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## TITLE

# Niche conservatism and spread of seaweed invasive lineages with different residence time in the Mediterranean Sea.

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# ABSTRACT

Marine algae invasions attract a lot of interest as they are altering the structure of marine ecosystems. However, niche dynamics and risk predictions of marine invasions integrating phylogeographic structure in the analyses have not yet been investigated. In this study, we perform a comprehensive analysis of two invasive lineages of *Caulerpa taxifolia* with different residence time in the Mediterranean Sea for a better understanding of their invasive processes. We performed lineage-based and species-based niche models to assess the risk of invasion, the spatial overlap, and the variables delimiting the distribution of the two lineages. We also compared the effect of using different extents on niche overlap and niche shift analyses. Intraspecific models with pooled occurrences accurately found two separate regions susceptible of invasion for each invasive lineage in the Mediterranean, while species-based predictions underestimated invaded regions. The invasive lineages spread across colder coastal areas than the species. Altogether, we provide evidence that different invasive lineages of algae show dissimilar environmental responses and invasive ranges that are not detectable by species-based analyses. Moreover, niche overlap and niche shift analyses seem to depend greatly on the geographical extent used. According to the most appropriate extent (worldwide), the invaded range did not show niche shift, and thus, no evidence of a post-introduction adaptation scenario was found as both lineages invaded habitats similar to their Australian native locations. Actions to prevent further spreading of the most recent invasive lineage are needed.

KEYWORDS

*Caulerpa*, lineage-based niche modelling, marine invasions, niche overlap, niche shift, vagile species

# INTRODUCTION

Advances in understanding mechanisms regulating the potential establishment and spread of alien species are paramount. The assessment of niche conservatism is fundamental for the prediction and understanding of the eco-evolutionary mechanisms underlying invasion scenarios (Petitpierre et al. 2012; Rey et al. 2012). In this context, the development of a framework including different niche overlap and niche shift metrics (Warren et al. 2008; Petitpierre et al. 2012; Broennimann et al. 2012) makes it feasible to test environmental dissimilarities between the invaded and native range, and it deepens our understanding of the adaptive potential of invaders. The combined use of niche shift analysis and species distribution models (SDMs) further enables the evaluation of the niche requirements of alien species, and the prediction of biological invasions. However, recent studies on niche shifts arising during invasions have reported dissimilar results depending on the taxa studied. Even under an unified framework, niche change has been identified in invasive reptiles, amphibians and fishes (Li et al. 2014;  Parravicini et al. 2015) in a higher proportion than in terrestrial plants, birds and a set of different genera of vertebrates (Petitpierre et al. 2012; Strubbe et al. 2013; Strubbe et al. 2015). These dissimilar conclusions highlight the intricacy of the niche dynamics involved in biological invasions. As a step towards further understanding, several studies have attempted to investigate if the integration of clade-level phylogeographic data is able to obtain more reliable estimates of the distribution of species (Schulte et al. 2012; Godefroid et al. 2016) and niche dynamics during invasions (Strubbe et al. 2015). Despite of the encouraging results shown by this lineage-based approach, its application is hampered by the limited availability of phylogeographic data for most of the species.

While most of the research focuses on the terrestrial realm, there is still very little scientific understanding of niche conservatism of marine invaders (but see Parravicini et al. 2015). To our knowledge, no single study has integrated phylogeographic structure among marine invasive lineages besides. The Mediterranean Sea is considered to be the most invaded marine basin in the world (Parravicini et al. 2015), where native and invasive congeneric species can coexist in sympatry, as it is the case of the marine green algal genus *Caulerpa*. These algae have drawn much attention in the last two decades, mostly because two tropical *Caulerpa* species, *C. taxifoli*a (M. Vahl) C. Agardh and *Caulerpa cylindracea* Sonder (previously *C. racemosa* var. *cylindracea* (Sonder) Verlaque, Huisman & Boudouresque), have rapidly spread into the Mediterranean (Meinesz et al 2001;Varela-Álvarez et al. 2006, 2011; Williams 2007). Another indigenous *Caulerpa* co-occurs in the Mediterranean Sea, *Caulerpa prolifera* (Forsskål) J.V. Lamouroux, a worldwide distributed species (Varela-Álvarez et al. 2012; Varela-Álvarez et al. 2015). *Caulerpa* spp. have the capacity to spread by fragmentation and they often show invasive behaviour when introduced outside their native ranges, acting as competitors of seagrasses (de Villèle and Verlaque 1995; Williams 2007) and corals (Kružić et al. 2008). According to sequence and phylogenetic analyses of both nuclear and chloroplast regions, Mediterranean populations of *C. taxifolia* are of Australian origin. In 1984, *C. taxifolia* was accidentally released from an aquarium in Monaco and spread throughout the Mediterranean Sea (Meinesz et al. 2001). *C. taxifolia* has also spread outside the Atlantic area (e.g. the California Coastline, Jousson et al. 2000), and it has been considered an introduced and opportunistic alga even in some regions of Australia (Glasby et al. 2005; Glasby 2013). Recently, a slender form of *C. taxifolia* has been reported in the Mediterranean (Jongma et al., 2013). With only a difference of one single nucleotide mutation on the cp16S rDNA intron, and slight morphological variations (slender thallus and lack of large rhizoidal pillars), Jongma et al., (2013) described this new variety/entity

