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Article

Phylogeny of *Cystoseira sensu lato* from SE Spain suggests a complex colonization of the Mediterranean

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Abstract: *Cystoseira* is a relevant alga, which forms dense meadows on rocky substrates up to 100 meters deep. They are widely recognized as bioindicators for their restricted habitat (sub-coastal zone) and low tolerance to pollution. We have monitored *Cystoseira sensu lato (sl)* spp. in abrasion platforms in Cabo de Las Huertas (Alicante, SE Spain), a total of seven species. A cladogram has been constructed using fifteen phenotypic taxonomic relevant characters. We have also optimized *Cystoseira* DNA extraction and sequenced amplicons from mt23S, tRNA-Lys, and psbA genes of five *Cystoseira sl* species from Cabo de Las Huertas. Our phylogenetic supertree, built with these sequences, backs three clades in the genus. Rare species *Treptacantha sauvageauana* and *Treptacantha algeriensis,* sequenced for the first time, are included with *T. elegans* and the Atlantic species *T. baccata* and *T. abies-marina* in Cystoseira-VI or Cystoseira-II clades. Our data supports a complex colonization of *Cystoseira (sl)* spp. in the Mediterranean, perhaps involving Atlantic clades.

Keywords: *Cystoseira*, Algal cartography, Abrasion platforms, SE Mediterranean, Phylogeny supertree, DNA sequencing.

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1. Introduction

The family *Sargassaceae* Kützing (*Phaeophyceae*) inhabits all oceans, from polar waters to the warmest tropical seas [1]. *Cystoseira sensu lato (sl)* species have its maximum diversity in the Mediterranean Sea, with two thirds of all species described found [2-4]. Some of them have habitats so reduced as coastal platforms [5]. Outside Mediterranean Sea they are mainly found in the Northeast Atlantic. This may explain why they were reintroduced into the Mediterranean Sea six million years ago, after Messiniense's crisis [6,2,7]. Recent research corroborates the polyphyletic nature of these algae, distinguishing three taxonomic genera [8,9,10]. They comprise *Cystoseira sensu stricto, Carpodesmia* and *Treptacantha.*

As an engineering species they form dense meadows on rocky substrates up to 100 meters deep [2,5,11,12]. They are widely recognized as bioindicators for their restricted habitat in the sub-coastal zone and low tolerance to pollution [13,14]. Most Mediterranean species are protected by the Barcelona (Annex II, COM/2009/585), the Bern Conventions (Annex I) and the INDEMARES project [15], contributor to the expansion of Natura 2000 Network. They are also used to assess the ecological quality of the coast (CARLIT), a requirement under Water Framework Directive (2000/60/EU) for the conservation of good water status [13].

Cystoseira spp. have a high morphological plasticity which, with recurrent hybridization processes [16,2] makes taxonomic assignment of some species rather difficult [10,17,18]. *Cystoseira sl* communities are currently declining due to anthropic pressures [10,19-22]. Reforestation projects have been developed [20,22,23]. Even their economic value has even been quantified to promote their preservation [24]. However, there is little public knowledge on the value of these ecosystems outside the phycological community.

SE Spain has a great diversity of *Cystoseira sl* populations due to the presence of rocky platforms from Quaternary Period, which generate adequate niches for their development [25,26]. However, there is little research on the distribution patterns of *Cystoseira sl* in these areas, since most studies of

Spanish Mediterranean populations have been carried out in coasts of Catalonia and Balearic Islands [27-33]. Without light limitations the main factor modifying the abundance of coastal communities is nutrient availability, which depends largely on hydrodynamism [13,34]. Geomorphological characteristics of the substrate (lithology, slope, depth) may account for the environmental heterogeneity of the system, a key factor in algal distribution [35]. Inclination of the substrate can affect vertical zonation [36]. Previous studies indicate that type of substrate and depth can also affect the distribution of *Cystoseira sl*. Decimetric blocks and pebbles displaced by storms can affect these communities and lead to their replacement by other species [37]. These authors also reported variation in patterns of distribution with the degree of exposure to waves. On the other hand, intraspecific variability and environmental conditions to which they are exposed may cause variations in the concentration of polyphenols, polysaccharides and pigments. These compounds can affect DNA extraction protocols [38]. Sequencing of various genes (e.g. mt23S, tRNA-Lys, psbA, COI) has clarified the phylogenetic relationships of these genera [2,9,10,39,40].

Our hypothesis stands that the width and the location of the platform of *Cystoseira sl* communities, and the degree of wave exposure will affect their distribution. These factors are directly related to hydrodynamics and therefore, to nutrient availability. There will be a differentiation in horizons depending on the width of the platform and the degree of exposure to waves to which it is subjected. The aim of this study is to increase our knowledge of *Cystoseira sl* in SE Spain using ecological, morphological and molecular tools. We have chosen El Cabo de las Huertas, a good preserved natural coasts amidst largely touristic developed shores. This area is a Site of Community Importance proposed in 2001 (ESZZ16008) because of the presence of sandy seabed with well-preserved seagrasses (*Posidonia oceanica, Zostera marina* and *Cymodocea nodosa)* despite the extensive touristic development. This makes this site interesting to revaluate its degree of protection [41]. Unfortunately, a management plan for this area does not exist nor has progressed its declaration as a Specially Protected Area [42]. Concluding, our work could be a sound foundation to develop an ambitious *Cystoseira sl* protection plan in SE Spain.

2. Materials and Methods

2.1. Cartography of Cystoseira sensu lato.

Our sampling method consisted on a walk along 14 km of Alicante city coast (38º21’26.48” N, 0º24’31.37" W – 38º19’29.64" N, 0º30’40.41" W). We divide the coastal strip into 3 horizons parallel to the coastline: The proximal is next to the midlittoral zone. The distal horizon is hit by waves and it is sometimes emerged. The medium horizon is between these two. *Cystoseira sl* species have been identified *in situ* and their semi-quantitative abundance scored visually as in Ballesteros *et al*., (2007). The lowest value (1) corresponds to isolated individuals. Several individuals forming no patches score 2. For isolated patches the abundance value is 3. For patches forming a discontinuous horizon, the value is 4. A continuous horizon of the same species of *Cystoseira sl* scores as 5. Sixty-one linear transects (30-80m) were performed along the coastline of study (2018, May-July) recording the abundance of *Cystoseira sl* communities per horizon.

