



## Research Article CORRECTED PROOF

## 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)

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### Abstract

A new alien species of *Caulerpa*, corresponding morphologically to *Caulerpa taxifolia* (Vahl) C. Agardh var. *distichophylla* (Sonder) Verlaque, Huisman and Procaccini, already known from Turkey, Sicily, and Cyprus, was first discovered in Malta in June 2013. The original record was of but a few fronds, and surveys made during winter at the same site did not locate any plants. A survey conducted during July 2014, however, located two patches of the alga occupying a total area of ca 125m<sup>2</sup> in the 4–6m depth range, with a mean frond density of 276.3 (± 51.21 SD) per m<sup>2</sup>. Also in July 2014, two patches with a total of 520 fronds in an area of 0.37m<sup>2</sup> at 14–15m depth were found at a second site ca 7.4 km along-coast distance from the first site. The alien algae formed part of a photophilic assemblage growing on rock in clearings adjacent to *Posidonia oceanica* (Linnaeus) Delile stands. The most likely source of the Maltese populations is Sicily, and the most likely vector is recreational and/or commercial shipping.

**Key words:** alien species, invasive species, Malta, Sicily, *Caulerpa cylindracea*, photophilic algae, *Posidonia oceanica*

### Introduction

The genus *Caulerpa* within the family Caulerpaceae includes coenocytic chlorophytes with stolons that spread horizontally over the substratum, anchored by means of rhizoids. At intervals, the stolons give rise to upright photosynthetic fronds. The AlgaeBase database currently lists some 92 taxonomically valid taxa at specific and infraspecific level within the genus (Guiry 2014); however, the taxonomy is confused as a number of species complexes exist, and some species have variable morphology that is determined primarily by environmental conditions. The genus has a pan-tropical/pan-subtropical distribution with highest species richness in southern Australia (Guiry 2014); a few species occur in temperate waters including the two indigenous Mediterranean species, *C. prolifera* (Forsskål)

J.V. Lamouroux, and *C. ollivieri* Dostál, which may, however, be a dwarf ecotype of *C. prolifera* (UNEP/IUCN/GIS Posidonie 1990).

In the Mediterranean, *Caulerpa* achieved great notoriety when, in 1984, the alien species *Caulerpa taxifolia* (Vahl) C. Agardh was reported from inshore waters off Monaco, apparently an escape from the aquaria of the local Oceanographic Museum (Meinesz and Hesse 1991; Jousson et al. 1998). From Monaco, this aquarium strain, which was subsequently shown to be genetically different from the ancestral wild-type native to northeastern Australia (Meusnier et al. 2004), spread westwards (France, Spain, the Balearic Islands) and eastwards (Italy, Croatia) as well as south to Tunisia (Meinesz et al. 2001). The Tunisian population seems to represent a second, independent, introduction (Meusnier et al. 2004). Dramatically dubbed the ‘killer alga’ by the media because of

its invasive nature and because of concern about its potential displacement, or at least degradation, of indigenous Mediterranean species, especially seagrass (Meinesz 1999; see review by Boudouresque et al. 2009), this species gave rise to both public interest on the impact of alien species on native ecosystems, and to scientific research on its origin, ecology and impact as well as on methods of control. Paradoxically, the sudden spread of a second introduced species of *Caulerpa* in the early 1990s, *Caulerpa racemosa* (Forrskål) J. Agardh, did not generate much public interest although it was even more invasive than *C. taxifolia*, and spread much further and faster, and it had the potential for as much, if not more, environmental impact (Verlaque et al. 2000; Verlaque et al. 2004; Piazzini et al. 2005; Klein and Verlaque 2008; Boudouresque et al. 2009). Although *Caulerpa racemosa* had been present in the Mediterranean since the 1920s, Verlaque et al. (2000) showed that the invasive strain was different from the previously recorded populations, and Verlaque et al. (2003) eventually identified this invasive strain as *Caulerpa racemosa* var. *cylindracea* (Sonder) Verlaque, Huisman and Boudouresque. This taxon was recently elevated to specific rank as *Caulerpa cylindracea* Sonder, on the basis of molecular and morphological evidence (Belton et al. 2014). The originally present Mediterranean strains of *Caulerpa racemosa*, referred to by Verlaque et al. (2000) and subsequent authors as *C. racemosa* var. *turbinata* (J. Agardh) Eubank + var. *uvifera* (C. Agardh) J. Agardh, and as *C. racemosa* var. *lamourouxii* (Turner) Weber-van Bosse f. *requienii* (Montagne) Weber-van Bosse, were respectively assigned by Belton et al. (2014) to a species for which the oldest available name is *Caulerpa chemnitzia* (Esper) J. V. Lamouroux, and to *Caulerpa lamourouxii* (Turner) C. Agardh, which was reinstated as a full species.