(*Caulerpa taxifolia* var. *distichophylla* (Sonder) Verlaque, Huisman and Procaccini) which is spreading in the Eastern Mediterranean Basin and it is also of South Western Australian origin (Jongma et al. 2013). While this new strain seems to be in an early stage of invasion and actively spreading, for reasons that are not well understood, the regression of *C. taxifolia* meadows is a fact, and the species has disappeared or declined in abundance at almost all locations colonized in the Mediterranean Sea (Jaubert JM, personal communication to ALGAE-L, 30TH of October, 2011). On the other hand, *C. prolifera* is considered a native species in the Mediterranean Sea, though two hypotheses on its origin were given: an Atlantic origin of Mediterranean populations (because the western Atlantic area contained the highest genetic diversity and also the most ancestral sequence types), or an ancient Indo-Pacific origin (because of closely related sequences) (Varela-Álvarez et al. 2015). Because of the worldwide distribution of native and invasive species, *Caulerpa* spp. provide a good model to test potential expansions into new geographical areas and the effect of global climatic and environmental changes. Though a potential spreading of *C. cylindracea* has been predicted (Verbruggen et al. 2013), to our knowledge no model has been assessed for *C. taxifolia* and/or *C. prolifera*. Moreover, the information available on these two species permits the integration of the phylogeographic structure into invasive SDMs and niche shift analyses. With respect to the methodological approach, most research to date has tended to predict invasions using presence-absence algorithms rather than presence-only methods. However, model uncertainty arises from the lack of reliable absence data as it could underpredict the potential suitable habitat of the invader (Hirzel et al. 2002; Chefaoui & Lobo, 2008; Jiménez-Valverde et al. 2011; Václavík & Meentemeyer, 2012), especially in the case of recent invasions. Presence-only methods are not so affected by the geographical extent chosen -unlike techniques using absences, pseudo-absences or background data (Acevedo et al. 2012; Mateo et al. 2015)-

allowing the use of a greater extent that includes both native and invaded ranges, even if they are located in different hemispheres or continents. This approach could include all accessible area for the species over time to calibrate the model, as suggested by Barve et al. (2011), and also prevents the extrapolation of models to conditions outside the calibration range, a practice which involves uncertainties and should be avoided (Fitzpatrick and Hargrove 2009; Jiménez-Valverde et al. 2011).

Here, we used distributional data of the two invasive lineages of *C. taxifolia* with different residence time in the invaded range to perform the first comprehensive study of this highly invasive marine species involving assessment of niche overlap, niche shift, and the potential spread of the invasive clades. This study explores different analytical approaches, under the expectation that measures of niche shift and niche overlap might be sensitive to the area used to calibrate the analyses (Mateo et al. 2015; Kirchheimer et al. 2016; Qiao et al. 2017). Since our model species is an invasive alga widely dispersed by anthropogenic vectors, such as vessels and escapes from aquaria, areas susceptible to invasion could be worldwide distributed. Thus, we compared the effect of using a worldwide extent (all accessible area) versus using projections from the native to the invaded range (a common practice) on niche overlap and niche shift analyses. Under the supposition that each invasive lineage has dissimilar environmental requirements, and also different from the entire species, we also tested the ability of lineage-based against species-based SDMs to assess the risk of invasion of these coastal species. Some research exists comparing projections from SDMs fit using pooled data from invasive and native ranges with those fit using just the native occurrences (e.g. Broennimann & Guisan, 2008), but little is known in the case of invasive lineage-based predictions. Thus, we also tested the ability of native against pooled occurrences to predict the spread of each invasive lineage in the Mediterranean Sea. With this comparative approach using different datasets and extents, we explored: (1) the potential spread and establishment of the two invasive lineages in the Mediterranean Sea regarding their different stage of invasion; (2) the spatial and environmental niche overlap between the invasive lineages coexisting in the Mediterranean; and (3) the existence of realized niche shift between the invaded and native ranges. By means of these analyses we try to provide a better understanding of the invasive process and assist the progress of monitoring and management of these alien species in the Mediterranean Sea.