Trails were georeferenced using GPSies^{+} (Klaus Bechtold, ©2017) and distribution maps drawn using QGIS v 2.18 with WGS84 (EPSG:4326) coordinate system. An orthophoto of the region provided by the National Plan for Aerial Orthophotography (PNOA) of the National Geographic Institute [43] was used as model to draw a map according to the real geography.

2.2. Abiotic factors and spatial variability of Cystoseira spp.

Platform width, wave exposure and the sublittoral horizons were recorded as main hydrodynamism variables (Table S1). Geomorphological characteristics of the substrate (lithology, slope, presence of pools or rifts) were also scored. Records have been analysed with Primer software (v.6.1) [44]. A 3 fixed factor PERMANOVA analysis has also been carried out with Bray Curtis similarity matrix to study the influence of the main variables related to hydrodynamism on the abundance of *Cystoseira sl* species. A reduced model with 4999 permutations has been chosen. The distribution patterns of the samples have been analysed with a Principal Coordinates Analysis (PCO), built with the same Bray-Curtis matrix.

2.3. Morphological characterization.

Specimens were collected in spring (2018) using chisel and hammer, avoiding damaging the basal structure and kept in 10% alcohol in seawater until analysis. A qualitative matrix using Primer 7 software [45] with 15 phenotypic characters of 37 *Cystoseira sl* was constructed (Table S2). Morphological data from samples described in Gómez-Garreta *et al.* (2000) [45], Cormaci *et al.* (2012) [46] and Orellana *et al.* (2019) [9] were recorded as reference algal groups. A cladogram was constructed using a dissimilarity Bray-Curtis matrix with 'simple matching' method and a SIMPROF test carried out to distinguish statistical differences between individuals, adding a 'dummy variable' to improve the robustness of the cladogram.

2.4. DNA extraction.

Individuals collected were kept in cold seawater. They were cleaned of epiphytes and lyophilized in less than 24h. Fine lyophilized thallus ground in liquid nitrogen was used for DNA extraction. DNA extractions were performed using a protocol based on the pre-treatment of Lane *et al.* (2006) [47] in combination with the cleaning steps of Rogers and Bendich (1989) [48]. All treatments were performed on ice to avoid DNA degradation. Lyophilized thallus is added to Buffer A (1.65M sorbitol, 50mM MES (sulphonic acid) pH 6.1, 10mM EDTA, 2% (w/v) PVP-40, 0.1% (w/v) BSA (Bovine Serum Albumin) and 5mM ß-mercaptoethanol) while stirring for 2-3 min. The mixture is filtered with Miracloth® and centrifuged at 3000g. Buffer A is removed, pellet is resuspended in Buffer B (Buffer A without PVP) and centrifuged at 3000g until the pellet was compact. Buffer B is discarded, and pellet is resuspended in DNA extraction buffer [48]. Subsequent centrifugations will be at 15000g. After 1-hour incubation on ice, centrifugation is done for 10min. Aqueous phase is transferred into new tubes for phenol, chloroform and isoamyl alcohol extraction for 5 min and then centrifugation. This step was performed again without phenol for 2min. DNA is treated with -20° isopropyl alcohol on ice for 20 minutes. After centrifugation for 10 min supernatant is discarded and pellet washed with ethanol at -20ºC. Pellets containing DNA are resuspended in nuclease free water and stored at -80ºC.

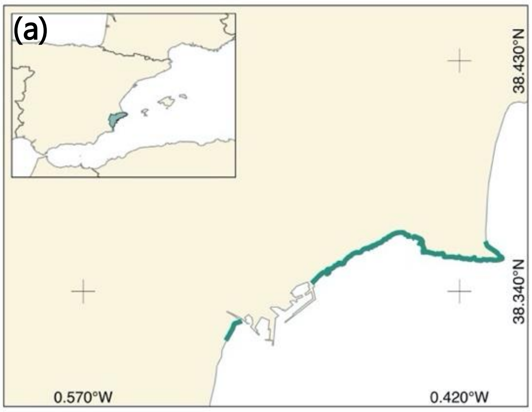
2.5. Gene amplification, sequencing and phylogenetic analysis.

Six sets of primers from previous work were used (Table S3). Some of them (psbA-FR2 and psbA-F2R) can be used in three different combinations [2]. PCR reactions included a preheating stage of 2 min at 92ºC, nine cycles of 30 s at 94ºC, 1 min at 45ºC with a final extension of 1 min at 72ºC plus 29 cycles of 30 s at 94ºC, 30 s at 50ºC with a final extension of 1 min at 72ºC. With psbA-FR primer final extensions were increased from 1 to 2 min. PCR reactions were repeated when required to supply enough DNA for Sanger sequencing. Amplicons were run in 2% agarose gels for quality testing, then treated with a DNA purification kit (Qiagen). Sequencing was carried out by Macrogen Inc. (Korea). Consense sequences were determined using Bioinformatics 'Reverse complement' [49] and Omega Cluster tools [50]. A sequence database has been set up based on previous studies [2,9]. Phylogeny analyses were performed using Mega X software [51], through a Muscle type alignment [52]. Both supertree and isolate trees for individual genes (mt23S, tRNA-Lys and psbA) have been built using 'Neighbor-Joining' method [53,54] and phylogenetic test 'Bootstrap' with 1500 interactions [55]. All taxa used in the phylogenetic analyses are listed in Supplementary table S4. Sequences were obtained from Draisma *et al.* (2010) [2], Orellana *et al.* (2019) [9] and Bruno de Sousa *et al.* (2019) [10], among others.

3. Results

3.1. Cartography of communities associated with the coastal fringe

Cystoseira sl species were found over more than 4km of the 14 km sampled (Fig. 1a, 1b), in nearly uninterrupted communities at Cabo de las Huertas (Cape area) and separate individuals or patches on La Almadraba beach, Paseo Marítimo and La Playita bay (Fig 1c). *Cystoseira compressa, C. humilis, C. foeniculacea, Carpodesmia amentacea* var. *stricta, C. brachycarpa* var. *balearica, Treptacantha algeriensis* and *T. sauvageauana* were the species found in the area of study.