In addition to these taxa, two additional, alien, species of *Caulerpa* have been reported from the Mediterranean: *Caulerpa mexicana* Sonder ex Kützing, limited to the coasts of Israel, Lebanon, Syria and Turkey; and *C. scalpelliformis* (Brown ex Turner) C. Agardh, with a similar distribution but also with isolated records from Egypt and Turkey (Ukabi et al. 2012; Verlaque et al. in press). Both *C. mexicana* and *C. scalpelliformis* are actually species complexes (Belton et al. 2014) but the Mediterranean strains have not yet been genetically characterised.

In 2006, a gracile form of *Caulerpa taxifolia* was reported from the Gulf of Iskenderun,

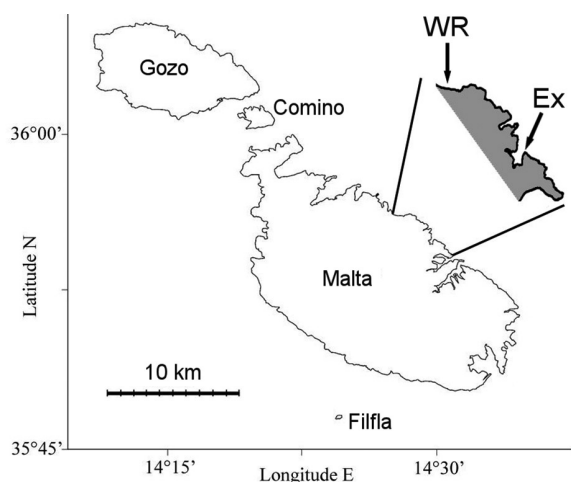
southeast Turkey (Cevik et al. 2007), and a year later the same form was recorded from the coasts of Sicily (Cormaci and Furnari 2009; Meinesz et al. 2010); more recently it has been found in Cyprus (Çiçek et al. 2013). Morphological and molecular studies have shown this gracile form to be different from the aquarium strain of *Caulerpa taxifolia*, and very close to the Australian species, *Caulerpa distichophylla* Sonder (Jongma et al. 2013); however, molecular analysis of *Caulerpa distichophylla* and *Caulerpa taxifolia* only revealed slight genetic differences between the two, leading Jongma et al. (2013) to consider *Caulerpa distichophylla* as a gracile variety of *Caulerpa taxifolia* and to propose the name *Caulerpa taxifolia* (Vahl) C. Agardh var. *distichophylla* (Sonder) Verlaque, Huisman and Procaccini.

*Caulerpa taxifolia* var. *distichophylla* appears to have been introduced into the Mediterranean from southwestern Australia and, unlike the already present aquarium strain of *Caulerpa taxifolia*, it seems to be limited by winter temperatures of less than 15°C (Jongma et al. 2013). Sicily, therefore, seems to represent the westward and northern limit of spread of *C. t.* var. *distichophylla* (Jongma et al. 2013; Musco et al. 2014). Obviously, there are no thermal limits to its spread southwards and here we record such a range extension to the Maltese Islands, which now represents the southernmost station for this relatively new alien species in the Mediterranean.

We were alerted to the presence of this species when, during surveys made in the summer of 2013 to monitor selected invasive alien species, including *Caulerpa cylindracea*, in Maltese marine protected areas (MPAs) (MEPA 2013) as part of an IUCN sponsored project (Otero et al. 2013), six fronds of a gracile *Caulerpa* previously unknown from the Maltese Islands were collected together with other algae from a site on the northeast coast of the island of Malta.

## Material and methods

Systematic surveys for the gracile *Caulerpa* were conducted during November 2013, January 2014, and July 2014 at the site where the original specimen of this species was collected in June 2013, the White Rocks area on the northeast coast of the island of Malta (Figure 1), as well as in adjacent areas along the same shore. A second site at Exiles in Sliema, also on the northeast coast of the island of Malta and at some 7.4 km along-coast distance from the White Rocks site



**Figure 1.** Map of the Maltese islands showing the location of the two sites where *Caulerpa taxifolia* var. *distichophylla* was found. Ex, Exiles; WR, White Rocks.