## METHODS

### *Species data, genetic lineages, and study area*

Occurrence records of *C. taxifolia* and *C. prolifera* were compiled from the Global Biodiversity Information Facility (GBIF; http://www.gbif.org/), Algaebase (http://www.algaebase.org/), the Australia's Virtual Herbarium (http://avh.chah.org.au/), and references from the literature. All records were checked to remove duplicates, aquarium specimens, and dubious locations due to misidentifications. To reduce the effect of sampling bias, just one observation per 0.083°×0.083° pixel was considered (~ 9.2 $km^2$; resolution of the environmental variables).

After the filtering process, we assigned the occurrences to each lineage. We considered 359 occurrences of *C. taxifolia* for species-level analyses. In addition, following Meusnier et al. (2002) and Jongma et al. (2013), we segregated those pertaining to the two genetically different lineages (*C. taxifolia* invasive lineage and *C. taxifolia var. distichophylla*) for intraspecific analyses. As a result, 65 occurrences were assigned to *C. taxifolia* "invasive strain" and 45 occurrences to *C. taxifolia var. distichophylla*. Both lineages are located either in the Mediterranean Sea (invaded range) or along the Australian coast (mostly their native range, but with some invaded regions by the “invasive strain”). For *C. prolifera*, we assigned the occurrences to the genetically distinct lineages found in Varela-Álvarez et al. (2015): West Atlantic, East Atlantic and a larger area representing the Mediterranean Sea, the Mediterranean/Atlantic transition zone, and some Indo Pacific sites. From these, only the Mediterranean native lineage (with 87 occurrences in the Mediterranean Sea) was considered for comparison purposes.

We defined the extent of the study as the subpolar region, to cover the full appropriate range of these worldwide-distributed species. Finally, the study area was determined by the general depth limit of the species (-70 m), which due to the resolution used, allowed a margin to cover the maximum depth found for *C. taxifolia* var. *distichophylla* in one location (-100 m; Aplikioti et al. 2016).

### *Selection of environmental variables*

Sea surface temperature (SST) measures (minimum SST, maximum SST, mean SST, and range of SST), nutrients (nitrate and phosphate), salinity, and the minimum, mean, and maximum diffuse attenuation coefficient (Kd) at 490 nm ($m^{-1}$) were obtained from Bio-ORACLE (Tyberghein et al. 2012). This selection was based on general knowledge of seaweeds requirements (Lüning 1990) and previous studies on *Caulerpa* species (Verbruggen et al. 2013). Variables were tested for the study area using the Pearson correlation coefficient ($r \geq |0.7|$), which resulted in only five uncorrelated variables: mean SST, range of SST, phosphate, salinity, and mean Kd.

