Stressful Conditions Give Rise to a Novel and Cryptic Filamentous Form of *Caulerpa cylindracea*

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Morphological plasticity can enable algae to adapt to environmental change and increase their invasibility when introduced into new habitats. Nevertheless, there is still a lack of knowledge on how such plasticity can affect the invasion process of an invasive species. In this context, the high plasticity in the genus *Caulerpa* is well documented. However, after an extremely hot summer, a previously unreported filamentous morphology of *Caulerpa cylindracea* was detected; indeed, this morphology could only be confirmed taxonomically after in-depth morphological characterization and molecular analysis with the genetic marker *tuf*A. We describe an *ex situ* culture experiment which showed that stressful conditions, such as high temperatures, can trigger this morphological change. Almost all of the thalli maintained at a constant extreme temperature of 29°C died, but after being returned to optimum temperature conditions, the filamentous morphology began to develop from the surviving microscopic tissue. In contrast, thalli at a control temperature of 21°C maintained the regular morphology throughout the experiment. When *C. cylindracea* develops this filamentous morphology, it may act as a cryptic invader because it is difficult to detect in the field. Furthermore, the filaments likely improve *C. cylindracea*'s invasive capabilities with regard to resistance, persistence and dispersion and may have an important role in the re-colonization process, after a population disappears following a period of stressful conditions. Possibly, *C. cylindracea*'s ability to respond plastically to stressful conditions might explain its remarkable success as an invasive species.

Keywords: cryptic invasions, morphological plasticity, resistance form, Mediterranean Sea, *Caulerpa cylindracea*

INTRODUCTION

Biological invasions refer to the process by which different organisms, commonly known as invasive species, can arrive and establish in a new habitat, where they disrupt the normal functioning of the system. Currently, these invasions are considered one of the main drivers of global change due to their adverse effects on biodiversity, habitat structure and native ecosystem functioning (Mack et al., 2000; Stachowicz and Byrnes, 2006; Simberloff et al., 2013; Bellard et al., 2016). Additionally, the establishment of invasive species it is often associated to great economic costs (Pimentel et al., 2001, 2005) due to their alteration of several ecosystem services (Pejchar and Mooney, 2009; Vilà et al., 2010). The impacts of invasive species are especially important and noticeable in marine

ecosystems, where biological invasions are on the rise due to the increases in their main vectors of introduction, such as shipping traffic, the aquarium trade, or the opening and widening of new corridors (Katsanevakis et al., 2013; Seebens et al., 2013; Galil et al., 2017); and in the future, the establishment of non-native species is expected to continue increasing due to climate change (Stachowicz et al., 2002; Lejeusne et al., 2010).

Species invasiveness depends on the features that enable a non-native organism to invade a certain habitat (Richardson et al., 2011) with the main influence being the life-history traits of the invader (Grotkopp et al., 2002; Pyšek and Richardson, 2008; Van Kleunen et al., 2010). Several studies have suggested that phenotypic plasticity is one of the most important of such features for invasive species (Richards et al., 2006; Davidson et al., 2011). Plants and algae can adapt by modifying, among other things, photosynthetic traits (Molina-Montenegro et al., 2012; Zanolla et al., 2015), leaf-area and shoot allocation (Arenas et al., 2002; Liu and Su, 2016) and growth form (Van Kleunen and Fisher, 2001; Monro and Poore, 2009) allowing them to respond to changes in light, temperature or herbivory pressure (Lewis et al., 1987; Monro and Poore, 2005; Nicotra et al., 2010). In this sense, understanding how this phenotypic plasticity affects the success of an invasive species is crucial to our understanding of its invasion process (Schaffelke et al., 2006; Theoharides and Dukes, 2007) and might have important implications for the successful management of the species (Hobbs, 2000; Simberloff et al., 2005).

Caulerpa cylindracea is a siphonaceous green macroalga, native to Western Australia, that has become one of the most widespread non-native algae in the Mediterranean Sea (Piazzi et al., 2005; Klein and Verlaque, 2008; Montefalcone et al., 2015). Indeed, *C. cylindracea* is currently considered the most invasive species within the Mediterranean basin (Katsanevakis et al., 2016) and has also invaded areas in the Atlantic Ocean (Verlaque et al., 2004) and in Southern Australia (Klein and Verlaque, 2008). Still, it is not clear which has been the source of the primary introduction in the Mediterranean Sea, although it is quite likely that it was through the aquarium trade, with shipping traffic and fishing gear being the main sources of the secondary introductions within the basin (Verlaque et al., 2003). Ecologically, *C. cylindracea* spread causes the homogenization of native communities due to the formation of dense and continuous meadows (Klein and Verlaque, 2008), which has negative implications for the native macroalgal assemblages and the diversity of the communities (Piazzi et al., 2001; Piazzi and Ceccherelli, 2006; Klein and Verlaque, 2011). Also, this species affects the sedimentation rates, the carbon turnover, the organic matter composition and the quality of the invaded sediments (Piazzi et al., 2007; Holmer et al., 2009; Pusceddu et al., 2016; Rizzo et al., 2017). Morphologically, *C. cylindracea* is characterized by a simple morphology, formed by creeping stolons and erect shoots with grape-like ramuli (also called branchlets) that can be arranged radially or distichously (Klein and Verlaque, 2008). In addition, it has been reported that species in the genus *Caulerpa* show a high degree of morphological plasticity in response to environmental conditions (Peterson, 1972; Calvert, 1976; Coppejans and Beeckman, 1989; Collado-Vides, 2002b), allowing these species to adapt

to different environments and thus increasing their invasive potential (Collado-Vides, 2002b; Raniello et al., 2004; Smith, 2009). Several factors such as temperature, light or depth, can trigger subtle morphological changes in stolon and ramuli shape (Peterson, 1972; Calvert, 1976; Ohba and Enomoto, 1987; Ohba et al., 1992), photosynthetic traits (Raniello et al., 2004, 2006) and the interspace between erect axes (Collado-Vides, 2002b; De Senerpont Domis et al., 2003). However, more acute morphological changes have been detected for the first time in *C. cylindracea* during a recent field survey (**Figure 1**). The thalli of these specimens consisted only of thin vertical filaments, which were impossible to identify as *Caulerpa* species until morphological and molecular characterization confirmed their identity. At present, the exact conditions that trigger this morphological change are unknown but, considering that the filamentous form was found in Montenegro after the extremely warm summer of 2018 (**Figure 2**), it would appear that stressful conditions brought about by high temperatures could be involved. A better understanding of the conditions that trigger this morphological shift—which allows *C. cylindracea* to become a cryptic invader—will greatly enhance our understanding of the invasive process, the collapses and the recoveries of this species.