**Figure 2.** *Caulerpa taxifolia* var. *distichophylla* collected from Exiles, Sliema.

(3.9 km linear distance) (Figure 1) (where small patches were reported in July 2014) was also surveyed.

Shore-normal belt transects spaced 5 m apart were laid out from the shore to a distance of 50 m and SCUBA divers swam along the transects looking for the alga. When patches of the alga were uncounted, the approximate diameter of the patch (the distance between the extreme peripheral fronds) was measured using an underwater tape-measure and the density of fronds was estimated, either by counting all the fronds present in the patch if the patch was small, or by counting the fronds present in 20 cm × 20 cm quadrats placed randomly on the patch.

Specimens of the gracile *Caulerpa* and the accompanying flora were collected and transported fresh to the laboratory for examination. Underwater photographs were also taken.

The morphometry of the specimens was described through measurement of the diagnostic structural features used by Jongma et al. (2013) to characterise specimens from Sicily. The diagnostic features of a sample of specimens from each site were captured in photographs and micrographs and subsequently measured using the Measurement Tool of ImageJ v. 1.46r (Rasband 1997–2012).

Voucher specimens of plants from both Maltese sites have been deposited in the herbarium of the Department of Biology, University of Malta, as dry and wet-preserved material.

## Results

The gracile *Caulerpa* was originally found in a transect at White Rocks (Table 1) and was collected specifically because it co-occurred with *Caulerpa cylindracea* but was obviously different from the latter. No other specimens of the gracile *Caulerpa* were collected from other transects in the White Rocks area or in other Maltese MPAs during these surveys.

The White Rocks site (Figure 1) was specifically searched for the gracile *Caulerpa* on three other occasions: 24 November 2013, 04 January 2014 and 19 July 2014; the species was only found during the last of these surveys when two patches were located (Table 1). Independently of these surveys, a gracile *Caulerpa* was reported from the ‘Exiles’ area, Sliema (Figure 1) on 09 July 2014, and dedicated surveys in this area were made on 16 July 2014 (Table 1).

Plants from all sites listed in Table 1 were examined in the laboratory and measured (Table 2). The Maltese plants (Figure 2) had the following characteristics.

Thalli green; stolons slender, creeping, 0.30 – 0.84 mm in diameter (mean: 0.60±0.12 SD); rhizoidal pillars 0.63–3.48 mm long (mean: 1.65±0.78); fronds simple, erect 5.90–170.33 mm long (mean: 34.32±32.08) and 3.60–1.25 mm wide (mean: 1.95 ±0.48), feather-like; rachis 0.28–0.68 mm wide (mean: 0.50±0.09), with opposite distichous pinnules; pinnules 0.74–2.04 mm long (mean: 1.13 ±0.27) and 0.25–0.46 mm wide (mean: 0.38±0.05).

Morphologically the Maltese plants fall within the morphometric range of *Caulerpa taxifolia* var. *distichophylla* given by Jongma et al. (2013);

**Table 1.** Occurrence and characteristics of *Caulerpa taxifolia* var. *distichophylla* patches in the Maltese Islands.

| Locality       | Coordinates of shore end of transect | Date         | Linear distance of patch from shore (m) | Depth range of patch (m) | Dimension of patch | Standardized frond density (N/m <sup>2</sup> ) |
|----------------|--------------------------------------|--------------|---|--------------------------|--------------------|--|
| White Rocks    | 35°56'31.64"N<br>14°27'18.78"E       | 11 June 2013 | 30                                      | 3.6                      | Not recorded.      | 30 (estimated; no quadrat counts)              |
| White Rocks    | 35°56'14.05"N<br>14°27'47.46"E       | 19 July 2014 | 35                                      | 4.5                      | 13.2 × 2.8m        | 235.0 (± 21.25 SD)                             |
| White Rocks    | 35°56'15.26"N<br>14°27'47.13"E       | 19 July 2014 | 44                                      | 5.5                      | 22.0 × 4.0m        | 317.5 (± 24.75 SD)                             |
| Exiles, Sliema | 35°55'14.31"N<br>14°29'56.98"E       | 16 July 2014 | 190                                     | 14.5                     | 0.50 × 0.50m       | 340 (all fronds in patch counted)              |
| Exiles, Sliema | 35°55'13.76"N<br>14°29'56.14"E       | 16 July 2014 | 165                                     | 15.0                     | 0.35 × 0.35m       | 180 (all fronds in patch counted)              |