### *Environmental niche modelling and spatial overlap*

We firstly assessed the environmental similarity between the native (Australia) and the invaded range (Mediterranean Sea) using the Multivariate Environmental Similarity Surfaces (MESS; Elith et al. 2010) analysis in the “ecospat” package for R (Broennimann et al. 2015). MESS analysis reports the similarity of any point in the invaded range to the reference points (native range) according to the variables selected. MESS maps revealed a high dissimilarity between the native and invaded ranges (MESS ranging from -497  to -165; see Fig. S1 in Supporting Information). Thus, we trained the models using the whole study area (a worldwide geographic extent) to avoid the extrapolation of models from the native (Australia) to a novel scenario (i.e. Mediterranean Sea). To estimate the geographic range susceptible to invasion we used Mahalanobis distance, a presence-only method not sensitive to changes in extent, which produces an elliptic envelope for the species by calculating the distance between a given point and the mean of the niche (Clark et al. 1993; Calenge et al. 2008). Though Mahalanobis distance achieves a more consistent visualization of the niche in the geographical space (habitat suitability map) than Environmental Niche Factor Analysis (ENFA; Hirzel et al. 2002), both can be used complementarily to explore the niche structure (Calenge et al. 2008). Thus, ENFA was used to assess the relevance of each variable to predict the species by means of the marginality factor (which explains the difference between the mean conditions in the study area and the niche of the species) and one specialization factor (which measures the breadth of the niche) (Hirzel et al. 2002). Before Mahalanobis distance computation, the variables were scaled to equate their variance. Analyses were fit using the “adehabitat” package (Calenge 2006) in R.

We modelled the worldwide potential distribution of the species and the intraspecific lineages separately. For comparison purposes, predictions were produced using pooled datasets (occurrences from the invaded and native ranges), and occurrences from the native range of each lineage. The performance of the models was assessed using the Boyce index ($B_i$; Boyce et al. (2002)) to obtain a continuous value varying from -1 to 1, where a zero score means a random model and positive values indicate consistent predictions (Hirzel et al. 2006). Finally, we explored the spatial overlap between the binary predictions of suitable habitats for the two invasive lineages of *C. taxifolia* in the Mediterranean Sea, by previously reclassifying the maps according to the Boyce index.

### *Environmental niche overlap and niche change*

We measured environmental niche overlap among native and invaded ranges of the lineages using Schoener's *D* index (Schoener 1968; Warren et al. 2008) according to the $PCA_{env}$ method proposed by Broennimann et al. (2012). *D* metric compares the overlap of the environment between pairs of species with a value ranging from 0 (no overlap) to 1 (identical niches). The occupancy in the cells of a kernel smoothing density (resolution = 100) was used to determine *D* index between datasets. The Mediterranean lineage of the native *C. prolifera* was also analysed for comparison purposes. To explore the effect of the geographical extent on niche overlap and niche shift measures, we calibrated the PCA on the entire environmental space (worldwide), and on the native range (Australia), in both cases using the same variables than in the SDMs. The same duplication was performed in the remaining analyses.

The niche equivalency and similarity tests described by Warren et al. (2008) were also performed for the two invasive lineages between the invaded and native ranges according to the framework proposed by Broennimann et al. (2012). To test the niche equivalency, the occurrences are randomly reallocated in two datasets and *D* is computed in a process repeated 100 times. An histogram of simulated values shows if the observed *D* falls inside the 95% of reshuffled values (then, the null hypothesis of niche equivalency cannot be rejected). The niche similarity test compares random occurrences from one range with the observed niche in the other range by means of *D* through 100 iterations. Niches more similar than expected by chance will show an observed *D* greater than 95% of the resampled values.

We applied the methodology proposed by Petitpierre et al. (2012) to assess niche change between the native and invaded ranges of the invasive lineages of *C. taxifolia*. Three components of niche change were measured: unfilling (niche space inhabited only in the native range), stability (niche space occurring in both ranges) and expansion (niche space colonized only in the invaded range). Niche change indices were computed on the whole environmental extent (native and invaded) and also at the intersection between the native and invaded range (analogue conditions), which avoids an overestimation of niche shift (Guisan et al. 2014). All analyses were carried out using the "ecospat" package (Broennimann et al. 2015) in R.

## RESULTS

### *Environmental niche modelling, risk of invasion, and spatial overlap*

All variables used accounted for the discrimination between the species requirements and the environmental space of the study area (see marginality and specialization ENFA scores in Table S1; Supporting Information). The variables which contributed the most to the marginality factors were salinity, phosphate and the mean Kd. The two invasive lineages of *C. taxifolia* (as well as the species itself) and the native lineage of *C. prolifera* in the Mediterranean Sea are present in coastal areas with higher salinity and lower phosphate and Kd than the mean worldwide conditions. All lineages occur in locations with lower mean SST than *C. taxifolia* (species set) and the study area. In contrast, there were considerable differences among lineages and species regarding the range of SST. The niche breadth (specialization factor) also differed among datasets and variables. A visualization of the differences in niche position and breadth for the two invasive intraspecific lineages of *C. taxifolia* regarding the native and invaded ranges and each variable is shown in Fig. 1. The invaded niche of both lineages shows a wider range of SST and salinity than the native.