In this study, our aim was to determine whether extreme temperature conditions can trigger the formation of a filamentous morphology in *Caulerpa cylindracea* similar to that observed in the field. To do so, the morphological plasticity of this species was studied through culture experiments at contrasting temperatures. In addition, in order to confirm the taxonomical identity of the specimens, all thalli (from both cultured and natural populations) were genetically characterized with a chloroplast molecular marker (*tuf*A), which had been used previously for the genus *Caulerpa* (Famà et al., 2002; Kazi et al., 2013; Sauvage et al., 2013).

MATERIALS AND METHODS

Study Sites and Culture
Extreme Temperature Laboratory Experiment

To study the effects of extreme temperatures on *C. cylindracea*, specimens from a population in Spain (Roses: 42° 14′ 18.26″N; 3° 12′ 25.74″E) were sampled in February 2019. Once in the laboratory, samples were cleaned with sterilized seawater to remove all the epiphytes and detritus (such as dead *Posidonia oceanica* rhyzomes and dead shells). For acclimation, *Caulerpa* samples were placed in aquariums (12 L) with sterile seawater and in a *Radiber AGP-360* growth chamber at 12°C and a 12:12 (L:D) cycle at 200 µmol photons m^{-2} s^{-1} to simulate natural conditions of irradiance and temperature for 1 week. After the acclimation period, algal cultures were prepared for a period of 170 days under either control conditions or extreme (i.e., very warm) conditions (**Figure 3**). Six fragments of *C. cylindracea* (≈4 cm^2 each) were randomly transferred to six plastic beakers (1 L): three control treatments and three extreme-temperature treatments, each containing 200 g of sterilized gross sand and 0.5 L of sterilized seawater to which was added 5 ml/l of K-medium (Keller et al., 1987). The temperature treatments for

FIGURE 1 | Macroscopic picture of a rock covered by a thick patch of *Womersleyella setacea* turf with the filamentous form of *Caulerpa cylindracea* growing from beneath it (black arrows).

the experiment were as follows: "control" (21°C) based on the average summer seawater temperature recorded in the sampled area and "extreme" (29°C) based on abnormally high summer seawater temperatures recorded in the Mediterranean Sea[1]. After an adaptation period of 7 days at 12°C in the growth chamber, the temperature was progressively increased (by 1°C every 2 days) in all six treatment beakers for 18 days until a temperature of 21°C

was reached in the growth chamber. At this point, the beakers were split into two *Radiber AGP-360* growth chambers, one to keep the "control" beakers and the other to keep the "extreme" beakers throughout the experiment. Following this, the "control" beakers were maintained at 21°C for the remaining 152 days of the experiment in the growth chamber; whereas, in the case of the "extreme" beakers, the temperature was raised in the other growth chamber by 1°C every 5 days for the next 40 days until a temperature of 29°C was reached; this temperature was then
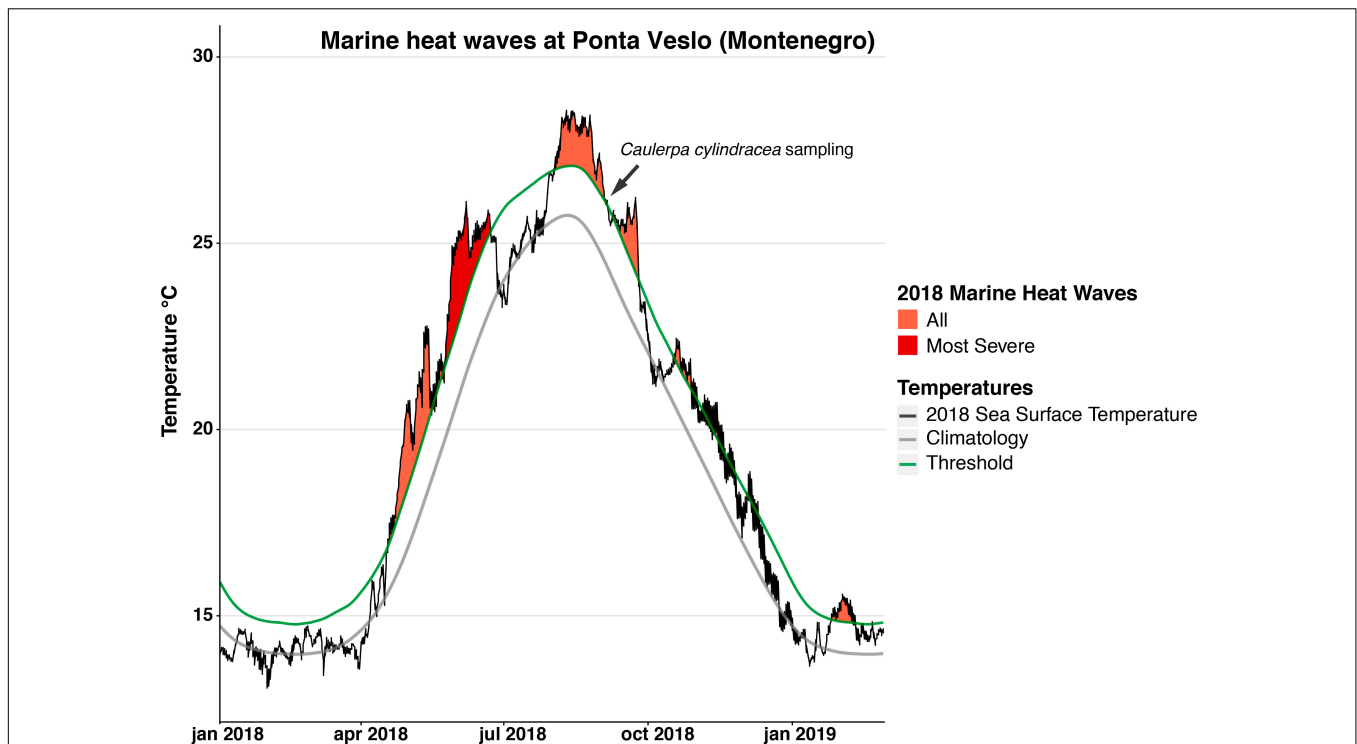
FIGURE 2 | Marine heat wave (MHW) events during 2018 at Ponta Veslo, Montenegro (42° 22′ 5.15″N; 18° 36′ 22.50″E), calculated with the *heatwaveR* package (Schlegel and Smit, 2018) using Reynolds Optimally Interpolated Sea Surface Temperature (OISST) data (Reynolds et al., 2007). The gray line represents the SST climatology for the last 35 years; the green line indicates the 90th percentile MHW threshold; and the black line shows the SST during 2018. The dark red filled area indicates the most severe MHW event during 2018, while the orange filled areas indicate all the other MHW events identified over the same time period. The dark gray arrow indicates the day of the year when the filamentous morphology of *Caulerpa cylindracea* was sampled from the field (5th of September).
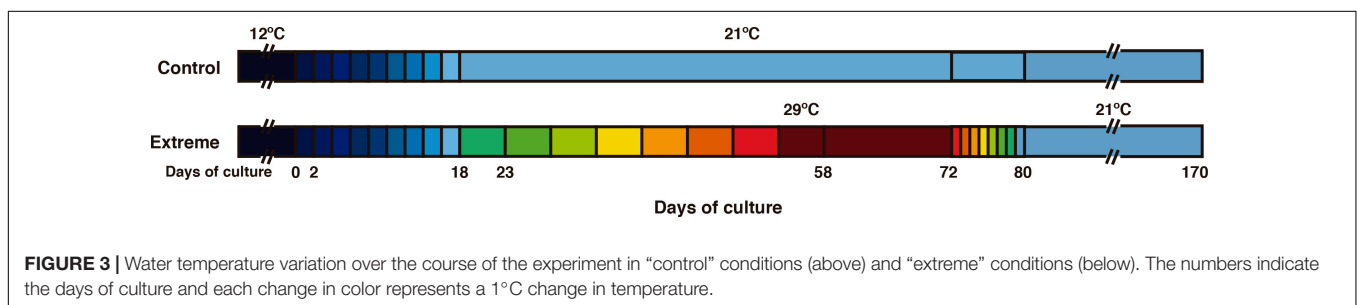
FIGURE 3 | Water temperature variation over the course of the experiment in "control" conditions (above) and "extreme" conditions (below). The numbers indicate the days of culture and each change in color represents a 1°C change in temperature.

