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Alejandro Montecinos

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Université Pierre et Marie Curie

Universidad Austral de Chile

Sciences de la Nature et de l'Homme : Evolution et Ecologie

UPMC-MNHN

Station Biologique de Roscoff / UMI 3614, Evolutionary Biology and Ecology of Algae

Species delineation and hybridization in the brown seaweed *Ectocarpus* complex

Par Montecinos Alejandro

Thèse de doctorat

Dirigée par Myriam Valero et Marie-Laure Guillemin

Présentée et soutenue publiquement le 08 Novembre 2016

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UNIVERSIDAD AUSTRAL DE CHILE
FACULTAD DE CIENCIAS

SPECIES DELINEATION AND HYBRIDIZATION IN THE BROWN SEAWEED *ECTOCARPUS* COMPLEX

TESIS DOCTORAL

ALEJANDRO E. MONTECINOS
ROSCOFF – FRANCIA

2016

Acknowledgments

Firstly, I would like to express my gratitude to my two supervisors: Myriam Valero and Marie-Laure Guillemin. Thank you very much for the continuous support of my PhD and specially for your patience, motivation, and immense help. Their guidance helped me in all the time of research and writing of this thesis. Besides my advisors, I would like to thank the rest of my thesis committee: Didier Jollivet, Susana Coelho, Pierre-Alexandre Gagnaire, Nicolas Bierne and specially to Akira F. Peters. Thank you very much for their insightful comments and encouragement.

Thank you very much Akira for the nice *Ectocarpus* sampling through the Chile coast.

Thank you very much Komlan and Stephan for your help in the development of the second chapter of this PhD Thesis.

Thank you very much to all my co-authors, it was really nice to work together. Also, thank you very much Christophe Destombe for all your help in the academic and the personal sense and for your jokes as well.

I thank my fellow labmates from BEDIM and DIVCO: Marie, Chloé, Bertrand, Jérôme, Lucie, Jaro, Sarah, Aga, etc... For sure, I forgot more people but it is usual in me...but I will improve it, is a promise. I want to thank my friends that I meet in the Station Biologique de Roscoff. Thank you very much for especially for the soirées!!

I would like to thank my family: my parents, my sister, my brother in law and my brothers/nephews for supporting me throughout this thesis.

Last but not the least, I want to thank to Katy, you were my partner in this adventure. Thank you very much for supporting me and be part of this experience.

Finally, I want to thank to Becas Chile and all the founding involved in this PhD Thesis.

Well, no more to add.
Thank you very much again.

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Introduction

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INTRODUCTION

I. Hybridization, reproductive isolation and speciation processes

Speciation, the evolutionary process by which new biological species arise, is one of the most fascinating and controversial processes in evolutionary biology (Mayr 1942, Otte and Endler 1989, Coyne an Orr 1998, Feder et al. 2013a). This process of divergence between taxa is generally described as complete when the reproductive isolation induced by reproductive barriers is established (Dobzhansky 1937, Mayr 1942, Coyne and Orr 2004). The numerous reproductive barriers described have been classified into two types: (I) prezygotic barriers such as ecogeographic, temporal, and behavioral differences between species that limit the formation of zygotes and (II) postzygotic barriers such as hybrid inviability, hybrid sterility, and F2 breakdown leading to lower fitness in hybrids than in the parental species (Dobzhansky 1937, Mayr 1942).

During the last 50 years, a major area of debate among biologists working on speciation was the geographical context in which these reproductive barriers are evolving (reviewed in Turelli et al. 2001, Fitzpatrick et al. 2009). While in allopatry both pre and postzygotic reproductive isolating mechanisms arise as inevitable by-products of genetic divergence without gene flow between divergent taxa, in sympatry and parapatry, speciation occurs in spite of gene flow (i.e. speciation-with-gene-flow, see review of Smadja and Butlin 2011 and Figure I.1). Processes of 'speciation-with-gene-flow' have been described to explain how, at a given – non – null – level of

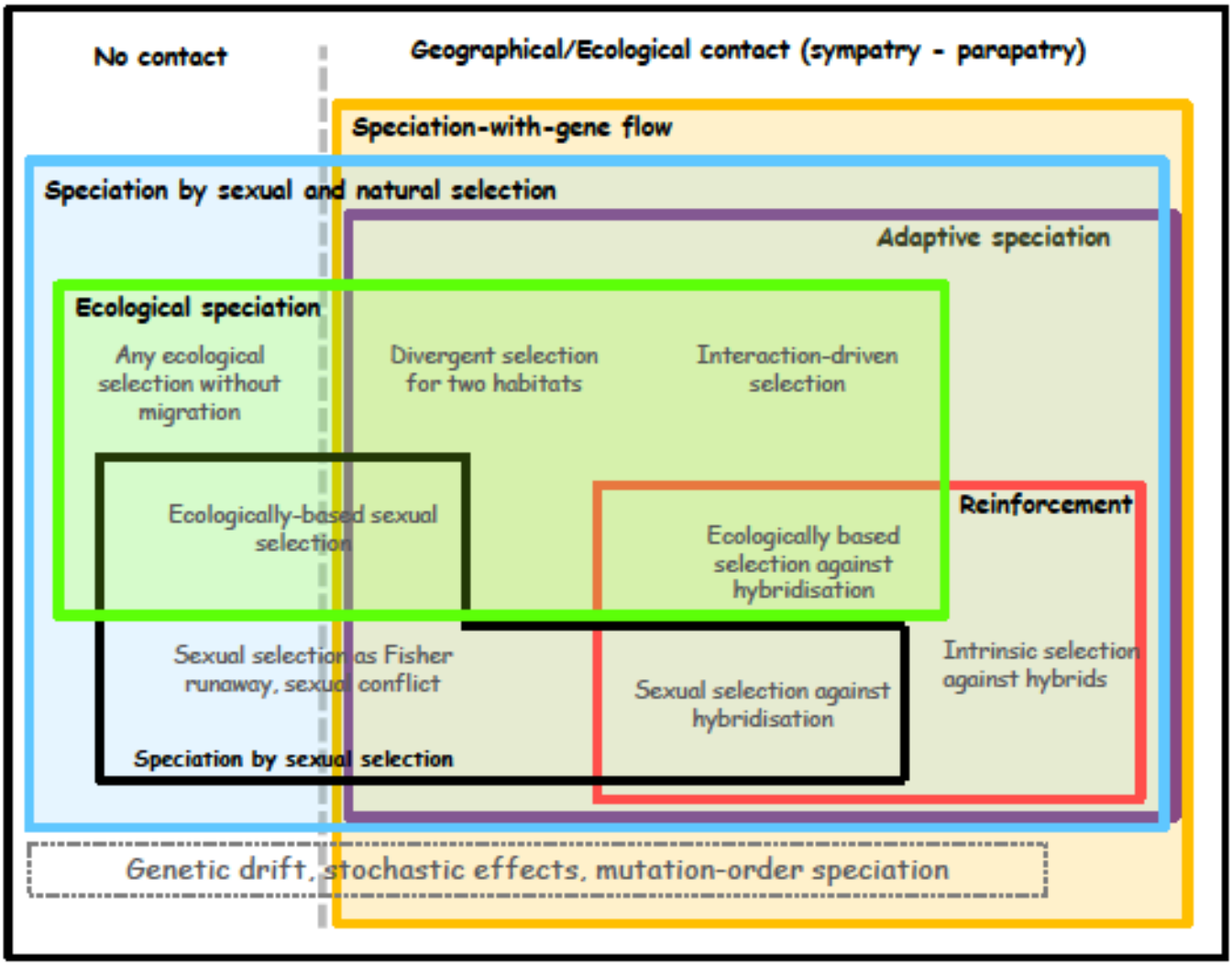


Figure I.1. Different scenario of speciation (from Smadja and Butlin, 2011).

The Figure distinguishes scenarios of speciation with no contact at all between diverging populations (left of dashed line) from those with geographical or ecological contact at least at some point in time and space (right of dashed line). The different types of evolutionary and selective forces potentially involved in each scenario (grey text) are used to define and delimit different modes and mechanisms of speciation (coloured frames).

it is possible for increased reproductive isolation to evolve (Feder et al. 2013b). Hybridization was already known by taxonomists in the 18th century and has remained an active and productive area of research up to the present days (see reviews of Arnold et al. 1999, Rieseberg and Carney 1998, Coyne and Orr 2004, Mallet 2005, Abbot et al. 2013). In nature, hybridization occurs in hybrid zones, these areas may have two different origins depending on the geographical context in which divergence between

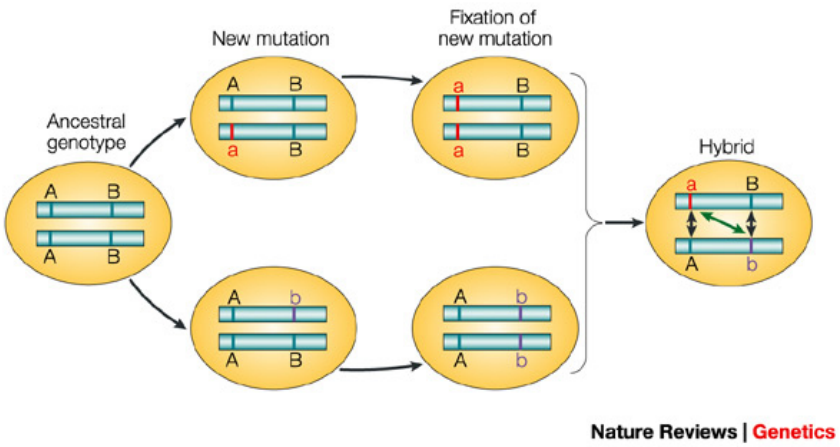
the parental species occurred. (1) Primary hybrid zones, where divergence is taking place between adjacent populations of a previously homogeneous species, for example through ecological speciation. (2) Secondary hybrid zones, where hybridization arise in turn from a secondary contact between two populations that were previously allopatric.

In the last decades, the debate has shifted away from geographic modes of speciation, towards the difficult challenge of assessing the frequency of speciation processes involving gene flow in nature, and recent efforts have been made in understanding and elucidating the factors that facilitate the occurrence of speciation with gene flow (Smadja and Butlin 2011). Research on speciation has allowed to pinpoint the main mechanisms causing divergence among organisms and some have effectively been related to particular geographical modes of speciation (Smadja and Butlin 2011). Figure I.1 is taken from this paper and summarizes the different modes and mechanisms of speciation. It shows that the same mechanisms are involved whatever the geographic/ecological contexts, except for speciation by reinforcement, wich involves gene flow. In addition, Figure I.1 shows that each mechanism is not exclusive and can sometimes act in synergy. Adaptive or ecological speciation has been recognized as a central mechanism of evolutionary change within species, which ultimately results in reproductive isolation between the diverging populations as a by-product (Figure I.1). One well-studied example of incipient ecological speciation is the case of the marine gastropod *Littorina saxatilis*, a common inhabitant of intertidal shores of the north Atlantic (Johannesson 2003, Rolan-Alvarez 2007, Galindo et al. 2010). The intertidal zone represents a gradient of contrasted micro habitats in which ecotypes can be formed multiple times along the coast by differential selection. In the snail *L. saxatilis* in several sites along the coast, different ecotypes were shown to be the result of repeated processes of local divergence, with reproductive barriers having

evolved in situ in the face of gene flow and forming primary rather than secondary contact zones (see for review Johannesson 2016). Models including sexual selection (Panhuis et al. 2001, Ritchie 2007) have also suggested that differential patterns of trait variation related to reproductive success within populations contribute to reproductive isolation among populations (Figure I.1). A compelling example is related to the explosive radiation of cichlid fishes in the African Rift Lakes, where populations with overlapping distributions are diverging as a function of the differential preference of male color in mate selection (Seehausen et al. 2008). Other models of speciation based on the action of random processes and drift do not include a role of selection of any sort but rather invoke a key role of stochastic events (Figure I.1). Such models include speciation by polyploidization, genetic drift and founder-events/population bottlenecks. Although such models have a long history in speciation research (for example the classical example of small populations colonizing different islands leading to species formation, Thorpe et al. 1994), clear empirical support for such models in nature or in laboratory is relatively limited except for the cases of speciation by polyploidization (Coyne and Orr 2004). Polyploidy, or the presence of three or more complete sets of chromosomes, has been documented in a wide variety of taxa and is particularly prevalent in plants where between 47 and 70% of all angiosperms are described to be of possible polyploid origin (Ramsey and Schemske 1998). Polyploidy is a mechanism that has caused many rapid speciation events in sympatry because offspring of, for example, tetraploid x diploid crosses often result in triploid sterile progeny (Ramsey and Schemske 1998). Polyploidy is viewed as a mechanism that can rapidly lead to the formation of new species, potentially without selection for the divergence of other characters (Mallet et al. 2013).

Two complementary but different approaches were used to study the genetic basis of reproductive isolation in the literature. First, barriers to gene flow were investigated using laboratory crosses of well-established model species. These studies have been successful in identifying genes that contribute to classical traits linked to the establishment of reproductive barriers, such as hybrid sterility or unviability, and ultimately to speciation processes. For example, crossing experiments and genetic mapping in different *Drosophila* species have allowed to demonstrate that hybrid incompatibility was due to Dobzhansky-Muller interaction (see Box 1). Indeed, Brideau et al. (2006) proved that lethal hybrid incompatibility resulted from the epistatic interaction between the *D. melanogaster* allele of Hmr (Hybrid male rescue gene identified by Barbash et al. 2003) and the *D. simulans* allele of Lhr (Lethal hybrid rescue gene identified by Presgraves et al. 2003).

Box 1: Dobzhansky-Muller model of hybrid incompatibility (modified From Wu and Ting, 2004).



Dobzhansky (1937) and Muller (1942) independently formulated a model of how hybrid incompatibility could evolve. In the ancestral population, the genotype is AA BB. When the population is split into two, A evolves into a in one population and B evolves into b in the other. a and b are mutually incompatible. As the a–b interaction is not present in the pure species, the evolution of incompatibility is possible.
This model explains intrinsic post-zygotic isolation reducing hybrid fitness in all environments and involves epistatic interactions among genes (green arrow). This mechanism of intrinsic post-zygotic isolation should be distinguished from extrinsic or environment-dependent reproductive isolation.