Models calibrated for each invasive lineage achieved better performance ($B_i$ *C. taxifolia* var. *distichophylla* = 0.92; $B_i$ *C. taxifolia* “invasive strain” = 0.86) than using the complete set of occurrences for *C. taxifolia* ($B_i$ = 0.54). Lineage-based models with pooled occurrences predicted better the invaded region in the Mediterranean Sea than the species-based model, which even underestimated already invaded locations (Fig. 2). According to this worldwide prediction, potential suitable habitats for the invasive strain are also found in the southern region of the Brazilian coast (Santa Catarina state). On the other hand, lineage-based models calibrated using just the native occurrences completely failed to predict both invasions in the Mediterranean region, resulting in predictions of 0 km$^2$ of suitable habitat (Fig. S2 and S3 in Supporting Information). After a binary classification of the habitat suitability maps, the Mediterranean area environmentally suitable for invasion was approximately four times higher for *C. taxifolia* “invasive strain” (24048.8 km$^2$) than for *C. taxifolia* var. *distichophylla* (6136.4 km$^2$) (Fig. 3). Though certain overlapping regions existed, the risk of invasion of the latter lineage was higher in the Eastern Mediterranean Basin. There is still a large extension of coast susceptible to invasion by both invasive lineages.

### *Environmental niche overlap and niche change*

Niche overlap and niche shift results differed considerably between geographic extents used to calibrate the PCAs. Niche overlap between invaded and native ranges, and also between lineages, tended to be higher when measured using the worldwide extent (Fig. 4). The higher overlap was shown between the native niche of *C. prolifera* and the exotic ranges of the invasive lineages of *C. taxifolia* at a worldwide scale. The hypothesis of niche equivalency was rejected for both invasive lineages and extents used, given that the observed overlap between invaded and native ranges was significantly lower than 95% of simulated overlaps (Table 1 and Fig. S4 and S5 in Supporting Information). Despite the fact that niches were not identical, niche similarities between ranges were found (Table 1 and Fig. S4 and S5). Interestingly, similarity was less significant when measured in the native range, and even a dissimilar niche of *C. taxifolia* var. *distichophylla* resulted when measured from the invaded to the native range.

Depending on the extent used, both invasive lineages showed considerable discrepancies in the components of niche change between invaded and native ranges (Table 1 and Fig. 5). The contribution of the variables to the PCA axes varied with the range used as extent (Fig. 5). Niche conservation (i.e. stability) between the ranges was predominant and the niche shift (i.e. expansion) was null using the worldwide extent. By contrast, niche change analyses performed using the Australian extent provided evidence of niche shift (expansion > 10%) for both invasive lineages.

## DISCUSSION

### *Niche overlap and niche change analyses: the effect of the extent*

We found that the extent of the geographic background used to calibrate niche overlap and niche change analyses affected most of niche measures. The niche equivalency test reported non-equivalent niches between the invaded and native ranges of the two invasive lineages of

*C. taxifolia*. However, niche equivalency was the only niche measure which obtained a consistent result across extents. When analysed on the worldwide extent, it could be highlighted that the niches did not experience change during the invasion process, since evidence of niche conservatism, niche overlap and similarity between the invaded and native ranges were found. On the contrary, the use of the native range as extent (i.e. Australia) produced lower overlap and similarity between the niches, and the niche expansion values indicated the existence of niche shifts. According to these dissimilar results, niche overlap and niche shift analyses greatly depend on the geographical extent used, so caution must be applied with the choice of the extent. In our particular case, the use of the native extent is not recommended because the accessible regions via dispersal over relevant periods of time should be the ideal extent for model calibration (Barve et al. 2011; Broennimann et al. 2012; Qiao et al. 2017). Thus, results are more conclusive at a worldwide extent (excluding inappropriate habitats such as polar climates or the deep sea) for invasive and highly dispersive species and lineages whose reachable areas are widespread due to the human action (e.g. shipping routes, aquaria). The extent has previously been found to influence niche shift analyses (Mateo et al. 2015; Kirchheimer et al. 2016; Qiao et al. 2017). Further research is needed to investigate the “zoom effect” of the extent on the PCA's ordination and derived niche measures, and to explore if there is a general tendency among species.