maintained for a further 14 days and then gradually lowered by 1°C each day back to 21°C and kept at this temperature for the remaining 90 days of the experiment (**Figure 3**). Throughout the experiment, the seawater and growth medium mixture was renewed once a week.

Field Sampling for Morphological and Taxonomical Characterization

Samples of *C. cylindracea* were collected from natural populations in Spain (Roses: 42° 14′ 18.26″N; 3° 12′ 25.74″E); Croatia (Funtana: 45° 10′ 40.16″N; 13° 35′ 32.31′ E and Split: 43° 30′ 28.79″N; 16° 23′ 17.56″E); Montenegro (Ponta Veslo: 42° 22′ 5.15″N; 18° 36′ 22.50′E); and Albania (Kallm: 41° 19′ 27.88″N; 19° 25′ 19.31″E) by scuba-divers at depths of between 5 and 12 m based on previous knowledge on the presence of the invasive alga

in these locations. All samples were transported in zip bags within a thermal box to maintain a constant seawater temperature until the morphological characterization was performed in the laboratory. All samples showed the typical morphology of the species (**Figure 4B**). These samples were also used for the taxonomic characterization of the populations (see "Molecular Analysis" section).

Data Collection and Analysis
Extreme Temperature Laboratory Experiment

The effects of the extreme temperatures we applied to *C. cylindracea* were assessed by measuring the macroscopic morphometric changes. The structure and area of *Caulerpa* were measured by means of macroscopic photographs taken with an
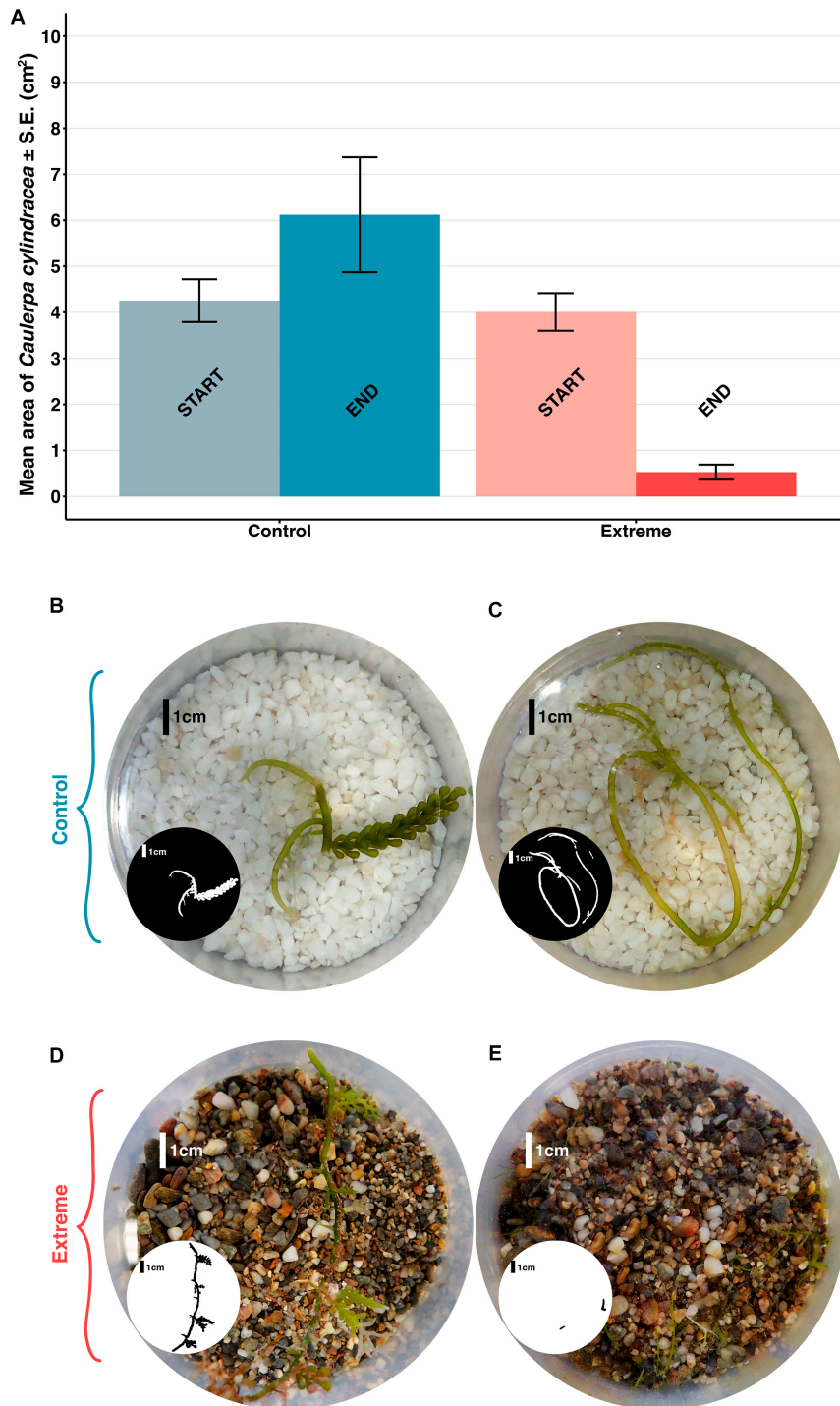
FIGURE 4 | (A) Mean area ±S.E. of *Caulerpa cylindracea* in the beakers (*n* = 6, 3 control beakers and 3 extreme temperature beakers), at the beginning of the experiment, and after 72 days. Photographs **(B,C)** show control beakers at the beginning of the treatment and after 72 days, respectively. Photographs **(D,E)** show extreme temperature beakers at the beginning of the treatment and after 72 days, respectively. The small black and white circles highlight the *C. cylindracea* present in each beaker.

