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Concern about the spread of the invader seaweed *Caulerpa taxifolia* var. *distichophylla* (Chlorophyta: Caulerpales) to the Western Mediterranean

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Abstract

The Australian alien seaweed *Caulerpa taxifolia* var. *distichophylla*, after being established along the Turkish Mediterranean coast in 2006, was recorded in Southern Sicily in 2007. Since then local fishermen asked for support to counteract the effects of entanglement of large amounts of the alien strain wrack in their trammel nets, causing the gear to become ineffective. The further northward and westward spread of this new alien strain is believed to be limited by winter temperature. We present novel data confirming that the new alien strain is fully naturalized in the Central Mediterranean and is expanding its range beyond such limit (i.e. the 15°C February isotherm), thus becoming potentially able to colonize the western basin. Based on a preliminary estimation of the effects on native polychaete assemblages, and considering some peculiarities of Sicily (mostly linked to its geographical position in the Mediterranean Sea), the risk linked to the increasing range of distribution of the invasive algae is highlighted.

Keywords: Mediterranean Sea, Alien species, Benthic assemblages, Invasive, *Posidonia oceanica*, Polychaetes.

Introduction

Among the ca. 1000 alien species recorded up to now in the Mediterranean Sea (Zenetos *et al*., 2012) some invasive macroalgae have raised serious concern due to their capability to modify the physical and chemical features of the invaded habitats (e.g. sediment deposition rate, redox potential depth, etc.) and to compete with resident flora, thus potentially interfering with biodiversity and ecosystem functioning (Williams & Smith, 2007). The invasion of *Caulerpa taxifolia* (Vahl) C. Agardh and *C. cylindracea* (Sonders), previously known as *C. racemosa* var. *cylindracea* (Sonders) Verlaque, Huisman *et* Boudouresque (see Belton *et al*., 2013), is particularly significant due to the amplitude of the affected area and the impact on native biota also mediated by the production of deterrent compounds (Boudouresque *et al*., 1995; Dumay *et al*., 2002; Klein & Verlaque, 2008; Papini *et al*., 2013). Both *Caulerpa* species colonized an array of Mediterranean habitats, including the endemic *Posidonia oceanica* (Linnaeus) Delile meadows. The so called *P. oceanica* "*matte*", a complex structure formed by intertwined living or dead rhizomes, together with the sediment filling the interstices, appears to be particularly suitable for the colonization of these alien algae (Piazzi & Balata, 2009). The *matte* usually hosts rich invertebrate communities that can be affected by the presence of *C. cylindracea* and *C. taxifolia* (Francour *et al*., 2009; Box *et al*., 2010).

Recently, Jongma *et al.* (2013) demonstrated that a slender featherlike *Caulerpa* taxon discovered in Southern Turkey in 2006 by Cevik *et al.* (2007) and recorded in South Eastern Sicily in 2007 (as *C. distichophylla* in Meisnez *et al*., 2010) is in fact a morphologically slender variety of *C. taxifolia*, *C. taxifolia* (Vahl) C. Agardh var. *distichophylla* (Sonder) Verlaque, Huisman *et* Procaccini (hereafter *Ctvd*), resulting from recent introduction from South Western Australia where the taxon is endemic. Until now, the new alien strain has been reported from the southernmost part of Sicily, namely, from Punta Braccetto to Capo Passero (Fig. 1), in the area between Iskenderun Bay and Antalya Bay in Turkey (Meisnez *et al*., 2010; Jongma *et al*., 2013), and Cyprus (Çiçek, 2013). According to Jongma *et al.* (2013), the Mediterranean distribution of this variety and its ecological requirements in the area of origin in Australia suggest that winter water temperatures might limit its spread to the northern and western parts of the Mediterranean Sea. Specifically, the affected area in Sicily is located within the 15° C winter isotherm, which is considered to be the boundary limit of the northward and westward establishment of *Ctvd*.