*Niche analysis on a worldwide extent*

As explained above, we considered the use of the entire extent susceptible of invasion (i.e. the worldwide extent) to be more adequate to calibrate niche change analyses than projecting from the native to the invaded range. Despite the different residence time of both invasive lineages in the Mediterranean Sea, their niches were significantly similar (but non-equivalent) in reference to their worldwide potential range. The two invasive lineages also showed high niche conservation both under analogous and non-analogous conditions. Thus, both clades seem to have found similar environmental conditions in the Mediterranean Sea than those existing in their native habitat; there is no evidence of a post-introduction adaptation scenario. In fact, the Mediterranean coasts seem to offer the most suitable conditions for these lineages outside their native range, together with specific coastal areas in Brazil (discussed below). According to the marginality factors derived from ENFA (Table S1), localities where both lineages are found, both in native and invaded ranges, show less Kd and lower phosphate concentration than the mean conditions of the study area. These conditions may be related to less eutrophicated and turbid habitats. Interestingly, lineage-based models revealed that the coasts where the invasive lineages occur show colder mean SST than those occupied by all the lineages grouped in *C. taxifolia* as one single species (conditions that are present in the Mediterranean Sea, and which are also shared by the native lineage of *C. prolifera*). On a more detailed scale (Fig. 1), slight differences regarding the requirements of both lineages arise, which may be related to their disjunct distribution between the Eastern and Western Mediterranean basins. Niche breadths also differed slightly, since the invaders seem to occupy a wider range of salinity and higher ranges of SST in the Mediterranean than in the native range.

### *Predicted spread and establishment of the invasive lineages in the Mediterranean Sea*

Lineage-based models with pooled occurrences from both ranges offered more realistic predictions of the risk of invasion in the Mediterranean Sea than species-based models and also than models trained exclusively with native occurrences. The specific niche requirements of the invasive lineages could not be captured by a species-based model, given that it underestimated the risk of invasion of both lineages and also failed in the prediction of Mediterranean regions where the invasion already occurred. Intraspecific models altered species-based predictions. They were more accurate and with these models we found two separate regions susceptible of invasion for each lineage in the Mediterranean. Other studies also showed a better performance and capacity to discern between subtaxa using lineage-based models (Pearman et al. 2010), as well as enhanced invasion risk predictions (Godefroid et al. 2016). Suitable habitats for *C. taxifolia* var. *distichophylla* were found mainly in the Eastern Mediterranean Sea, except for overlapping regions of both invasive lineages in Sicily, Sardinia, Greece and Morocco. On the contrary, suitable habitats for the invader with a longer residence time (*C. taxifolia* “invasive strain”) were found mainly in the Western Mediterranean Sea. The different stage of invasion may account for the larger area predicted for the oldest invader, since the number of occurrences was higher and, therefore, a wider niche breadth was shown regarding the set of variables used in the prediction. On the other hand, the distribution of *C. taxifolia* var. *distichophylla* might still be in non-equilibrium with the environment due to its recent introduction, this being a source of uncertainty to our models (Jiménez-Valverde et al. 2011). Therefore, we should be alert to the potential risk of invasion of the most recent invasive lineage, as a consequence of ongoing dispersal events.

The use of pooled occurrences from both ranges also enhanced predictions when compared to the use of only native-range data. Unreliable models were obtained by using just native occurrences as they failed to predict any suitable habitat for both lineages in the Mediterranean Sea. These results are consistent with other studies (Broennimann and Guisan 2008; Jiménez-Valverde et al. 2011) and they have important implications for developing pest risk predictions.

Though these lineages have been able to reach the Mediterranean Sea and find suitable environmental conditions, the success of the invasions may depend on other factors such as the competition with native species (or even with other invaders). Taking into consideration predictive models and niche analyses performed using the worldwide extent, spatial overlap exists between the native lineage of *C. prolifera* and *C. taxifolia* “invasive strain”, as well as environmental niche overlap (*D*= 0.59). It has been suggested that those aliens with niche requirements more dissimilar to the native species have the highest chance  of establishing themselves (van Kleunen et al. 2015). In fact, there are hypotheses about different invasive *Caulerpa* spp. (e.g. *C. taxifolia* and *C. racemosa*) competing among each other in the Mediterranean Sea (Piazzi and Ceccherelli 2002; Piazzi et al. 2016), though the mechanisms (e.g. allelochemical, overgrowth, etc.) that control these presumed interactions, if any, remain unknown (Piazzi et al. 2016). This may be one of the possible explanations why *C. taxifolia* is in regression in the Mediterranean Sea. Of course, this interpretation is limited by the comparison with just phylogenetically related lineages, as other competitors may be present and biotic constrains are difficult to be estimated.