Olympus TG-5 camera, which were then analyzed with Adobe Photoshop CC 2018. Living parts of *C. cylindracea* (green color) were manually selected and measured using the "analysis tool."

Then, to assess whether there were differences in the area of *C. cylindracea* between treatments at the end of the experiment, a linear mixed effect model (LMM) was fitted with "*C. cylindracea*

area" as the response variable and "treatment" and "time" as the explanatory variables. The interaction between both explanatory variables was included in the model and a random term for "replicate" was used to take into account the repeated measures design (Harrison et al., 2018). To fit this model, the package *lme4* (Bates et al., 2015) in the statistical environment R was used (R version 3.6.3) (R Core Team., 2018). Finally, to compare the effects between levels in the treatment factor ("control" and "extreme") at each time observation ("beginning" and "end"), Tukey *post hoc* tests were performed using the functions "pairs" and "emmeans" from the package *emmeans* (Lenth, 2018).

Morphological Characterization and Comparison

The morphological characterization of the cultured stolons and filaments was assessed by means of microscopic photographs, taken using a Zeiss AXIO Imager A.2 (Carl Zeiss, Berlin, Germany) equipped with an AxioCam MRc5 camera and a stereomicroscope Stemi 2000-C (Carl Zeiss, Berlin, Germany) equipped with an AxioCam ERc 5s camera; and the images were analyzed with Zen2011 software (Blue Edition). Also, to account for regional morphological variability, the mean stolon thickness of 10 randomly sampled stolons from each of the natural populations (Roses, Funtana, Split, Ponta Veslo, and Kallm) was compared to the thickness of filaments obtained at extreme temperatures.

To assess whether mean thickness of filaments differed from that of stolons, a linear model was fitted with "*C. cylindracea* thickness" as the response variable and "location_morphology" as the explanatory variable, in the statistical environment R. Finally, to compare between location_morphology levels ("Roses_filaments," "Roses_stolons," "Funtana_stolons," "Split_stolons," "Ponta Veslo_stolons," and "Kallm_stolons"), Tukey *post hoc* tests were performed using the functions "pairs" and "emmeans" from the package *emmeans*.

Molecular Analysis

To determine species identity, a genetic analysis was performed on all sampled and incubated thalli, including both filamentous and typical morphology. DNA extraction was performed following the DNeasy Plant Mini Kit (QiaGen) protocol in order to obtain the best DNA quality. The primer used to amplify the genetic material was *tuf*A (elongation factor A, from the chloroplast). The reactions were performed in 25 µL volumes and the master mix contained 5 mM of $MgCl_2$, 0.3 mM of each primer, 0.2 mM of dNTPs, 0.5 units of Taq DNA polymerase and 1.0 µL of the extracted DNA. The PCR reaction consisted of 40 cycles of 94°C for 1 min (denaturation), 52°C for 1 min (primer annealing) and 72°C for 2 min (extension) (Famà et al., 2002). The PCR reaction was finalized with a final 5 min step at 72°C. The PCR products were purified and sequenced by Macrogen Spain.

Sequences analyses were performed using different R (R Core Team., 2018) packages: *MUSCLE* (Edgar, 2004) to align the sequences, and *APE* (Paradis et al., 2004) and *PHANGORN* (Schliep et al., 2017) to create phylogenetic trees based on statistical analyses (Bio neighbor-joining tree, k80 distance, with 10,000 replicates).

RESULTS

Extreme Temperature Laboratory Experiment

In the model fitted to the data from the extreme temperature experiment, both "treatment" and the interaction term between "treatment" and "time" showed a significant effect on *C. cylindracea* area ($p < 0.05$, **Table 1**), being the area of *C. cylindracea* equal between treatments at the beginning of the experiment but being significantly different between "control" and "extreme" conditions at the end ($p < 0.05$, **Table 2**). Actually, after 72 days, the mean surface area of the *Caulerpa cylindracea* thalli under "control" conditions had increased by 44%, whereas that of the specimens exposed to the "extreme temperature" treatment (at 29°C for final 14 days of this 72 days period) had decreased significantly by 87% (**Figure 4A**). Furthermore, while the control specimens maintained the typical morphology of the species—characterized by thick rhizomes with some vertical vesicular fronds (**Figure 4C**)—almost all the thick parts of the specimens in the "extreme temperature treatment" had disappeared after 14 days at 29°C (**Figure 4E**).

When the cultures were returned to optimum conditions, new living parts emerged from the sediment in all the "extreme treatment" beakers, after 20 days. However, these new parts did not resemble the original *C. cylindracea*'s morphology. Instead, they presented a new type of structure characterized by erect thin filaments (**Figure 5A**), which for the next 3 months continued growing vertically and extending throughout the liquid in the beakers (**Figure 5B**).

Macroscopically, these filaments, which grow vertically from the substrate, are long (between 5 and 10 cm) and thin, and occasionally branched. Each filament has rhizoids in its basal part (which is without plastids) that serve to attach the

**TABLE 1 |** ANOVA summary for the LMM fitted to the extreme temperature culture data.

Dependent variable	Predictor	*F* value	Df	Pr (>F)
Caulerpa cylindracea area	Treatment	18.965	1	0.005*
	Time	1.446	1	0.275
	Treatment × Time	15.896	1	0.007*

Caulerpa cylindracea area was modeled as a function of Treatment ("control" and "extreme"), Time (start and end) and their interaction, with Replicate as a random effect to account for the repeated measures over time. The asterisk (*) denotes significant p-values.