On the other hand, the field of population genomics with the emerging of new sequencing technologies (Luikart et al. 2003, Nosil and Feder 2013) provides a unique

opportunity to characterize the genetic basis of speciation (for a recent review see Payseur and Rieseberg, 2016). Population genomics approaches are expected to point out candidate loci possibly associated with components of reproductive isolation. When the speciation process is incomplete, theory predicts that linkage between alleles that favor reproductive isolation will increase and thus the question arises of how these associated alleles are maintained in spite of recombination in sexually reproducing organisms (Smadja and Butlin, 2011). Genomic divergence is thus expected to be highly heterogeneous during the process of population divergence because genetic differentiation associated with divergent natural selection could accumulate in some regions of the genome while the homogenizing effects of gene flow or random differentiation by genetic drift preclude divergence in other regions (Abbott et al. 2013, Smadja and Butlin, 2011). During the speciation process, gene flow might be reduced either at individual "barrier loci" or across a greater fraction of the genome through associations with these loci depending on the genome architecture, the importance of recombination and level of gene flow (Abbott et al. 2013). In their review, Abbott et al. (2013) highlight three pressing questions that remain to be answered by biologists studying speciation in order to better understand the evolutionary significance of hybridization and gene flow in the speciation process: (I) How many genomic regions differentiate during speciation?", (II) "How small are regions where divergence significantly exceeds the genomic average?" and (III) "How are regions of exceptional divergence dispersed around the genome?".

Taken together, major advances to current knowledge in plant and animals speciation have been made using different population genomics approaches such as whole genome sequencing of new promising study systems (i.e. in the butterfly, *Heliconius* Genome Consortium, 2012); powerful analyses based on genome scans (i.e.

in the Cichlids of the genus *Pundamilia*, Keller et al. 2013) and the observation of genomic architecture evolution after hybridization (i.e. in the North American lake whitefish, *Coregonus clupeaformis*, Gagnaire et al., 2013). A comprehensive overview of the recent advances in plant speciation research on the genetic basis of reproductive isolation were published by Lafon-Placette et al. 2016 (see Figure I.2). These advances have allowed to pinpoint the genetic targets of selection, natural or sexual, that drive the establishment of reproductive barriers between species (for example the genetic basis of

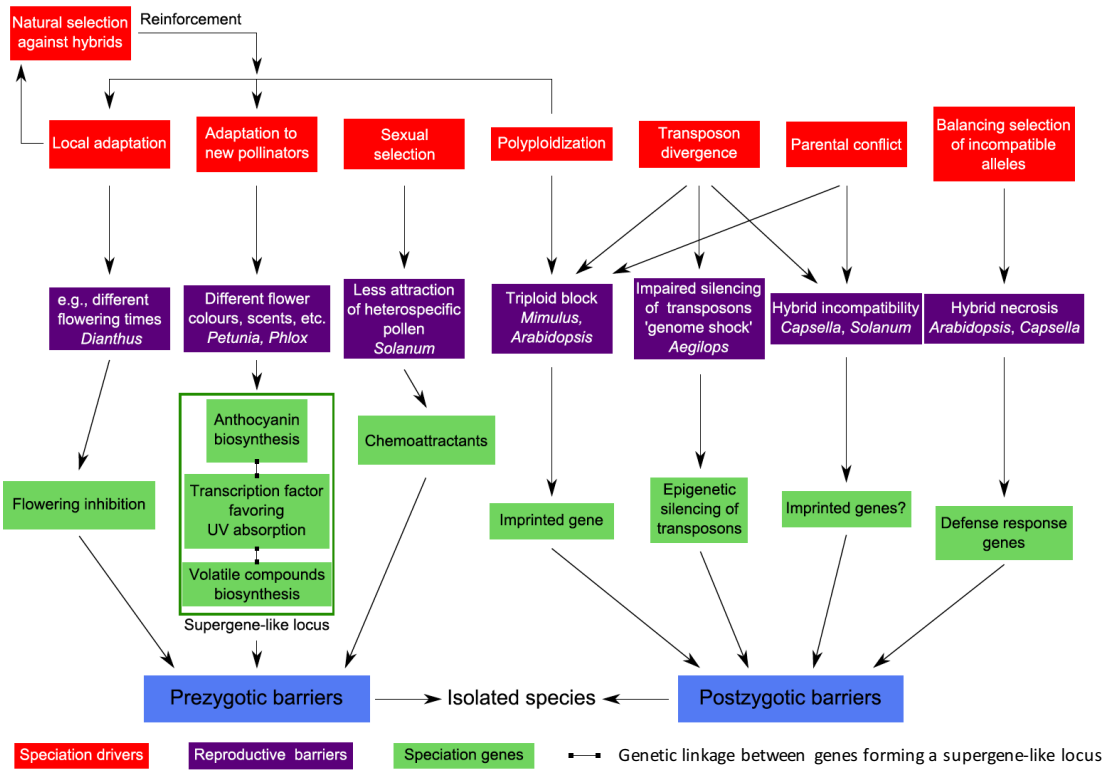


Figure I.2 – Non exhaustive examples of genetic elements underlying reproductive barriers in plants (Figure taken from Lafon-Placette et al. 2016), illustrating the advances made to unravel the genetic bases for different types of hybridization barriers. Forces driving the establishment of reproductive barriers are in red, the barriers in purple and the underlying genetic elements in green. The arrow loop from 'local adaptation' to 'adaptation to new pollinators' illustrates reinforcement: the hybrids between two populations, adapted to different local environments, are expected to be negatively selected in parental environments. Traits preventing costly hybridization will therefore be selected, for example, flower color change and pollinator shift. The arrows between 'polyploidization' 'local adaptation'/'adaptation to new pollinator' show that, besides instant reproductive isolation (triploid block), polyploids adapt to ecological niches/pollinators that differ from that of their parental species.

traits such as flowering time and flower color contributing to prepollination barriers, Sheehan et al., 2012; or the supergene-like incompatible loci responsible of pollination

syndromes in *Petunia* species, Hermann et al., 2013; see Figure I.2). They have also confirmed the role of hybridization as a promoter of speciation rather than an inhibitor of reproductive isolation, since it can acts as an additional source of adaptive genetic variation.

Most data on hybridization in the wild come from analyses of vascular plant or animal species. Investigating a greater diversity of biological models will help unravel the general importance of hybridization in evolution. Nevertheless, data are accumulating for species belonging to other phyla such as fungi (see for reviews Kohn 2005; Giraud et al. 2008), red algae (Zuccarello et al. 2005; Destombe et al., 2010; Maggs et al. 2011; Hind and Saunders 2013; Niwa and Kobiyama 2014; Savoie and Saunders 2015) and brown algae (Coyer et al. 2002; Engel et al. 2005; Peters et al. 2010a; Geoffroy et al. 2015 and references herein) which differ substantially in their life cycle and life history traits.

In this PhD thesis, we will study species of the genus *Ectocarpus,* a filamentous brown alga characterized by a haploid-diploid life cycle (see section III below about the study model). The haploid-diploid life cycle of this study model gives the advantage that both haploid and diploid independent phases could be studied separately allowing to determine at which level reproductive barriers occur (Figure I.3). In diploid life cycles, mitotic cell division and somatic development occur entirely in the diploid phase. The haploid stage is reduced to a single-cell (i.e. gamete) produced through meiosis in the diploid phase and fertilization occurs immediately after release of gametes to re-establish the diploid phase. Therefore, population genetics studies based on diploid life cycles are focused only on diploid individuals. On the other hand, in haploid-diploid life cycles, somatic development occurs in both haploid and diploid phases and there is an alternation between two types of independent functional

individuals: haploid gametophytes produced by meiosis and diploid sporophytes resulting from fertilization. The direct access to the haploid part of the life cycle allows to untangle the effect of reproductive barriers preventing fertilization (i.e. an absence of diploid hybrid genotypes is expected) or preventing meiosis (i.e. an absence of recombinant haploid genotypes is expected). Conversely, in diploid species, such processes cannot be distinguished directly using population genetics in the field and they require more complexes garden studies.

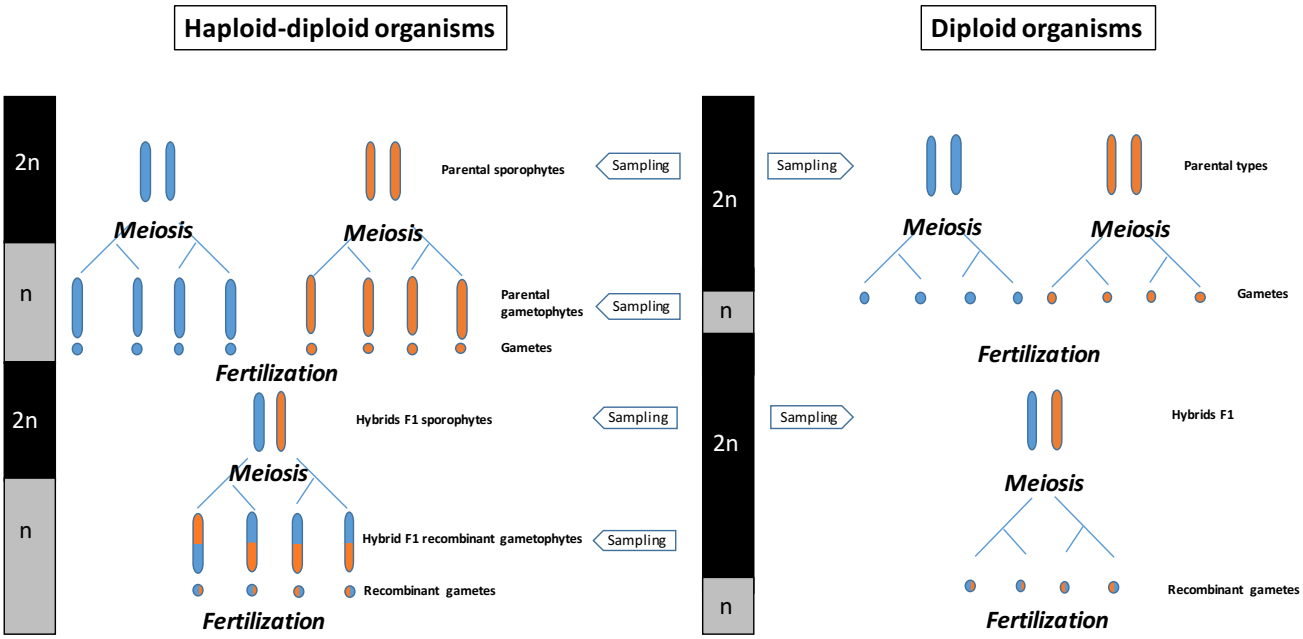


Figure I.3 – (from Montecinos et al., in prep, Chapter 3) The haploid-diploid life cycle model to study hybridization and reproductive barriers.

Recently, the consequences of various ploidy levels on the dynamics of reproductive isolation were studied in the yeast *Saccharomyces cerevisiae* by M. Rescan (2016). She showed that reproductive isolation evolved more rapidly in haploid than in diploid yeast populations. The explanation is as follow, while reproductive incompatibilities are fully expressed in the first generation (F1) in haploid hybrids, diploid hybrid F1 may benefit from heterosis and thus reproductive incompatibilities

will evolve slower. Thus in haploid-diploid species, we may expect reproductive isolation to evolve faster than in diploid species since, as in haploid species, reproductive incompatibilities should be fully expressed in the F1 (Figure I.3). In fact, the same mechanisms are involved in the classical phenomenon called Haldane's rule (Haldane 1922). In diploid species with chromosomic sex determination systems, the heterogametic sex (XY or ZW) suffer from fully exposed incompatibilities involving alleles on the X or the W chromosome, leading to the preferential sterility and inviability of hybrids of the heterogametic sex. Note that in the haploid-diploid species, sex is expressed in the haploid phase and the chromosomal sex determination system is haploid (U, V, Ahmed et al., 2015) and thus asymmetry between sexes in hybrid fitness is not expected.

II. Species delimitation

Numerous definitions of speciation have been proposed that have led to the existence of various criteria to delimit species (e.g. Mayden 1997, 1999 listed 24 species concepts but see also Pante et al. 2015). In an attempt to solve the controversy among the multiple species definitions already proposed, De Queiroz (1998, 1999, 2007) advocated that as species are evolving dynamic entities and that they should be considered as segments of population-level lineages (ancestral-descendent sequence of populations, Figure I.4).

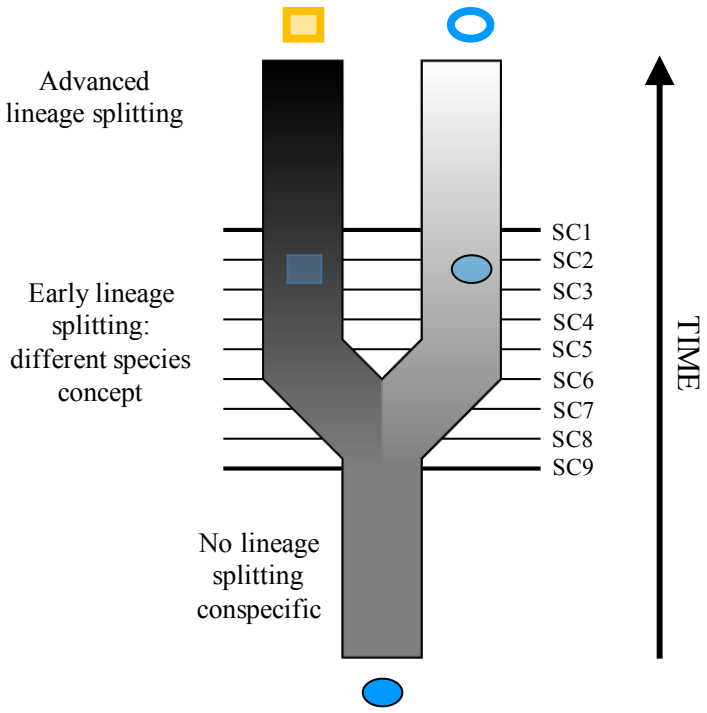


Figure I.4 - Simplified sketch showing a speciation event. (from de Queiroz, 1998, 2007). A single lineage (gray) splits into two divergent lineages (dark gray and light gray). Below the first thick black line, a single species is recognized unambiguously, while above the second thick black line, two species are recognized unambiguously. The zone between the two thick black lines corresponds to possible disagreement over the number of species (1 vs. 2), depending on which species concept is applied. SC (species criterion) 1 to 9 represents the times at which the lineages acquire different properties (i.e., when they become phenetically distinguishable, diagnosable, reciprocally monophyletic, reproductively incompatible, ecologically distinct, etc.). Ovals and squares represent phenotypical traits of individuals.

De Queiroz (1998, 2007) has then proposed that contemporary species delimiting criteria act as secondary proprieties that arise at different moments of the speciation process (e.g. reproductive isolation, niche differentiation, monophyly, etc., SC1 to SC9 in Figure I.4). Species taxa could then be considered as scientific hypotheses (species hypotheses) and species delimitation is only a process of falsification based on the acquisition of new evidence on various species delimiting criteria (Pante et al. 2015).