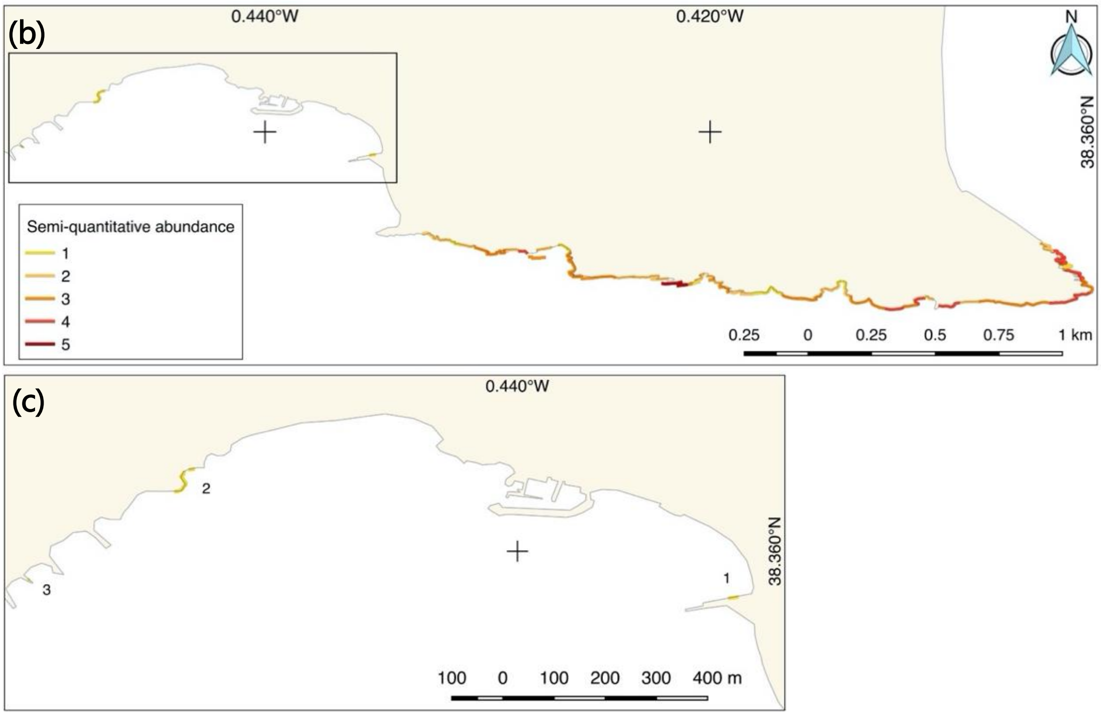


Figure 1: **(a)** Area of study: El Cabo de las Huertas (Alicante, Valencian Community, Spain). **(b)** Semi-quantitative abundance found in *Cystoseira sensu lato* in El Cabo de las Huertas. Legend: 1: One individual (isolated algae). 2: Several isolated individuals. 3: *Cystoseira sensu lato* patches. 4: Discontinuous belts of at least one species. 5: Continuous belts of one or more species. **(c)** Individuals or isolated patches found near El Cabo de las Huertas. 1: La Almadraba beach. 2: Paseo Marítimo. 3: Playa La Calita.

3.2. *Distribution of* Cystoseira sensu lato

Cystoseira sl communities are frequent in the area of study, in rocky shores with high hydrodynamism (Fig 2a). They usually disappear abruptly when the rock substrate changes from platform to metric stones (Fig. 2b). *Cystoseira sl* communities on narrow platforms and medium wave exposure are different from those under low or high wave exposure (Tables 1, 2 and S5). In wide platforms the degree of wave exposure conditions *Cystoseira sl* communities. The abundance of *Cystoseira sl* species depends on their location at the platform, since populations from proximal and distal horizons also differ significantly.

A PCO *Cystoseira sl* abundance with environmental factors was analysed using 183 samples from 61 transects recorded (Fig. 3). Axes explain 79.9% of the total variability and vectors with high correlation are represented (cor>0.2). Main hydrodynamism variables vectors are located to lower left quadrant indicating the direction of higher values of hydrodynamism. Two geomorphologic variables have close correlation with hydrodynamism, 'Inclination' negatively and 'Rifts' positively. Canopy height is also directly linked. The lowest heights of *Cystoseira sl* have been located in La Calita, which is a cove with high anthropic impact (Figure S6). *Cystoseira sl* general abundance cannot be explained by a single environmental factor.

Figure 2: **(a)** Exposed platform of El Cabo de las Huertas with *C. amentacea* var. *stricta* and *T. algeriensis* community. **(b)** Metric stones in front of the exposed platform without presence of *Cystoseira sl*.

Source	df	SS	MS	Pseudo-F	P(perm)	Uniq. perms
Width	3	15532	5177.3	3679	**0.0002**	4986
Wave exposure	2	14298	7148.9	5.08	**0.0002**	4983
Horizon	2	10849	5424.7	3.8548	**0.0016**	4985
Width*WaveExp	5	20474	4094.9	2.9098	**0.0002**	4972
Width*Horizon	6	5638.4	939.73	0.66778	0.8234	4974
Width*WaveExp	4	6531.8	1632.9	1.1604	0.3108	4982
Width*WaveExp*Horiz	10	6083.7	608.37	0.43231	0.9948	4982
Res	150	$2.1109*10^5$	1407.3			

Table 2: PERMANOVA analysis of variables related with hydrodynamism. Bold numbers indicate significative differences (p-value<0.05)

Width*Wave Exposure			
Wave Exposure	*t*	*P(perm)*	*perms*
Width = 0 (No platform)			
Med - Low	1.9482	**0.0438**	2246
Width = Narrow			
Med - Low	1.8362	**0.0408**	4981
Med - High	2.5084	**0.0008**	4990
Low - High	1.0787	0.3138	4983
Width = Medium			
Med - Low	1.2407	0.2272	4994
Med - High	1.7067	0.0532	4989
Low - High	1.0416	0.3604	4992
Width = Wide			
Med - Low	2.1585	**0.0034**	4985
Med - High	2.1066	**0.0052**	4993
Low - High	2.7538	**0.0002**	4992

Horizon			
Horizon	*t*	*P(perm)*	*perms*
Prox - Med	1.4607	0.0978	4985
Prox - Distal	2783	**0.0004**	4986
Med - Distal	1314	0.1656	4987