TABLE 2 | Effect of temperature treatment on the area of *Caulerpa cylindracea*, at the beginning and end of the culture experiment.

Dependent variable	Time	Comparison	df	t ratio	*p*-value
Caulerpa cylindracea area	Start	Control—Extreme	6	0.260	0.803
	End	Control—Extreme	6	5.899	0.001*

Tukey tests were applied to the fitted mixed model to compare the area of Caulerpa cylindracea between temperature treatments at each time observation. The temperature treatments are: Control (n = 3) and Extreme (n = 3). The asterisk (*) indicates that the p-value is significant.
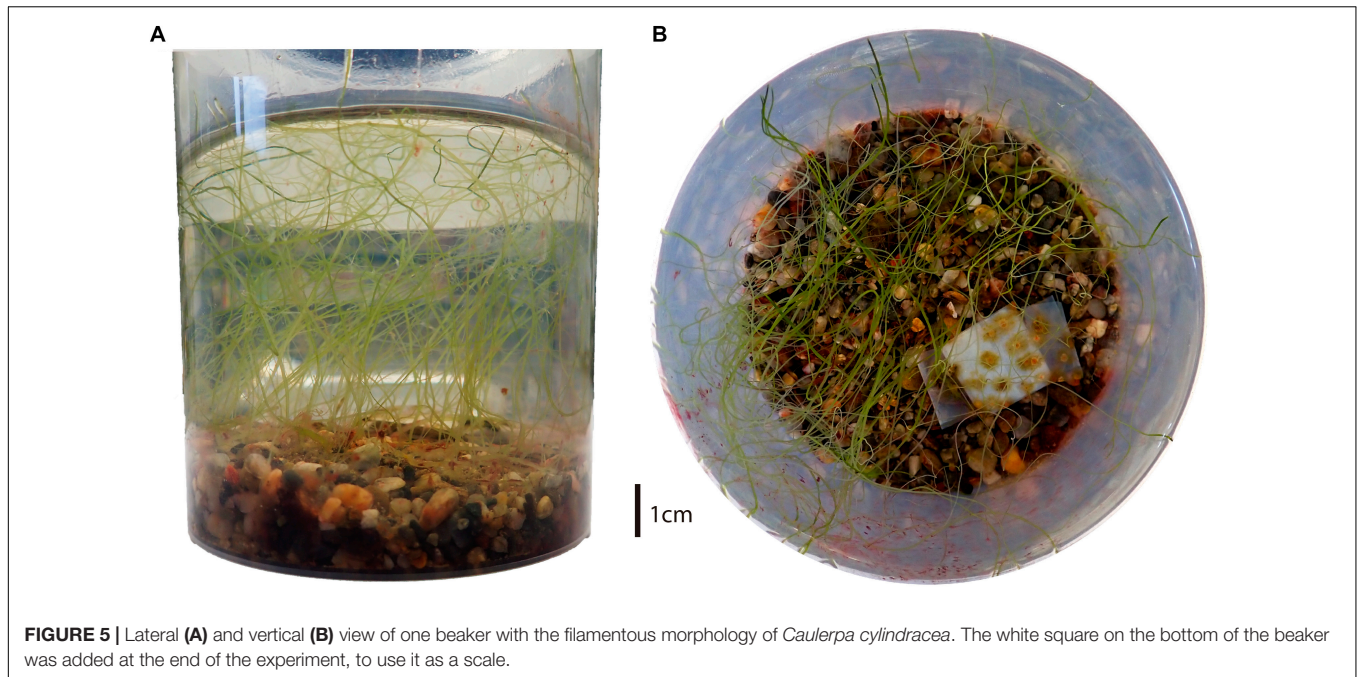
FIGURE 5 | Lateral **(A)** and vertical **(B)** view of one beaker with the filamentous morphology of *Caulerpa cylindracea*. The white square on the bottom of the beaker was added at the end of the experiment, to use it as a scale.

filament to the substrate. The upper part of the filament (with plastids) is light green and in all cases devoid of rhizoids or vesicles, which contrasts with the typical morphology of *C. cylindracea* (**Figures 4B,D**).

These filaments contrasted with the *C. cylindracea* thalli cultured under control conditions which, throughout the experiment (**Figures 4B,C**), maintained the typical morphology described for this species: the thalli were characterized by thick, prostrate stolons with interspaced rhizoids for anchoring, even where they grew unattached to the substrate. Vesicular fronds could also be observed, although these were smaller than in the field.

Morphological Comparison

In the model fitted to the morphological data, the variable "location_morphology" showed a significant effect on *C. cylindracea* thickness ($p < 0.05$, **Table 3**), with filament thickness being significantly different to stolon thickness ($p < 0.005$, **Table 4**) while stolon thickness was similar between populations (**Table 4**). Actually, microscopically, there was an almost 10-fold difference in mean thickness between the

filaments (222.42 ± 9.78 μm) in the extreme temperature beakers and the stolons ($2,093.38 \pm 31.50$ μm) sampled at different natural populations. This is amply illustrated in **Figure 6**.

TABLE 4 | Effect of location and morphology on the thickness of *Caulerpa cylindracea*.

Dependent variable	Morphological comparison	Population comparison	t ratio	p-values
Caulerpa cylindracea thickness	Filament vs. Stolon	Filament vs. Funtana	−18.893	< 0.001*
		Filament vs. Kallm	−18.770	< 0.001*
		Filament vs. Ponta Veslo	−20.082	< 0.001*
		Filament vs. Roses	−17.226	< 0.001*
		Filament vs. Split	−17.881	< 0.001*
	Stolon vs. Stolon	Funtana vs. Kallm	−0.123	1
		Funtana vs. Ponta Veslo	1.189	0.840
		Funtana vs. Roses	−1.667	0.559
		Funtana vs. Split	1.012	0.912
		Kallm vs. Ponta Veslo	−1.312	0.777
		Kallm vs. Roses	1.544	0.638
		Kallm vs. Split	0.889	0.948
		Ponta Veslo vs. Roses	2.856	0.064
		Ponta Veslo vs. Split	2.201	0.254
		Roses vs. Split	−0.654	0.986

Tukey tests were applied to the fitted mixed model to compare the thickness of Caulerpa cylindracea between locations and morphologies. The asterisk (*) indicates that the p-value is significant.

TABLE 3 | ANOVA summary for the LM fitted to the *Caulerpa cylindracea* thickness data.