In 2007, the fishermen from south eastern Sicily asked for support to counteract the effects of entanglement of large amounts of *Ctvd* wrack in their trammel nets, causing the gear to become ineffective. Increasing concern expressed by stakeholders have prompted local authorities to ask for scientific support to assess the magnitude of the new, unexpected phenomenon. A few years later, the invader was mostly reported in shallow waters (F. Andaloro, unpublished data).

We hereby present the results of a two-year (2012-2013) monitoring program aimed at: 1) assessing the geographic distribution of the new alien strain along the Sicilian coast, and 2) detecting the affected shallow water habitats. Moreover, assuming that the new alien strain might have detrimental effects on native biota, we also provide a first estimation of *Ctvd* effects on polychaete assemblages associated to *P. oceanica*.

Material and Methods

Analysis of Ctvd *distribution*

In May 2012, a monitoring program was initiated with the purpose of surveying the coasts of Sicily. The aim was to assess the invasion of Southern Sicily and survey the westward and northward spread of *Ctvd*. In particular, 30 randomly chosen sites were studied: 1) 8 sites located in the area reported as invaded, from Punta Braccetto (PB) to Donnalucata (DL) and 2) 22 sites scattered in other areas of the Sicilian shoreline, in order to check the spread of *Ctvd* outside the affected area. Specifically, 11 sites were located to the west of the area of the first record of the invader algae (PB) and 10 along the northern coast of Sicily facing the Tyrrhenian Sea (Fig. 1).

At each site, the shallow water habitats (0-5 m depth) within 100 m from the shoreline were surveyed between May and July 2012 and 2013 by snorkelling. Such habitats encompassed sandy bottoms, *P. oceanica* and *Cymodocea nodosa* (Ucria) Ascherson meadows, shallow rocky bottoms and breakwaters.

Analysis of assemblages

In order to get a quick estimation of the effects of the alga invasion on the resident benthic assemblages, polychaetes were analyzed at family level following