Table 3: *A posteriori* PERMANOVA analysis of significative interactions of variables related with hydrodynamism. Bold numbers show significative differences (p-value<0.05)

When *Cystoseira sl* individual species are considered different patterns of distribution appear (Fig. 4). For instance, *C. compressa* has a wide distribution, since this species is the most environmentally tolerant of all found. With the largest ecological range, it is the most abundant and is nearly homogeneously distributed in all horizons of El Cabo de las Huertas (Fig. 4a). *C. amentacea* var. *stricta* and *T. algeriensis* are distributed positively with vectors, therefore to hydrodynamism. They have a slightly smaller ecological range, with irregular distribution (Figs. 4b, 4c). Meanwhile, *C. humilis*, *T. sauvageauana* and *C. foeniculacea* have less correlation with hydrodynamism, with reduced distribution usually located at proximal horizon (low wave exposure) (Figs. 4d, 4e). The proximal horizon has a discontinuous presence of *C. compressa*, *C. amentacea* var. *stricta*, *T. algeriensis*, *T. sauvageauana* and *C. humilis* while more exposed horizons have a continuous community of these species (Fig. S7).

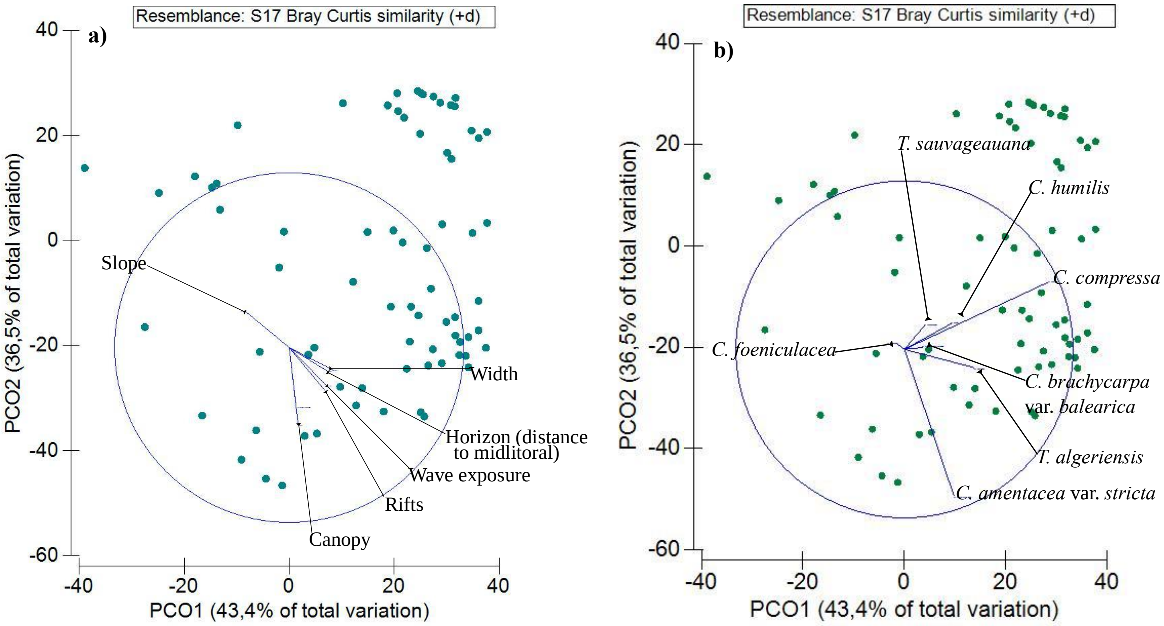


Figure 3: (**a**) Ordination PCO plot of *Cystoseira sl* communities in SE Spain related to environmental variables. (**b**) Ordination PCO plot of *Cystoseira sl* communities in SE Spain with *Cystoseira sl* species (n=183).