Dependent variable	Predictor	*F*-value	Df	Pr (>Chisq)
Caulerpa cylindracea thickness	Location_Morphology	116.84	5	<0.001*

Caulerpa cylindracea thickness was modeled as a function of Location_Morphology ("Roses_filaments," "Roses_stolons," "Funtana_stolons," "Split_stolons," "Ponta Veslo_stolons," and "Kallm_stolons"). The asterisk (*) denotes significant p-value.

FIGURE 6 | (A) Mean width ±S.E. of *Caulerpa cylindracea* filaments (left) and stolons (right) from different populations (*n* = 10 for each population). (B) View of two filaments under the stereomicroscope a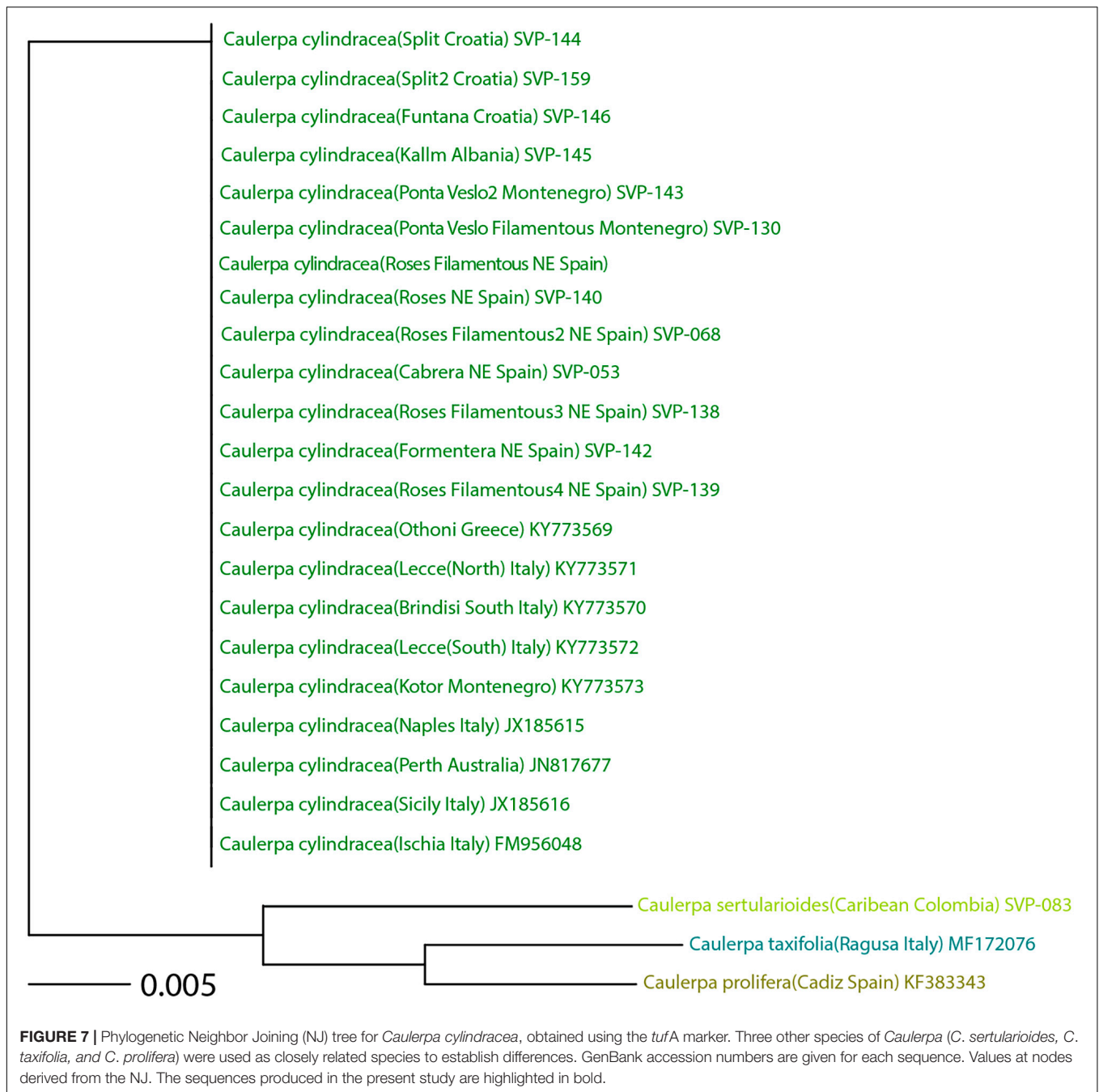t 1.0×. (C) View of a stolon with a vertical vesicular frond under the stereomicroscope at 1.0×. (D) (1) Macroscopic view of *C. cylindracea* with the regular morphology and (2) macroscopic view of the filamentous form of *C. cylindracea*. The white arrow shows the rhizoids that the filaments use for attaching themselves to the substrate.

Although erect filaments are rarely ascribed to *Caulerpa* genus, the presence of trabeculae—slender strands traversing the lumen of the thallus (Lamouroux, 1809; Womersley, 1984; Wynne and Bold, 1985)—confirms the identity of the thalli. The anatomical morphology of this structure in the filaments is identical to that of the regular stolons, as they traverse the interior of the filament going from one side to the other of a circular section and attach to the wall with a thickened structure that resembles a suction cup. However, the trabeculae in the filaments are much thinner and less numerous than in the stolons, which might explain why the filaments are so weak.

FIGURE 7 | Phylogenetic Neighbor Joining (NJ) tree for *Caulerpa cylindracea*, obtained using the *tuf*A marker. Three other species of *Caulerpa* (*C. sertularioides, C. taxifolia, and C. prolifera*) were used as closely related species to establish differences. GenBank accession numbers are given for each sequence. Values at nodes derived from the NJ. The sequences produced in the present study are highlighted in bold.

Taxonomical Characterization

For the genetic analysis, a total of 14 high quality *tuf*A sequences of up to 820 bp. were obtained and amplified from five specimens with the filamentous shape, eight specimens from the Mediterranean Sea with the common morphology of *Caulerpa cylindracea* and an additional sequence from *C. sertularioides*, which was obtained from the Caribbean Sea (**Table 5**). In addition, several *C. cylindracea* sequences were obtained from GenBank, together with sequences from *C. taxifolia* and *C. prolifera*, in order to establish differences in the phylogenetic tree. In the Neighbor Joining tree (**Figure 7**), all sequences from

C. cylindracea formed a highly supported cluster, grouped close together, which included both filamentous and typical thalli, thus indicating that they are the same entity.

