) Location of Madeira archipelago in the North-eastern Atlantic; Madeira archipelago; *Caulerpa ashmeadii* occurrence area (dotted, dark-grey polygon) off Porto Santo island. Circles 1 and 10 (black dots) represent the locations surveyed where specimens were present and collected, whereas the white dots are the locations that have been surveyed since 2016 but where *C. ashmeadii* was not detected.

long, 0.5 mm diameter) with rounded (obtuse) apices devoid of apiculi. DNA barcodes generated for the gene *tufA* for the 4 specimens were all 100% identical (i.e. no base pair variation among specimens). BLASTn analyses of the barcodes on NCBI GenBank matched *C. ashmeadii* from Florida (type locality, specimen TS1824, KF977086) with 100% identity. Logically, phylogenetic reconstruction positioned the Porto Santo specimens among previously sequenced *C. ashmeadii* (Fig. 2) and closely related to sister species considered as Western Tropical Atlantic (WTA) endemics, *C. floridana* W.R.Taylor 1960, *C. wysorii* T.Sauvage & M.J. Wynne 2021 and an unknown *Caulerpa* species. These putative WTA endemics are closely related to the ampho-Atlantic species *C. prolifera*. The molecular identification is fully congruent with the morphological attributes described above and the description of this species (Harvey, 1858), which further confirms the identity of our new record from Porto Santo as *C. ashmeadii*.

3.2. Habitat characterization

Individuals of the green macroalga *C. ashmeadii* were first detected at Porto Santo subtidal soft-bottom seafloor (33.05° N, 16.30° W) in November the 7th 2020 at 20 m depth. Subsequent surveys (November 12th and 13th) revealed that the species formed scattered patches growing among the green calcareous bryopsidalean macroalgae *Halimeda incrassata* f. *gracilis* Børgesen 1913 (see Verbruggen et al., 2006 for this species molecular confirmation locally) and *Penicillus capitatus* Lamarck 1813 and occasionally *C. prolifera* (Fig. 3b, c, and e). However, some isolated patches were also monospecific (i.e., *C. ashmeadii* only) (Fig. 3f). At least 41 patches were detected, spreading for more than 1 km NW and 500 m SE from the first observed patch, at depths ranging from 18 to 22 m (Fig. 1). The biggest patch observed during the above surveys had an estimated area of 3–4 m². All specimens from these patches corresponded to the morphology of the sequenced specimens

confirmed as *C. ashmeadii*.

Eight months later (July 2021), a complementary survey showed that the area of occurrence of *C. ashmeadii* had increased, with many patches found especially east and south of the original area of occurrence (November 2020), and with a slightly broader bathymetry (18–26 m). Subsequent observations made during the fall of 2021, detected a small patch further southeast at 24 m depth in a soft-seabed at the boundaries of the rocky reef of “Pedra do Jota” (Fig. 1, circle 10). In March 2022, resurvey of this area identified few small individuals (i.e., stolon/thallus not yet forming a dense patch). Overall, based on our combined assessment conducted from 2020 to 2022, we estimate that *C. ashmeadii* currently occupies an area of approximately 0.22 km² in southern subtidal soft-bottom of Porto Santo Island (Fig. 1).

4. Discussion

Identification of the genus *Caulerpa* is often challenging due to the difficulty of assessing morphological boundaries for some species (Belton et al., 2014; Pattarach et al., 2019). In this context, DNA-assisted identification (e.g. Fernández-García et al., 2016; Darmawan et al., 2021) is generally recommended for species confirmation, especially in the case of new records or potential introductions in remote floras (i.e. distant from the known distribution of a species). Indeed, early in this study, the collected specimens were identified as *C. sertularioides*, however upon re-examination it turned out that this epithet did not fit the specimens, which, rather corresponded to *C. ashmeadii* (lack of apiculi, obtuse cylindrical ramuli and overall habit with large feathery assimilators). Thanks to collaborative efforts, this identification was later confirmed to *C. ashmeadii* via DNA barcoding of the gene *tufA* (Fig. 2).

Several feather-like *Caulerpa* species occur in the Eastern Tropical Atlantic (ETA), including the pantropical species *C. mexicana*,