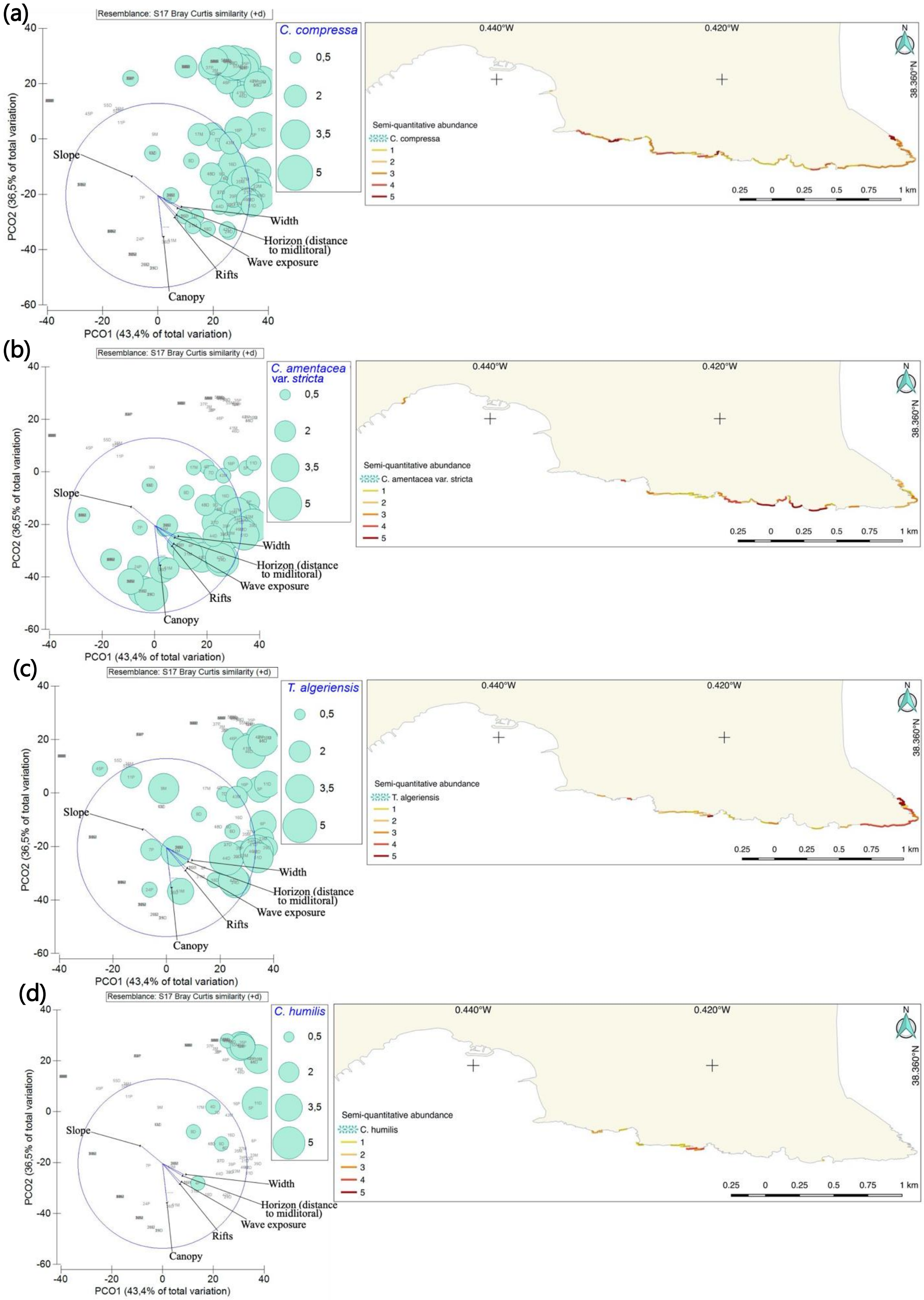


Figure 4: Bubble plots overlaying PCO plot of *Cystoseira sl* in El Cabo de las Huertas. The size of the values determines the abundance of *Cystoseira sl* in each sample. **(a)** *C. compressa.* **(b)** *C. amentacea* var. *stricta.* **(c)** *T. algeriensis.* **(d)** *C. humilis.*

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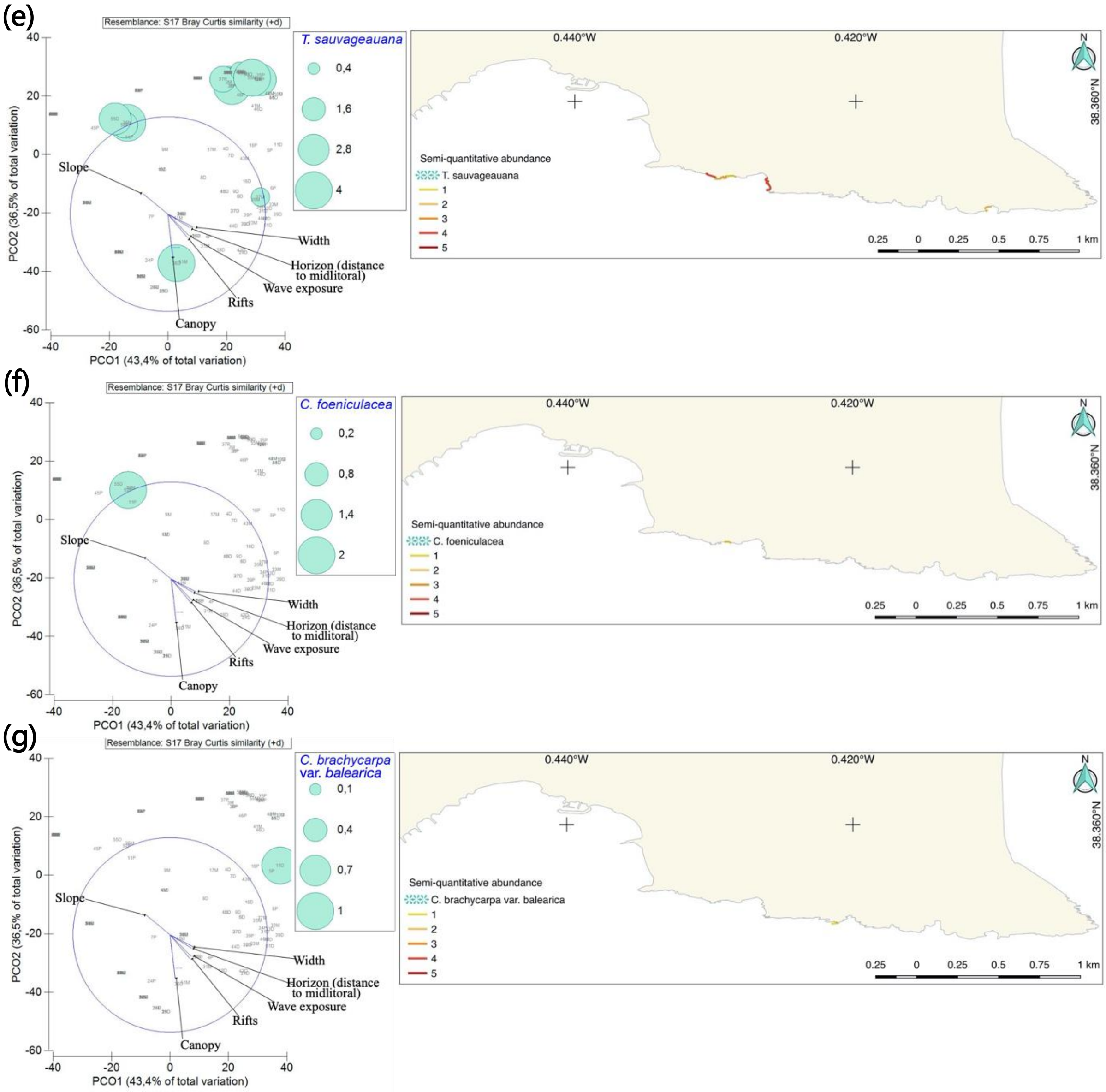

Figure 4: Cont. Bubble plots overlaying PCO plot of *Cystoseira sl* in El Cabo de las Huertas. The size of the values determines the abundance of *Cystoseira sl* in each sample. **(e)** *T. sauvageauana.* **(f)** *C. foeniculacea.* **(g)** *C. brachycarpa* var. *balearica.*

3.3. Morphological analysis

Our morphological cladogram includes eight clades (*Cystoseira* I-VIII) (Fig. 5). *Cystoseira* VIII is the clade with least similarity, followed by *Cystoseira* VII. From *Cystoseira* VI to I, branches can be differentiated with increasing similarity values. Similarities of more than 85% prevents species assignments. Most of our samples close to their counterparts included in the previous reference studies. However, several species (*T. sauvageauana, C. foeniculacea, C. compressa*) display more variability within a given clade or are located in different clades.