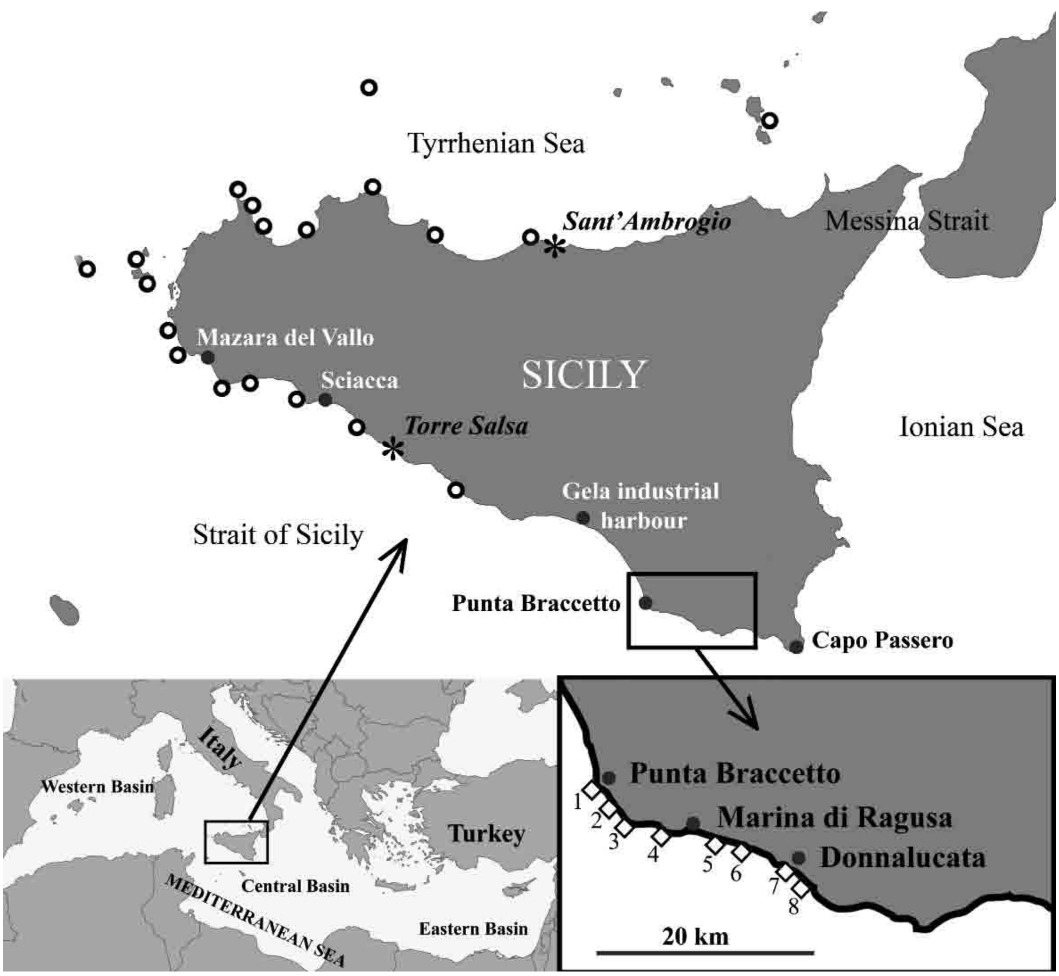

Fig. 1: Map of the study area. Circles represent surveyed sites where *Caulerpa taxifolia* var *distichophylla* was not observed, diamonds represent 8 invaded sites where the alga was monitored in 2012 and 2013 in the invaded area, and asterisks represent new records outside the invaded area.

Musco *et al.* (2011). Since *P. oceanica* (particularly *matte* at meadow border) appeared to constitute the most suitable habitat for *Ctvd* in the invaded area (see results below), it was chosen as the target habitat for impact assessment. Polychaete distribution patterns at the invaded DL (Fig. 1) and not invaded Sciacca (SC) meadows were analyzed.

The experimental design encompassed two factors: meadow (ME), fixed, 2 levels (invaded and non-invaded); and site (SI), random and nested within meadow, 2 levels (1 and 2). There were 3 replicates for each combination of factor levels.

The non-invaded *P. oceanica* meadow was haphazardly selected among those located upstream of the impacted area in the Strait of Sicily and having similar features in terms of distance from the shoreline (c.a. 100 m), surrounding bottom (fine sand), and meadow phenological variables, including shoot density, average leaf length, leaf area index, height of the meadow (unpublished data).

Underwater, 20x20x10 cm of *P. oceanica* dead *matte* samples were taken at 3 m depth with hand metal corers on the borders of *P. oceanica* meadows, after using an airlift sampler to collect epibenthic vagile individuals. At DL, only *matte* with *Ctvd* percent coverage higher than 95% of total cover was sampled. The collected material was transferred to 0.4 mm mesh bags underwater and further sieved through 0.5 mm mesh; 10% buffered formalin was used for sample fixation. After rinsing, polychaetes were transferred to 70% alcohol, sorted and identified at family level following Fauchald (1977).

In addition, at both meadows (DL and SC) 6 replicate samples were randomly collected using PVC corers (ø 3.5 cm) within the first 5 cm layer in order to measure the redox potential depth (RPD) of dead *matte* at meadow borders.

Data were analyzed by standard techniques including univariate (ANOVA) and multivariate approaches (MANOVA, SIMPER).