Previous studies have suggested the importance of genetic and chromosomal traits in biological invasions. Diploids and polyploids of some plant species often occupy different landscape regions, and polyploids typically replace their diploid parents along ecological gradients (e.g. Maherali et al. 2009; Treier et al. 2009; te Beest et al. 2012). Differences in ploidy levels and genome sizes among *C. prolifera*, *C. taxifolia* and *C. cylindracea* in the Mediterranean have been associated to invasive behaviour (Varela-Álvarez et al. 2012). Besides, it has been reported that niche differentiation readily occurs between different ploidy levels within a species (te Beest et al. 2012). Given that we did not find evidence of a post-introduction adaptation scenario, the hypothesis of a pre-adaptation of invaders in different life history stages with different ploidy levels may play an important role in determining the success of the invasions. Further research to test this hypothesis on different ploidy levels of both invasive lineages in the Mediterranean and also in the native areas is strongly recommended.

We can conclude that the integration of subtaxa information in niche modelling and niche shift analyses seems to be a recommendable practice for risk invasion assessment, provided that phylogeographic information exists. Intraspecific models altered species-based predictions and they were more accurate finding two separate regions susceptible of invasion. With the exception of some overlapping regions, the Eastern Mediterranean coasts were more appropriate for *C. taxifolia* var. *distichophylla*, while larger suitable coastlines were found in the Western Mediterranean for the oldest invasive strain. The niches were conserved across a worldwide extent and no evidence of a post-introduction adaptation scenario was found, because both lineages invaded habitats similar to their native locations (low eutrophic, clear and cold waters). The current findings imply that different invasive lineages of algae may show dissimilar environmental response and invasive ranges, and it could serve as a model for other marine invaders in the Mediterranean Sea. There is an urgency to define appropriate management plans for a large extension of coast susceptible to invasion in the Mediterranean and also the Southern hemisphere (e.g. the Brazilian coast). Monitoring already invaded regions would be needed to estimate any change in *C. taxifolia "invasive strain"* and its possible spread. Furthermore, actions are needed to control a rapid spread of *C. taxifolia* var. *distichophylla*, the most recent and unpredictable invasive lineage, to prevent its spread and avoid an invasion similar to that of its closely related strain. In the past, several treatment options for the eradication of *C. taxifolia* have been investigated, such as physical removal by hand, pumping, and smothering with jute matting and rubber conveyor belts (Glasby et al. 2005). The use of chlorine in California (Williams and Schroeder 2004; Anderson 2005) and the application of coarse sea salt in Australia (Glasby et al. 2005) seemed to be the most succesful methods (although expensive and time consuming). A rapid response -such as that of California- based on early detection, coordinated actions among the environmental agencies, and studies to compare the efficacy of different treatments (Anderson 2005) would be desirable for this recent seaweed invasion.

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## Disclosure of potential conflict of interests

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Conflict of Interest: The authors declare that they have no conflict of interest

“This article does not contain any studies with human participants or animals performed by any of the authors.”

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# TABLES

**Table 1** Results of niche equivalency and similarity tests, and measures of niche change (expansion, stability and unfilling) derived from the PCA calibrated using the worldwide extent and the native range (Australia) for the two invasive lineages of *Caulerpa taxifolia*. Equivalency and similarity were calculated comparing the observed Schoener's *D* value with simulated niche overlaps. We show the niche similarity of the native to the invaded range (Similarity 1 → 2), and of the invaded to the native range (Similarity 2 → 1), indicating if the assumption of niche similarity cannot be rejected ("Similar") or can be rejected ("Dissimilar"). Niche equivalency and similarity tests graphs are shown in Fig. S4 and S5. Nonequiv: the assumption of niche equivalency is rejected. Significant *P*-values ($P < 0.05$) are shown in bold. Asterisks represent niche change indices computed under analogous conditions.