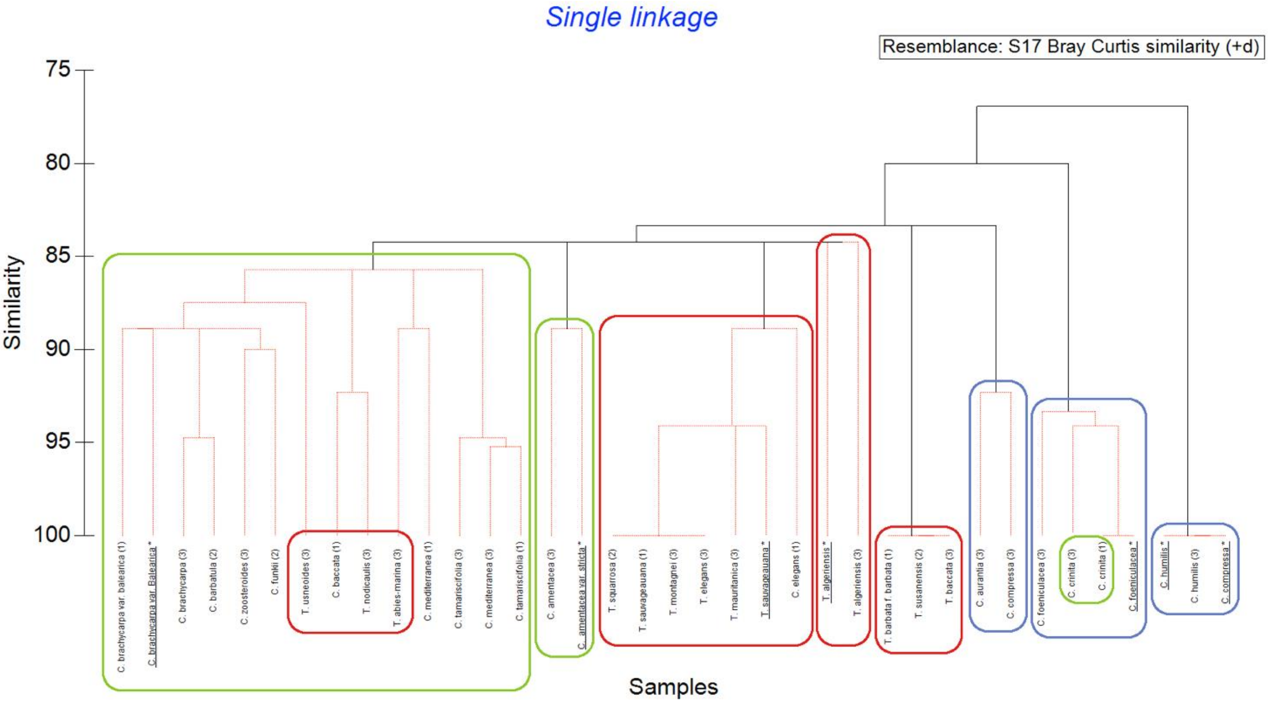

Figure 5: Single linkage cladogram based on data from *Cystoseira sl* built using Bray Curtis similarity matrix. Boxes correspond to current genus classification by Orellana *et al.*, 2019 [9]: Blue = *Cystoseira sensu stricto* or Cystoseira-I; Red= *Treptacantha* or Cystoseira-II. Green= *Carpodesmia* or Cystoseira-III. Label legend: (1): Gómez-Garreta *et al.,* 2010 [45]. (2):Cormaci *et al.*, 2012 [46]. (3): Orellana *et al.*, 2019 [9]. (*) underlined: This study.

3.4. Phylogenetic analysis

Our DNA protocol completely removed *Cystoseira sl* secondary metabolites and allowed template amplification with all primers used (Figs. S8-S11). In this study 30 *Cystoseira sl* DNA samples have been sequenced, corresponding to 3 genes. We have built a concatenated tree (supertree) with a total of 98 *Fucaceae* sequences from 41 species, 22 of them are *Cystoseira sl* species (Fig. 6). The supertree is more robust (higher node stats values) than those trees built with single genes (Figs. S12-S14).

Three clades (I-III) have been obtained. Cystoseira-I comprises *C. foeniculacea*, in a separate subclade, *C. compressa* and *C. humilis* together in a further subclade. Cystoseira-II includes four differentiated branches with *Treptacantha* spp. species (*T. abies-marina, T. baccata, T. usneoides, T. susanensis* and *T. barbata*). The latter two species clustered in a separate subclade. Cystoseira-II includes sequences of *T. sauvageauana* and *T. algeriensis* for the first time, both sequences separated from the rest. Cystoseira-III includes *C. amentacea, C. brachycarpa, C. crinita, C. funkii, C. mediterranea, C. tamariscifolia,* and *C. zosteroides*, this last species as an outlier. Within clade III *C. crinita* and *C. brachycarpa* can be clearly differentiated from the rest. Cystoseira-I and Cystoseira-II appear closer phylogenetically unlike Cystoseira-III.

4. Discussion

Hydrodynamism plays a key role in the *Cystoseira sl* populations distribution from SE Spanish coast. This has also been found in [13,31,34,56]. In our study, *Cystoseira sl* -in general terms- is most abundant and widely distributed in zones with large exposure to waves. For instance, *C. amentacea* var. *stricta*, *T. algeriensis* and *C. compressa,* with large productive potential, require light saturation [57] and high hydrodynamism [13,27,31,37,58,59]. Consequently, they are frequently found in rifts and other exposed places. Also, there are seasonal species which have their optimal development during