Results

Distribution, spread and patterns of abundance of **Ctvd** *along the Sicilian coast*

In May 2012, *Ctvd* was observed in all the screened habitats along the south-eastern coast between PB and DL (Table 1; Fig. 2). The invader occasionally colonised sandy bottoms as sparse short fronds (8-10 cm), which did not appear dense enough to form typical mats. In contrast, *Ctvd* colonized both natural and artificial rocky bottoms, where it often co-occurred with *C. cylindracea*. In particular, on breakwaters, *Ctvd* appeared more abundant and with longer fronds (ca 15 cm) under sheltered and shaded conditions. The invader alga was most abundant on dead *matte* as well as along the *P. oceanica* meadow borders. Incidentally, some small mats of the invader alga were located in those meadow spots where the seagrass shoots were less dense.

Neither non-invaded meadows nor uncolonized *P. oceanica* dead *matte* were found at the checked south-eastern sites. The invader strain appeared to be the dominant alga in the regular algal assemblage associated to the dead *matte*, often monopolizing space availability in this habitat (Table 1; Fig. 2).

It is worth noting that the slender fronds appeared generally longer (up to 35 cm, 20 cm on average) in areas shaded by *P. oceanica* leafs than in areas exposed to direct light (c.a. 20 *vs.* 8 cm on average). Notably, in one of the localities checked, Marina di Ragusa (Fig. 1), the new alien algal strain was particularly abundant in the marina, where it flourished on the lines used to moor the boats. *Ctvd* was not observed in the other localities checked along the Sicilian coast in 2012 (Fig. 1).

In 2013, *Ctvd* was established at all the sites where it was previously found along the south-eastern coast, with patterns of abundance similar to those observed in 2012 (Table 1). In addition, the invader was recorded for the first time on the south-western coast at (a) Torre Salsa (Strait of Sicily), municipality of Siculiana, and on the northern coast at (b) Sant'Ambrogio (Tyrrhenian Sea,

Table 1. Level of Ctvd invasion in different habitats at 8 sites checked in spring-summer 2012 in south-eastern Sicily: *** = heavily invaded (dense fronds forming mats, cover >50%), ** = moderately invaded (numerous fronds, cover <50% and >10%), * = slightly invaded (few sparse fronds, cover <10%), / = habitat not present/observed. PB = Punta Bracceto; TdM = Torre di Mezzo; PS = Punta Secca; MdR = Marina di Ragusa; DL = Donnalucata; *Po* = *Posidonia oceanica*; RS = rocky shore; *Cn* = *Cymodocea nodosa*, AHS = artificial hard substrate.

		Sites							
		1 (PB)	**2 (TdM)**	**3 (PS)**	**4 (MdR)**	**5 (MdR)**	**6 (MdR)**	**7 (DL)**	**8 (DL)**
Latitude		*36°48'56.70"N*	*36°48'16.36"N*	*36°47'18.90"N*	*36°46'56.46"N*	*36°46'48.48"N*	*36°46'16.26"N*	*36°45'17.82"N*	*36°44'53.06"N*
Longitude		*14°27'31.08"E*	*14°28'38.18"E*	*14°29'32.22"E*	*14°31'42.36"E*	*14°34'02.34"E*	*14°35'41.10"E*	*14°38'35.13"E*	*14°39'09.16"E*
Habitat	*Po*	***	**	***	***	**	***	***	***
	RS	**	*	*	*	*	/	/	/
	Sand	*	*	**	/	*	*	*	*
	Cn	*	/	*	/	/	/	*	*
	AHS	/	/	*	/	/	/	***	***

