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SPECIES DELIMITATION AND PHYLOGEOGRAPHIC ANALYSES IN THE *ECTOCARPUS* SUBGROUP *SILICULOSI* (ECTOCARPALES, PHAEOPHYCEAE)[1]

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The genus *Ectocarpus* (Ectocarpales, Phaeophyceae) contains filamentous algae widely distributed in marine and estuarine habitats of temperate regions in both hemispheres. While *E. siliculosus* has become a model organism for genomics and genetics of the brown macroalgae, accurate species delineation, distribution patterns and diversity for the genus *Ectocarpus* remain problematic. In this study, we used three independent species delimitation approaches to generate a robust species hypothesis for 729 *Ectocarpus* specimens collected mainly along the European and Chilean coasts. These approaches comprised phylogenetic reconstructions and two bioinformatics tools developed to objectively define species boundaries (General Mixed Yule Coalescence Method and Automatic Barcode Gap Discovery). Our analyses were based on DNA sequences of two loci: the mitochondrial cytochrome oxidase subunit 1 and the nuclear internal transcribed spacer 1 of the ribosomal DNA. Our analyses showed the presence of at least 15 cryptic species and suggest the existence of incomplete lineage sorting or introgression between five of them. These results suggested the possible existence of different levels of reproductive barriers within this species complex. We also detected differences among species in their phylogeographic patterns, range and depth distributions, which may suggest different biogeographic histories (e.g., endemic species or recent introductions).

Key index words: **barcode; brown alga; COI-5P; cryptic species; *Ectocarpus*; hybridization; introgression; ITS1; species delimitation**

***Abbreviations*: ABGD, Automatic Barcode Gap Discovery; BI, Bayesian Inference; COI-5P, 5′-partial cytochrome c oxidase subunit 1; GMYC, General Mixed Yule Coalescent; ITS1, internal transcribed spacer 1; ML, Maximum Likelihood; mtDNA, mitochondrial DNA; NEA, North European Atlantic; nrDNA, nuclear ribosomal DNA; NW France, North-West France; SEP, South-East Pacific**

Delineating species boundaries is a long-standing methodological and conceptual challenge, especially in algal systems. Some of the problems arise from the fact that species are dynamic entities that change with time (Sites and Marshall 2003, 2004) and a plethora of species concepts has been proposed (Mayden 1997, 1999, Coyne and Orr 2004). Recently, a new line of thinking was put forth among biologists whereby species are considered as

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scientific hypotheses (species hypotheses) and species delineation is a process of refutation based on the acquisition of new evidence (Pante et al. 2015). DNA-based methods, such as single-gene barcoding, have been proven especially useful to uncover cryptic species where classical taxonomy has been problematic in organisms characterized by simple morphology and/or by high phenotypic plasticity (e.g., in animals: Yamashita and Rhoads 2013, in plants: Carstens and Satler 2013, in seaweeds: Tronholm et al. 2010). However, problems are linked with this single-gene approach. Indeed, single-locus data represent the history of a single gene that might not be representative of organismal history. Differences in the overall amount of differentiation between loci or in how these loci reconstruct the relationships among groups generate discordance in species boundaries delineation among markers and the importance of using multiple independent loci to generate robust species hypotheses has been repeatedly emphasized (Dupuis et al. 2012).

Distance-based approaches have classically defined species using arbitrary thresholds (universal or defined visually using a barcode gap in a particular group of species, Hebert et al. 2003). Species have also been defined based on the existence of well-supported monophyletic groups (Wiens and Penkrot 2002). However, monophyly, while a discrete criterion, is arbitrary with respect to taxonomic level (Goldstein and DeSalle 2011). Methods characterized by an increased statistical rigor and better objectivity in delimiting species, such as the General Mixed Yule Coalescent (GMYC) (Pons et al. 2006, Monaghan et al. 2009) and the Automatic Barcode Gap Detection (ABGD) (Puillandre et al. 2012a), were recently developed to detect discontinuities in DNA sequence variation associated with species boundaries. GMYC uses a pre-existing phylogenetic tree to determine the transition signal from speciation to coalescent branching patterns. While, ABGD detects the breaks in the distribution of genetic pair-wise distances, referred to as the "barcode gap," relying exclusively on genetic distance between DNA sequences. GMYC and ABGD analyses combined with searches for well-supported monophyletic groups in phylogenetic reconstructions have been used to detect the existence of cryptic species in many taxa (e.g., snails: Prevot et al. 2013, fish: Alò et al. 2013, copepods: Cornils and Held 2014, red algae: Payo et al. 2012 and Pardo et al. 2014 or brown algae: Vieira et al. 2014).

The genus *Ectocarpus* Lyngbye (Ectocarpales, Phaeophyceae) is widely distributed in marine and estuarine habitats of temperate regions in both hemispheres (Stache 1990). *Ectocarpus* spp. is found as a short-lived annual and often colonizes abiotic substrata or grows as an epiphyte on macrophytes; the habitat of *Ectocarpus* spp. includes the subtidal up to high intertidal pools (Russell 1967a,b, 1983a, b). Members of *Ectocarpus* spp. complex have been described as important contributors to biofouling and are frequently encountered as epiphytes in mariculture settings (Stache-Crain et al. 1997). The genus *Ectocarpus* has a long research history, starting in the XIX century with the first taxonomic descriptions of this genus (Dillwyn 1809, Lyngbye 1819). Despite being a model organism (Peters et al. 2004, Cock et al. 2010), basic knowledge concerning species delineation, distribution patterns, diversity and differentiation remains elusive (Peters et al. 2010a).

Initial morphology-based descriptions of species diversity have a long and controversial history. For example, Hamel (1931-1939) recognized five species along the European Atlantic coast, which he classified into two major groups based on branching pattern and sporangium shape: the section "*siliculosi*" and the section "*fasciculati*." Later, Cardinal (1964), using field material from the French Channel, proposed another classification and distinguished four species with seven varieties in the *Ectocarpus* subgroup *siliculosi* and three varieties in the *Ectocarpus* subgroup *fasciculati*. Conversely, Russell (1966, 1967a) using isolates from around the British Isles, demonstrated that sporangium morphology was not an informative species character. His proposal to reduce the number of European species to two (*E. fasciculatus* and *E. siliculosus*) was later supported by crossing and chemical studies (Müller and Eichenberger 1995) and was the most widely accepted classification system until recently (but see Peters et al. 2010a). Nonetheless, within *E. siliculosus*, reproductive barriers have been reported between isolates from different geographic areas (reviewed in Stache-Crain et al. 1967). Prezygotic barriers have been described for populations from NE America (Müller 1976); likewise, reduced development or normal sporophyte development with inhibition of meiosis (post-zygotic barriers) has been observed for strains isolated from different hemispheres (Müller 1977, 1979, 1988, Stache 1990). Despite these observations, Müller and Kawai (1991) proposed to collapse *E. siliculosus* isolates into a single species arguing that full or slightly reduced interbreeding patterns could be explained by the geographic isolation between populations in this world-wide distributed species. However, this explanation has been questioned by different studies where sequence-based analyses have identified cryptic diversity within the *Ectocarpus* genus. First, phylogenetic analyses using ITS1 (nrDNA) and the Rubisco spacer of choloroplast DNA (cpDNA) of 43 *Ectocarpus* strains isolated from all continents except Antarctica, showed several lineages within the *Ectocarpus* subgroup *siliculosi* (Stache-Crain et al. 1997). Second, using three additional markers (*cox*3 and *rps*14-*atp*8 [both from mtDNA] and ITS2 [nrDNA]) and including samples isolated from NW France, Peters et al. (2010a) suggested the existence of at least four different lineages within the *Ectocarpus* subgroup *siliculosi*; for one of the four lineages, they

proposed to reinstate the name *E. crouaniorum* Thuret coined by Thuret in Le Jolis. Third, the presence of most lineages described by Stache-Crain et al. (1997) was later confirmed for strains sampled along the South-East Pacific coast (Peters et al. 2010b). Finally, using a single-locus approach (COI-5P) on samples from NW France, Mediterranean Sea and Asia, Peters et al. (2015) reported again several lineages previously described in Stache-Crain et al. (1997) as well as 14 additional lineages possibly representing different species. However, this last study also warned against problems linked to a single-gene approach, such as incomplete lineage sorting or introgression.

The molecular findings discussed above support the probable occurrence of highly divergent genetic lineages, including cryptic species, within the *Ectocarpus* section "*siliculosi*." However, none of the previous studies has employed an integrative approach to clarify the species diversity within *Ectocarpus* and evaluate introgression levels within and among natural populations. This study uses two unlinked loci (i.e., COI-5P and ITS1 DNA-markers) and a set of methods developed to delimit species to clarify the number of cryptic species within this group using 729 specimens collected mainly along the European and Chilean coasts. The extent to which natural hybridization and introgression occur in the field was investigated by searching for incongruence between the independent nuclear and mitochondrial markers. Finally, phylogeographic patterns, range and depth distributions of the most common *Ectocarpus* species were studied.

MATERIALS AND METHODS

Field collections and isolation of Ectocarpus *strains.* Seven hundred and twenty-one *Ectocarpus* samples were collected from 37 sites located along the North-East Atlantic (NEA), Mediterranean and South-East Pacific (SEP) coasts. They were complemented with eight strains isolated from United States, South Korea, Australia and New Zealand (Table 1). Samples collected in the field were isolated and maintained as clonal cultures, as described in Couceiro et al. (2015). Position on the shore of the collected individuals was recorded using a coarse classification (high intertidal, H; mid intertidal, M; low intertidal, L; upper subtidal, US; subtidal, S; or drifting, Drift), to examine whether putative cryptic species occupy different tidal zones.

DNA extraction, sequencing and alignments. Total DNA was extracted from lyophilized samples using the NucleoSpinR 96 Plant Kit (Macherey-Nagel, Duren, Germany). Partial COI (COI-5P, mitochondrial) was amplified using the primers GAZF2 and GAZR2 (Lane et al. 2007) as described in Peters et al. (2015). A nuclear fragment containing the ITS1 region and 224 bp of the flanking genes 18S and 5.8S was amplified using the primers and PCR conditions described by Peters et al. (2010a). PCR amplicons for both markers were sequenced at Genoscope facilities (Evry, France) or at Eurofinis Genomics (Ebersberg, Germany). Individuals showing phylogenetic incongruences between markers were sequenced twice to discard contamination errors in the preparation of the samples before the sequencing. Sequences were aligned manually using MEGA v6.06 (Tamura et al. 2013) and checked by eye; only traces with high quality values and no ambiguities were retained for further analyses.