DISCUSSION

Our culture experiments showed that extreme environmental conditions, in this case high temperatures maintained for long periods of time (i.e., 14 days at 29°C), affected the survival of *Caulerpa cylindracea* and triggered the development of a new

TABLE 5 | *Caulerpa* sequences used to build the phylogenetic tree.

Species	Accession number	Herbarium code	Site
Caulerpa cylindracea	MT274435	Svp-144	Split, Croatia
Caulerpa cylindracea	MT274436	Svp-159	Split, Croatia
Caulerpa cylindracea	MT274442	Svp-146	Funtana, Croatia
Caulerpa cylindracea	MT274446	Svp-145	Kallm, Albania
Caulerpa cylindracea	MT274444	Svp-143	Ponta Veslo, Montenegro
Caulerpa cylindracea	MT274443	Svp-130	Ponta Veslo, Montenegro
Caulerpa cylindracea	MT274447	Svp-053	Cabrera, Spain
Caulerpa cylindracea	MT274445	Svp-142	Formentera, Spain
Caulerpa cylindracea	MT274440	Svp-068	Roses, Spain
Caulerpa cylindracea	MT274441	Svp-140	Roses, Spain
Caulerpa cylindracea	MT274439	Svp-138	Roses, Spain
Caulerpa cylindracea	MT274438	Svp-139	Roses, Spain
Caulerpa cylindracea	KY773569		Othoni, Greece
Caulerpa cylindracea	KY773571		Lecce, Italy
Caulerpa cylindracea	KY773570		Brindisi, Italy
Caulerpa cylindracea	KY773572		Lecce, Italy
Caulerpa cylindracea	KY773573		Kotor, Montenegro
Caulerpa cylindracea	JX185615		Naples, Italy
Caulerpa cylindracea	JX185616		Sicily, Italy
Caulerpa cylindracea	FM956048		Ischia, Italy
Caulerpa cylindracea	JN817677		Perth, Australia
Caulerpa sertularioides	MT274434	Svp-083	Colombia
Caulerpa taxifolia	MF172076		Ragusa, Italy
Caulerpa prolifera	KF383343		Cadiz, Spain

The underlined names show the sequences that were amplified in this study. The rest of the sequences were obtained from GenBank.

morphology characterized by long, thin vertical filaments from the surviving tissues. This new morphology of *C. cylindracea* has also been found in the field and is described here for the first time.
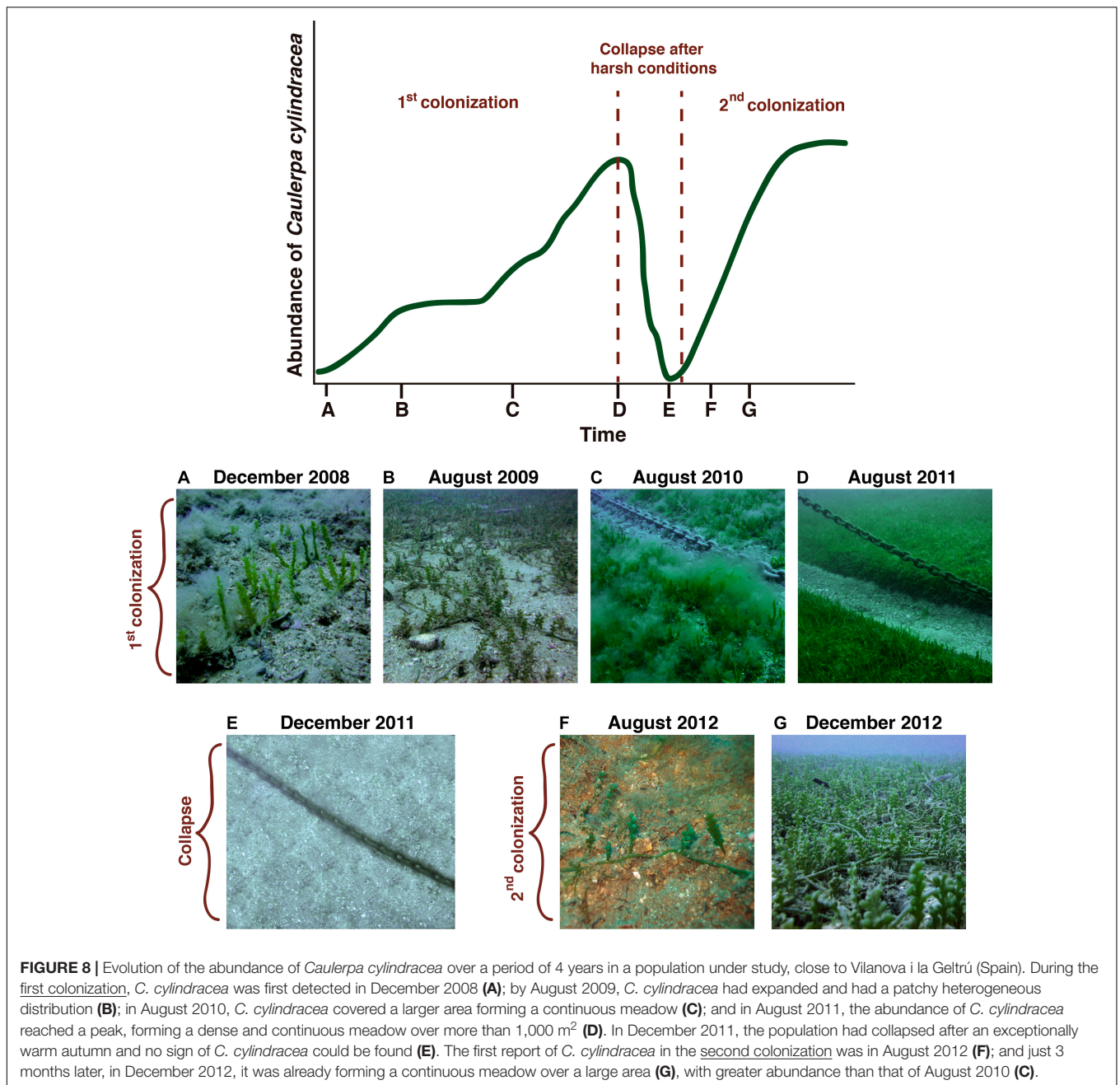
The laboratory experiment showed the remarkable capacity of *C. cylindracea* to survive under stressful culture conditions as the temperature treatment chosen (29°C) is much higher than the usual temperatures found at both the native and the invaded range (Klein and Verlaque, 2008). Additionally, *C. cylindracea* showed a great ability to adapt to environmental change, because it developed a new filamentous morphology from the damaged tissue that survived to the unfavorable culture conditions, i.e., extreme temperatures. These morphological changes during vegetative development are a common strategy in sessile organisms such as plants (Dorn et al., 2000; Puijalon et al., 2008; Nicotra et al., 2010) and algae (Kübler and Dudgeon, 1996; Garbary et al., 2004; Monro and Poore, 2005; Fowler-Walker et al., 2006) to tolerate environmental change, and to improve their competitive and survival capacity (Bradshaw, 1972; Harper et al., 1986; Price and Marshall, 1999; Collado-Vides, 2002a). Actually, more or less acute morphological changes have been previously observed and described in different algae species (e.g., some *Caulerpa* species, *Ulva prolifera*, *Chondrus crispus*, *Asparagopsis armata*, *Padina jamaicensis*, *Codium fragile,* or *Ecklonia radiata* among others) under different culture conditions (Ohba and Enomoto, 1987;