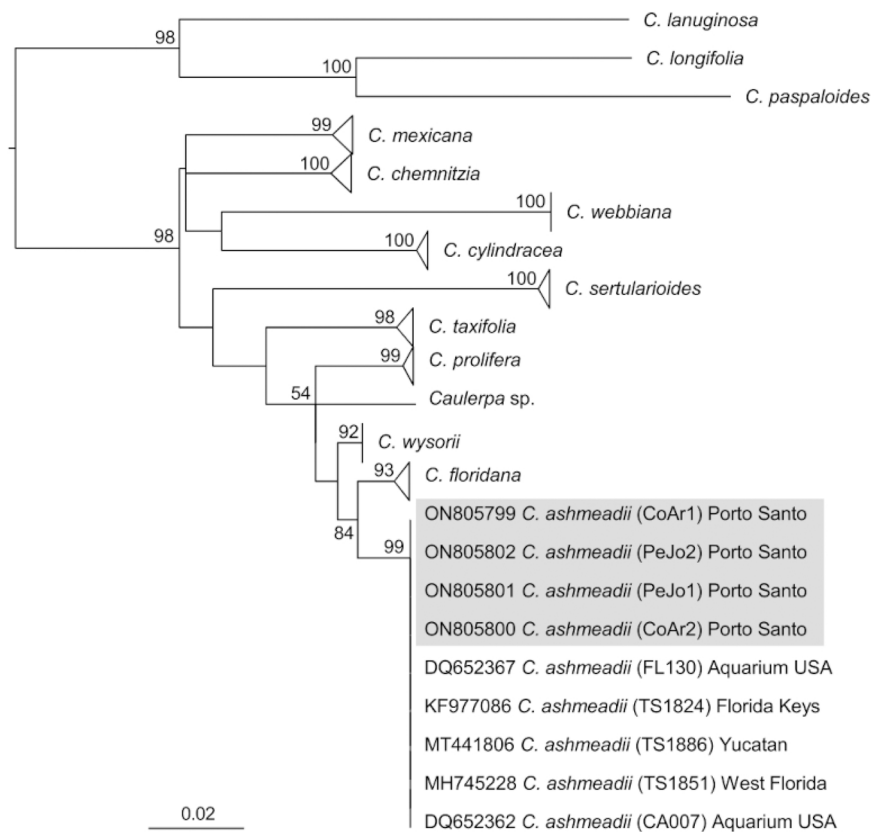


Fig. 2. Phylogenetic position of *Caulerpa ashmeadii* based on the chloroplast gene *tufA*. The topology and bootstrap support values were obtained with RAxML (values < 50% not reported). Labels shaded in grey correspond to newly generated sequences. The tree was rooted with *C. lanuginosa* J.Agardh, *C. longifolia* C.Agardh, and *C. paspaloides* (Bory) Greville.

C. sertularioides and *C. taxifolia* (Gil-Rodríguez and Afonso-Carrillo, 1980; Afonso-Carrillo et al., 1984; Cruz-Reyes et al., 2001). *C. webbiana*, which is also pantropical (Guiry and Guiry, 2022) and occurs throughout the Madeira archipelago, can occasionally develop distichous assimilators (as f. *disticha*, see Table 1), but is rather diminutive and quite different in its overall habit (Gonzalez Henriquez and Santos Guerra, 1983). In the WTA, another two distichous species are found, *C. floridana* and *C. wysorii*, which, along with *C. ashmeadii*, belong to a radiation of species considered endemic to this region (Fig. 2 and previously shown in Sauvage et al., 2014, 2021). However, aside from *C. ashmeadii*, all of the above species exhibit an apiculus on the branchlet. Hence, in any future observations in Porto Santo, or elsewhere in the Madeira archipelago or the Azores and Canary archipelagos, this key character should be examined to confirm taxonomic identification. Overall, so far, there is no indication that some of the ETA species listed above could represent misidentifications of *C. ashmeadii* in Webbnesia region. For example, the illustration and description of *C. sertularioides* in Afonso-Carrillo et al. (1984), (upcurved branchlet, ‘sharp’ apices, see their fig.7) is representative of that species and *C. mexicana* was also previously sequenced and confirmed from Gran Canaria based on the internal transcribed spacers (ITS1 & 2, accession AJ007816 in Genbank, Olsen et al., 1998).

Interestingly, at its type locality in the Florida Keys, *C. ashmeadii* generally occurs in sheltered calm areas on soft-bottom within seagrass beds or close to mangroves at shallow depths of 1–2 m (Pacheco-Cervera et al., 2009), but it has also been reported in deeper subtidal zones (e.g. down to 36 m, Littler and Littler, 2000, although Caribbean specimens are molecularly unverified to date). Contrastingly, in Porto Santo, *C. ashmeadii* is only found in deeper habitat where it forms dense patches from 18 m down to 26 m. Regular monitoring carried out by the authors in this island since 2016 allowed the observation of a significant change

in the studied mesophotic area depicted in Fig. 1. Initially, in the summer of 2016, the sandy seabed was mostly bare, with interspersed small *C. prolifera* meadows. The following year, in November 2017, a significant change was observed, with two additional bryopsidalean macroalgal species covering the seafloor, namely *H. incrassata* and *P. capitatus*. Later, between 2018 and 2022, our surveys revealed a progressive increase and dominance of the latter two species. Indeed, within a 5-year period, communities shifted from an almost unvegetated sandy habitat to a green macroalgae-dominated habitat, which now, also includes *C. ashmeadii* since 2020.