Because speciation is not always accompanied by morphological change (Agapow et al. 2004, Bickford et al. 2007, Lumbsch and Leavitt 2011), species delimitation using only the organism morphology is often inadequate in some taxonomic groups. Following the ever-growing facility in obtaining molecular data, DNA-based methods have then become the standard tools to identify species (e.g. barcoding, Hebert et al. 2003, Hajibabaei et al. 2007). Numerous methods have been developed to detect discontinuities in sequence variation associated with species boundaries (Sites and Marshall 2004, Pons et al. 2006, Monaghan et al. 2009, Puillandre et al. 2012a). Limitations and complementarity of these numerous methods have been discussed in the review of Carstens et al. (2013) entitled “How to fail at species delimitation”. In this review different useful methods were listed (see Box 2 below) and Carstens et al. (2013) recommended that a wide range of species delimitation analyses should be applied to each molecular data set in order to limit errors due to low power to detect cryptic lineages of some of these approaches or in study cases where assumptions of these methods could be violated. They propose to give confidence in the species delimitation suggested only when all or most of the methods used give congruent results for a specific data set and that researchers should only place their trust in delimitations that are congruent across methods.

Box 2: Some useful methods for species delimitation using or not a priori information of samples assignation to putative lineages (modified from Carstens et al. 2013).

1-Species discovery approaches assign samples to groups without a priori information

Structurama (Huelsenbeck et al. 2011) implements the clustering algorithm first described by Pritchard et al. (2000; for their program Structure) that clusters samples into populations by minimizing Hardy–Weinberg disequilibrium for a given partitioning level. Structurama includes the addition of reversible jump MCMC to identify the optimal partitioning level. Nearly any type of genetic data can be input into Structurama, and the program can assign individuals to population with or without the admixture. One shortcoming of genetic clustering approaches is that they do not assess the evolutionary divergence of population clusters (http://cteg.berkeley.edu/~structurama/index. html).

Gaussian Clustering (Hausdorf & Hennig 2010) groups samples into populations using genotypic data by searching for clusters that can be attributed to being mixtures of normal allele frequency distributions. Like Structurama, the method is flexible in terms of the data that can be analysed. This approach is implemented in R using the prabclus (Hausdorf & Hennig 2010) and mclust (Fraley & Raftery 2006) packages. As in other clustering approaches, temporal divergence among putative groups is not explicitly estimated.

The **general mixed Yule coalescent model** (GMYC; Pons et al. 2006) takes an ultrametric genealogy estimated from a single genetic locus as input. The method attempts to model the transition point between cladogenesis and allele coalescence by utilizing the assumption that the former will occur at a rate far lower than the later. This results in a shift in the rate of branching of the genealogy that reflects the transition between species-level processes (such as speciation and extinction) and population-level processes (allele coalescence). Reid & Carstens (2012) proposed a version of the GMYC that accounts for phylogenetic uncertainty gene tree estimates using a Bayesian analysis. Both implementations of the GMYC are likely to delimit well-supported clades of haplotypes as independent lineages and as such may be prone to over delimitation (http://r-forge.r-project.org/projects/splits, https://sites.google.com/site/noahmreid/home/software).

Choi & Hey (2011) describe two new methods for jointly estimating population assignment along with the parameters of an isolation-with-migration model. **Joint demography and assignment** (JDA) is applicable to an island or two population models, while joint demography and assignment of population tree (JDAP) is applicable to more than two diverging populations. Each takes sequence data as input and is implemented within IMa2 (Hey & Nielsen 2007) (http://genfaculty.rutgers.edu/hey/software).

Unlike other methods described here, the unified model of Guillot et al. (2012) can analyse nongenetic data (phenotypical, geographical, behavioural) in addition to genetic data. Their approach implemented a Bayesian clustering algorithm that assumes that each cluster in a geographical domain can be approximated by polygons that are centred around points generated by a Poisson process. Guillot et al.'s model is flexible in terms of the genetic data that it can utilize and capable of accurately delimiting species. Their model is available as an extension of the R GENELAND package (Guillot et al. 2005) (http://www2.imm.dtu.dk/~gigu/Geneland/).

O'Meara's heuristic method (O'Meara 2010) of species delimitation takes gene trees from multiple loci as input and operates under a similar assumption to the GMYC (namely that allelic coalescence occurs more rapidly than speciation). Provided that this assumption is true, the longest branches of gene trees are likely to represent species level differences, and thus, congruence across loci is indicative of both the species tree and the population assignments. O'Meara's method is implemented in the Brownie package (O'Meara 2008). Because this method takes gene trees as input, its accuracy will likely be correlated with the nodal support values in the gene trees (http://www.brianomeara.info/brownie).

Species delimitation analyses that use the multispecies coalescent model compare the probability of trees with differing numbers of OTUs to identify optimal partitions of the data (e.g. spedeSTEM, BPP). Salter et al. (2013) extend this strategy to its maximum extent by calculating the probability of the phylogeny that treats individual samples as putative lineages. The putative lineages are then sequentially collapsed on the basis of which samples are most closely related, the probability of the species tree is recalculated,

and information theory (Burnham & Anderson 2002) is used to identify the optimal model of lineage composition. Thus, **spedeSTEM discovery** can be used to simultaneously delimit evolutionary lineages and assign samples to these lineages (http://carstenslab.org.ohiostate.edu/software.html).

2- Species validation approaches require the user to assign samples to putative lineages

The popular validation method **BPP** (Yang & Rannala 2010) implements a reversible jump Markov chain Monte Carlo (rjMCMC) search of parameter space that includes h, population divergence and estimated distributions of gene trees from multiple loci. The method takes sequence data as input and also requires the user to define the topology of the species tree. Given this information, the algorithm implemented in BPP then traverses the parameter space to compute the posterior probability of the proposed nodes of the species tree. While inaccurately specified guide trees can lead to false-positive delimitations, the accuracy of BPP does not generally appear dependent on its ability to estimate gene trees. As this manuscript was in review, an improvement to the rjMCMC was described (Rannala & Yang 2013) (http://abacus.gene.ucl.ac.uk/software.html).

The validation approach **spedeSTEM** was developed to test species boundaries in a system with existing subspecies taxonomy (Carstens & Dewey 2010). The approach computes the probability of the gene trees given the species tree for all hierarchical permutations of lineage grouping, and therefore, complex cases such as that described by Carstens and Dewey (four species with 1–4 described subspecies) can be evaluated. Because the –lnL (ST|GTs) is computed directly by STEM (Kubatko et al. 2009), rather than estimated, phylogenetic uncertainty in the species tree does not affect species delimitations. However, accuracy of spedeSTEM is dependent on the quality of the genetree estimates (http://carstenslab.org.ohio-state.edu/software.html).

To avoid these problems of delimiting species boundaries, another strategy called the integrative taxonomy has been proposed (Samadi & Barberousse 2015; Pante et al., 2015). Integrative taxonomy consists in analyzing different characters, with different methods, and applying different criteria of species delimitation to propose species hypotheses that are as robust as possible (Figure I.5). Recently, two bioinformatics tools based on a single locus approach: 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) have been extensively used in combination to detect species boundaries in many taxa (e.g.: snails (Prevot et al. 2013), copepods (Cornils and Held 2014), algae (Payo et al. 2012, Pardo et al. 2014), fish (Alo et al. 2013). Both methods

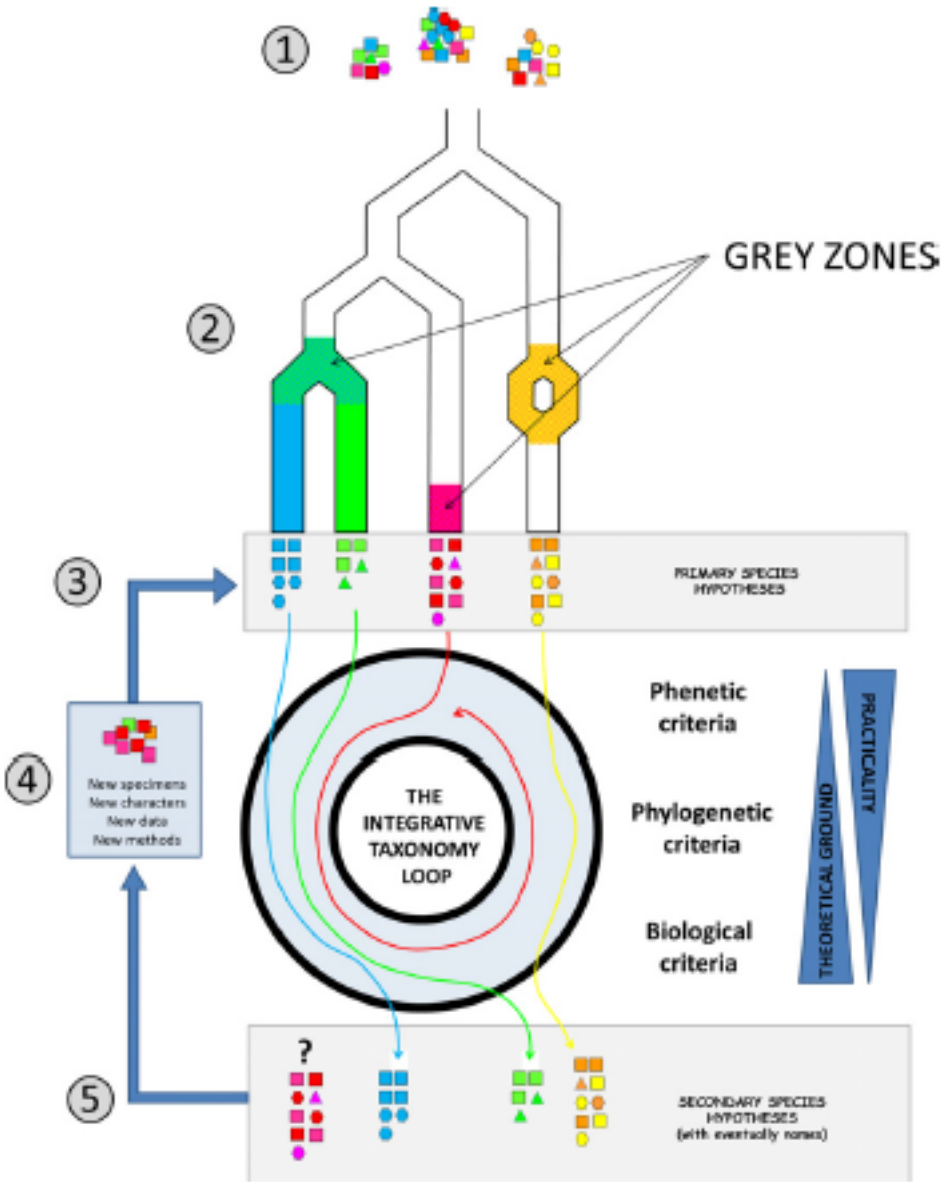


Figure I.5 - The integrative taxonomy loop (Figure taken from Pante et al., 2015). The different families of criteria are listed right of the loop, with the more theoretically grounded on the bottom, and the more operational (i.e. easy to test practically) on the top. Within each family of criteria, different kinds of characters (i.e. morphology, ethology, ecology, biochemical, genetic) and methods (i.e. distances, maximum parsimony, maximum likelihood, population genetics inferences, crossing experiments, observations) may be applied. The different steps are as follows. 1. Population and phylogenetic sampling. 2. Sampled species may be highly differentiated (blue and green), recently diverged species that are still in a 'grey zone' (see Figure I.4) with most characters undifferentiated (pink and red), or a single species that went through a temporal split into several temporary lineages (yellow/orange). 3. Primary species hypotheses (PSH) are proposed, for example using morphology or a single molecular marker. 4. PSH are engaged in the integrative taxonomy loop and are evaluated, possibly with the addition of new material, using different criteria for species delimitation. The more theoretically grounded biological criteria can be tested directly using cross-experiments or indirectly with unlinked markers, and complemented with more operational criteria. 5. When possible, taxonomic decisions are taken by turning PSH into secondary species hypotheses—SSH, and are named. Some lineages (i.e. the pink/red lineage) may stay in the loop, needing more conclusive data before being turned into SSHs. Most of the literature and methods for species delimitation focus on species that are currently in the grey zone (cf. Carstens et al. 2013, Box 2), even though most delimitation cases fall outside of this range.

have been described as complementary since they are based on different criteria to delineate species boundaries. Moreover, since all gene trees do not necessarily reflect the species tree (Wendel and Doyle 1998, Rubinoff and Holland 2005, Petit and Excoffier 2009), the study of disagreements between genes have often led to an enhanced understanding of the evolutionary history of taxa when various independent genes where examined. In sister taxa in which hybridization may be common, this pattern of incongruence due to introgression have been largely reported (e.g. see Cathey et al. 1998, Rieseberg et al. 1996, Neiva et al. 2010, Fehrer et al. 2007, Neiva et al. 2012).

DNA based methods associated with these bioinformatics tools for species delimitation and using various unlinked genes have been especially useful uncovering cryptic species in groups, such as algae, where classical taxonomy has been problematic, such as many algae (Leliaert et al. 2009, Tronholm et al. 2010, Payo et al. 2012, Tronholm et al. 2012, Leliaert et al. 2014, Pardo et al. 2014, Vieira et al. 2014). These newly discovered species have presented new opportunities to study important mechanisms of speciation, mate recognition and conservation planning.

However, next-generation sequencing tools can also generate significant advances in species delimitation and phylogenomics. Among the genomic approaches that are applicable to these fields, the usefulness of restriction site-associated DNA tag (RAD tag; Baird et al. 2008) sequencing has been recently investigated. This methodology allows gathering large-scale genome-wide data at moderate to low costs while only providing short sequences (~100–150 bp). These sequences flank the cutting sites of a restriction enzyme (or several enzymes) and RAD-seq generally yield thousands of loci distributed throughout the genome. This approach does not require a reference genome and can therefore be applied to non-model organisms. RAD-seq (Davey and Blaxter

2010) allows (1) creating phylogenetic datasets of unprecedented size (Eaton and Ree, 2013; Eaton, 2014; Escudero et al., 2014; Hipp et al. 2014; Takahashi et al. 2014), (2) genotyping thousands of SNP throughout the genome (Baird et al. 2008), (3) detecting hybridization and introgression among non-model organisms (Twyford and Ennos 2011, Eaton and Ree 2013), and (4) inferring species trees and species delimitation (Leaché et al. 2014). Therefore, RADseq may be a promising tool to assess species limits and phylogenetic relationships in closely related taxa for which traditional DNA sequence approaches have failed to provide well-supported solutions. RAD sequencing has proven useful in species delimitation and phylogeny reconstruction within recently and rapidly diverged groups (for example, Orobanchaceae flowering plants in Eaton and Ree, 2013; swordtails in Jones et al. 2013, *Heliconius* butterflies in Nadeau et al. 2013, fishes in Wagner et al. 2013, geckos in Leaché et al. 2014). Moreover, reconstructing the phylogeny of more distantly related taxa was also possible (for example, in Carabus beetles, Cruaud et al. 2014 and oak trees, Hipp et al. 2014). To date, only one study of phylogenomics in algae has been published (Fraser et al., 2016). This study aimed to clarify the species status of the sympatric species *Durvillaea chathamensis* and *D. antarctica* on Chatham Island (New Zealand). Previous studies based on molecular markers have suggested that the two species were genetically similar and the status of the former species was questioned. This study demonstrated a better resolution based on RAD-seq data identifying a case of recent or incipient speciation, which traditional approaches (e.g. Sanger sequencing of a few loci) were unable to detect or resolve.