| | *C. taxifolia* (invasive strain) | | *C. taxifolia distichophylla* | |
|---|---|---|---|---|
| | Worldwide range | Native range | Worldwide range | Native range |
| Equivalency | **Nonequiv**<br>***P* = 0.019** | **Nonequiv**<br>***P* = 0.019** | **Nonequiv**<br>***P* = 0.019** | **Nonequiv**<br>***P* = 0.019** |
| Similarity 1 → 2 | **Similar**<br>***P* = 0.049** | Similar<br>*P* = 0.069 | Similar<br>*P* = 0.059 | Similar<br>*P* = 0.544 |
| Similarity 2 → 1 | **Similar**<br>***P* = 0.049** | Similar<br>*P* = 0.584 | **Similar**<br>***P* = 0.029** | **Dissimilar**<br>***P* = 0.010** |
| Expansion | 0.000<br>0.000* | 0.680<br>0.662* | 0.000<br>0.000* | 0.995<br>0.995* |
| Stability | 0.999<br>0.999* | 0.319<br>0.337* | 0.999<br>0.999* | 0.004<br>0.004* |
| Unfilling | 0.002<br>0.002* | 0.001<br>0.000* | 0.106<br>0.106* | 0.999<br>0.999* |

## FIGURE LEGENDS

**Fig. 1** Occurrence density of the two invasive intraspecific lineages plotted against the five variables used in the analyses distinguishing between conditions in the invaded (inv) and the native range (nat). To allow a better visualization of the niche breadth, the range of each variable has been adjusted to its maximum and minimum values.

**Fig. 2** Comparison among habitat suitability maps predicted for *Caulerpa taxifolia* using the complete distribution for the species (upper panel), and the occurrences from the native and invaded ranges of the two intraspecific invasive lineages separately (middle and bottom panels). Presence of each strain are depicted by triangles, circles and squares. All models were calibrated with Mahalanobis distance algorithm using a worldwide geographic extent (in the left) and cropped to show better the invaded range (Mediterranean Sea; in the right). Lineage-based models reported more accurate risk invasion predictions than the species-based model. Lineage-based models calibrated with the occurrences from the native range failed to detect any suitable habitat in the invaded range (not shown, but see Figures S2 and S3).

**Fig. 3** Suitable habitats predicted for the two invasive lineages of *Caulerpa taxifolia* in the Mediterranean Sea (invaded range) according to Mahalanobis distance algorithm. Presences of each strain represented by circles. Models were calibrated in the whole study area (worldwide) using pooled occurrences from the invaded and native range. A binary classification of habitat suitability was produced using the Boyce index. “Overlap” represents suitable habitat for both invasive lineages. Circles: *C. taxifolia* (invasive strain); squares: *C. taxifolia* var. *distichophylla.*

**Fig. 4** Heatmaps showing the niche overlap metric (Schoener's *D*) among the different lineages of *Caulerpa* species between the invaded (inv) and native ranges (nat). The figure shows *D* metrics obtained with a PCA calibrated using the worldwide extent (left), against those calibrated in the native range (Australia; right). Dendrograms are used to visualize clusters of similarity among *D* values. *C. taxifolia* var. *distichophylla* (CTAX.DIST) and *C. taxifolia* (invasive strain; CTAX.INV) are invasive lineages, while CPRO.MEDIT is a non invasive lineage of *C. prolifera* native in the Mediterranean Sea. *D* values tended to be higher when measured using the worldwide extent.

**Fig. 5** Comparison of niche dynamics of the invasive lineages of *Caulerpa taxifolia* between the native (Australia) and invaded range (Mediterranean Sea), using the whole study area (worldwide; upper figures) and the native area (below) as calibration extents. Different results were obtained in expansion (red), stability (blue) and unfilling (green) depending on the extent. In the figures below solid lines show the available environment in the native (green) and in the invaded range (red), while dashed lines delimit 50% of the available (background) environment. Density of the species in the native range are shown as a grey shadow and the red arrow shows the change between the centre of the native and invaded niches. Correlation circle shows the PCA ordination diagram with the contribution of the environmental variables on the two axes. Sstmean: mean sea surface temperature, sstrange: range of sea surface temperature, damean: mean diffuse attenuation coefficient (Kd).