Species-delimitation procedure. First, 729 *Ectocarpus* COI-5P sequences were used to define putative species within the *Ectocarpus* section "*siliculosi*" group. Among them, 710 sequences were generated in this study and deposited in GENBANK (Table 1 and Table S1 in the Supporting Information) while 19 were published by Peters et al. (2015) and downloaded from the same public database (Table S1). To establish putative species, two species delineation methods (ABGD and GMYC) were combined with two phylogenetic inference methods (Maximum Likelihood, ML and Bayesian inference, BI). Thereafter, putative species delineated with the COI-5P were consolidated using 630 sequences of the nrDNA marker ITS1. Five hundred and eighty sequences were generated in this study (Table 1 and Table S2 in the Supporting Information) and 50 were downloaded from GENBANK (Table S2). A single alignment including all ITS1 sequences could not be generated due to the partly high sequence variability including the presence of indels (Stache-Crain et al. 1997); the ITS1 data set was therefore divided into four subgroups (see results on species consolidation below for more information about the composition of these four subgroups). Sequence alignment, tree reconstructions and ABGD tests were carried out independently for each subgroup. Sample groups were considered as species when all (or nearly all) the methods employed to test their boundaries and the results obtained for two independent genes were concordant.

Phylogenetic analyses of DNA sequences. Phylogenetic analyses were conducted separately for the COI-5P and ITS1 regions using both ML and BI methods. ML analyses were performed using RAxML v8 (Stamatakis 2014). We selected the best-fit substitution model using the Akaike information criterion implemented in jModelTest v2.1.8 (Darriba et al. 2012). The selected model was GTR I+G for COI-5P, GTR +G for the first, second, and fourth ITS1-subgroups and GTR I+G for the third ITS1-subgroup. Statistical support was estimated using 1,000 replicates and a rapid bootstrap heuristic (Stamatakis et al. 2008). BI analyses were conducted using MrBayes v3.2.3 (Huelsenbeck and Ronquist 2001). Two independent analyses were run using four chains each and 20 million generations. Trees and parameters were sampled every 1,000 generations and the default parameters for temperature and branch swapping were used. The first 20% of sampled trees were discarded as "burn-in" to ensure stabilization. The remaining trees were used to compute a consensus topology and posterior probability values. The split frequency (variance among the four independent runs) was below 0.003, confirming that the posterior probability distribution was accurately sampled.

Automatic Barcode Gap Discovery. ABGD identifies a limit between the frequency distribution of intra- and interspecific pair-wise genetic distances, even if they overlap, using several a priori thresholds of genetic distances chosen by the user (Puillandre et al. 2012a). Then, it is recursively applied to previously obtained groups to get finer partitions until there is no further partitioning. ABGD was remotely run at http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html. We computed Kimura two-parameter (K2P) genetic distances among specimens using default settings.

General Mixed Yule Coalescent. GMYC identifies a threshold value for the shift in branching rate from coalescent lineage branching to interspecific diversification on an ultrametric tree and explicitly delimits "independently evolving" clusters (i.e., putative species; Pons et al. 2006, Monaghan et al. 2009). Before the analysis, duplicated haplotypes were removed from our data set using DnaSP v5.10.1 (Librado and

TABLE 1. Sites and samples sequenced in this study. The number of site, the continent, country, name of site, code, year of sampling and the number of sequences for both markers (COI-5P and ITS1) are indicated.

No. of site	Continent	Country	Site	CODE	Year	COI-5P	ITS1
1	Europe	United Kingdom	Wick	WIC	2008	10	10
2	Europe	United Kingdom	Rattray Head	RAT	2008	1	0
3	Europe	United Kingdom	Dunstaffnage	DUN	2008	7	8
4	Europe	United Kingdom	Berwick	BER	2008	12	12
5	Europe	United Kingdom	Mull of Galloway	MUL	2008	14	14
6	Europe	United Kingdom	Pett level	PET	2008	11	13
7	Europe	United Kingdom	Gosport Marina	GOS	2008	1	3
8	Europe	United Kingdom	Plymouth	PLY	2010–2011	38	37
9	Europe	United Kingdom	Restronguet	RES	2010	13	12
10	Europe	France	Cherbourg	CHE	2006	2	0
11	Europe	France	Roscoff	ROS	2010–2012	48	31
12	Europe	France	Saint Malo	STM	2010	23	0
13	Europe	France	Traezh Hir	THZ	2010	28	24
14	Europe	France	Concarneau	CON	2010	1	0
15	Europe	France	Quiberon	QUI	2010–2012	30	27
16	Europe	Spain	Ribadeo	RIB	2013	27	25
17	Europe	Spain	Coruña	COR	2013	26	19
18	Europe	Spain	Ría de Arousa	RIA	2013	8	8
19	Europe	Portugal	Viana	VIA	2013	11	8
20	Europe	Italy	Naples	NAP	2012	42	27
21	Europe	Greece	Korinthos	KOR	2011	10	5
22	Europe	Greece	Lesbos	LES	2009	9	6
23	South America	Peru	Bahía Mendieta	BHM	2006	0	3
24	South America	Peru	San Juan	SJN	1988/2006	2	0
25	South America	Chile	Pisagua	PSG	2006	2	1
26	South America	Chile	Pan de Azúcar	PAN	2004–2005/2013	46	39
27	South America	Chile	Caldera	CAL	2013	67	62
28	South America	Chile	Quintay	QUI	2013	84	59
29	South America	Chile	Concepción	CON	2013	45	37
30	South America	Chile	Valdivia	VAL	2013	39	32
31	South America	Chile	Estaquilla	EST	2013	42	38
32	South America	Chile	Achao	ACH	2013	22	20
33	North America	United States	Oregon	ORE	2009	2	0
34	North America	United States	Massachusetts	MAS	2009	1	1
35	Asia	Korea	Kimnyung/Hanrim	KIM	2006	3	1
36	Oceania	New Zealand	Kaikoura	KAI	1988	1	0
37	Oceania	Australia	Victoria	VIC	1988	1	0
					Total	729	582

Rozas 2009). Branch lengths were estimated under a relaxed log-normal clock using the Bayesian analysis implemented in BEAST v1.8.2 (Drummond et al. 2012). A coalescent (constant size) prior was used and Markov Chains Monte Carlo (MCMC) were run for 20 million generations. Trees were sampled each 1,000 generations with a 10% burn-in. A visual inspection of MCMC progression using Tracer v1.6 was performed to corroborate stabilization. An ultrametric tree was constructed using TreeAnnotator v1.8.1 (Rambaut and Drummond 2010). Both the single-threshold (Pons et al. 2006) and the multiple-threshold (Monaghan et al. 2009) versions of GMYC were fitted on the ultrametric tree using the SPLITS v1.0-19 package for R (https://r-forge.r-project.org/projects/splits/).

Network reconstructions. Haplotype networks were reconstructed for the eight *Ectocarpus* species for which more than 15 sequences were available for each marker under study. The haplotype networks were reconstructed using the median-joining algorithm implemented in NETWORK v6.13 (Bandelt et al. 1999).

Genetic diversity. Genetic diversity indices were calculated for the three species, *E. siliculosus*, *E. crouaniorum* and *Ectocarpus* 6, that were sampled most frequently (i.e., >45 individuals for which both the COI-5P and ITS1 markers were sequenced). The number of haplotypes (*nH*); the number of polymorphic sites (*S*); gene diversity (*H*); and nucleotide diversity (π, Nei and Li 1979) were computed using ARLEQUIN v3.5.1.3 (Excoffier and Lischer 2010).

AMOVA analysis. For the two most widely geographically distributed species (i.e., *E. siliculosus* and *E. crouaniorum*), a nested analysis of molecular variance (AMOVA, Excoffier et al. 1992) was implemented using ARLEQUIN v3.5.1.3 (Excoffier and Lischer 2010) to test for the partition of genetic variance within locations, among locations within regions and among regions. Based on the geographic clustering of the sampled localities, four regions were defined: United Kingdom, France, NW Iberian Peninsula and Chile.

RESULTS

Ectocarpus *putative species delineation based on COI-5P.* The 729 COI-5P sequences (603 bp) from the *Ectocarpus* specimens included 90 unique haplotypes with 123 variable sites and a high level of haplotypic diversity (0.9093). The phylogenies inferred from these haplotypes using ML and BI (Fig. 1a) gave comparable topologies and suggested the presence of 15 putative species within the group *E. siliculosi.*

Eleven of these lineages were highly supported monophyletic groups (bootstrap values >84 for ML and >0.98 for BI, Fig. 1a) while the remaining four were singletons. Genetic pair-wise K2P distances ranged from 0 to 0.089 and the shape of the distribution was clearly bimodal with two conspicuous maxima at 0.0025 and 0.0550. ABGD located the barcode gap within the 0.011–0.037 distance range (Fig. S1a in the Supporting Informaiton) and primary partitions using this threshold suggested the existence of 15 genetic groups (Figs. 1a and S1b). The likelihood of the GMYC model, for both the single- and the multiple-threshold models (LGMYCsingle = 720.08 and LGMYCmultiple = 723.40), was significantly higher than the likelihood of the null model (L0 = 704.01). However, the partitions obtained were not identical for the different threshold limits; 16 groups were delimited with the single-threshold method (confidence limits, 15–18) and 22 groups were delimited with the multiple-threshold method (confidence limits, 18–26). The likelihood values of the single and multiple-threshold analyses were not significantly different, suggesting that application of the more complex multiple-threshold analysis did not result in a significant improvement of the results. Thus, we selected the single threshold over the multiple-threshold model to delimit putative species in our data set (Figs. 1a and S2 in the Supporting Information).

The eleven monophyletic groups recovered by tree reconstructions were supported as putative species by the ABGD results while only 10 of these 11 clades were supported as putative species by the GMYC single-threshold results. This discrepancy involved the split of two haplotypes (L211 and L206) from the rest of the *E. siliculosus* clade in the GMYC (Figs. 1a and S2). The four singletons observed in both phylogenetic reconstructions were defined as species by both ABGD and GMYC (Figs. 1a and S2). Integration of all species delimitation methods yielded 15 putative species in the *siliculosi* group. Two of them corresponded to *E. siliculosus* and *E. crouaniorum*, the rest were named using numbers 1–13 (Fig. 1a).