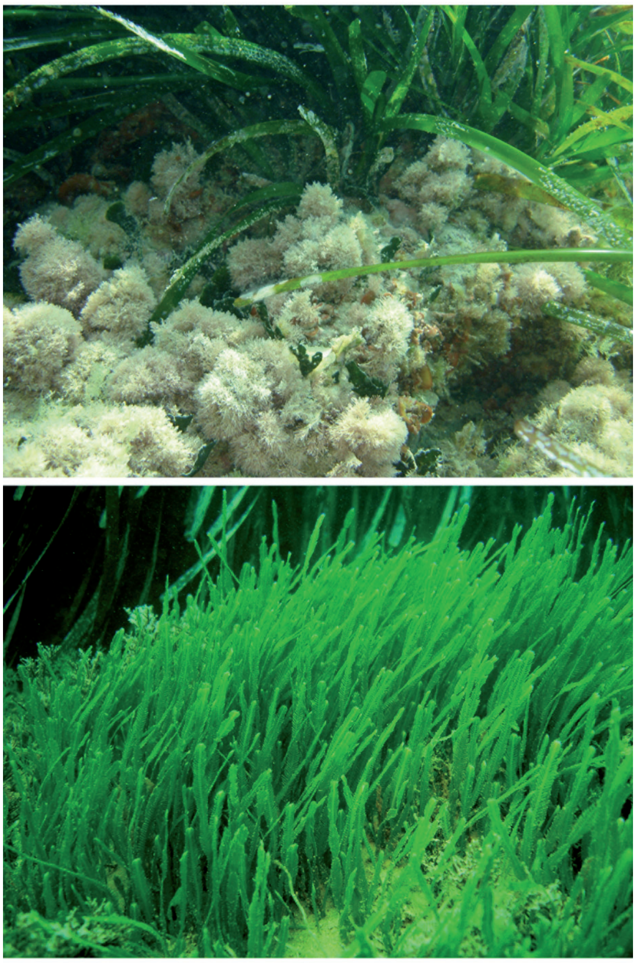

Fig. 2: Non-invaded *matte* at the *Posidonia oceanica* meadow border (top); *matte* invaded by *Caulerpa taxifolia* var *distichophylla* (down).

Western Mediterranean Basin), municipality of Cefalù (Fig. 1). Specifically:

a) The surveyed site at Torre Salsa (37° 20' 52.34'' N, 013° 21' 59.77'' E) is a small shallow sandy bay delimited by rocky cliffs. On the western side of the bay, a rocky platform runs parallel to the coast and is mostly covered by *P. oceanica*. There, large patches of *Ctvd* were found in the inner part of the bay, at the foot of the meadow, between 0.5 and 2 m depth. The patches grew on dead *matte*, intermingled with the brown algae *Padina pavonica* (Linnaeus) Thivy and *Halopteris* sp., as well as the green algae *Bryopsis* spp. Where present, the fronds of *Ctvd* formed dense mats covering roughly 30% of the available habitat. Fronds did not exceed 8 cm in length.

b) The coast at Sant'Ambrogio (38° 01' 10.25''N, 014° 04' 53.81'' E) is characterized by a mosaic of sandy beaches interspersed with beds of pebbles, cobbles and boulders. Sandy bottoms, which were the most frequent feature, presented rare rocky outcrops made of large blocks, flat on the top and covered by turf. The blocks lay at a depth of about 3 m and were roughly between 0.5 and 1 m high. At the base of the block, where the rock appeared eroded close to the sandy bottom, a slight overhang developed. There, the rocks were carpeted by patches of *Ctvd* surrounded by algal turf. Patches were a few hundred square centimetres in extent and characterized by dense and relatively short fronds, between 6 and 8 cm in length.

Analysis of polychaete assemblages and RPD

Altogether, 992 individuals belonging to 32 polychaete families were found (685 individuals and 31 families at the invaded meadow; 307 individuals and 26 families at the control one). Total abundance was significantly higher at the invaded meadow ($F_{1,2}$ = 26.58; p = *0.04*; COCHRAN'S = 0.60 NS, data log-transformed); while non significant differences in the number of families were observed ($F_{1,2}$ = 1.62; p = 0.33; C = 0.73 NS).

At the assemblage level, significant differences between meadows were confirmed by MANOVA (Table 2).

The SIMPER routine revealed that Bray-Curtis dissimilarity was high between meadows (72.9%). This result was mainly due to clear differences in the distribution patterns of the families Sabellariidae and Capitellidae. Both taxa were more abundant at the impacted

Table 2. PERMANOVA testing for differences in the multivariate polychaete assemblages. Significant p-values are given in italics. Analysis based on Bray-Curtis similarity matrix calculated from the original untransformed dataset; me = meadows, si(me) = sites in meadows.