**Table 2.** Means and associated standard deviation of morphometric parameters measured for specimens of *Caulerpa taxifolia* var. *distichophylla* from Maltese sites, with the range of the same parameters for the population from Punta Braccetto, Sicily, Italy, as reported by Jongma et al. (2013). All values are in mm.

| Locality   | Character       |                         |               |             |              |                |               |
|--|-----------------|-------------------------|---------------|-------------|--------------|----------------|---------------|
|  | Stolon diameter | Rhizoidal pillar length | Frond height  | Frond width | Rachis width | Pinnule length | Pinnule width |
| White Rocks transect 1<br>35°56'14.05"N<br>14°27'47.46"E | 0.58 ± 0.07     | 1.91 ± 1.07             | 19.54 ± 6.67  | 1.84 ± 0.39 | 0.50 ± 0.09  | 1.01 ± 0.21    | 0.37 ± 0.06   |
| White Rocks transect 2<br>35°56'15.26"N<br>14°27'47.13"E | 0.67 ± 0.10     | 1.55 ± 0.39             | 28.27 ± 9.38  | 1.70 ± 0.26 | 0.51 ± 0.05  | 1.03 ± 0.11    | 0.40 ± 0.02   |
| Exiles, Sliema<br>35°55'13.76"N<br>14°29'56.14"E         | 0.47 ± 0.15     | 1.28 ± 0.58             | 99.11 ± 53.57 | 2.37 ± 0.20 | 0.62 ± 0.05  | 1.28 ± 0.11    | 0.35 ± 0.03   |
| Sicily   | 0.5–1.10        | 0.1–0.5                 | 10 (maximum)  | 2.0–4.0     | 0.5–1.0      | 1.0–3.0        | 0.2–0.5       |

no statistically significant differences were found between measurements made of the Maltese specimens and those reported by these authors (One-Way ANOSIM,  $P > 0.05$ ). However, while specimens from the two White Rocks patches were not significantly different from one another, both differed significantly from the Exiles specimens (One-Way ANOSIM,  $P < 0.05$ ), and 97% of the difference was due to frond height (SIMPER), the White Rocks plants having shorter fronds than the Exiles plants.

All presently known Maltese stations for *C. taxifolia* var. *distichophylla* are in the shallow infralittoral (3–15 m depth) zone. At both White Rocks and at Exiles, the alga formed part of photophilic assemblages growing on a gently sloping rocky seabed with a thin veneer of sediment and colonised by sparse *Posidonia oceanica* (Linnaeus) Delile meadows or scattered clumps of the seagrass (Figure 3). These assemblages

occurred within clearings of varying size between the seagrass stands; the clearings were larger at White Rocks than at Exiles (Figure 4). At Exiles, the clearings with *C. taxifolia* var. *distichophylla* were located within 10m from the transition between the seagrass meadow and the unvegetated sandy seabed. The following macrophytes were present in the same assemblage as *C. taxifolia* var. *distichophylla* (E = Exiles; WR = White Rocks):

#### Fucophyceae

*Cystoseira foeniculacea* (L.) Greville f. *tenuiramosa* (Ercegovic) A. Gómez Garreta, M.C. Barceló, M.A. Ribera and J. Rull Lluch [WR]

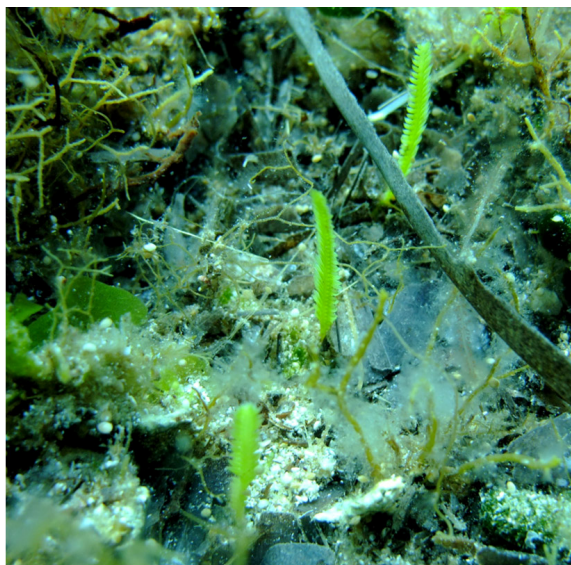
*Dictyopteris polypodioides* (A.P. De Candolle) J.V. Lamouroux [E, WR]