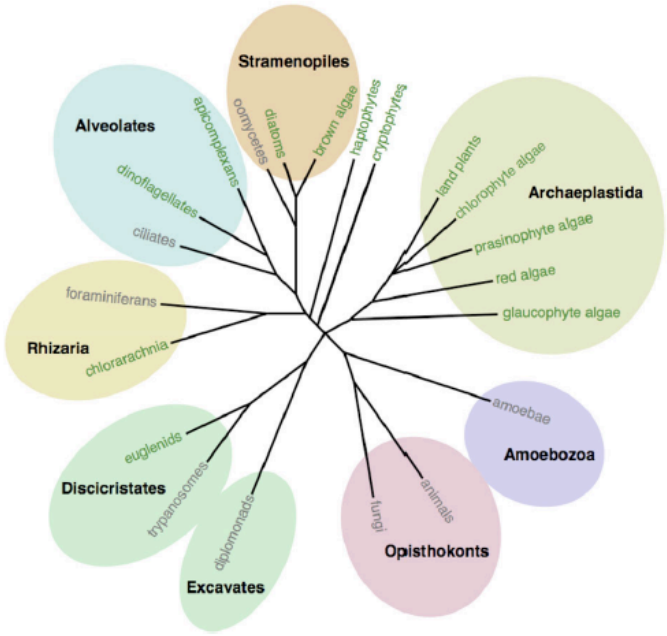


Figure I.6 - Simplified tree including all eukaryotic organisms (Figure taken from Cock and Coelho 2011 that was adapted from Baldauf 2008). Groups including photosynthetic organisms are indicated by green lettering. A single primary endosymbiotic event was at the origin of the plastids of all the members of the archaeplastida. Photosynthetic species within the stramenopiles, alveolates, haptophytes, cryptophytes, chlorarachnia, and euglenids obtained their plastids through multiple secondary or tertiary endosymbiotic events.

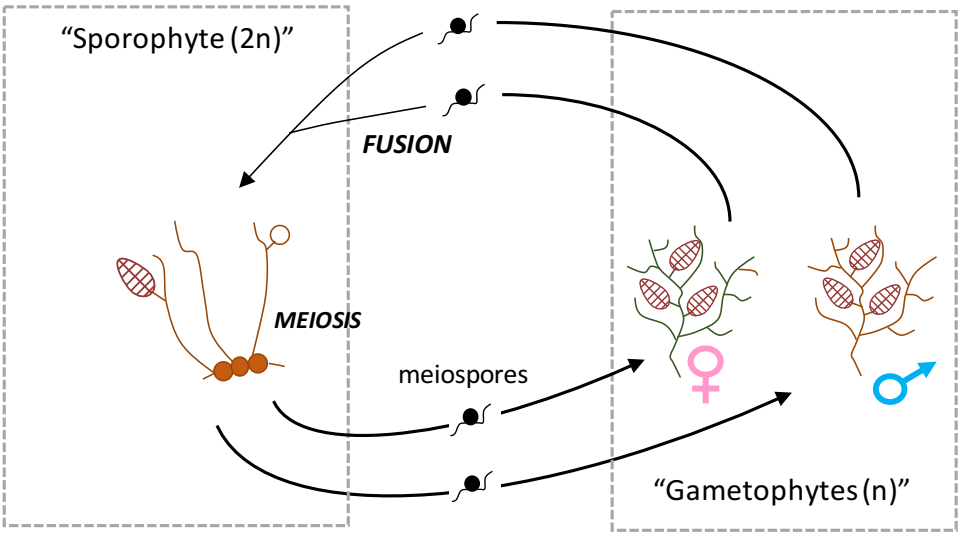


Figure I.7 – Sexual life cycle of *Ectocarpus spp.* modified from Le Bail et al. (2011). It involves an alternation between the diploid sporophyte and haploid, dioecious (male and female) gametophytes. The sporophyte produces meio-spores through meiosis in structures named unilocular sporangia. The meio-spores are released and develop as gametophytes. The gametophytes produce gametes by mitosis in structures named plurilocular gametangia. Fusion of male and female gametes produces a zygote, which develops as a diploid sporophyte, completing the sexual cycle.

III. Model of study: the genus *Ectocarpus*

The Phaeophyceae or brown algae, is a large group of mostly marine multicellular algae, including seaweeds of high economic and ecologic importance in both hemispheres. Brown seaweeds represent important resources with a wide range of uses in the food, cosmetic, and fertilizer industries and are attracting increasing attention as a source of active biomolecules (McHugh 2003). The brown algae represent one of only five eukaryotic lineages (the other four being animals, fungi, green plants, and red algae) where complex multicellularity have evolved independently. However, most biological research has been focused on biological models developed in vascular plants and animals and many gaps remain in other, much less studied lineages, including the brown algae (Figure I.6). Many interesting features of the brown algae stem from their phylogenetic distance from classical models of biology (See Stramenopiles, Figure I.6). Moreover, the alternation between gametophyte and sporophyte (Figure I.7), which involves sequential development of two independent complex multicellular organisms of different ploidy, represents a novel situation compared with the life cycles of classical model organisms, in which the gametophyte generation is usually highly reduced or absent (e.g., vascular plants and animals) (reviewed in Charrier et al. 2008).

During the last decades, genome sequencing of model organisms (*Arabidopsis thaliana* Kaul et al. 2000, *Caenorhabditis elegans* Kamath et al. 2003 and *Drosophila melanogaster* Adams et al. 2000) have largely benefited the biological research focusing on animals and higher plants. Recently, *Ectocarpus sp.,* a filamentous brown algae of the family Ectocarpaceae (Figure I.8) was selected as the model species for genetics and genomics of brown algae. In the field, species of the genus *Ectocarpus* are encountered in marine and estuarine habitats of temperate regions in both hemispheres (Stache

1990). They are found as short-lived annuals colonizing abiotic substrata or growing as epiphytes on macrophytes (Figure I.8c).

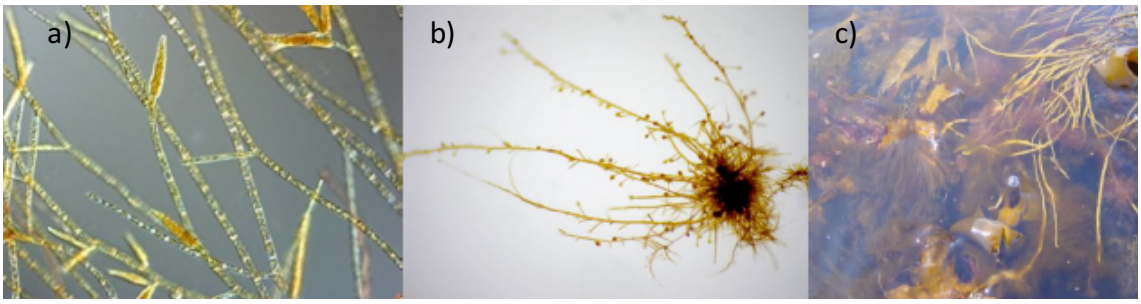


Figure I.8 – Morphology and habit of Ectocarpus spp. (in the laboratory a and b. In the field epiphyte on *Himanthalia elongata* and *Laminaria hyperborean*, c)

The habitat of *Ectocarpus* includes the subtidal up to high intertidal pools (Russell 1967a, b, 1983a, b). The choice of *Ectocarpus* as a biological model species (see reviews in Peters et al. 2004; Charrier et al. 2008) was based on its long history of use as an experimental organism in the laboratory because of its small size and because its entire life cycle can be completed, in Petri dishes within 3 months. The genome of one *Ectocarpus* species (200 Mbp), named at that time *E. siliculosus* (see following paragraph), has been fully sequenced and annotated (Cock et al. 2010). The establishment of this model has largely helped to increase our knowledge on brown algae, for example, in sex determination (e.g. Ahmed et al. 2014), sexual dimorphism (Lipinska et al. 2016), life cycle (Coelho et al. 2011), development (Le Bail et al. 2011), etc. However, despite all these advances, population genetics studies in this genus are still scarce (but see Couceiro et al. 2015) and many questions are still open, such as (1) how many different species can be distinguished within the genus *Ectocarpus*? (2) How are these species distributed? (3) Do they co-occur in the field? (4) What is the level of hybridization? (5) What are the mechanisms of reproductive isolation?

Descriptions of species in the genus *Ectocarpus*, was based on morphology and have been controversial. The first classification based on the branching pattern and the sporangium shape (Hamel 1931-1939), suggested the existence of five species clustered into two major groups: the *Ectocarpus* sections "*siliculosi*" and "*fasciculati*". Conversely, Cardinal (1964) described four species with seven varieties in *E. siliculosus* and three varieties in *E. fasciculatus* based on field material from the French Channel coast. On the other hand, Russell (1966, 1967b) using isolates collected along the French coast of Brittany, showed that sporangium morphology, formerly used to distinguish species in the *E. siliculosus* complex in previous studies, was not informative. However, branching pattern consistently revealed a difference between thalli with conspicuous main axes and thinner, often fasciculate, laterals (*E. fasciculatus*) and thalli showing subdichotomous branching (*E. siliculosus*). Based on the works of Russell (1966, 1967b), the number of species in *Ectocarpus* worldwide was then consensually reduced to two species. Laboratory crosses presented in the work of Müller and Eichenberger (1995) supported this proposal. Indeed, using gametophytes of *E. siliculosus* and *E. fasciculatu*s derived from sporophytes collected along the French coast of Brittany, Müller and Eichenberger (1995) showed that intraspecific crosses gave viable zygotes, which developed into fertile sporophytes. Conversely, interspecific crosses were unsuccessful. Gamete fusions did not occur between female gametes of *E. fasciculatus* and male gametes of *E. siliculosus* and the authors proposed the existence of a possible prezygotic barrier between the two species. However, hybrid zygotes were formed in the reciprocal crosses "female *E. siliculosus* x male *E. fasciculatus*" but died soon after germination. This outcome pointed to the existence of a porous prezygotic barrier with a level of leakage depending on the strains involved in the crosses and the existence of postzygotic barriers when fertilization occurs.

Biochemical studies comparing the two species reported the presence of the chemotaxonomic marker betaine-lipid diacetylglycerylhydroxymethyltrimethyl-/β-alanine in cultures of *E. fasciculatus* but not in the ones of *E. siliculosus*, seemingly supporting the biological findings based on crosses and morphology (Müller & Eichenberger 1995).

Even if all evidences supported the existence of these two highly diverging species/groups, doubts had been raised about their status: are there only two species of *Ectocarpus* worldwide or are *E. fasciculatus* and *E. siliculosus* in reality two clades including various cryptic species? Evidences supporting the second idea have been advanced. Indeed, complex patterns of cross-fertility have been reported between strains identified morphologically as *E. siliculosus* but characterized by different geographical origins (Müller 1976). *E. siliculosus* strains have been described as often but not always cross-fertile. For example, prezygotic barriers were evidenced among populations of *E. siliculosus* from NE America (Müller 1976). However, crossfertility experiments between strains of *E. siliculosus* isolated from different hemispheres suggested the presence of post-zygotic barriers. Zygotes from such crosses showed either a reduced development or normal growth but inhibition of meiosis (Müller 1977, 1979, 1988, Stache 1990). Based on these results Müller & Kawai (1991) proposed that all the isolates of *E. siliculosus* are part of a single species showing a world-wide distribution and encompassing many geographically separated populations that show full or slightly reduced interbreeding.

More recently, studies using molecular tools aimed to describe the species diversity within the genus *Ectocarpus*. First, Stache-Crain et al. (1997) carried out a phylogenetic analysis (Figure I.9) using 43 *Ectocarpus* strains (which were isolated on all continents except Antarctica) using the internal transcribed spacer (ITS1, nrDNA)

and the Rubisco spacer (cpDNA). This study supported the principal division of *Ectocarpus* into two major clades: *E. siliculosus* and *E. fasciculatus*. Within the clade *E. siliculosus*, various lineages separated by large genetic distances were evidenced, suggesting the existence of cryptic species in *Ectocarpus* (Figure I.9).