Source	df	SS	MS	F	*p*
me	1	9490	9490	4.44	*0.03*
si(me)	2	4279	2139	1.84	0.11
Res	8	9280	1160		
Total	11	23049			

Table 3. SIMPER output showing the main contributions of polychaete families to the Bray-Curtis dissimilarity between assemblages at invaded and control P. oceanica meadows (cut-off for lower contribution 70%). Inv: Invaded;Con: Control; AvA: average abundance; AvD: average dissimilarity; C%: percent contribution

	Inv	Con		
	AvA	**AvA**	**AvD**	**C%**
Sabellariidae	32.5	0.2	18.8	25.9
Capitellidae	24.0	2.8	14.0	19.2
Syllidae	8.8	11.7	5.7	7.9
Terebellidae	8.5	5.3	4.2	5.7
Maldanidae	4.8	2.5	3.1	4.3
Sabellidae	5.8	1.5	2.8	3.8
Nereididae	1.7	5.7	2.7	3.8

meadow, while Syllidae resulted more abundant at the control meadow (Table 3).

The average RPD (± SD) measured at the SC meadow (1.67 ± 0.26 cm) resulted significantly deeper than that observed at DL (0.83 ± 0.41 cm), as confirmed by the ANOVA ($F_{1,10} = 17.86$; $p < 0.01$; C = 0.71 NS).

Discussion

Records of the abundance, distribution and persistence of *Ctvd* allow inferring that this taxon is fully naturalized in southern Sicily. The spread along the northwestern coast of Sicily, beyond the distribution limit previously assumed (Jongma *et al.*, 2013), the capability to monopolize the space in certain habitats, the changes in polychaete assemblage structure and RPD observed during this study, as well as the changes in soft bottom invertebrate diversity observed in Turkey (Cevik *et al.*, 2012), and the economic damages claimed by local fisheries due to the huge amount of wrack produced, suggest that *Ctvd* has the potential to become a pest in the Western Mediterranean Basin.

The most evident difference between *Ctvd* and the conspecific aquarium strain is that the former is much more slender (Jongma *et al.*, 2013). This might imply differential dispersal potential between the two taxa. In fact, slender fronds are supposed to break, drift and disperse more easily. During our survey, it was common to observe sparse fronds of the new alien strain accumulating ashore (see also Fig. 1 in Jongma *et al.*, 2013). The debate concerning the vector of introduction of *Ctvd* in the Mediterranean Sea is still open; both intercontinental shipping and aquarium trade might be responsible for the arrival of the new alien (Jongma *et al.*, 2013). Geographic position and current regime make Sicily peculiar from a biogeographic point of view, being part of at least 3 Mediterranean biogeographic sectors, as well as being the natural crossroad between the Western and the Eastern Mediterranean basins (Bianchi, 2007). Using an elegant modelling approach Papini *et al.* (2013) recently suggested that the invasion of the entire Mediterranean area by *C. cylindracea* might have originated in Sicily instead of Libya (Nizamuddin, 1991). Strikingly, the authors identify the most probable start of the invasion along the Sicilian coast now experiencing the invasion of *Ctvd*. In fact, the Strait of Sicily is the main shipping route in the Mediterranean Sea, where about one third of global commercial shipping and trading is concentrated (Dobler, 2002). In addition, some important bunkering facilities are present, as well as a large fishing fleet based at Mazara del Vallo, and an increasing number of marinas hosting international cruising boats (e.g. Marina di Ragusa, Cefalù). According to Papini *et al.* (2013), transportation by anchors and fishing nets could have caused the impressive spread of *C. cylindracea*. Our observation of *Ctvd* growing on lines in the harbour of Marina di Ragusa, a popular destination for tourists, and its strains entrapped in fishing nets support the idea that the local spread is linked to (or at least enhanced by) secondary shipping, i.e. vessels used for tourism purposes and fishing boats. This might be the case for the first record in the Western Mediterranean Basin. Cefalù is among the most important tourism localities in Sicily while its geographic position suggests that current-driven transportation of *Ctvd* from the southeast would be improbable. In fact, as far as Sicily is concerned, Atlantic surface water reaches the island from the west and splits into two main currents, moving into the Sicily Strait and the Tyrrhenian Sea respectively, which do not meet downstream (Poulain & Zambianchi, 2007). These features do not facilitate the current-driven westward and northward spread of the alien algal propagules.