*Dictyota linearis* (C. Agardh) Greville [E, WR]

*Dictyota mediterranea* (Schiffner) G. Furnari [WR]

*Padina pavonica* (L.) Thivy [E, WR]





**Figure 3.** Assemblage of shallow infralittoral photophilic algae with *Caulerpa taxifolia* var. *distichophylla* from the White Rocks site, northeastern Malta.



**Figure 4.** Habitat of *Caulerpa taxifolia* var. *distichophylla* at White Rocks, northeastern Malta. The algae occurred within assemblages of photophilic algae adjacent to stands of *Posidonia oceanica* on rock at depths of 4-6m.

#### Ulvophyceae

*Anadyomene stellata* (Wulfen) C.Agardh [WR]  
*Caulerpa cylindracea* Sonder [E, WR]  
*Cladophora* cf. *nigrescens* Zanardini ex  
Frauenfeld [WR]  
*Dasycladus vermicularis* (Scopoli) Krasser [WR]  
*Flabellia petiolata* (Turra) Nizamuddin [E]

#### Rhodophyceae

*Jania rubens* (L.) J.V. Lamouroux [E, WR]  
*Peyssonnelia* sp. [E]  
*Sphaerococcus coronopifolius* Stackhouse [E]  
Unidentified non-geniculate coralline alga [WR]

#### Discussion

Morphologically, the gracile *Caulerpa* from Malta has the same characteristics as *C. t.* var. *distichophylla* from Sicily and fits within the range of variation for all structures measured by Jongma et al. (2013); we are confident that the Maltese plants belong to this taxon. Within the Maltese Islands, the morphometric differences between plants from the two collection sites could be due to environmental differences. In Sicily, Musco et al. (2014) noted *C. taxifolia* var. *distichophylla* from shallow water to have longer fronds in shaded conditions than when exposed to full light. We have observed a similar phenomenon in Maltese *C. cylindracea* where plants in shallow water have short fronds that grow longer, and more distichous, with depth (personal observations).

*Caulerpa taxifolia* var. *distichophylla* was first recorded from a very limited area at White Rocks where it had an extremely low frond density, which was so sparse as to suggest a single individual. This patch could not be located again in subsequent surveys, which suggests that the fronds at least may only be seasonally present. Since at White Rocks *C. taxifolia* var. *distichophylla* co-occurred and was admixed with *C. cylindracea*, which was abundant, it is quite likely that the former species may have persisted as stolons, which are not distinguishable in the field from those of its congener. Compared to the first record in June 2013, the second record at White Rocks is remarkable for the much larger area covered and the high frond density, as well as the occurrence of two patches, albeit in close proximity. This suggests that the species is spreading at White Rocks, either by extension of existing stolons or by new colonisation through fragmentation, possibly during the winter months.

At Exiles, both patches of *C. taxifolia* var. *distichophylla* occupied a limited area and had a reduced frond density, again suggesting that each may represent a single plant. However, the patches were distant from each other, suggesting independent colonisation. Moreover, the Exiles site is about 7.4 km along-coast distance from the White Rocks site, which may indicate the

presence of unknown source populations rather than connectivity between the two sites.

*Caulerpa taxifolia* var. *distichophylla* may have been present in Malta earlier than the first sighting in 2013. In spite of its distinctive morphology which is dissimilar from any other indigenous species, the diminutive size of the fronds (maximum length measured by us: 170.3 mm; mean:  $36.4 \pm 35.5$  SD mm) and the fact that the plant grows embedded within dense assemblages of taller-growing algae, makes it very difficult to spot, even when specifically being searched for.