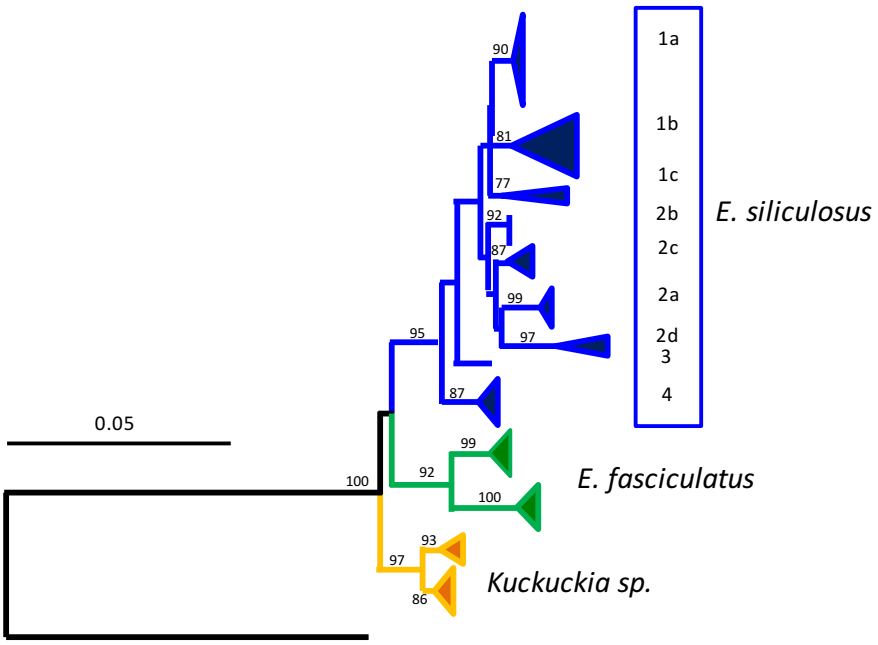


Figure I.9 – Simplified phylogenetic tree (modified from Stache-Crain et al. 1997, tree reconstructed using neighbor-joining distance) based on sequence alignments of the RUBISCO spacer region from 52 strains of *Ectocarpus*, *Kuckuckia*, and outgroup species sampled worldwide. *Pylaiella* sp. was used as an outgroup. Values on branches correspond to bootstrap support (majority rule ≥ 50% over 100 replicates). Blue branches represent the section siliculosi (codes 1a to 4 represent the names of each lineage), green and yellow branches represent *E. fasciculatus* and *Kuckuckia* clades respectively

Second, Peters et al. (2010a) using three additional markers (*cox*3 and *rps*14-atp8, both from mtDNA and ITS2, nrDNA) and samples isolated from Brittany (France) confirmed the existence of the two main clades (sections *fasciculati and siliculosi*) and evidenced at least five highly divergent lineages within *E.* section *siliculosi*. Peters et al. (2010a) proposed to reinstate the name *E. crouaniorum* for the samples of the clade 2c (see Stache-Crain et al. 1997, Figure I.9). *E. crouaniorum* had been recognized as a distinct species by Hamel (1931–1939) but was synonymized with *E. siliculosus* and considered only as morphotypes showing different growth habits by several authors since then (e.g. Cardinal 1964). Crossing experiments among *E. siliculosus* and *E.*

crouaniorum formed viable hybrids in only one out of 12 experiments, but the meiosis was not completed in the hybrids and abortive unilocular sporangia were formed (Peters et al. 2010b). These results are reminiscent of the ones described for crosses between *E. siliculosus* strains from geographically distant origins (Müller 1988). Peters et al. (2010b), using the same four markers as in Peters et al (2010a), confirmed the presence of most lineages obtained in Stache-Crain et al. (1997) in Southern Peru and Northern Chile. They have also shown that the sequenced strain (lineage 1c in Stache-Crain et al. 1997) is part of the *Ectocarpus* subclade '*siliculosi*' but forms a lineage clearly separated from *E. siliculosus* and *E. crouaniorum*. Finally, using a single-locus approach (COI-5P) on samples from North West France, Mediterranean Sea and Asia, Peters et al. (2015) reported again several divergent lineages including the ones previously described in Stache-Crain et al. (1997) and another 14 additional lineages, all 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. These molecular findings showed the occurrence of different lineages within *Ectocarpus* section "*siliculosi*" that probably represent cryptic species but the number, distribution and evolutionary history of such lineages is still unclear.

Increasing even more the complexity of species recognition and delimitation in the *Ectocarpus* section "*siliculosi*", natural hybridization has been evidenced between divergent lineages (Peters et al. 2010a). Putative field hybrids were identified based on PCR-amplification of ITS, which differs in length between the two species; hybrids showed both bands. However, the level and importance of hybridization and the mechanisms underlying speciation between these two species is still an open question. Indeed, it was not possible to study the potential level of hybridization among the

different lineages of *Ectocarpus* section "*siliculosi*" in previous studies due to the low number of markers and the low number of samples used.

Thus, we chose the genus *Ectocarpus* as a study model to investigate species delineation, hybridization and reproductive isolation in this complex of cryptic species. Unlike most model organisms to date (with the exception of the yeast *Saccharomyces cerevisiae* and the liverwort *Marchantia polymorpha*), species of the genus *Ectocarpus* are characterized by a haploid–diploid life cycle involving an alternation between two independent multicellular generations and both asexual and sexual reproduction are also possible in these species (Figure I.7). Undoubtedly, the study of speciation and hybridization based on a model with a complex life cycle, different from classical systems, will increase our knowledge in the evolution of reproductive isolation (see section II, above).

IV. Objectives of the PHD thesis

The general objective of my PHD thesis is to delineate species and study hybridization success and the process of speciation within the group *Ectocarpus* section *siliculosi*. The first chapter clarifies the number of species within the *Ectocarpus* section *siliculosi*. The second chapter studies phylogenomics and species delimitation at the whole genome level using NGS (i.e. ddRAD-seq) within this species complex. Finally, the third chapter studies the importance and level of hybridization among *E. siliculosus* and *E. crouaniorum*. In order to fulfill these objectives, I used several molecular markers and a combination of phylogenetic, population genetic and population genomic approaches. Moreover, an extensive sampling of *Ectocarpus* was realized along the Chilean and European coasts during this project and a comprehensive collection of *Ectocarpus* strains is now available for the scientific community.

In the first chapter, in order to clarify the number of cryptic species within the *Ectocarpus* section *siliculosi*, I used two unlinked loci (i.e. COI-5P, mitochondrial and ITS1, nuclear DNA markers) and an integrative approach developed to delimit species. A collection of 729 specimens sampled mainly along the European and Chilean coasts have been used. As a first approximation, we searched traces of natural hybridization and introgression in this group by examining incongruences between the independent nuclear and mitochondrial markers. In addition, phylogeographic patterns, range and depth distributions of the most common *Ectocarpus* species were reported. In the second chapter we have carried out RAD-sequencing on 322 strains of the different cryptic species identified in chapter 1 in order to better resolve their phylogenetics relationships using 13,859 polymorphic SNP. The idea was also to use SNP data to investigate the genomic pattern of introgression between these species. However, because of technical reasons, only the phylogenomics approach has so far been

completed yet. In the third chapter, I studied the potential importance of hybridization in the field between the two most common species of the *Ectocarpus* section *siliculosi* group: *E. siliculosus* and *E. crouaniorum* that belongs to two highly divergent clades. The study was based on a collection of more than 900 uni-algal cultures sampled in eight sites located along the European coast. Information from species-specific nuclear and cytoplasmic markers jointly with 9 microsatellite loci was combined to determine if sampled individuals belonged to one of the parental species or were potential hybrids. We then determined how common where haploid and diploid hybrids and described their genetic composition. Ultimately our results help in better understanding the geographical framework of hybridization between *E. siliculosus* and *E. crouaniorum* and the relative importance of pre and post-zygotic barriers in this haploid-diploid genus. In this study, we have not only used classical data treatments to test for the existence of hybrids but have also specifically developed a new Bayesian method, named XPloidAssignment, to assign genotyped individuals to parental species and different genetic classes of hybrids. The new method can be applied to data sets with varying degrees of ploidy between individuals or loci.

Chapter 1

Species delimitation and phylogeographic analyses in the *Ectocarpus* subgroup *siliculosi* (Ectocarpales, Phaeophyceae)

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J. Phycol. *, ***–*** (2016)
© 2016 Phycological Society of America
DOI: 10.1111/jpy.12452

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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Editorial Responsibility: M. Cock (Associate Editor)

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

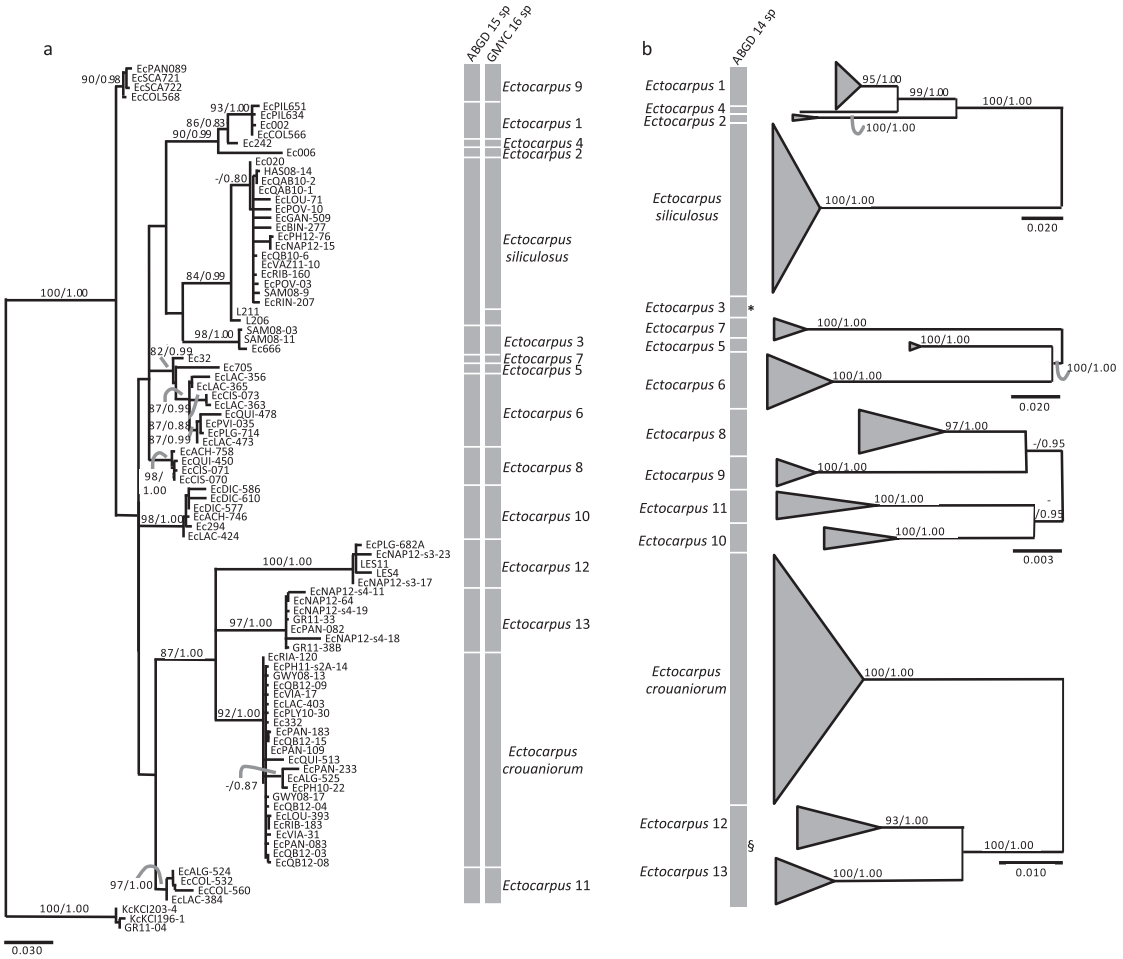

Fig. 1. Proposed species in the *Ectocarpus siliculosi* group using COI-5P (a) and their consolidation using ITS1 (b). Statistical support values >75 and posterior probabilities >0.80 are shown on branches. The asterisk in Figure 2b indicates the sequences of *Ectocarpus* 3 that were unalignable with 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.

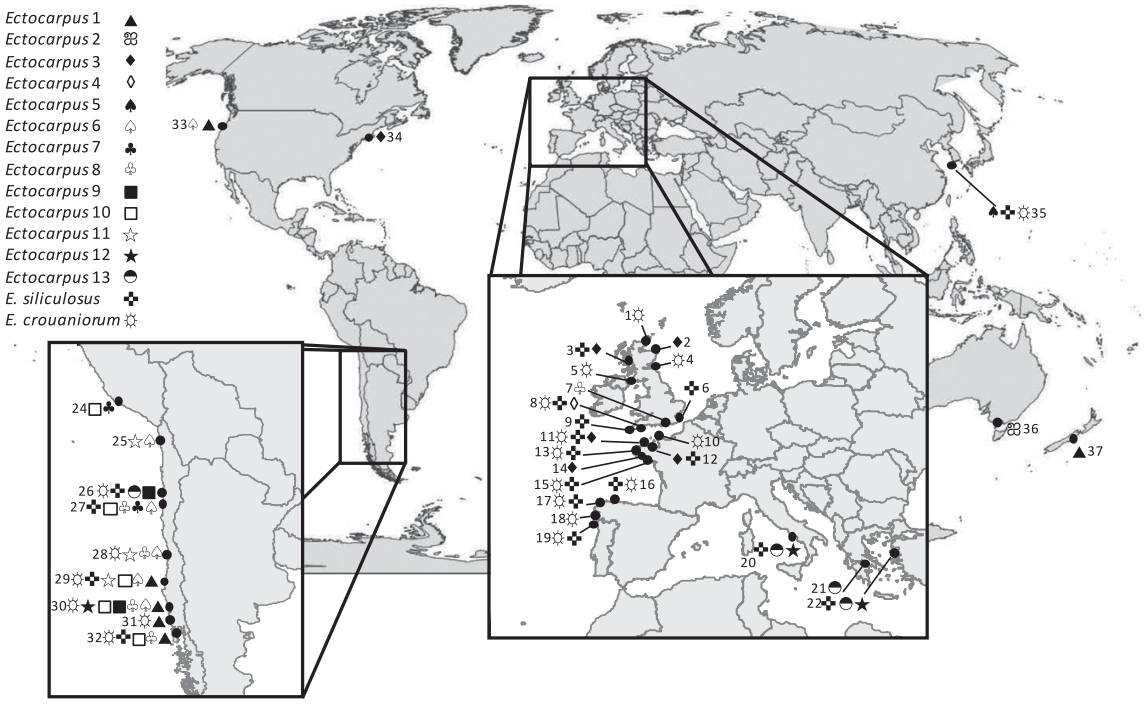

FIG. 2. Distribution of the 15 species of the *Ectocarpus* subgroup *siliculosi* as defined according to the mitochondrial marker COI-5P. Sites are numbered as in Table 1.

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).