Ectocarpus *putative species consolidation using ITS1 sequences.* As reported by Stache-Crain et al. (1997), the alignment of all the ITS1 sequences from the *siliculosi* group was not possible because of their high degree of divergence and the presence of numerous indels. Therefore, alignments were made for four subgroups that were established based on sequence similarity: (i) *Ectocarpus* 1, 2, 4 and *E. siliculosus* (alignment length 885 bp); (ii) *Ectocarpus* 5, 6, and 7 (alignment length 447 bp); (iii) *Ectocarpus* 8, 9, 10, and 11 (alignment length 456 bp); (iv) *Ectocarpus* 12, 13 and *E. crouaniorum* (alignment length 686 bp). The ITS1 sequences of *Ectocarpus* 3 did not align well with any other putative species and were not included in these analyses. The topology of the unrooted ITS1 trees built for the alignment subgroups were congruent with the putative species defined using the COI-5P sequences (Fig. 1b). The ABGD analyses conducted within these four subgroups of alignments were also remarkably congruent with the putative species defined using the COI-5P sequences (Figs. 1b and S3 in the Supporting Information). The only discrepancy was the merging of *Ectocarpus* 12 and *Ectocarpus* 13 in the ABGD results (Fig. 1b). Since both *Ectocarpus* 12 and *Ectocarpus* 13 were nonambiguously defined as two separated putative species with the COI-5P and were retrieved as monophyletic groups in both tree reconstructions for ITS1, we decided to retain 15 consolidated species within the *siliculosi* group (Fig. 1).

Cases of incongruences between markers. Even though results were largely concordant between markers and methods in delineating 15 consolidated species within the *siliculosi* group, incongruences between the nuclear and the mitochondrial markers were observed in several individuals collected in Chile (Table 2). In particular, a total of 20 individuals collected in Pan de Azúcar (site 26) and nine individuals collected in Quintay (site 28), were identified as *E. crouaniorum* using the mitochondrial marker COI-5P but as *Ectocarpus* 12 using the nuclear marker ITS1. Moreover, one individual sampled in Quintay (site 28), which was identified as *Ectocarpus* 10 using the COI-5P mitochondrial marker, was identified as *Ectocarpus* 11 based on the nuclear ITS1 marker and one individual collected in Concepción (site 29), which was identified as *Ectocarpus* 9 using the COI-5P was also identified as *Ectocarpus* 11 based on the nuclear marker.

Geographic distribution of the 15 Ectocarpus *species.* We chose to display the delimitation of the geographic distribution of the 15 species using the results of the mitochondrial COI-5P marker alone. However, it should be noted that a few discrepancies existed between the mitochondrial and the nuclear markers used in this study (see previous paragraph). Distribution patterns varied greatly among the species (Fig. 2). Concerning the 10 most commonly sampled species, one seemed to be restricted to a single biogeographic region (*Ectocarpus* 7, $n = 32$, found only in the Peruvian Province); four species showed a distribution limited to one ocean (*Ectocarpus* 1, $n = 30$, and *Ectocarpus* 6, $n = 48$, both found in North and South Pacific; *Ectocarpus* 10, $n = 39$, distributed only in the South Pacific and *Ectocarpus* 3, $n = 29$, observed only in the North Atlantic); and five species were encountered in more than one ocean. Among these five species, *Ectocarpus* 12 ($n = 16$) and *Ectocarpus* 13 ($n = 34$) were distributed in both the South Pacific and the North Atlantic. In the case of *Ectocarpus* 8 ($n = 14$), the species was present in both oceans but only one sample was found in the Atlantic. *E. siliculosus* and *E. crouaniorum* were the most common species in our data set. *E. siliculosus* ($n = 206$) was distributed

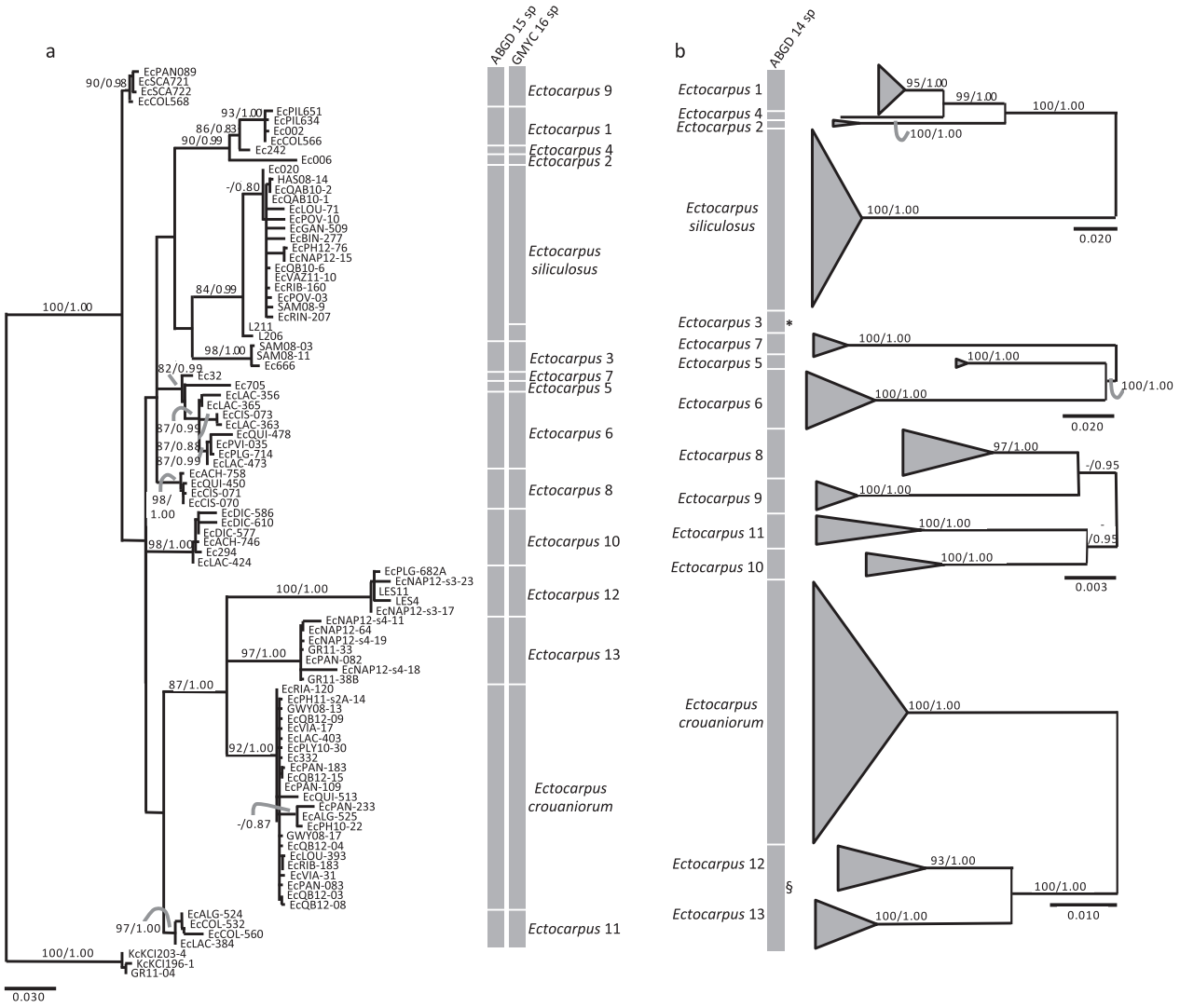

FIG. 1. Proposed species in the *Ectocarpus siliculosi* group using COI-5P (a) and their consolidation using ITS1 (b). Statistical support values >75 and posterior probabilities >0.80 are shown on branches. The asterisk in Figure 2b indicates the sequences of *Ectocarpus* 3 that were unalignable with any other of the four alignment subgroups, and § indicates the two consolidated species that were merged using ABGD. The outgroup for the COI-5P analyses were three sequences of *Kuckuckia spinosa*.

in the Mediterranean Sea, the Pacific and the North Atlantic Oceans, and *E. crouaniorum* ($n = 258$) was distributed in the South Pacific and the North Atlantic Oceans.

Haplotype network. Haplotype networks were created for the eight species for which >15 sequences were available for both markers (Fig. 3). A star-like network pattern, which is usually associated with a recent population expansion, was obtained for six of the eight species studied. For example, *E. siliculosus* and *E. crouaniorum* had a single, frequent and widespread haplotype together with several less frequent haplotypes generally restricted to oceanic regions (Northern Atlantic, Lusitanian region, Mediterranean Sea or Peruvian province, Fig. 3); this topology was consistent for both COI-5P and ITS1. In contrast, a more reticulate and complex haplotype network was observed for both markers with *Ectocarpus* 6.

Genetic diversity of E. siliculosus, E. crouaniorum *and* Ectocarpus *6.* Genetic diversity estimates (nH, H, π and S) were generally much lower for the mitochondrial marker than for the nuclear marker (Tables S3–S5 in the Supporting Information for *E. siliculosus*, *E. crouaniorum* and *Ectocarpus 6*, respectively). Regardless of the gene considered, no clear pattern of genetic diversity distribution could be detected within each of the three species (Tables S3–S5).

COI-5P data for *E. siliculosus* (Table S3) indicated that the highest number of haplotypes was on the NW Iberian Peninsula ($nH = 6$, although close values, i.e., $nH = 5$, were also found for the United Kingdom, France and Chile) whereas the highest

TABLE 2. Association between mitochondria (COI-5P) and nrDNA (ITS1) sequences in the *Ectocarpus* specimens in which both markers were sequenced. Individuals showing incongruence among markers are indicated in bold.

ITS1/COI-5P	*Esil*	*Ecro*	*Ec* 1	*Ec* 2	*Ec* 3	*Ec* 4	*Ec* 5	*Ec* 6	*Ec* 7	*Ec* 8	*Ec* 9	*Ec* 10	*Ec* 11	*Ec* 12	*Ec* 13
Esil	178														
Ecro		152													
Ec 1			1												
Ec 2				1											
Ec 3					3										
Ec 4						1									
Ec 5							1								
Ec 6								46							
Ec 7									31						
Ec 8										13					
Ec 9											10				
Ec 10												31			
Ec 11											**1**	**1**	3		
Ec 12		**29**												11	
Ec 13															17

Esil, *E. siliculosus*; *Ecro*, *E. crouaniorum*; *Ec* 1–12, *Ectocarpus* 1–12.