As far as the possible impact on Mediterranean biota is concerned, it could be argued that the slenderer tallus of *Ctvd* can form a peculiar mat with a different texture compared to the thicker conspecific strain. This might imply differential detrimental effects between the two varieties. The presence of this invader in the Mediterranean region requires scientific effort to answer an array of open questions concerning its biology in the new invaded area (reproduction, ecological requirements, etc.) as well as its impact on key Mediterranean habitats and associated communities (i.e. *P. oceanica,* hard bottoms). In particular, the *matte* is a peculiar secondary habitat (Piazzi & Balata, 2009) hosting highly diverse assemblages. Significant difference in RPD between meadows suggests that *Ctvd* might deteriorate the quality of sediment associated to *matte* by reducing oxygen penetration. This could in turn affect the resident biota. In fact, the polychaete assemblages investigated during this study showed significant structural differences between invaded and non-invaded meadows. Tolerant and opportunistic polychaetes (Sabellaridae and Capitellidae) characterized the impacted meadow, while sensitive polychaetes (Syllidae) characterised the control one (Simboura & Zenetos, 2002; Giangrande *et al.*, 2005). Moreover, higher abundance of individuals was recorded in the invaded *matte*, but no differences in taxonomic richness were detected. Higher polychaete species richness was instead observed as a consequence of *Ctvd* muddy sand bottom invasion along the Turkish coast (Cevik *et al.*, 2012). Regarding other invasions of *P. oceanica* meadows by closely related algae, Francour *et al.* (2009) did not observe any significant difference in polychaete abundance between sites invaded by *C. taxifolia* and controls, while Box *et al.* (2010) observed a positive correlation between *C. cylindracea* biomass and polychaete abundance and taxonomic richness in invaded meadows. The difference among the published responses of Mediterranean benthic assemblages to invasions by *Caulerpa* species might be related to distinct ecological traits among these invaders and substrate characteristics (*matte*, sand, mud, etc.).

Further analyses encompassing the entire invertebrate community at finer taxonomic level and the structure of fish assemblages associated to *P. oceanica* will enable a better understanding of the ecological effects of *Ctvd* and possible differences with the congeneric alien strains. In this respect, it is worth noting that the harmful indole alkaloid caulerpin, produced by both the aquarium strain of *C. taxifolia* and by *C. cylindracea,* which is directly responsible for detrimental biological effects on native Mediterranean fauna (e.g. Felline *et al.*, 2012), was not observed in *Ctvd* collected in its area of origin (Waterhouse Island, Australia) (Schwede *et al.*, 1986). If confirmed for the Mediterranean populations, the absence of caulerpin might imply further biological differences between the two *C. taxifolia* alien strains, which might in turn correspond to different ecological effects on the resident biota.

This pilot study indicates that the new alien *Ctvd* is a potential threat for the benthic assemblages of a common, endemic coastal Mediterranean habitat. The spread of *Ctvd* along the Sicilian coast raises serious concern about its invasive potential. Specifically, our data confirm that *Ctvd* is fully naturalized (*sensu* Williamson, 1996) in the Central Mediterranean and is expanding its range beyond this limit, thus becoming potentially able to colonize the Western Mediterranean basin

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