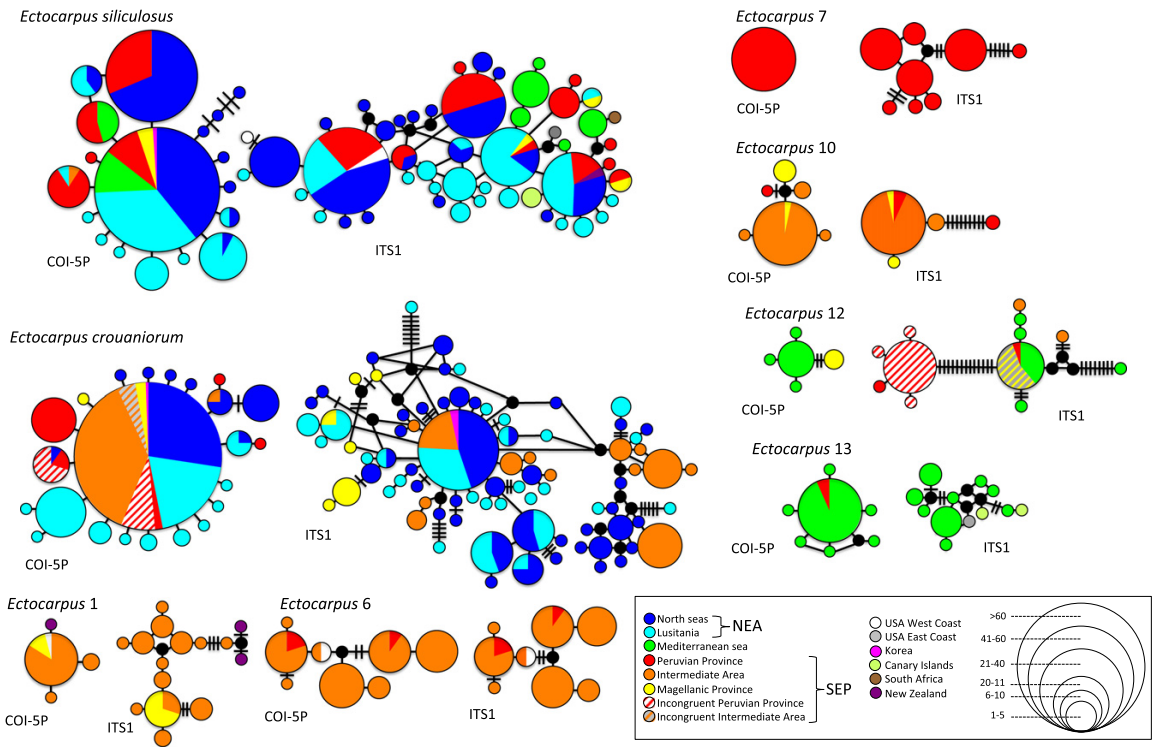

FIG. 3. Haplotype networks of for COI-5P and ITS1 for eight *Ectocarpus* spp. Biogeographical region of origin (within the South-East Pacific [SEP] as defined by Camus 2001 and within the North-East Atlantic [NEA] as defined by Spalding et al. 2007) of the samples are represented as different colors 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

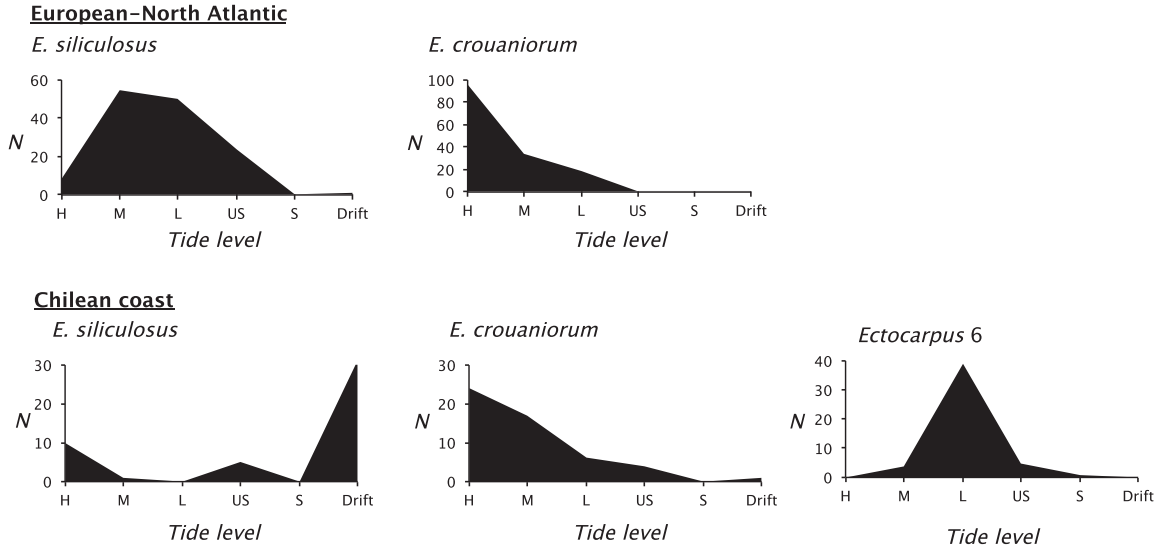

Fig. 4. Distribution on the shore of the commonest *Ectocarpus* species in the North Atlantic-European and Chilean coasts. The numbers of samples and the zones where the samples were collected are indicated (H, high intertidal; M, mid intertidal; L, low intertidal; US, Upper subtidal; S, Subtidal; Drift,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

We are particularly grateful to C. Destombe, S. Faugeron, A. Mann, D.G. Müller, P. Murúa, D. Patiño, D. Schroeder, J. West and R. Westermeier, who helped by collecting samples. The authors thank Biogenouest Core Facility for technical assistance and the members of Grupo BioCost of Coruña University for advice and logistic support during sampling in Galicia and North Portugal. We also thank to S. Mauger for assistance in the molecular laboratory and L. Dartevelle and J. Coudret for help in maintaining strains in the Station Biologique de Roscoff. We particularly appreciated insightful and constructive comments from C. Destombe and J. M. Cock. Principal funding came from a doctoral grant to A. E. Montecinos (Becas-Chile, CONICYT, advanced human resources program), additional support from the projects IDEALG (France: ANR-10-BTBR-04), "Bibliothèque du vivant" (France: INRA-MNHN-INEE-CNRS) and Bi-Cycle (France: ANR10-BLAN-1727), the EU FP7 "capacities" specific program ASSEMBLE (grant no. 227799), the EU Interreg program France (Channel)—England (project Marinexus), the TOTAL Foundation (Project "Brown algal biodiversity and ecology in the Eastern Mediterranean Sea"), and a Ray-Lankester Fellowship (UK: Marine Biological Association, Plymouth) to A. F. Peters. The work was carried out within the context of the international research network "Diversity, Evolution and Biotechnology of Marine Algae" (GDRI No. 0803). The authors have declared that no competing interests exist.

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Appendix I.1

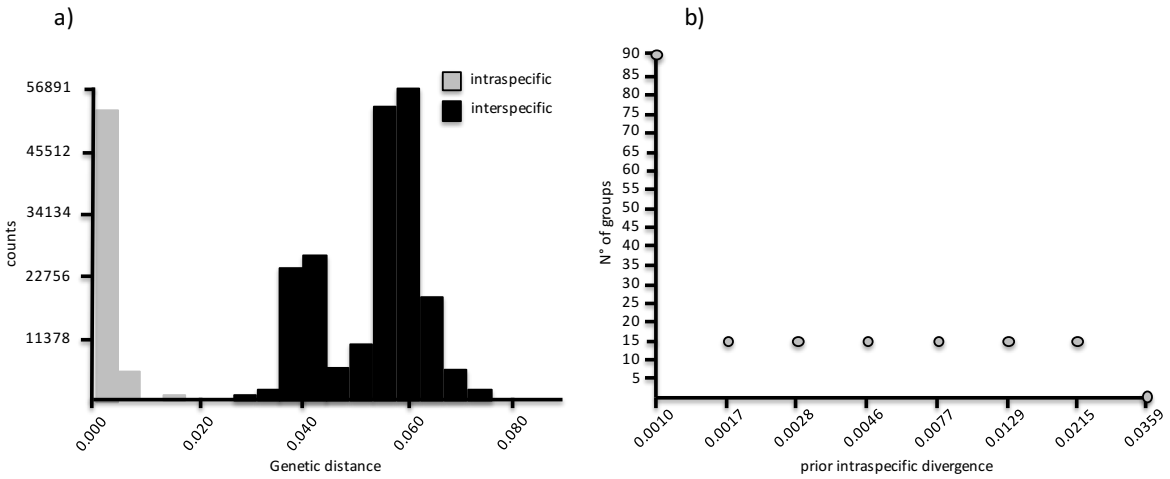

Figure S1. Distribution of pairwise distances for the marker COI-5P and automatic barcode gap discovery (ABGD) results. (a) Frequency distribution of K2P distances between haplotype pairs for the marker COI-5P. (b) ABGD results showing the number of groups (primary partitions) obtained for a range of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

Appendix I.2

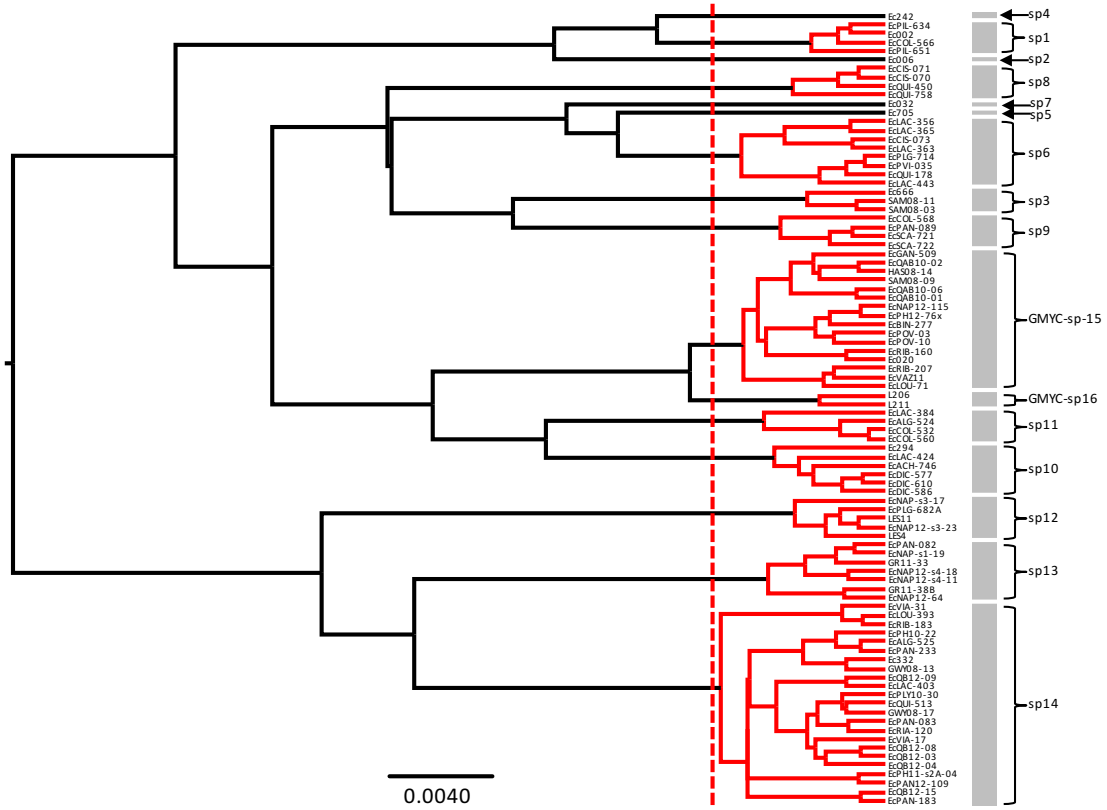

Figure S2. Ultrametric Bayesian tree showing the results obtained using the GMYC single threshold based on COI-5P. The vertical dotted red line shows the point of transition between coalescence and speciation processes.

Appendix I.3

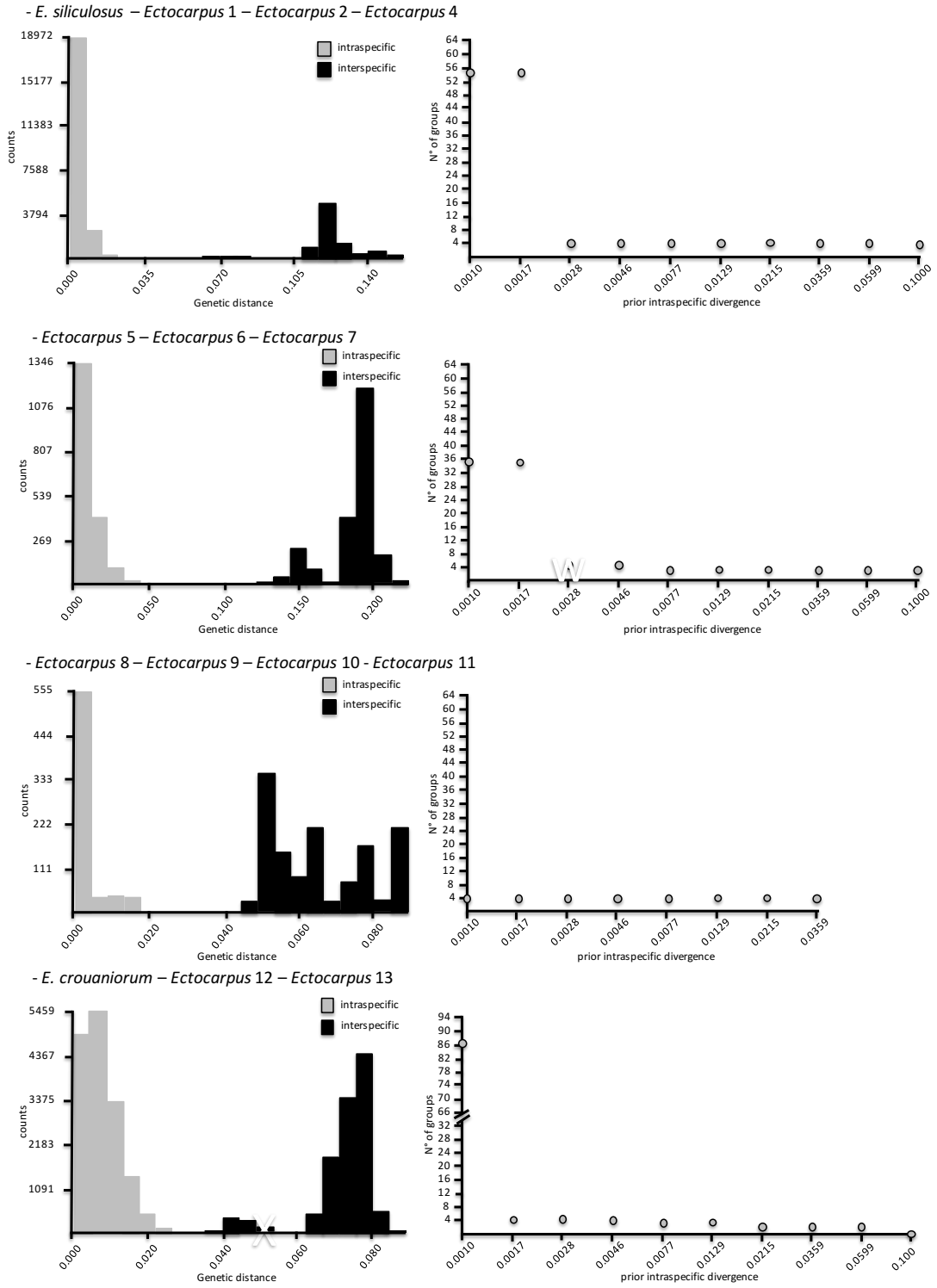

Figure S3. Distribution of pair-wise distances between haplotype pairs for the marker ITS1 (left) and automatic barcode gap discovery (ABGD, primary partitions) results for the four different alignments (right). Sub-alignment 1: *E. siliculosus*, *Ectocarpus* 1, *Ectocarpus* 2, *Ectocarpus* 4; sub-alignment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

Appendix I.4

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.