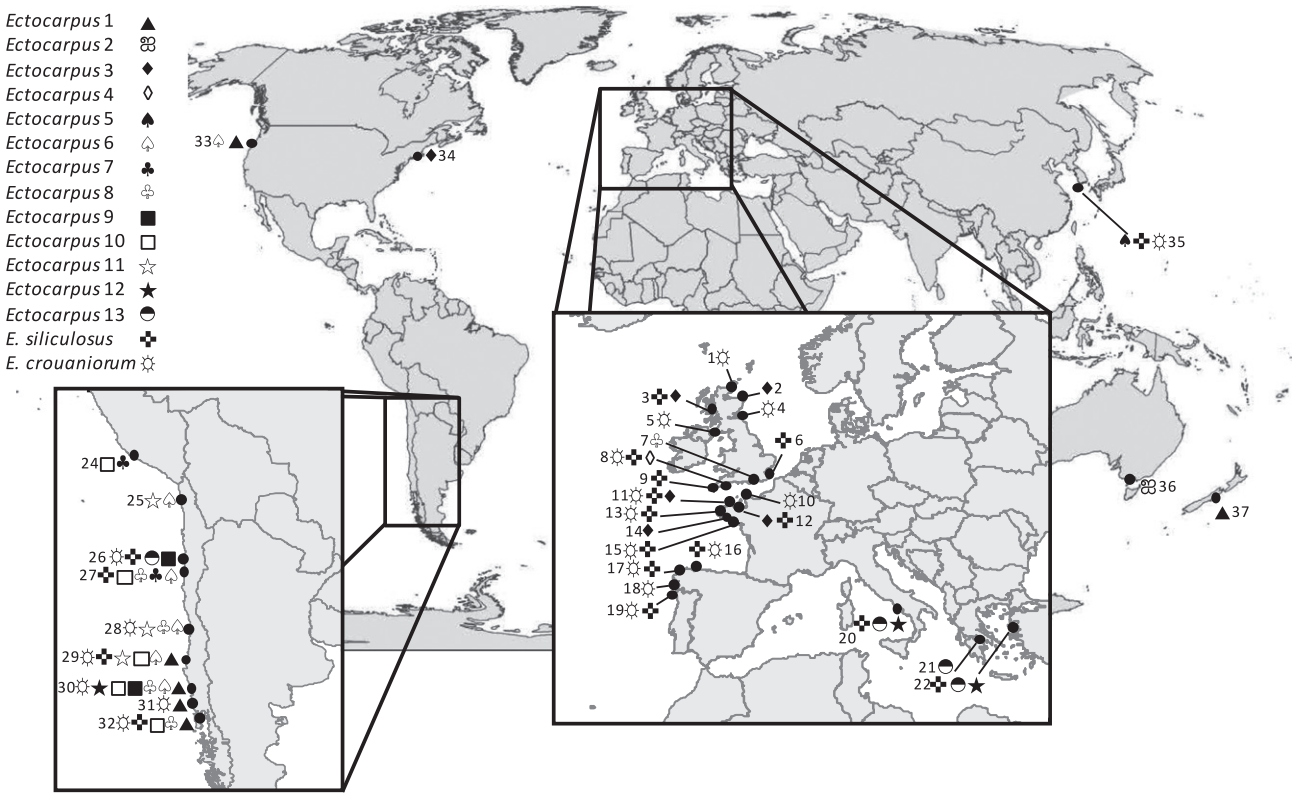

FIG. 2. Distribution of the 15 species of the *Ectocarpus* subgroup *siliculosi* as defined according to the mitochondrial marker COI-5P. Sites are numbered as in Table 1.

values of genetic diversity and nucleotide diversity were in Chile ($H = 0.748 \pm 0.028$ and $\pi = 0.237 \pm 0.164$). ITS1 data gave similar results with the highest value of genetic diversity for the NW Iberian Peninsula ($H = 0.862 \pm 0.045$), the highest nucleotide diversity for Chile ($\pi = 0.653 \pm 0.364$) and the highest number of polymorphic sites for France ($S = 19$; Table S3). On the other hand, COI-5P data for *E. crouaniorum* (Table S4) indicated that the highest number of haplotypes as well as the highest values of nucleotide diversity and polymorphic sites were in France ($nH = 10$, $\pi = 0.170 \pm 0.130$, $S = 11$, Table S4) while the highest values of genetic diversity were in France and in the NW Iberian Peninsula ($H = 0.624 \pm 0.092$ and 0.679 ± 0.080 respectively). ITS1 data for this same species indicated, however, that the highest number of haplotypes was in the United Kingdom ($nH = 23$) and Chile ($nH = 23$)

while all values of genetic diversity were close to one, whatever the region (Table S4). Within *Ectocarpus* 6, because of the distribution pattern of this species (Fig. 3), estimations of genetic diversity were carried out only along the Chilean coast (Table S5). The population of Las Cruces (LAC, Table 1), the largest population of *Ectocarpus* 6 ($n = 28$), showed the highest number of haplotypes, genetic diversity, and polymorphic sites for both markers: $nH = 6$, $H = 0.791 \pm 0.048$ and $S = 8$ for COI-5P, and $nH = 19$, $H = 0.934 \pm 0.0343$ and $S = 38$ for ITS1 (Table S5).

AMOVA analysis of the cosmopolitan species E. siliculosus *and* E. crouaniorum. Results of the nested AMOVA for both markers are given in Tables 3 and 4 for *E. siliculosus* and *E. crouaniorum*, respectively. These analyses suggested that the total genetic variance was mainly explained by variance within sites: 55.81% and 65.18% in *E. siliculosus*, 59.07% and 77.39% in *E. crouaniorum*, for the COI-5P and the ITS1, respectively. The variances among regions (<13% in *E. siliculosus* and <11% in *E. crouaniorum*) and among sites within regions (<32% in *E. siliculosus* and <31% in *E. crouaniorum*), although significant, were lower than the variance within sites (Tables 3 and 4).

Tide-level distribution of E. siliculosus, E. crouaniorum, *and* Ectocarpus *6. Ectocarpus* species occurred from the upper subtidal up to intertidal pools (Fig. 4). Along the North Atlantic coast, different tide-level distributions were observed for the two most abundant *Ectocarpus* species (see Fig. 4). *E. crouaniorum* occurred from the high intertidal to the high subtidal but was most abundant within higher intertidal pools (Fig. 4). *E. siliculosus*, which also occurred from the high intertidal to the high subtidal, was most abundant in the lower tidal areas (Fig. 4). The distribution of *E. crouaniorum* followed the same pattern in Chile as on the North Atlantic coast. For *E. siliculosus*, no clear pattern of distribution could be inferred in Chile since most samples were collected as drifting thalli that had been washed ashore. *Ectocarpus* 6 was found from medium intertidal to subtidal levels and was more abundant in the low intertidal (Fig. 4).

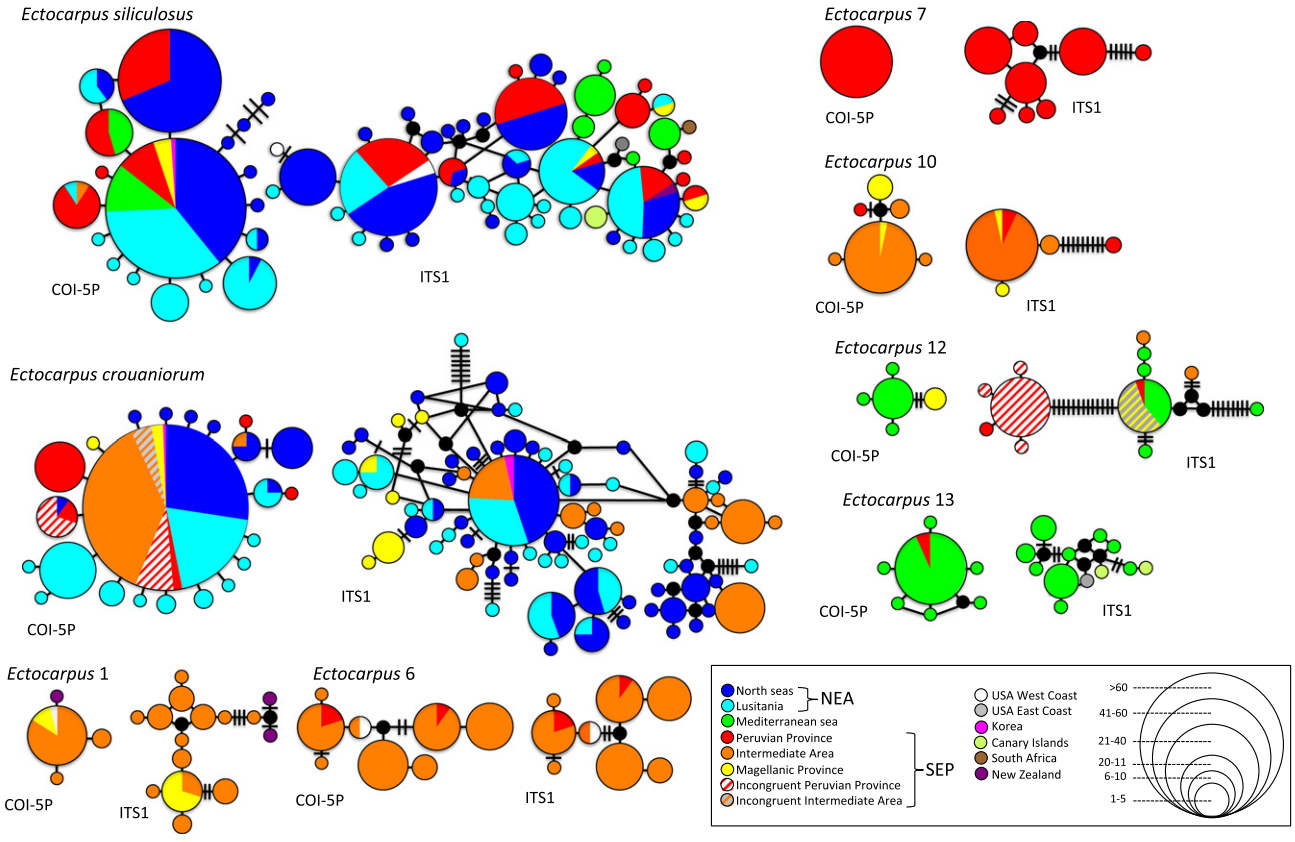

FIG. 3. Haplotype networks of for COI-5P and ITS1 for eight *Ectocarpus* spp. Biogeographical region of origin (within the South-East Pacific [SEP] as defined by Camus 2001 and within the North-East Atlantic [NEA] as defined by Spalding et al. 2007) of the samples are represented as different colors identified in the box. The individuals of *E. crouaniorum* and *Ectocarpus* 12, for which incongruent results were obtained for the two sequenced markers, are indicated by stripes. In the networks, each circle represents a haplotype and its size is proportional to its frequency (correspondence between circle sizes and numbers of individuals is indicated in the box). Black circles represent hypothetical unsampled haplotypes. For haplotypes separated by more than one mutational step, black bars indicate the number of mutational steps (i.e., substitutions and/or gaps).