The confirmation of the presence of *C. ashmeadii* in Porto Santo represents a remarkable expansion of the species known distribution by more than 4200 km from the WTA to the Eastern Atlantic waters of the Madeira archipelago. The new record of *C. ashmeadii* discovered in the mesophotic soft-bottom habitats of Porto Santo island is unexpected and appears cryptogenic, considering the published distributional range of this species in the WTA (Guiry and Guiry, 2022). Indeed, the nearest location from Madeira archipelago where *C. ashmeadii* has previously been reported is the Atlantic island of Bermuda (Schneider, 2003). However, according to this author (personal communication), this record could never be verified and will be excluded in future species list of Bermuda algae. To date, in the WTA, *C. ashmeadii* has only been verified molecularly from its type locality in the Florida Keys and vicinity in the Gulf of Mexico (Yucatan-Mexico, and Western Florida, Stam et al., 2006, Sauvage et al., 2014, 2021). None of the records of *C. ashmeadii* from the Caribbean and down to Brazil (e.g., Taylor, 1979; Littler and Littler, 2000) have thus far been sequenced. Moreover, records from Brazil are also morphologically ambiguous (Sauvage et al., 2014, see their fig. 9 & 10). Some records of the epithet exist from Southeast Asia (see listing from Guiry and Guiry, 2022), but correspond to distichous growth of *C. macrodisca* Decaisne (confirmed from Thailand by Pattarach et al.,

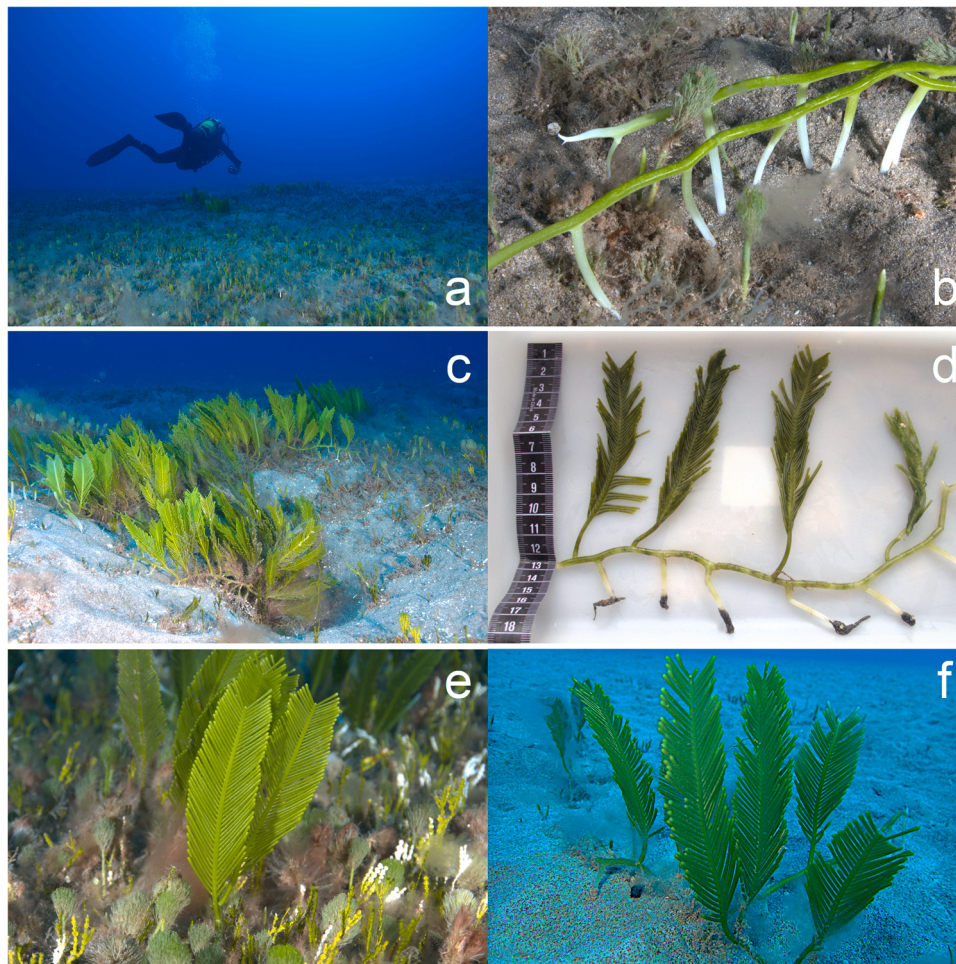


Fig. 3. General view from the habitat (a); detail of *C. ashmeadii* rhizoids (b); view of a *C. ashmeadii* patch (c); *C. ashmeadii* sample in the lab (measuring tape in centimetres) (d); Close-up of fronds: inside *Halimeda incrassata* and *Penicillus capitatus* meadow (e); and in bare sand (f).