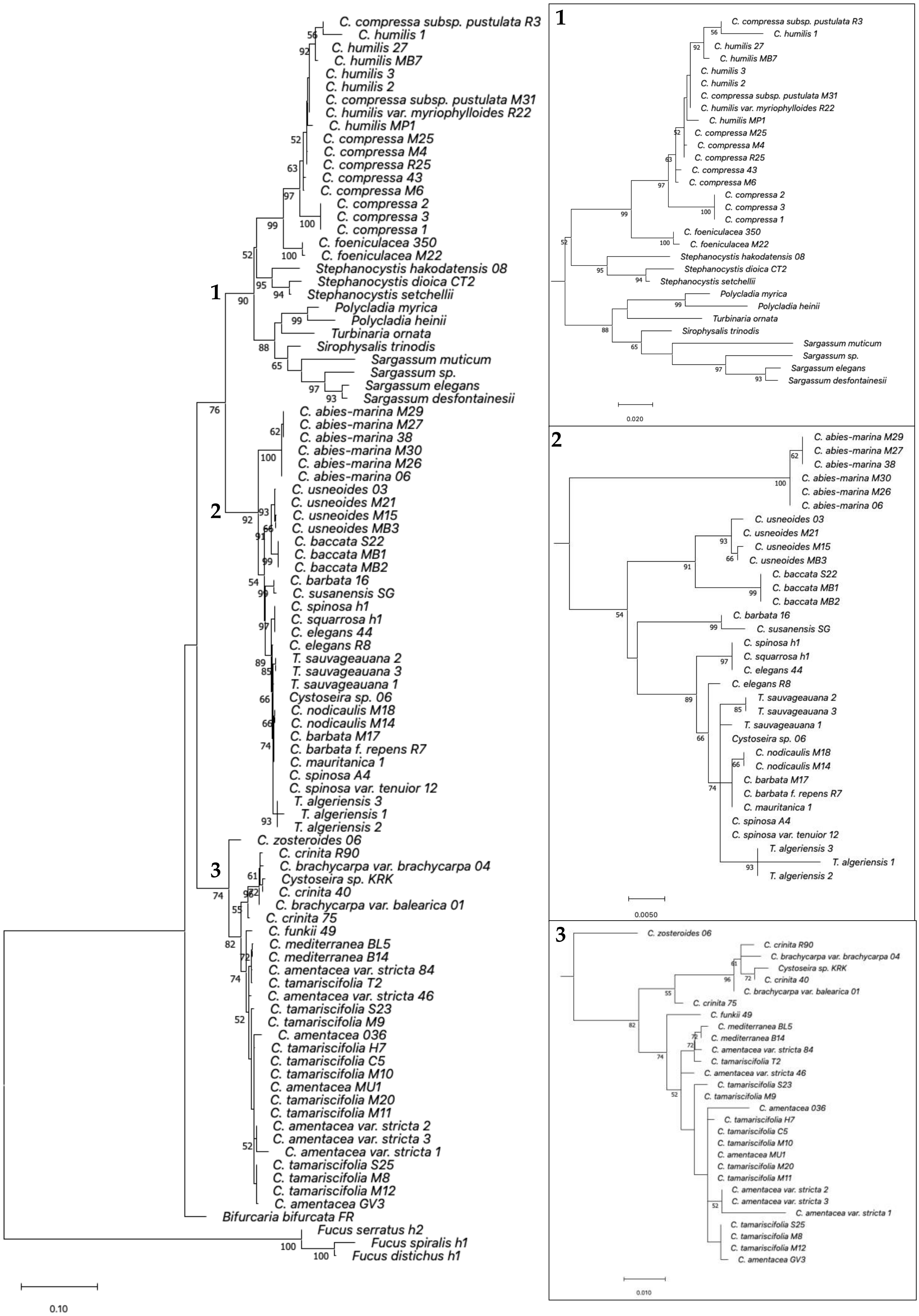

Figure 6: Maximum likelihood phylogenetic subtree obtained with Nearest Neighbor Interaction on concatenated mt23S-psbA-tRNA-Lys sequences of samples from *Sargassaceae* family. Values of branches represent maximum likelihood bootstrap support values (>50%). Samples sequenced in this study labelled as 1, 2 or 3. Labels: 1. Cystoseira-I. 2. Cystoseira-II. 3. Cystoseira-III.

spring [13]. The widespread distribution of *C. compressa* may reflect its high ecological tolerance, even to anthropic impact. This is perhaps the reason why it is the only *Cystoseira sl* species found in urban areas such as La Almadraba beach [13,19,60]. Other species (e.g. *T. sauvageauana, C. foeniculacea*) are distributed preferentially in more sheltered environments [61,62] where hydrodynamism is less relevant. They have a perennial behavior, maintaining their thalluses all year [30,63]. Although *C. brachycarpa* inhabits sheltered environments [45,64], in our study it was found in an abrasion platform. The orientation on the coast, facing south, receives less impact from the dominant eastern waves [65] allowing *C. brachycarpa* to survive under these conditions. Coastline slope does not favour the presence of *Cystoseira sl* populations, as found in Thyrrenian islands [66]. Slope degree has a close inverse relationship with those populations since above 60° can highly reduce the probability of settlement of these communities. Human trampling is to be considered because it takes place on coastal platforms during summer season. Those coves with high tourist influx have lower values of abundance and canopy of *Cystoseira sl*, indicating they are very sensitive to this anthropic disturbance. Even low intensities of trampling can highly affect the spatial distribution of algal communities [60,67] causing the simplification of the platform communities [12]. Therefore, future efforts could be applied studying this relation in order to apply management plans for *Cystoseira sl* protection.

Recent studies show that natural hybridization is operating in seaweed forests of *Cystoseira sl* [2,8,16]. In our study the high variability of *T. sauvageauana, C. foeniculacea, C. compressa* in our morphological cladogram would agree with this. Each *Cystoseira sl* gender has morphological characters with various phenotypes [9], usually one of them more common than the rest. So, perhaps species that exhibit uncommon morphotypes could not be classified appropriately in the cladogram. Numerical taxonomy techniques are useful to find this intraspecific variability. For instance, the lack of spines in secondary and higher order branches of *C. crinita* produces the translocation of this species from Cystoseira-III into Cystoseira-I, in which all species are characterized by the absence of this type of appendages. Last studies indicate that there are many exceptions to this character, reinforcing the idea of the need to combine morphological with phylogenetical studies [9]. Like any other method of morphological identification, it needs to rely on molecular taxonomy to clarify the classification. Therefore, morphological data has been completed with phylogenetic studies [2,10].