TABLE 3. Analysis of molecular variance of *E. siliculosus* for each molecular marker (COI-5P and ITS1). Regions, not including the Mediterranean for which only a single site was sampled, as in Table S3.

Source of variation	df	SS	Variance components	% variation	*P*-value
COI-5P					
Among regions	3	18.982	0.07307	12.54	<0.0001
Among sites within regions	10	22.304	0.18446	31.65	<0.0001
Within site	154	50.095	0.32529	55.81	0.0351
Total	167	91.381	0.58283		
ITS1					
Among regions	3	51.486	0.15194	7.14	<0.0001
Among sites within regions	10	74.673	0.58877	27.68	<0.0001
Within site	154	213.502	1.38638	65.18	0.1476
Total	167	339.661	2.12709		

df, degree of freedom; SS, sum of squares.

TABLE 4. Analysis of molecular variance of *E. crouaniorum* for each molecular marker (COI-5P and ITS1). Regions as in Table S4.

Source of variation	df	SS	Variance components	% variation	*P*-value
COI-5P					
Among regions	3	11.404	0.05270	10.74	<0.0001
Among sites within regions	12	19.118	0.14818	30.19	<0.0001
Within site	134	38.851	0.28993	59.07	0.0088
Total	149	69.373	0.49082		
ITS1					
Among regions	3	37.469	0.08576	2.67	<0.0001
Among sites within regions	12	97.254	0.63927	19.93	<0.0001
Within site	134	332.610	2.48217	77.39	0.37634
Total	149	69.373	0.49082		

df, degree of freedom; SS, sum of squares.

DISCUSSION

In this study, we have characterized the species diversity, geographic distribution, and phylogeographic patterns within the group *siliculosi* of the genus *Ectocarpus*. The results presented are based on the most extensive sampling of this group available to date. Using a mitochondrial and a nuclear marker, two complementary species delineation techniques and two tree reconstruction methods, we propose the recognition of 15 putative species within the *siliculosi* group. *E. siliculosus* and *E. crouaniorum*, the only two named species within the *siliculosi* group (Peters et al. 2010a,b, 2015, Couceiro et al. 2015), were recovered as different species belonging to the most highly divergent clades in our study. We confirmed also that the genome-sequenced species (that we referred as *Ectocarpus* 7) is different from *E. siliculosus*, as recently suggested by Peters et al. (2015). Moreover, we found individuals showing incongruences between the nuclear and mitochondrial markers suggesting introgression, hybridization or incomplete lineage sorting between some of the newly delineated closely related species. Finally, our extensive sampling along the NEA and SEP coasts revealed that the 15 *Ectocarpus* species showed different patterns of distribution varying from rare to common cosmopolitan species. Haplotype network topologies for the commonest species showed different patterns of genetic structure suggesting different evolutionary histories.

High species diversity within the siliculosi *group.* Concordance across results obtained with different methods (monophyly in tree reconstruction, ABGD and GMYC) and the use of unlinked molecular markers (COI-5P and ITS1) are now widely acknowledged methods of supporting the delimitation of previously undescribed species (Carstens et al. 2013, Modica et al. 2014). Indeed, one could expect that unlinked selectively neutral genes will attain concordant genealogical histories when taxa have undergone species-level divergence (i.e., no gene flow for a sufficient amount of time), while reticulate genealogical patterns across those unlinked loci will be observed when genetic exchange exists between taxa (Sites and Marshall 2004). Our results showed an 80% concordance between methods, a result congruent with studies undertaken in hyper-diverse taxa such as insects (Kekkonen and Hebert 2014). Previous studies have shown that GMYC can lead to an overestimation of group partitioning while ABGD is considered as a more conservative method to delimit species (Puillandre et al. 2012a,b, Kekkonen and Hebert 2014), a result concordant with that observed in the *siliculosi* group. Despite few discordances detected between our ABGD and GMYC

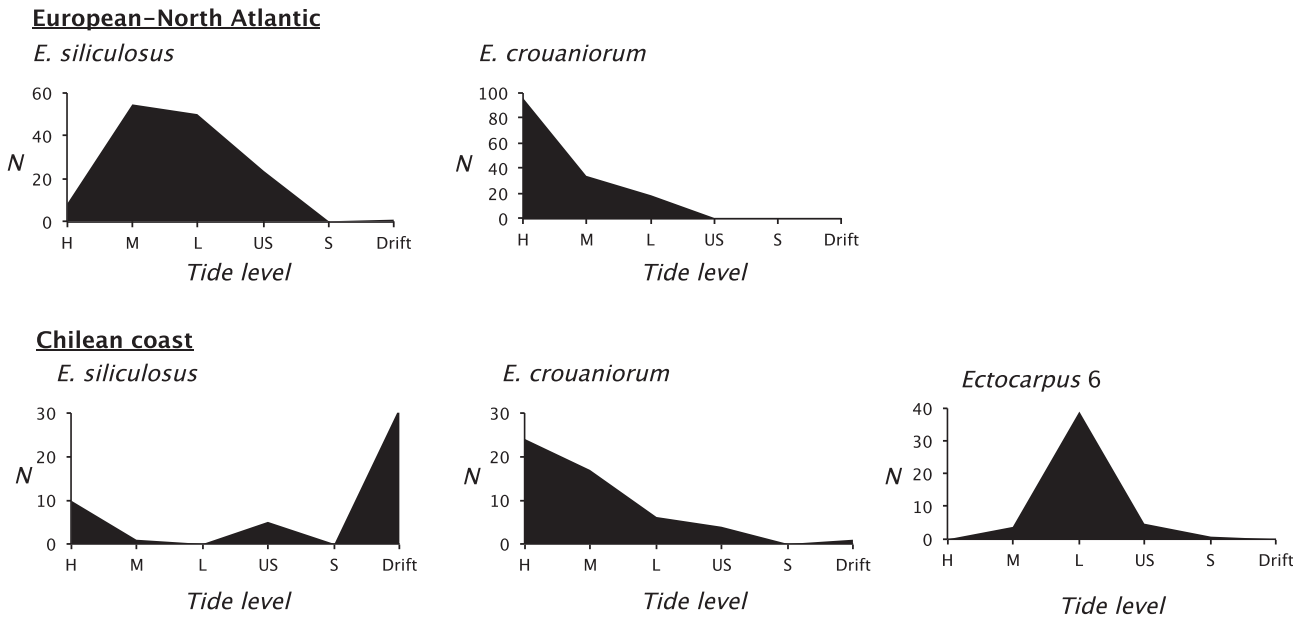

FIG. 4. Distribution on the shore of the commonest *Ectocarpus* species in the North Atlantic-European and Chilean coasts. The numbers of samples and the zones where the samples were collected are indicated (H, high intertidal; M, mid intertidal; L, low intertidal; US, Upper subtidal; S, Subtidal; Drift, drifting).

analyses, all putative genetic groups formed highly divergent singletons or monophyletic groups for both markers. For the COI-5P marker, the barcode gap ranged from 0.011 to 0.037 K2P pair-wise genetic distance, which included the cut-off value (0.018) proposed empirically (by eye) by Peters et al. (2015) for *Ectocarpu*s and other genera of Ectocarpales.

The phylogenetic relationships among the 15 species revealed the occurrence of a monophyletic group composed of *E. crouaniorum*, *Ectocarpus* 12, *Ectocarpus* 13, and a paraphyletic assemblage composed of the remaining 12 other species within the *siliculosi* group. This branching pattern was retrieved in all previous phylogenetic studies despite a disagreement between the tree topologies depending on the marker used (ITS1 and rubisco spacer: Stache-Crain et al. 1997, ITS1, ITS2, Rubisco spacer region, *cox*3 and *rps*14-*atp*8: Peters et al. 2010a, ITS1, ITS2, Rubisco spacer and *cox*3: Peters et al. 2010b, COI-5P: Peters et al. 2015, COI-5P and ITS1: this study). Incomplete and/or uneven taxon sampling could produce different tree topologies. Previous studies have reported that the inclusion of additional taxa in a phylogenetic analysis can increase (on average) the accuracy of the inferred topology (Lecointre et al. 1993, Hillis et al. 2003, Hedtke et al. 2006).

A problem generated by the various attempts at resolving the *Ectocarpus* phylogeny is the use of various species codes (Stache-Crain et al. 1997, Peters et al. 2010a,b). In our work, some clades previously described in the literature were retained as different species (clades 1a, 1c, 2a, 2b, 2c, and 3; Stache-Crain et al. 1997, Peters et al. 2010a,b), while other clades were split into different species (1b and 4) (Stache-Crain et al. 1997, Peters et al. 2010a,b; see Table S6 in the Supporting Information for the correspondence between previously distinguished "lineages" and the species code proposed in this study).

Our study detected high levels of cryptic species diversity in the *siliculosi* group, as suggested in previous studies (Stache-Crain et al. 1997, Peters et al. 2010a,b). However, it is highly probable that more species exist within this species complex. Indeed, within our data set, two species comprised more than 62% of the samples sequenced (*E. siliculosus* and *E. crouaniorum*) while three species (*Ectocarpus* 2, *Ectocarpus* 4 and *Ectocarpus* 5) were rare and were represented by less than 5 individuals. It will be necessary to carry out additional population sampling including a better representation of different biogeographic regions to better estimate species diversity and distribution of *Ectocarpus* in a worldwide context. The temperate waters of the southern Australia and the NW Pacific require particular scrutiny since the few sequenced samples from this region ($n = 12$, 1.5% of the samples sequenced) included two species not encountered in other regions (*Ectocarpus* 2 and *Ectocarpus* 5).