			COI-5P					ITS1				
Region	**Site**	**Code**	**N**	***nH***	***H* (SD)**	***π* (SD) (.10^{-2})**	***S***	**N**	***nH***	***H* (SD)**	***π* (SD) (.10^{-2})**	***S***
United Kingdom	Dunstaffnage	DUN	4	2	0.500 (0.265)	0.166 (0.164)	2	4	3	0.833 (0.222)	0.343 (0.279)	4
	Pett level	PET	10	3	0.378 (0.181)	0.066 (0.076)	2	10	4	0.778 (0.091)	0.450 (0.288)	7
	Plymouth	PLY	23	2	0.403 (0.091)	0.067 (0.072)	1	23	6	0.739 (0.064)	0.344 (0.217)	8
	Restronguet	RES	12	3	0.591 (0.108)	0.116 (0.106)	2	12	6	0.849 (0.074)	0.333 (0.221)	9
	Total UK		**49**	**5**	**0.501 (0.060)**	**0.102 (0.091)**	**5**	**49**	**12**	**0.850 (0.029)**	**0.468 (0.273)**	**13**
France	Roscoff	ROS	16	2	0.125 (0.106)	0.021 (0.038)	1	16	10	0.892 (0.063)	0.445 (0.274)	13
	Traezh Hir	THZ	15	4	0.619 (0.120)	0.221 (0.163)	4	15	4	0.619 (0.120)	0.438 (0.271)	11
	Quiberon	QUI	14	2	0.363 (0.130)	0.060 (0.069)	1	14	4	0.648 (0.116)	0.344 (0.224)	6
	Total France		**45**	**5**	**0.549 (0.065)**	**0.123 (0.103)**	**5**	**45**	**16**	**0.848 (0.040)**	**0.534 (0.305)**	**19**
NW Iberia	Ribadeo	RIB	13	3	0.615 (0.078)	0.140 (0.119)	3	13	4	0.692 (0.115)	0.314 (0.209)	7
	Coruña	COR	13	3	0.295 (0.156)	0.051 (0.063)	2	13	8	0.808 (0.113)	0.321 (0.213)	12
	Viana	VIA	4	2	0.500 (0.265)	0.166 (0.164)	2	4	4	1.000 (0.177)	0.293 (0.246)	4
	Total Iberia		**30**	**6**	**0.529 (0.095)**	**0.121 (0.103)**	**7**	**30**	**13**	**0.862 (0.045)**	**0.338 (0.211)**	**18**
Mediterranean	**Naples**	**NAP**	**9**	**2**	**0.389 (0.164)**	**0.065 (0.075)**	**1**	**9**	**2**	**0.389 (0.164)**	**0.057 (0.066)**	**1**
Chile	Pan de Azúcar	PAN	10	2	0.356 (0.159)	0.118 (0.109)	2	10	2	0.200 (0.154)	0.029 (0.045)	1
	Caldera	CAL	29	5	0.665 (0.067)	0.169 (0.130)	5	29	10	0.796 (0.062)	0.647 (0.366)	16
	Concepción	CON	1	1	0	0	0	1	1	0	0	
	Achao	ACH	4	1	0	0	0	4	4	1.00 (0.177)	0.606 (0.453)	7
	Total Chile		**44**	**5**	**0.748 (0.028)**	**0.237 (0.164)**	**5**	**44**	**12**	**0.853 (0.030)**	**0.653 (0.364)**	**18**
		TOTAL	**177**	**15**	**0.706 (0.026)**	**0.177 (0.013)**	**15**	**177**	**41**	**0.911 (0.011)**	**0.596 (0.330)**	**47**

Appendix I.5

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.

				COI-5P				ITS1				
Region	**Site**	**Code**	**N**	***nH***	***H* (SD)**	**π (SD) (.10^{-2})**	**S**	**N**	***nH***	***H* (SD)**	**π (SD) (.10^{-2})**	**S**
United Kingdom	Wick	WIC	8	1	0	0	0	8	7	0.964 (0.077)	0.846 (0.507)	19
	Berwick	BER	11	2	0.436 (0.133)	0.072 (0.079)	1	11	5	0.855 (0.066)	0.234 (0.162)	5
	Mull of Galloway	MUL	12	3	0.318 (0.164)	0.055 (0.067)	2	12	9	0.939 (0.058)	0.626 (0.367)	20
	Plymouth	PLY	11	3	0.618 (0.104)	0.302 (0.212)	4	11	7	0.891 (0.074)	0.946 (0.538)	20
	Total UK		**42**	**6**	**0.474 (0.087)**	**0.164 (0.126)**	**6**	**42**	**23**	**0.938 (0.024)**	**0.728 (0.393)**	**36**
France	Roscoff	ROS	15	5	0.562 (0.143)	0.190 (0.145)	6	15	12	0.962 (0.040)	0.663 (0.379)	28
	Traezh Hir	THZ	9	3	0.556 (0.165)	0.101 (0.100)	2	9	7	0.917 (0.092)	0.493 (0.308)	16
	Quiberon	QUI	12	6	0.758 (0.122)	0.183 (0.145)	5	12	6	0.849 (0.074)	0.269 (0.176)	8
	Total France		**36**	**10**	**0.624 (0.092)**	**0.170 (0.130)**	**11**	**36**	**22**	**0.916 (0.037)**	**0.523 (0.295)**	**38**
NW Iberia	Ribadeo		8	1	0	0	0	8	5	0.893 (0.086)	0.694 (0.423)	14
	Coruña	RIB	5	3	0.800 (0.164)	0.166 (0.154)	2	5	5	1.000 (0.127)	0.753 (0.504)	15
	Ría de Arousa	COR	5	2	0.600 (0.175)	0.100 (0.109)	1	5	4	0.900 (0.161)	0.243 (0.191)	4
	Viana	VIA	2	2	1.000 (0.500)	0.332 (0.406)	2	2	2	1.000 (0.500)	1.453 (1.512)	12
	Total Iberia		**20**	**5**	**0.679 (0.080)**	**0.148 (0.121)**	**4**	**20**	**14**	**0.963 (0.026)**	**0.711 (0.396)**	**40**
Chile	Las Cruces	LAC	6	3	0.600 (0.215)	0.111 (0.112)	2	6	4	0.800 (0.172)	0.543 (0.359)	11
	Concepción	CON	7	2	0.571 (0.120)	0.095 (0.099)	1	7	5	0.905 (0.103)	0.984 (0.596)	16
	Valdivia	VAL	11	2	0.327 (0.153)	0.054 (0.066)	1	11	6	0.800 (0.114)	0.466 (0.286)	18
	Estaquilla	EST	24	1	0	0	0	24	8	0.808 (0.053)	0.668 (0.371)	14
	Achao	ACH	4	1	0	0	0	4	4	1.000 (0.177)	0.364 (0.285)	5
	Total Chile		**52**	**3**	**0.429 (0.059)**	**0.073 (0.073)**	**2**	**52**	**23**	**0.928 (0.019)**	**0.836 (0.444)**	**31**
		TOTAL	**150**	**18**	**0.583 (0.046)**	**0.154 (0.118)**	**18**	**150**	**68**	**0.953 (0.010)**	**0.759 (0.401)**	**80**

Appendix I.6

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.

		COI-5P					ITS1				
Site	**Code**	***N***	***nH***	***H* (SD)**	**π (SD) ($.10^{-2}$)**	***S***	***N***	***nH***	***H* (SD)**	**π (SD) ($.10^{-2}$)**	***S***
Pisagua	PIS	1	1	0	0	0	1	1			0
Caldera	CAL	2	2	1.000 (0.500)	0.829 (0.908)	5	2	2	1.000 (0.500)	0.267 (0.377)	1
Quintay	QUI	9	4	0.750 (0.112)	0.525 (0.340)	7	9	5	0.806 (0.120)	0.781 (0.511)	11
Las Cruces	LAC	29	6	0.791 (0.048)	0.440 (0.269)	8	29	19	0.934 (0.034)	1.953 (1.046)	38
Concepción	CON	2	1	0	0	0	2	2	1.000 (0.500)	0.533 (0.653)	2
Valdivia	VAL	3	3	1.000 (0.272)	0.663 (0.564)	6	3	2	0.667 (0.314)	2.667 (2.104)	15
TOTAL		**46**	**8**	**0.822 (0.022)**	**0.511 (0.300)**	**10**	**46**	**25**	**0.897 (0.037)**	**1.615 (0.868)**	**45**

Appendix I.7

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

Montecinos et al.	Stache-Crain et al. 1997	Peters et al. 2010a	Peters et al. 2010b	Peters et al. 2015
E. siliculosus	1a	*E. siliculosus*	GT6-1a	*E. siliculosus*
E. crouaniorum	2c	*E. crouaniorum*	GT8-2c	*E. crouaniorum*
Ectocarpus 1	4	*E. siliculosus* (?)	4	4
Ectocarpus 2	4	---	---	4
Ectocarpus 3	3	---	---	3
Ectocarpus 4	---	---	---	---
Ectocarpus 5	---	---	---	1d
Ectocarpus 6	---	---	GT1	1c
Ectocarpus 7	1c	*E. siliculosus*	GT4-1c	1c
Ectocarpus 8	1b	---	---	---
Ectocarpus 9	1b	---	---	---
Ectocarpus 10	1b	---	GT2-1b	1b
Ectocarpus 11	---	---	GT3	1b
Ectocarpus 12	2b	---	GT8-2b	2b
Ectocarpus 13	2a	---	---	2a

Chapter 2

Phylogenetic inferences in the *Ectocarpus* subgroup *siliculosi* using a next-generation sequencing approach

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CHAPTER 2.

Phylogenetic inferences in the *Ectocarpus* subgroup *siliculosi* using a next-generation sequencing approach

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I. Abstract

The genus *Ectocarpus* (Lyngb.) Hamel (Ectocarpales, Phaeophyceae) is widely distributed in marine and estuarine habitats of temperate regions of both hemispheres. Recent studies based on few molecular markers and phylogenetic reconstructions have shown that the group *Ectocarpus siliculosi* forms a complex of sibling species. All studies agree in the occurrence of a strongly supported monophyletic group including *E. crouaniorum*, *Ectocarpus* 12 and *Ectocarpus* 13 but the phylogenetic relationships of the remaining 12 species were not fully resolved. In this work we aimed to test the capacity of unlinked nuclear loci obtained via RAD sequencing in resolving phylogenetic relationship within the *Ectocarpus* subgroup *siliculosi*. Our results retrieved a branching pattern concordant with the patterns recovered in the previous studies. Phylogenetics trees retrieved all eight species involved in this study as well-separated genetic groups. In addition, RAD sequencing was able to precise close relationships between species described before as paraphyletic. Moreover, occurrence of potential hybridization between closely related and sympatric species was suggested. Different patterns of geographic structure were demonstrated for two cosmopolitan species suggesting different scenarios of introduction. Together, these findings confirm the high power of RAD sequencing to resolve phylogenetics and population level structure.

II. Introduction

Sampling as many informative data from the genomes of as many taxa as possible to accurately and comprehensively infer phylogenetic relationships is one of the typical goals of molecular systematics. Thus, the selection of appropriate and sufficiently informative molecular markers has always been considered fundamental to phylogenetic reconstruction (Patwardhan et al. 2014). Generally, molecular systematic targets loci having desirable properties for phylogenetic inference that include (1) a high reliability of amplification and sequencing across the taxa of interest, (2) a confirmed orthology and (3) an appropriate rate of nucleotide substitution across the clades of interest. Historically, the study of only a few orthologous genes relative to the number of taxa was the rule. Traditional Sanger sequencing (Sanger et al. 1977) of plastid or mitochondrial DNA markers or nuclear ribosomal markers such as the internal transcribed spacer (ITS) have been widely applied to reconstruct phylogenies at the species and genus level of many taxa (e.g. plants, see review of Alvarez and Wendel 2003). However, due to the commonly uni-parental inheritance of plastids and mitochondria, phylogenies inferred from cytoplasmic markers are limited in their capacity to reflect the evolutionary history of a lineage (Hou et al. 2015). On the other hand, multi-copy nuclear markers such as ITS can mislead phylogenetic inferences because of concerted evolution (Alvarez and Wendel 2003). Searching for phylogenetically informative low-copy orthologous nuclear genes using traditional Sanger sequencing is a costly and laborious work and, even if they have been successfully applied to generate interspecific phylogenetic inference in some taxa (Zimmer and Wen 2013), their use remains limited.

Advances in next-generation sequencing (NGS) technologies are rapidly enhancing our capacity to study a plethora of markers over the whole genome (Andrews

and Luikart 2014). These advances present new opportunities and challenges for molecular systematics (Lemmon and Lemmon 2013, McCormack et al. 2013). In contrast to the traditional gene-centric approach, second generation short-read sequencing technology (e.g. Illumina and SOLiD) offers alternative methods to sample genome-wide nucleotide variation in the form of restriction site associated DNA (RAD) sequencing (Baird et al. 2008). RAD sequencing targets specific flanking regions of restriction sites. By producing short sequence reads (50–300 bp) with a wide genomic distribution, the method is expected to provide a genomewide view (Miller et al. 2007a, b, Baird et al. 2008, Davey et al. 2011, McCluskey and Postlethwait 2014). Since the data are sequence-based, RAD-seq loci can be mapped back to genetic or physical maps if appropriate reference genomes are available, potentially allowing researchers to assess the genomic distribution of divergence and introgression in a phylogenetic context (Nadeau et al. 2012, Hipp et al. 2014). RAD sequencing is one of the so-called "reduced representation" approaches that allow sampling a small but repeatable portion of the genome and have been proven a very cost effective methodology to generate high quality information of genetic diversity at thousands of identical loci for population level comparisons (Davey and Blaxter 2010). The ability of RAD sequencing in targeting repetitively the same portion of the genome, adjacent to the restriction sites, enables to select loci without access to previously develop genomic resources and to assess their orthology in silico (e.g. Catchen et al. 2011, Lu et al. 2013, Eaton 2014).