Our molecular supertree of combined sequences of psbA, mt23S and tRNA-Lys spacer genes from *Cystoseira sl* from SE of Spain mostly agrees with previous studies [2,9,10]. In agreement with Orellana *et al.* [9] our clades Cystoseira-II (*Treptacantha*) and Cystoseira-I (*Cystoseira sensu stricto*) are closer phylogenetically and with other genera such as *Polycladia*, *Stephanocystys*, *Sargassum* and *Sirophysalis*, and far from Cystoseira-III (*Carpodesmia*). This supports the lack of a common ancestor of these genera [2,9,10]. Cystoseira-III (*Carpodesmia*) is the most chemically complex genus [68,69] and the majority of its members are Mediterranean [2,70], suggesting that the seasonality of this sea [71] may be driving a pressure on these algae enhancing the production of their metabolites to deal with stressors like UV and temperature [72]. Neither Bruno de Sousa *et al.* [9] nor this study have succeeded in the resolution on *C. tamariscifolia*/*C. amentacea* clade. Therefore, future studies should be aimed to resolve these phylogenetic relationships. We have generated for the first time sequences data from *T. algeriensis* and *T. sauvageauana* which supports that both species are well differentiated and separated from the rest. These would also agree with their different morphology and ecological preferences [45,46]. The challenge of understanding their taxonomy may reside in the recent and continuous speciation of these genera in Mediterranean Sea [2,61,73], their adaptative convergence or possible genetic constraints [10]. There is an urgent need to keep investigating to understand their evolution and ecology in order to enhance their protection and maintain conservation efforts that help to preserve marine ecosystems for future generations.

This study provides the first bionomic cartography and evaluation of conservation of *Cystoseira sl* populations in SE Spain. Out of the 14 *Cystoseira sl* species cited in this area [74] half of them have been found in this study in a coastal fringe of just 4 km. In view of the diversity and abundance found, this region displays a relevant ecological quality [75]. The relative continuous algal canopy would also imply a good water status, as reported [76]. Therefore, because of its environmental characteristics and high algal diversity in the shores, this place should be a Special Conservation Area (SCA), integrated in the Natura 2000 Network (EU Directive 92/43/EEC). Also, the inclusion of *Cystoseira*, *Treptacantha* and *Carpodesmia* in El Cabo de las Huertas Community Importance Site would be a great achievement for the conservation of marine natural habitats of the Spanish Levant coast. The site may be useful for future extraction of specimens for transplants to former habitats which have undergone local extinctions [23]. There is also a pressing need to develop a management plan in view of the increasing disappearance of the prairies of eastern Mediterranean *Fucaceae* [19-22]. Long-term monitoring of these biocenosis would be desirable, which it could be carried out using CARLIT methodology, that employs *Cystoseira sl* populations as a key species establishing the health of the water body [13]. *Cystoseira* sl evolution data would generate time series that allows to know the state of these communities, evaluating the health of our coast. This type of study is already being carried out in different areas of Mediterranean Sea [20,31,59,77].

5. Conclusions

Cystoseira sl populations of the SE Spanish coast are distributed positively according to hydrodynamism. The bionomic cartography of these species assesses the good ecological quality and good water body status of El Cabo de las Huertas. The first sequences of psbA, mt23S and tRNA-Lys spacer genes of *T. algeriensis* and *T. sauvageauana* confirms that are included in Bruno de Sousa *Cystoseira* II group (2019), in agreement with morphological studies.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1. **S1: Table.** Geomorphological characteristics of the substrate and its levels selected to PERMANOVA (*) and Principal Component Analysis (PCO) analyses. **S2: Table.** List of qualitative phenotypical characters used for the building of Bray-Curtis dissimilarity matrix. **S3: Table.** List of primers used for phylogenetic analysis. **S4: Table**. GenBank accession numbers used in this study. **S5: Table**. Abundance of *Cystoseira sl* dominant species attending on Wave exposure, Width of the platform and Horizons of the platform. Percentage of contribution of each species to the dissimilarity. **S6: Figure**. Canopy height of *Cystoseira sl* recorded in the area of study. **S7: Figure.** Semiquantitative abundance of *Cystoseira sl* by horizons in the area of study. **(a)** Proximal horizon **(b)** Medium horizon **(c)** Distal horizon. Legend: 1: One isolated algae. 2: Several isolated individuals. 3: *Cystoseira sl* patches. 4: Discontinuous belts of at least one specie. 5: Continuous belts of one or more species. **S8: Figure.** Quality electrophoresis DNA gel on 2% agarose. Legend: CA: *T. algeriensis*. CC: *C. compressa*. CH: *C. humilis*. CS: *T. sauvageauana*. CST: *C. amentacea* var. *stricta*. C^-: Negative control. **S9: Figure. (a)** Quality electrophoresis DNA gel of mt23S primer. **(b)** Quality electrophoresis DNA gel of mt23SB primer. Legend: CA: *T. algeriensis*. CC: *C. compressa*. CH: *C. humilis*. CS: *T. sauvageauana*. CST: *C. amentacea* var. *stricta*. **S10: Figure.** Quality electrophoresis DNA gel of tRNA-LysB primer. Legend: CA: *T. algeriensis*. CC: *C. compressa*. CH: *C. humilis*. CS: *T. sauvageauana*. CST: *C. amentacea* var. *stricta* **S11: Figure. (a)** Quality electrophoresis DNA gel of psbA-FR primer. **(b)** Quality electrophoresis DNA gel of psbA-FR2 primer. **(c)** Quality electrophoresis DNA gel of psbA-F2R primer. Legend: CA: *T. algeriensis*. CC: *C. compressa*. CH: *C. humilis*. CS: *T. sauvageauana*. CST: *C. amentacea* var. *stricta*. **S12: Figure.** Maximum likelihood phylogenetic tree obtained with NNI on mt23S sequences of samples from *Sargassaceae* family. Values of branches represent maximum likelihood bootstrap support values (>50%). *Cystoseira sl* samples sequenced in this study labelled with 1 or 2. **S13: Figure.** Maximum likelihood phylogenetic tree obtained with NNI on tRNA-Lys spacer sequences of samples from *Sargassaceae* family. Values of branches represent maximum likelihood bootstrap support values (>50%). Samples sequenced in this study labelled with 1. **S14: Figure.** Maximum likelihood phylogenetic tree obtained with NNI on psbA sequences of samples from *Sargassaceae* family. Values of branches represent maximum likelihood bootstrap support values (>50%). Samples sequenced in this study labelled with 1, 2 or 3.

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