The high number of cryptic species present in sympatry within the same locality, especially in Chile, raises the question of what evolutionary mechanisms could reduce interspecific competition and promote such patterns. Peters et al. (2010a), Couceiro et al. (2015) and Geoffroy et al. (2015) showed that tide level, substratum, and season are important factors that have to be taken into account

when studying filamentous Ectocarpales. They reported that different species could occupy different spatio-temporal ecological niches related to different tide levels and/or host specificity. For example, Peters et al. (2010a) and Couceiro et al. (2015) showed that *E. crouaniorum* was located higher on the shore than *E. siliculosus* in NW France. In our study, this difference in tide-level distribution between *E. crouaniorum* and *E. siliculosus* was corroborated for additional sites in the North Atlantic. Moreover, among the Chilean coast, *Ectocarpus* 6 seemed more restricted to midintertidal pools. These first results provide a good opportunity to study the importance of ecological differentiation between the cryptic species of *Ectocarpus*.

Incongruence between markers. The huge variability in the level of genetic divergence between species revealed in this study will allow the correlation between reproductive incompatibility and degree of species divergence to be investigated in this genus. Cross-compatibility experiments have been carried out between laboratory strains of *E. siliculosus* and *E. crouaniorum* (Peters et al. 2010a), between *E. siliculosus* and *Ectocarpus* 7, between *Ectocarpus* 7 and *Ectocarpus* 1 (Peters et al. 2004), and between *E. siliculosus* and *Ectocarpus* 1 (Stache-Crain et al. 1997). Hybrid sporophytes from the four crosses were viable but incapable of meiosis. Müller and Kawai (1991), in contrast, crossed an *Ectocarpus* from Japan, which is closely related to the genome-sequenced strain based on its ITS sequence but for which COI-5P sequences are so far unavailable, with *E. siliculosus*, and obtained meiosis-competent hybrid sporophytes. This cross-definitively needs confirmation.

The situation in the field is less well studied. Peters et al. (2010a,b) revealed the presence of field hybrids between *E. siliculosus* and *E. crouaniorum* in Chile and France but nothing is known about the proportion of hybrids in natural populations. Inspection of tree topologies obtained for several loci from different compartments have been successfully applied to identify potential cases of introgression and ancient hybridization events in natural populations (Peters et al. 2007). In our data set, a low percentage of incongruence (6%) was observed between results obtained with the mitochondrial and the nuclear marker. The species involved were phylogenetically more closely related than the crosses mentioned in the last paragraph (Fig. 1, a and b). Incongruent individuals were found exclusively among Chilean samples collected at sites where the respective species were in contact. Taken together, our results suggest the existence of different levels of reproductive barriers within the *E. siliculosi* complex, leading to mtDNA introgression only between some species pairs. Incomplete reproductive isolation may have an important bearing on the evolutionary trajectories of species by decreasing divergence between species but also by allowing new favorable mutations and allelic combinations to transgress species boundaries (Allendorf et al. 2001, Mallet 2005). However, both incomplete lineage sorting and hybridization lead to similar gene tree incongruence signatures and distinguishing between those two processes has proven difficult (Knowles 2004). Hybridization may be high between closely related species, unfortunately, incomplete lineage sorting is also likely to be at least partly responsible of the gene tree incongruences in species complexes of recent origin. New statistical frameworks, which allow testing for hybridization despite incomplete lineage sorting, have been developed recently (see Yu et al. 2011 and references therein). More extensive sampling, adapted genetic tools and analyses are needed to estimate the extent and importance of hybridization between the species of the *siliculosi* group in the field.

Species distribution. During our study, we extensively sampled two coasts where strong biogeographic boundaries are recognized. Along the Chilean coast, two biogeographic boundaries have been described (Camus 2001). The first is located at 30–33°S and separates the Peruvian Province from the Intermediate Area; the second is located at 42°S and separates the Intermediate area from the Magellanic Province. Along the European coast, the Celtic-Sea/Brittany area has been described as a biogeographical transition zone between the Northern European Sea and the Lusitanian Province while the front Almería-Oran separates the Mediterranean coasts from the Atlantic ones (Spalding et al. 2007). Several phylogeographic studies have reported a concordance between genetic discontinuities and biogeographic boundaries, attributing this pattern to the existence of historical barriers caused by oceanographic or climatic features (for Chile see the reviews Haye et al. 2014 and Guillemin et al. 2016, for Europe see the reviews Maggs et al. 2008 and Neiva et al. 2016). Interestingly, the species for which the genome has been sequenced, *Ectocarpus* 7 ($n = 32$), is apparently restricted to the Peruvian Province; Peters et al. (2010b) previously found similar results using a smaller sampling scheme. The Peruvian Province is characterized by continuous upwelling of cool water (16°C–20°C at the sea surface) and is also affected by recurrent El Niño events, causing several weeks of higher sea surface temperatures (more than 10°C of amplitude) as a result of the southward incursion of warm waters (Peters and Breeman 1993). The distribution range of *Ectocarpus* 7 may reflect an adaptation of individuals to this specific oceanographic environment. Apart for *Ectocarpus* 7, our results did not support the existence of extensive biogeographic or phylogeographic breaks for other *Ectocarpus* species. This lack of phylogeographic structure has been reported for species that have high dispersal capacities and/or for invasive species (Cárdenas et al. 2009, Guillemin et al. 2014, Haye et al. 2014). Short dispersal

distances of spores, gametes, or zygotes coupled with rare events of long-distance colonization seem to be the rule for the Phaeophyceae (Reed 1990, Raimondi et al. 2004, Neiva et al. 2012, Robuchon et al. 2014). However, *Ectocarpus* is described as an important contributor to biofouling and long-distance dispersal might be associated with human transport (Stache-Crain et al. 1997). Indeed, we detected two truly cosmopolitan species (*E. siliculosus* and *E. crouaniorum*) showing a star-like haplotype network and shared haplotypes between continents, a pattern characteristic of a recent expansion that may be facilitated by human activities. *Ectocarpus* 1 and *Ectocarpus* 6 occur in both the northern and the southern Pacific Ocean. Despite the large distances separating the sampled populations of these two species, no genetic structure was detected in their haplotype networks, a pattern suggestive of recent dispersal events between the North and South Pacific coasts. Similarly, no phylogeographic structure was observed for three South Pacific species (*Ectocarpus* 9, *Ectocarpus* 10 and *Ectocarpus* 11) nor for one North Atlantic species (*Ectocarpus* 3). It has been suggested that recent colonization events can eliminate genetic structure linked to historical barriers (Smith et al. 2011, DiBattista et al. 2012), thus the introductions of *Ectocarpus* species through shipping activities could explain the lack of phylogeographic structure in our study. Stache-Crain et al. (1997) have indeed reported that strains belonging to *E. siliculosus* sensu stricto (referred as lineage 1a) sampled from different continents show a maximum of five substitutions for the ITS marker; these authors suggest that recent dispersal events could have shaped the genetic diversity in this species. Both natural dispersal after the Pleistocene and transport via shipping have been proposed for this species (Stache-Crain et al. 1997). In our study, the distribution of *Ectocarpus* 8 seems related to dispersal associated with human transport. This species was present in the South Pacific and a single sample was found in the North Atlantic. This sample corresponds to an individual collected in the Kingsbridge Estuary (Devon, England). This could suggest a recent arrival of this species in the English Channel in ballast water or attached to a ship hull. Even if the dispersal capacity of *Ectocarpus* might be favored by shipping, when the number of samples was sufficient to perform within-species genetic differentiation analyses, we always found a slight but significant hierarchical pattern of genetic differentiation (i.e., see Tables 3 and 4; results of the nested AMOVA). Consequently, the pattern of genetic differentiation may be more complex than what we found in this study and sampling effort needs to be improved for all species to get a comprehensive idea of species distribution, species phylogeography and population connectivity.

CONCLUSION

Using DNA sequence data and species delimitation methods, we have observed the presence of at least 15 species within the *Ectocarpus siliculosi* group. Species showed different patterns of distribution and suggested different evolutionary histories. Further scrutiny of individuals cultivated in controlled laboratory conditions may reveal consistent morphological differences between species. However, future research on speciation in these filamentous brown algae will have to take into account that in the field, it is impossible to distinguish between species within the *E. siliculosi* group. This contrasts clearly with the significant morphological differences observed between recently diverging species in other Phaeophyceae, such as *Fucus* (Cánovas et al. 2011, Coyer et al. 2011). In any case, the complex pattern of phylogenetic relationships among the 15 species revealed in this study, opens a very interesting field of research deciphering the process of evolution and diversification in this group using the tools available from the model organism for genomics and genetics of the brown macroalgae.

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Allendorf, F. W., Leary, R. F., Spruell, P. & Wenburg, J. K. 2001. The problems with hybrids: setting conservation guidelines. *Trends Ecol. Evol.* 16:613–22.

Alò, D., Correa, C., Arias, C. & Cárdenas, L. 2013. Diversity of *Aplochiton* fishes (Galaxiidea) and the taxonomic resurrection of *A. marinus*. *PLoS ONE* 8:e71577.

Bandelt, H. J., Forster, P. & Röhl, A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.* 16:37–48.

Camus, P. 2001. Biogeografía marina de Chile continental. *Rev. Chil. Hist. Nat.* 74:587–617.