It is interesting to note that at White Rocks, *C. cylindracea* was abundant and growing vigorously during the winter but had regressed during the summer, when, conversely, *C. taxifolia* var. *distichophylla* started showing strong growth. *Caulerpa cylindracea* was first found in Malta in 1997 (Borg et al. 1997), and by the mid-2000s had spread along almost the entire coast of the Maltese Islands, in places forming abundant populations (Mifsud and Lanfranco 2007); from 2006, these populations regressed and the plant practically disappeared from some coastal sites where it was previously common (Barbara and Borg 2013), only to reappear again in some sites in the past two years (authors' personal observations; J.A. Borg, personal communication). Moreover, on a regional scale, the aquarium strain of *C. taxifolia* currently seems to be in regression in many areas (Meinesz et al. 2010). It seems that inter- and intra-annual cycles of growth and regression are a common feature of these invasive species in the Mediterranean and it remains to be seen what patterns will be shown by *C. taxifolia* var. *distichophylla*.

So far *C. taxifolia* var. *distichophylla* in Malta is only known from two sites where it occupies very small areas and has a low frond density. This is in contrast with the situation in Sicily, where the invader is known from ca. 25 km of coastline in the original area of colonisation (Punta Braccetto, SE Sicily; Jongma et al. 2013), and from four additional sites along the southeastern coast of the island from Mazara del Vallo to Capo Passero; it is also known from a single site on the northern (Tyrrhenian) coast (see map in Fig. 1 of Musco et al. 2014). At most of these sites, the alien has a high cover (> 50%) in some habitats, especially at the periphery of *Posidonia oceanica* meadows (Musco et al. 2014).

In both Malta and Sicily, *C. taxifolia* var. *distichophylla* occurs in shallow water close to the shore, admixed with assemblages of photophilic

algae including *C. cylindracea*. In Malta *C. taxifolia* var. *distichophylla* has so far only been found on rocky bottoms occupying spaces between stands of *Posidonia oceanica*. In Sicily this species occurs on rocky and sandy bottoms as well as on artificial substrata, and is particularly abundant on matte at the periphery of *Posidonia oceanica* meadows, the latter appearing to be a preferred habitat (Jongma et al. 2013; Musco et al. 2014). No *Posidonia* matte occurs at the sites colonised by *C. taxifolia* var. *distichophylla* in Malta, and while this species occurs in close proximity to *Posidonia oceanica* meadows or stands, it does not occur within the meadows, suggesting that at present there is no spatial overlap between the two. The vigorous growth of the accompanying photophilic assemblage and the sparse cover of *C. taxifolia* var. *distichophylla* indicates that at present at least, other than a possible limited competition for space, the alien species has had little obvious impact on the previously resident assemblages, which also include the invasive *C. cylindracea*.

In the Mediterranean *C. taxifolia* var. *distichophylla* is a recent introduction, or series of introductions, from Australia. At Punta Braccetto in Sicily, Jongma et al. (2013) found plants that clustered in different clades, suggesting independent introductions from outside the Mediterranean and from the already established population in Turkey. The original introduction into the Mediterranean seems likely to have been due to the aquarium trade or shipping, although more likely the former (Cevik et al. 2007; Jongma et al. 2013). Within the Mediterranean, transport by currents or shipping or with fishing gear have been suggested to spread the alga (Musco et al. 2014). For the Maltese populations, the most likely source of the new colonisers is Sicily and the most likely vector is recreational and/or commercial shipping. The southern coast of Sicily is slightly less than 100 km directly north of the shores where *C. taxifolia* var. *distichophylla* was found in Malta and there is a regular, and at times heavy, two-way traffic of pleasure craft and other vessels between the two islands; it is also relevant to note that most yacht marinas and harbours in Malta are situated along the northeastern coast.

By most definitions, *C. taxifolia* var. *distichophylla* is invasive in Sicily where it is not only established, but is also extending its range and has had significant ecological impact on the ecosystems it has colonised; moreover, it has also affected fishing activities and therefore also

has pest status (Musco et al. 2014). In Malta, it seems that this species is in its initial stages of colonisation. While it has not yet become invasive or seems to have had any large ecological (or economic) impact, the situation needs to be very carefully monitored, and management of the species considered, especially since, like the aquarium strain of *C. taxifolia*, *C. taxifolia* var. *distichophylla* seems to readily propagate clonally by fragmentation. The shallow water stations where it has been found offer great potential for the alga to be ripped off the bottom by boat anchors and chains, propellers or winter storms and therefore for the species to spread. Any management measures, however, need to take into account the ease with which the alga propagates through fragmentation, lest management actions themselves inadvertently lead to spreading of the invader.

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