2019).

The isolated nature of the *C. ashmeadii*'s record in southern Porto Santo in the direct proximity of a harbour, suggests a recent introduction to be a valid hypothesis. Nonetheless, since the current distribution of this species in the Atlantic remains unclear and is potentially broad (i.e. unclear records from the Caribbean and Brazil, see above), mesophotic investigations should be conducted throughout the Madeira archipelago and vicinity (e.g. Canary Islands and Cape Verde) to determine whether deep, overlooked populations, may exist. The fact that in Porto Santo, *C. ashmeadii* shares the habitat with ampho-Atlantic species (*C. prolifera*, *P. capitatus* and *H. incrassata*) that are also common in the WTA (e.g. Taylor, 1979) is intriguing. *Penicillus capitatus* was recently observed in tide pools of the northern coast of Madeira island, in Porto Moniz in 2019 (Gonçalo Gomes, see www.youtube.com/watch?v=dGf5Y0cNvjs) and in Seixal in July 2021 (C. Ribeiro, P. Neves and P. Wirtz pers. observations, voucher MADM 4121). Likewise, the recent appearance of *P. capitatus* in shallower waters of Porto Santo (Neves et al., 2018) from its initial record below 50 m – by Audiffred and Prud'homme van Reine (1985), may indicate a potential expansion from deeper algal communities. Therefore, floristic surveys should probably be conducted also in the lower mesophotic zone to look for the presence of these accompanying species to understand the putative origin of *C. ashmeadii* in Porto Santo.

Caulerpa ashmeadii was found near the marina and port of Porto Santo (Fig. 1), suggesting that boat traffic may represent a potential vector for the species introduction from the WTA. In this regard, surveys were conducted inside the marina and port of Porto Santo in July 2021

but no evidence of the species presence was found. Aside from the above putative vector of introduction, the occurrence of *C. ashmeadii* in Porto Santo (as well as *P. capitatus*, *H. incrassata* and other macroalgae species found within the Madeira archipelago, e.g. Ferreira et al., 2012; Ribeiro et al., 2019), could also be a sign of climate change over the composition and structure of benthic communities in the Webbsnesia region. Regardless, the fact that new patches of *C. ashmeadii* were detected between November 2020 and March 2022, suggests an ongoing phase of active colonization, whose monitoring should be continued. Therefore, further studies should assess the potential spreading rate of this species and evaluate the environmental factors that drive population dynamics, in order to understand its ecological role (e.g., invasive nature and added complexity and biodiversity in soft-bottom habitats).

Monitoring will be crucial to determine whether the colonization of southern Porto Santo by *C. ashmeadii* (as well as *P. capitatus* and *H. incrassata*) represents a spatial/temporal fluctuation in macroalgal cover or a longer lasting phase shift of this benthic habitat (i.e. a new ecological state). Our findings further stress the importance of implementing long-term monitoring programmes, to assess and catalogue marine biodiversity and habitat changes. Ultimately, genomic studies comparing *C. ashmeadii* from Porto Santo to the genome of this species from Florida in the WTA (Sauvage et al., 2019) are foreseen in order to gain further insights into its potential geographical origin.

CRediT authorship contribution statement

C. Ribeiro: Conceptualization, Methodology, Investigation, Data

curation, Formal analysis, Visualization, Writing – original draft. **T. Sauvage**: Formal analysis, Investigation, Methodology, Writing – review & editing. **S. Ferreira**: Methodology, Investigation. **R. Haroun**: Data curation, Writing – review & editing. **J. Silva**: Conceptualization, Methodology, Writing – review & editing. **P. Neves**: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Molecular data available on Genbank ([ON805799-ON805802](https://doi.org/10.26434/chemrxiv-2023-0n805)).

Acknowledgements

The authors wish to thank Mar Dourado and Porto Santo Sub for assistance during diving operations and to IFCN park rangers for all the help during Porto Santo survey campaigns. Pedro Neves was financially supported by the Oceanic Observatory of Madeira Project (M1420-01-0145-FEDER-000001-Observatório Oceânico da Madeira-OOM). This study received funding from Programa de Monitorização da CORDECA and from the Portuguese national funds from FCT - Foundation for Science and Technology through project UIDB/04326/2020 and LA/P/0101/2020 granted to Centro de Ciências do Mar. Thomas Sauvage thanks Veronique Verrez-Bagnis for facilitating the preparation and sequencing of the specimens at the Ifremer Centre Atlantic. We are also grateful to Dr. Stefano Draisma for reviewing earlier version of the manuscript.

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