Cánovas, F. G., Mota, C. F., Serrao, E. A. & Pearson, G. A. 2011. Driving south: a multi-gene phylogeny of the brown algal

family Fucaceae reveals relationships and recent drivers of a marine radiation. *BMC Evol. Biol.* 11:371–96.
Cárdenas, L., Castilla, J. C. & Viard, F. 2009. A phylogeographical analysis across three biogeographical provinces of the southeastern Pacific: the case of the marine gastropod *Concholepas concholepas*. *J. Biogeogr.* 36:969–81.
Cardinal, A. 1964. *Étude sur les Ectocarpacées de la Manche*. J. Cramer, Weinheim, 86 pp.
Carstens, B. C., Pelletier, T. A., Reid, N. M. & Satler, J. D. 2013. How to fail at species delimitation. *Mol. Ecol.* 22:4369–83.
Carstens, B. C. & Satler, J. D. 2013. The carnivorous plant described as *Sarracenia alata* contains two cryptic species. *Biol. J. Linn. Soc.* 109:737–46.
Cock, J. M., Sterck, L., Rouze, P., Scornet, D., Allen, A. E., Amoutzias, G., Anthouard, V. et al. 2010. The *Ectocarpus* genome and the independent evolution of multicellularity in brown algae. *Nature* 465:617–21.
Cornils, A. & Held, C. 2014. Evidence of cryptic and pseudocryptic speciation in the *Paracalanus parvus* species complex (Crustacea, Copepoda, Calanoida). *Front. Zool.* 11:19.
Couceiro, L., Le Gac, M., Hunsperger, H. M., Mauger, S., Destombe, C., Cock, J. M., Ahmed, S., Coelho, S. M., Valero, M. & Peters, A. F. 2015. Evolution and maintenance of haploid-diploid life cycles in natural populations: the case of the marine brown alga *Ectocarpus*. *Evolution* 69:1808–22.
Coyer, J., Hoarau, G., Costa, J., Hogerdijk, B., Serrão, E. A., Billard, E., Valero, M., Pearson, G. A. & Olsen, J. L. 2011. Evolution and diversification within the intertidal brown macroalgae *Fucus spiralis/F. vesiculosus* species complex in the North Atlantic. *Mol. Phylogenet. Evol.* 58:283–96.
Coyne, J. A. & Orr, H. A. 2004. *Speciation*. Sinauer Associates, Sunderland, Massachusetts, 545 pp.
Darriba, D., Taboada, G. L., Doallo, R. & Posada, D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nat. Methods* 9:772.
DiBattista, J. D., Rocha, L. A., Craig, M. T., Feldheim, K. A. & Bowen, B. W. 2012. Phylogeography of two closely related Indo-Pacific butterflyfishes reveals divergent evolutionary histories and discordant results from mtDNA and microsatellites. *J. Hered.* 103:617–29.
Dillwyn, L. W. 1809. *British Confervae; or Colored Figures and Descriptions of the British Plants Referred by Botanists to the Genus Conferva. In* Phillips, W., Tard, G. & Street, L. [Eds.] W. Phillips, London, pp. 1–87, 1–6 (Index and Errata), Plates 69, 100–109, A–G (with text).
Drummond, A. J., Suchard, M. A., Xie, D. & Rambaut, A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol. Biol. Evol.* 29:1969–73.
Dupuis, J. R., Roe, A. D. & Sperling, F. A. 2012. Multi-locus species delimitation in closely related animals and fungi: one marker is not enough. *Mol. Ecol.* 21:4422–36.
Excoffier, L. & Lischer, H. E. L. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Resour.* 10:564–7.
Excoffier, L., Smouse, P. E. & Quattro, J. M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131:479–91.
Geoffroy, A., Mauger, S., De Jode, A., Le Gall, L. & Destombe, C. 2015. Molecular evidence for the coexistence of two sibling species in *Pylaiella littoralis* (Ectocarpales, Phaeophyceae) along the Brittany coast. *J. Phycol.* 51:480–9.
Goldstein, P. Z. & DeSalle, R. 2011. Integrating DNA barcode data and taxonomic practice: determination, discovery, and description. *BioEssays* 33:135–47.
Guillemin, M. L., Valero, M., Faugeron, S., Nelson, W. & Destombe, C. 2014. Tracing the trans-Pacific evolutionary history of a domesticated seaweed (*Gracilaria chilensis*) with archaeological and genetic data. *PLoS ONE* 9:e114039.
Guillemin, M. L., Valero, M., Tellier, F., Macaya, E. C., Destombe, C. & Faugeron, S. 2016. Phylogeography of seaweeds in the South East Pacific: complex evolutionary processes along a latitudinal gradient. *In* Hu, Z. M. & Fraser, C. I. [Eds.] *Seaweed Phylogeography*. Springer, Berlin, Germany, pp. 251–77.
Hamel, G. 1931-1939. *Phéophycées de France*. Wolf, Paris, XLVII + 431 pp., 9 plates.
Haye, P. A., Segovia, N. I., Muñoz-Herrera, N. C., Galvez, F. E., Martínez, A., Meynard, A., Pardo-Gandarillas, M. C., Poulin, E. & Faugeron, S. 2014. Phylogeographic structure in benthic marine invertebrates of the southeast Pacific coast of Chile with differing dispersal potential. *PLoS ONE* 9: e88613.
Hebert, P. D. N., Cywinska, A., Ball, S. L. & deWaard, J. R. 2003. Biological identifications through DNA barcodes. *Proc. R. Soc. B Biol. Sci.* 270:313–21.
Hedtke, S. M., Townsend, T. M. & Hillis, D. M. 2006. Resolution of phylogenetic conflict in large data sets by increased taxon sampling. *Syst. Biol.* 55:522–9.
Hillis, D. M., Pollock, D. D., McGuire, J. A. & Zwickl, D. J. 2003. Is sparse taxon sampling a problem for phylogenetic inference? *Syst. Biol.* 52:124–6.
Huelsenbeck, J. P. & Ronquist, F. 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* 8:754–5.
Kekkonen, M. & Hebert, P. D. N. 2014. DNA barcode-based delineation of putative species: efficient start for taxonomic workflows. *Mol. Ecol. Resour.* 14:706–15.
Knowles, L. 2004. The burgeoning field of statistical phylogeography. *J. Evol. Biol.* 17:1–10.
Lane, C. E., Lindstrom, S. C. & Saunders, G. W. 2007. A molecular assessment of northeast Pacific *Alaria* species (Laminariales, Phaeophyceae) with reference to the utility of DNA barcoding. *Mol. Phylogenet. Evol.* 44:634–48.
Lecointre, G., Philippe, H., Vân Lê, H. L. & Le Guyader, H. 1993. Species sampling has a major impact on phylogenetic inference. *Mol. Phylogenet. Evol.* 2:205–24.
Librado, P. & Rozas, J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25:1451–2.
Lyngbye, H. C. 1819. *Tentamen hydrophytologiae danicae; continens omnia hydrophyta cryptogama Daniae, Holsatiae, Faeroae, Islandiae, Groenlandiae hucusqve cognita, systematice disposita, descripta et iconibus illustrata, adjectis simul speciebus norvegicis. Opus, praemio ab Universitate regia hafniensi ornatum, et sumtu regio editum*. Hafniae, typis Schultzianis, in commissis Librariae Gyldendaliae, Copenhagen, 440 pp.
Maggs, C., Castilho, R., Foltz, D., Hensler, C., Jolly, M. T., Kelly, J., Olsen, J. L. et al. 2008. Evaluating signatures of glacial refugia for North Alantic benthic marine taxa. *Ecology* 89:108–22.
Mallet, J. 2005. Hybridization as an invasion of the genome. *Trends Ecol. Evol.* 20:229–37.
Mayden, R. L. 1997. A hierarchy of species concepts: the denouement in the saga of the species problem. *In* Claridge, M. F., Dawah, H. A. & Wilson, M. R. [Eds.] *Species: The Units of Diversity*. Chapman and Hall, London, UK, pp. 381–423.
Mayden, R. L. 1999. Consilience and a hierarchy of species concepts: advances toward closure on the species puzzle. *J. Nematol.* 31:95–116.
Modica, M. V., Puillandre, N., Castelin, M., Zhang, Y. & Holford, M. 2014. A good compromise: rapid and robust species proxies for inventorying biodiversity hotspots using the Terebridae (Gastropoda: Conoidea). *PLoS ONE* 9:e102160.
Monaghan, M. T., Wild, R., Elliot, M., Fujisawa, T., Balke, M., Inward, D. J. G., Lees, D. C., Ranaivosolo, R., Eggleton, P., Barraclough, T. G. & Vogler, A. P. 2009. Accelerated species inventory on Madagascar using coalescent-based models of species delineation. *Syst. Biol.* 58:298–311.
Müller, D. G. 1976. Sexual isolation between Europe and an American population of *Ectocarpus siliculosus* (Phaeophyta). *J. Phycol.* 12:252–4.
Müller, D. G. 1977. Sexual reproduction in British *Ectocarpus siliculosus* (Phaeophyta). *Br. Phycol. J.* 12:131–6.
Müller, D. G. 1979. Genetic affinity of *Ectocarpus siliculosus* (Dillw.) Lyngb. from the Mediterranean, North Atlantic and Australia. *Phycologia* 18:312–8.

Müller, D. G. 1988. Studies on sexual compatibility between *Ectocarpus siliculosus* (Phaeophyceae) from Chile and the Mediterranean Sea. *Helgoländer Meeresun.* 42:469–76.

Müller, D. G. & Eichenberger, W. 1995. Crossing experiments, lipid composition, and the species concept in *Ectocarpus siliculosus* and *E. fasciculatus* (Phaeophyceae, Ectocarpales). *J. Phycol.* 31:173–6.

Müller, D. G. & Kawai, H. 1991. Sexual reproduction of *Ectocarpus siliculosus* (Ectocarpales, Phaebphyceae) in Japan. *Jpn. J. of Phycol.* 39:151–6.

Nei, M. & Li, W. H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. USA* 76:5269–73.

Neiva, J., Pearson, G. A., Valero, M. & Serrão, E. A. 2012. Fine-scale genetic breaks driven by historical range dynamics and ongoing density-barrier effects in the estuarine seaweed *Fucus ceranoides* L. *BMC Evol. Biol.* 12:78.

Neiva, J., Serrão, E. A., Assis, J., Pearson, G. A., Coyer, J. A., Olsen, J. L., Hoarau, G. & Valero, M. 2016. Climate oscillations, range shifts and phylogeographic patterns of North Atlantic Fucaceae. *In* Hu, Z. M. & Fraser, C. I. [Eds.] *Seaweed Phylogeography.* Springer, Berlin, Germany, pp. 279–308.

Pante, E., Puillandre, N., Viricel, A., Arnaud-Haond, S., Aurelle, D., Castelin, M., Chenuil, A. et al. 2015. Species are hypotheses: avoid connectivity assessments based on pillars of sand. *Mol. Ecol.* 24:525–44.

Pardo, C., Lopez, L., Peña, V., Hernández-Kantún, J., Le Gall, L., Bárbara, I. & Barreiro, R. 2014. A multilocus species delimitation reveals a striking number of species of coralline algae forming maerl in the OSPAR maritime area. *PLoS ONE* 9: e104073.