Ohba et al., 1992; Kübler and Dudgeon, 1996; De Senerpont Domis et al., 2003; Garbary et al., 2004; Monro and Poore, 2005; Gao et al., 2016) and in the field (Lewis et al., 1987; Meinesz et al., 1995; Collado-Vides, 2002b; Garbary et al., 2004; Fowler-Walker et al., 2006), as a response to changes in temperature, salinity, hydrodynamism, light, or herbivory pressure.

Taking into account that *Caulerpa cylindracea* may be one of the most widely studied invasive algae species around the world, the lack of a previous description of the filamentous morphology demonstrates that when *C. cylindracea* adopts this form, it clearly goes undetected. For instance, the filamentous morphology was sampled—accidentally—in the field within a macroalgae benthic community (**Figure 1**) and was only identified as *Caulerpa* after the samples were analyzed under the microscope. Thus it is clear that *C. cylindracea* filaments also develop under natural conditions. The variables that trigger such filaments in the field may, however, be multiple and in general, remain uncertain. Nevertheless, the culture experiments and field observations described here strongly indicate that stressful conditions may induce the formation of these structures.

The phenotypic plasticity observed in *C. cylindracea* and its ability to withstand extreme conditions (high temperatures) for a long time are characteristics that clearly influence the invasiveness of this species, and improve its persistence and resistance to stress. Actually, this resistance, together with the difficulty of observing the filaments underwater, might explain why *C. cylindracea* populations sometimes seem to disappear only to bounce back after a few months (García et al., 2016). In such situations, it may be that while most of the population dies following unfavorable conditions (**Figure 8E**; Klein and Verlaque, 2008), some small remnants survive, most probably hidden and undetectable in the sediment or sand. Then, as was the case in our culture experiment, the surviving parts may produce filaments that go unnoticed by divers and researchers (**Figure 1**), and, subsequently, the regular morphology of *C. cylindracea* returns when favorable conditions allow the population to recover (**Figure 8G**). Typically, colonization of a new area by *C. cylindracea* can take approximately 3 years, but the presence of this cryptic and resistant form of the species would explain the marked reduction—from 3 years to less than 1 year—in the time it takes *C. cylindracea* to re-colonize areas in which it had disappeared (García et al., 2016), thus highlighting the importance of this morphology in the re-colonization process (**Figure 8**). Furthermore, when adopting this cryptic phase, identification is extremely difficult or simply impossible, which has further implications for the management of the species, since early detection is one of the most important requirements for the successful management of invasive species (Lodge et al., 2006; Vander Zanden et al., 2010; Giakoumi et al., 2019). This misidentification of the filaments can also affect current estimations of *C. cylindracea* in the Mediterranean Sea as this species may be present at several locations in this latent, cryptic form. Since traditional methods of direct observation may not always

FIGURE 8 | Evolution of the abundance of *Caulerpa cylindracea* over a period of 4 years in a population under study, close to Vilanova i la Geltrú (Spain). During the first colonization, *C. cylindracea* was first detected in December 2008 **(A)**; by August 2009, *C. cylindracea* had expanded and had a patchy heterogeneous distribution **(B)**; in August 2010, *C. cylindracea* covered a larger area forming a continuous meadow **(C)**; and in August 2011, the abundance of *C. cylindracea* reached a peak, forming a dense and continuous meadow over more than 1,000 m² **(D)**. In December 2011, the population had collapsed after an exceptionally warm autumn and no sign of *C. cylindracea* could be found **(E)**. The first report of *C. cylindracea* in the second colonization was in August 2012 **(F)**; and just 3 months later, in December 2012, it was already forming a continuous meadow over a large area **(G)**, with greater abundance than that of August 2010 **(C)**.

be reliable in detecting filaments of *C. cylindracea*, as they can be misidentified or missed entirely, methods involving environmental DNA (Taberlet et al., 2012; Kelly et al., 2014; Thomsen and Willerslev, 2015) could be useful in detecting this species, as has been the case with the detection of other invasive species (Dejean et al., 2012; Ardura et al., 2015; Simmons et al., 2015).

Dispersion is another process that might also be favored by *C. cylindracea* filaments, because these long and weak vertical structures are more easily broken upon contact or by water movement than the regular thallus. This will release living fragments that can be transported by currents or

attached to drifting objects, favoring dispersal of *C. cylindracea* and the potential for secondary introductions. Actually, in *Caulerpa* species, the fragmentation process is one of its most important reproductive strategies (Fralick and Mathieson, 1972; Ceccherelli and Cinelli, 1999; Smith and Walters, 1999; Ceccherelli and Piazzi, 2001) and allows these species to spread really fast.

To conclude, this newly identified filamentous morphology of *C. cylindracea* could act as a potential jack-of-all-trades that further improves this species' capacity as an invader. The filaments described here are involved in the persistence, resistance, and dispersion of this invasive

species and have characteristics that allow this species to withstand harsh abiotic conditions and which may help to explain its successful expansion in the Mediterranean Sea.

DATA AVAILABILITY STATEMENT

The datasets generated for this study and the code to analyse the data can be found here: https://github.com/JorgeSantamaria/Filamentous-Morphology-Caulerpa. The genetic sequences amplified in this study are deposited in the GenBank repository and can be accessed with the accession numbers shown in **Table 5**.

AUTHOR CONTRIBUTIONS

JS, EC, and AV conceived the ideas and the experimental design. JS, RG, and MG collected the samples. JS and RG analyzed the data. All authors drafted the manuscript, contributed substantially to revisions, and accepted the final version before submission.

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