Payo, D. A., Leliaert, F., Verbruggen, H., D'Hondt, S., Calumpong, H. P. & De Clerck, O. 2012. Extensive cryptic species diversity and fine-scale endemism in the marine red alga *Portieria* in the Philippines. *Proc. R. Soc. B Biol. Sci.* 280:20122660.

Peters, A. F. & Breeman, A. M. 1993. Temperature tolerance and latitudinal range of brown algae from temperate Pacific South America. *Mar. Biol.* 115:143–50.

Peters, A. F., Couceiro, L., Tsiamis, K., Küpper, F. C. & Valero, M. 2015. Barcoding of cryptic stages of marine brown algae isolated from incubated substratum reveals high diversity in Acinetosporaceae (Ectocarpales, Phaeophyceae). *Cryptogamie Algol.* 36:3–29.

Peters, A. F., Mann, A. D., Cordova, C. A., Brodie, J., Correa, J. A., Schroeder, D. C. & Cock, J. M. 2010b. Genetic diversity of *Ectocarpus* (Ectocarpales, Phaeophyceae) in Peru and northern Chile, the area of origin of the genome-sequenced strain. *New Phytol.* 188:30–41.

Peters, A. F., Marie, D., Scornet, D., Kloareg, B. & Cock, J. M. 2004. Proposal of *Ectocarpus siliculosus* (Ectocarpales, Phaeophyceae) as a model organism for brown algal genetics and genomics. *J. Phycol.* 40:1079–88.

Peters, A. F., Van Wijk, S. J., Cho, G. Y., Scornet, D., Hanyuda, T., Kawai, H., Schroeder, D. C., Cock, J. M. & Boo, S. M. 2010a. Reinstatement of *Ectocarpus crouaniorum* Thuret in Le Jolis as a third common species of *Ectocarpus* (Ectocarpales, Phaeophyceae) in Western Europe, and its phenology at Roscoff, Brittany. *Phycol. Res.* 58:157–70.

Peters, J. L., Zhuravlev, Y., Fefelov, I., Logie, A. & Omland, K. E. 2007. Nuclear loci and coalescent methods support ancient hybridization as cause of mitochondrial paraphyly between gadwall and falcated duck (*Anas* spp.). *Evolution* 61:1992–2006.

Pons, J., Barraclough, T. G., Gomez-Zurita, J., Cardoso, A., Duran, D. P., Hazell, S., Kamoun, S., Sumlin, W. D. & Vogler, A. P. 2006. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Syst. Biol.* 55:595–609.

Prevot, V., Jordaens, K., Sonet, G. & Backeljau, T. 2013. Exploring species level taxonomy and species delimitation methods in the facultatively self-fertilizing land snail genus *Rumina* (Gastropoda: Pulmonata). *PLoS ONE* 8:e60736.

Puillandre, N., Lambert, A., Brouillet, S. & Achaz, G. 2012a. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Mol. Ecol.* 21:1864–77.

Puillandre, N., Modica, M. V., Zhang, Y., Sirovich, L., Boisselier, M. C., Cruaud, C., Holford, M. & Samadi, S. 2012b. Large-scale species delimitation method for hyperdiverse groups. *Mol. Ecol.* 21:2671–91.

Raimondi, T., Reed, D. C., Gaylord, B. & Washburn, L. 2004. Effects of self-fertilization in the giant kelp, *Macrocystis pyrifera. Ecology* 85:3267–76.

Rambaut, A. & Drummond, A. J. 2010. TreeAnnotator version 1.6.1. Available at: http://beast.bio.ed.ac.uk/treeannotator (last accessed 05 January 2015).

Reed, D. C. 1990. The effects of variable settlement and early competition on patterns of kelp recruitment. *Ecology* 71: 776–87.

Robuchon, M., Le Gall, L., Mauger, S. & Valero, M. 2014. Contrasting genetic diversity patterns in two sister kelp species co-distributed along the coast of Brittany. *Mol. Ecol.* 23:2669–85.

Russell, G. 1966. The genus *Ectocarpus* in Britain. I. The attached forms. *J. Mar. Biol. Assoc. UK* 46:267–94.

Russell, G. 1967a. The genus *Ectocarpus* in Britain II. The free-living forms. *J. Mar. Biol. Assoc. UK* 47:233–50.

Russell, G. 1967b. The ecology of some free-living Ectocarpaceae. *Helg. Wiss. Meer.* 15:155–62.

Russell, G. 1983a. Formation of an ectocarpoid epiflora on blades of *Laminaria digitata. Mar. Ecol. Prog. Ser.* 11:181–7.

Russell, G. 1983b. Parallel growth patterns in algal epiphytes and *Laminaria* blades. *Mar. Ecol. Prog. Series* 13:303–4.

Sites, J. W. & Marshall, J. C. 2003. Delimiting species: a renaissance issue in systematic biology. *Trends Ecol. Evol.* 18:462–70.

Sites, J. W. & Marshall, J. C. 2004. Operational criteria for delimiting species. *Annu. Rev. Ecol. Evol. Syst.* 35:199–227.

Smith, B. T., Escalante, P., Hernández Baños, B. E., Navarro-Sigüenza, A. G., Rohwer, S. & Klicka, J. 2011. The role of historical and contemporary processes on phylogeographic structure and genetic diversity in the Northern Cardinal, *Cardinalis cardinalis. BMC Evol. Biol.* 11:136.

Spalding, M. D., Fox, H. E., Allen, G. R., Davidson, N., Ferdaña, Z. A., Finlayson, M., Halpern, B. S. et al. 2007. Marine ecoregions of the world: a bioregionalization of coastal and shelf areas. *Bioscience* 57:573–83.

Stache, B. 1990. Sexual compatability and species concept in *Ectocarpus siliculosus* (Ectocarpales, Phaeophyceae) from Italy, North Carolina, Chile, and New Zealand. *In* Garbary, D. J. & South, G. R. [Eds.] *Evolutionary Biogeography of the Marine Algae of the North Atlantic.* Springer Verlag, Berlin, Germany, pp. 173–86.

Stache-Crain, B., Müller, D. G. & Goff, L. J. 1997. Molecular systematics of *Ectocarpus* and *Kuckuckia* (Ectocarpales, Phaeophyceae) inferred from phylogenetic analyses of nuclear and plastid-encoded DNA sequences. *J. Phycol.* 33:152–68.

Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30:1312–3.

Stamatakis, A., Hoover, P. & Rougemont, J. 2008. A fast bootstrapping algorithm for the RAxML web-servers. *Syst. Biol.* 57:758–71.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol. Biol. Evol.* 30:2725–9.

Tronholm, A., Steen, F., Tyberghein, L., Leliaert, F., Verbruggen, H., Ribera Siguan, M. A. & De Clerck, O. 2010. Species delimitation, taxonomy, and biogeography of *Dictyota* in Europe (Dictyotales, Phaeophyceae). *J. Phycol.* 46:1301–21.

Vieira, C., D'hondt, S., De Clerck, O. & Payri, C. E. 2014. Toward an inordinate fondness for stars, beetles and *Lobophora*? Species diversity of the genus *Lobophora* (Dictyotales, Phaeophyceae) in New Caledonia. *J. Phycol.* 50:1101–19.

Wiens, J. J. & Penkrot, T. A. 2002. Delimiting species limits in spiny lizards (*Sceloporus*). *Syst. Biol.* 51:69–91.

Yamashita, T. & Rhoads, D. D. 2013. Species delimitation and morphological divergence in the scorpion *Centruroides vittatus* (Say, 1821): insights from phylogeography. *PLoS ONE* 7: e68282.

Yu, Y., Than, C., Degnan, J. H. & Nakhleh, L. 2011. Coalescent histories on phylogenetic networks and detection of hybridization despite incomplete lineage sorting. *Syst. Biol.* 60:138–49.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Figure S1. Distribution of pair-wise distances for the marker COI-5P and automatic barcode gap discovery (ABGD) results. (a) Frequency distribution of K2P distances between haplotype pairs for the marker COI-5P. (b) ABGD results showing the number of groups (primary partitions) obtained for a range of prior maximum divergence of intraspecific diversity.

Figure S2. Ultrametric Bayesian tree showing the results obtained using the GMYC single threshold based on COI-5P. The vertical dotted red line shows the point of transition between coalescence and speciation processes.

Figure S3. Distribution of pair-wise distances between haplotype pairs for the marker ITS1 (left) and automatic barcode gap discovery (ABGD, primary partitions) results for the four different alignments (right). Sub-alignment 1: *E. siliculosus*, *Ectocarpus* 1, *Ectocarpus* 2, *Ectocarpus* 4; sub-alignment 2: *Ectocarpus* 5, *Ectocarpus* 6, *Ectocarpus* 7; sub-alignment 3: *Ectocarpus* 8, *Ectocarpus* 9, *Ectocarpus* 10, *Ectocarpus* 11; sub-alignment 4: *E. crouaniorum*, *Ectocarpus* 12, *Ectocarpus* 13.

Table S1. COI-5P sequences used in this study. Details of the date, geographic origin, Genbank accession number and reference of each COI-5P sequence used in this study.

Table S2. ITS1 sequences used in this study. Details of the date, geographic origin, Genbank accession number and reference of each ITS1 sequence used in this study.

Table S3. Mitochondrial (COI-5P) and nuclear (ITS1) DNA sequence variation in *E. siliculosus*. Molecular diversity indices were calculated for the two molecular markers (COI-5P and ITS1). *N*, number of sequences; *nH*, number of haplotypes; *H*, gene diversity; π, nucleotide diversity; *S*, number of polymorphic sites. Standard deviations (SD) in parentheses.

Table S4. Mitochondrial (COI-5P) and nuclear (ITS1) DNA sequence variation in *E. crouaniorum*. Molecular diversity indices were calculated for the two molecular markers (COI-5P and ITS1). *N*, number of sequences; *nH*, number of haplotypes; *H*, gene diversity; π, nucleotide diversity; *S*, number of polymorphic sites. Standard deviations (SD) in parentheses.

Table S5. Mitochondrial (COI-5P) and nuclear (ITS1) DNA sequence variation in *Ectocarpus* 6. Molecular diversity indices were calculated for the two molecular markers (COI-5P and ITS1). *N*, number of sequences; *nH*, number of haplotypes; *H*, gene diversity; π, nucleotide diversity; *S*, number of polymorphic sites. Standard deviations (SD) in parentheses.

Table S6. Species nomenclature used in literature for the species of the *Ectocarpus siliculosi* group.