The use of RAD sequencing, which increases the number of unlinked molecular markers studied, can dramatically improve the accuracy of phylogenetic reconstruction when compared to traditional Sanger sequencing and gene-centric approaches (Rokas and Carroll 2005). Individual loci may have different evolutionary histories due to incomplete lineage sorting, gene duplication or loss, and processes of admixture such as

hybridization and introgression (Wendell 1998). RAD sequencing is then a promising tool to resolve phylogenetic relationships in taxa challenging the classical methods, as it is the case for recently diverging clades where reproductive barriers are incomplete. Indeed, a recent study based on a RAD-seq data set of more than 3 million base pairs (Wagner 2013), have successfully resolved the phylogenetic relationships among sympatric cichlid species in the Lake Victoria despite the fact that this group is characterized by recent adaptive radiation, incomplete lineage sorting and ongoing hybridization. However, RAD sequencing is not without limitations and new challenges have arisen when inferring phylogenies using these tools. Indeed, both sampling error (stochastic failure of a locus to be genotyped due to low read coverage) and the disruption of restriction sites by mutation lead to the recovering of sparse genotype matrices where missing data abound. The number of missing sites increases with the phylogenetic distance among the studied samples and the problem generated by missing data could be challenging for phylogenetic reconstruction using RAD sequencing (Rubin et al. 2012; Cariou et al. 2013; Hipp et al. 2014; Viricel et al. 2014). New methods to circumvent this problem have been proposed in recent studies. For example, the evaluation of phylogenetic reconstructions based on several matrices composed by different levels of missing data has been shown to be an excellent approach to evaluate the power of RAD-seq in phylogeny (e.g., Wagner et al. 2013, Hipp et al. 2014).

The genus *Ectocarpus* (Lyngb.) Hamel (Ectocarpales, Phaeophyceae) is widely distributed in marine and estuarine habitats of temperate regions of both hemispheres (Stache 1990). On the shore, *Ectocarpus* occurs from high intertidal pools to the subtidal and is found on abiotic substrata (rocks, wood, plastic, ship hulls), epiphytic on macroalgae or free-floating (Russell 1967a, b, 1983a, b). It has been described as an important contributor to biofouling and is frequently encountered as epiphyte in

mariculture (Stache-Crain et al. 1997). *Ectocarpus* has a long research history and the genome of one species of this genus (referred as *Ectocarpus* 7 in Montecinos et al 2016, Chapter 1, and the current study) has been completely sequenced, annotated (Cock et al. 2010) and mapped (Heesch et al. 2010).

Recent studies based on molecular markers and phylogenetic reconstructions have shown that the group *Ectocarpus siliculosi* forms a complex of sibling species adapted to different hosts and/or habitats along the shore gradient (Peters et al. 2010a,b, Peters et al. 2015, Montecinos et al 2016). The work of Montecinos et al. (2016) was able to uncover 15 cryptic species in the group *Ectocarpus siliculosi* using two loci (the mitochondrial COI - cytochrome oxidase subunit 1 and the nuclear ITS1) and a combination of bioinformatics tools specifically developed to define species boundaries. However, discrepancies between tree topologies are observed depending on the markers used (ITS1 and rubisco spacer: Stache-Crain et al. 1997, ITS1, ITS2, Rubisco spacer region, cox3 and rps14-atp8: Peters et al. 2010a, ITS1, ITS2, Rubisco spacer and cox3: Peters et al. 2010b, COI-5P: Peters et al. 20151, COI-5P and ITS1 Montecinos et al. 2016) when comparing results from different studies, and thus the phylogenetic relationships within the group *Ectocarpus siliculosi* have remained elusive. All studies agree only in the presence of a strongly supported monophyletic group consisting of *E. crouaniorum, Ectocarpus* 12 and *Ectocarpus* 13 (designations as in Montecinos et al. 2016). The study of Montecinos et al. (2016) included a large number of *Ectocarpus* specimens (N=729) but the markers chosen were not able to fully resolve the phylogenetic relationships of the remaining 12 species in the *E. siliculosi* group. Indeed, the 12 species formed an unresolved paraphyletic assemblage when using the mitochondrial marker. For ITS1, due to the presence of various large indels (a problem recurrent in this group, see Stache-Crain et al. 1997), it was not possible to generate a

unique alignment including all species and test for their relationship (Montecinos et al. 2016).

In this chapter we aimed to test the capacity of unlinked nuclear loci obtained via RAD sequencing in resolving phylogenetics relationship within the *Ectocarpus* subgroup *siliculosi*. The impact of historical biogeographical barriers along the Pacific coast on the process of divergence and speciation and the possible introduction events among the Pacific and Atlantic coasts will also be discussed.

Table II.1 – Individuals selected to generate the distinct dataset matrices. All 120 individuals were used to construct the M50-120 matrix while only 75 individuals (the ones in parentheses) remain in the M20-75 matrix.

Population	Country	Esil	Ecro	Ec1	Ec6	Ec7	Ec8	Ec9	Ec12	Ec13	Total
Roscoff	France	4 (3)	5 (4)								9 (7)
Ribadeo	Spain	4 (3)	4 (4)								8 (7)
Naples	Italy	4 (2)							8 (7)	14 (4)	26 (13)
Pan de Azúcar	Chile	4 (4)						5 (3)			9 (7)
Caldera	Chile	4 (1)			1 (1)	12 (11)	1 (0)				18 (13)
Las Cruces	Chile		3 (2)		12 (3)		1 (0)				16 (5)
Quintay	Chile				6 (6)		1 (0)				7 (6)
Valdivia	Chile		4 (2)	8 (3)							12 (5)
Estaquilla	Chile		4 (4)	11 (8)							15 (12)
Total		**20 (13)**	**20 (16)**	**19 (11)**	**19 (10)**	**12 (11)**	**3 (0)**	**5 (3)**	**8 (7)**	**14 (4)**	**120 (75)**

Ec1- Ec13 = *Ectocarpus* 1 – *Ectocarpus* 13, Esil= *E. siliculosus*, Ecro = *E. crouaniorum*.

III. Material and Methods

- *Collections of* Ectocarpus *strains*

We selected 120 (19 haploids and 101 diploids) *Ectocarpus* unialgal strains that were identified at the species level using various molecular markers (COI5P, ITS1, microsatellite loci, see Chapter 1 and Chapter 3). Nine of the fifteen putative species belonging to the *Ectocarpus* subgroup *siliculosi* (Montecinos et al. 2016) were selected; including: *Ectocarpus* 1 (n = 19), *Ectocarpus* 6 (n = 19), *Ectocarpus* 7 (n = 12), *Ectocarpus* 8 (n = 3), *Ectocarpus* 9 (n = 5), *Ectocarpus* 12 (n = 8), *Ectocarpus* 13 (n = 14), *E. siliculosus* (n = 20), *E. crouaniorum* (n = 20) (Table II.1). Only the more common species for which various samples were available were considered for this study. Samples were collected in different biogeographical regions in Europe and in Chile (the temperate South East Pacific). In Europe, phylogeographic studies have shown that populations from the Atlantic are highly differentiated from the Mediterranean ones and that in the Atlantic, the Celtic-Sea/Brittany area is a transition zone between the Northern European Seas and Lusitanian provinces (see for review Maggs et al 2008). Three sites were sampled in this study, one located in the Celtic-Sea/Brittany area (Roscoff), the second in Lusitanian region (Ribadeo) and the third in the Mediterranean Sea (Naples). In Chile, phylogeographic studies of seaweeds have confirmed that genetic breaks are broadly congruent with two major biogeographic boundaries along the South East Pacific (30-33°S and 42°S, see for review Guillemin et al., 2016a). The Peruvian biogeographic lies north of the 30-33°S boundary, the Magellan province south to the 42°S boundary and the Intermediate Area between the two boundaries (Camus et al. 2001). Six sites were sampled in Chile, of which two were located in the Peruvian Province (Pan de Azúcar and Caldera), three in the Intermediate

Area (Las Cruces, Quintay, Valdivia) and one at the northern limit of the Magellan Province (Estaquilla) (Table II.1).

- *DNA extraction and generation of RAD-seq data*

All individuals were grown in sterile standard seawater medium (Coelho et al. 2012) for 8 weeks under 12-hours light photoperiod at 13°C in a growth chamber. To ensure that almost fully axenic material will be obtained, 5 ml of a mix of antibiotics (9 mg/ml of Penicillin G, 4.5 mg/ml of Streptomycin and 0.9 mg/ml of chloramphenicol) (Coelho et al. 2012) were added to each liter of seawater used for the culture three days before the DNA extraction to reduce the bacterial load. Harvested individuals were lyophilized and DNA was extracted using the NucleoSpin® 96 Plant II kit (Macherey-Nagel GmbH & Co. KG, Germany) according to the manufacturer's instructions. DNA quality was checked on agarose gels and the quantity measured by PicoGreen® (Fisher Scientific).

Preparation of the double digest RAD library (ddRAD-seq) was carried out following Peterson et al. (2012) with a slight modification since sample tracking was ensured by using individual-specific nucleotide barcodes of 6 bp long (instead of the 5 bp long used in Peterson et al. 2012). The restriction enzymes *Pst*I and *Hha*I (New England Biolabs, https://www.neb.com/) were selected based on *in silico* digestion simulations carried out using the published genome of *Ectocarpus* 7 (Cock et al. 2010). Samples individually barcoded with a unique adapter were pooled prior to 150-600 bp size selection using a Pippin Prep 1.5% agarose gel (Sage Science) and AMPure XP beads (Agencourt, Beckman Coulter Genomics, Danvers, MA) cleaning. The generated libraries were quantified by both an *Agilent® 2100 Bioanalyzer®* (Agilent Technologies) and real-time PCR using the KAPA Library Quantification Kit (KAPA

Biosystems). Libraries with distinct multiplexing indices were then combined in equimolar ratios to compose a final pool of libraries for paired-end sequencing in two lanes of an Illumina Hiseq 2500 platform. Our targeted yield was to sequence ~27,000 fragments to a depth of coverage of at least 30X (Komlan Avia, com. pers.).

- *Analysis of ddRAD-seq data*

The ddRAD sequencing data were analyzed using the Stacks pipeline (Catchen et al. 2011, 2013). Raw sequence reads with ambiguous barcodes or restriction enzyme sites were discarded. The program Cutadapt (Martin 2011) was used to remove adapter sequences from raw reads. A sliding window of 25% of the length of a read was set to check sequence average quality and reads under 90% base call accuracy (phred score of 10) were discarded. We first used the program PEAR (Zhang et al. 2014) to identify paired-end non-overlapping reads and to merge reads from overlapping read pairs in a single consensus sequence. Unpaired reads (i.e. missing their mates) were leaved as singletons. For each individual, all those singletons and the new single consensus sequences were gathered in a unique group of singleton sequences and then trimmed to 94 bp with the program TRIMMOMATIC (Bolger et al. 2014). The paired-end read mates were also trimmed to 94 bp. Sequences were then aligned to the *Ectocarpus* reference genome (Cock et al. 2010; i.e. *Ectocarpus* 7 in Montecinos et al. 2016) using Bowtie 2 (Langmead and Salzberg 2012). End-to-end alignment with the "sensitive" mode was performed. Loci were discarded when two or more reportable alignment existed (i.e. potential paralogous sequences in the data). Loci that did not align with the reference genome (missing data) were also discarded. The obtained .sam files were imported into the Stacks pipeline (Catchen et al. 2013) to generate a final VCF file containing all observed SNPs, or the SAMtools package (Li et al. 2009) was used to call

variants with the mpileup function in conjunction with bcftools for multiallelic calling model. SAMtools was configured to generate VCF files that retain both variable and invariables sites. We used this configuration because models of molecular evolution used in maximum likelihood-based phylogenetic inferences are intended for sequence data, not SNPs alone, and the complete sequence of each fragment is better suited to reconstruct phylogenetic trees (Wagner et al. 2013). Indeed, while some studies still filter out invariant sites (e.g. Coghill et al. 2014), it has been observed that likelihood based tree inferences does not typically condition on all characters being variable, and thus may reconstruct biased branch lengths and topologies if invariant sites are excluded (Felsenstein 1992, Lewis 2001).

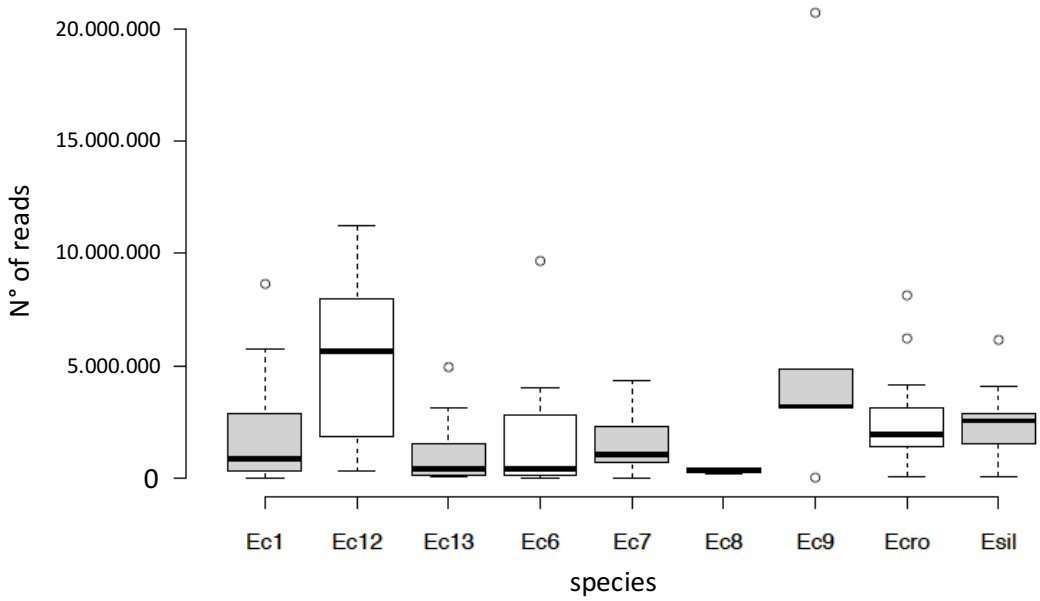

Figure II.1 – Boxplots depicting median, first and third quartile and standard deviation of the number of reads obtained per species. The number of haploid individuals varied from 1 (in *Ectocarpus* 8 and 13) to 11 (in *E. siliculosus*) and in diploid varied from 2 (in *Ectocarpus* 8) to 60 (in *E. siliculosus*).
Ec1- Ec13 = *Ectocarpus* 1 – *Ectocarpus* 13, Esil= *E. siliculosus*, Ecro = *E. crouaniorum*.

Depending on the percentage of missing data across samples allowed (0%, 10%, 30%, 50%, 70% and 90%, Table II.2), six data matrices were built using all 120

individuals. The matrix allowing 50% of missing data was chosen in the first analyses (M50-120) in order to minimize the quantity of missing data and maximize the number of sites included in the matrix. However, in our dataset, 45 individuals were characterized by a low number of reads (Figure II.1) and/or a low number of loci that were successfully aligned to the reference genome. Thus, a new matrix was built (M20-75) by excluding those 45 individuals from the previous matrix allowing 20% of missing data for phylogenetic reconstructions (Table II.1). Please note that the M20-75 matrix did not include individuals of *Ectocarpus* 8 (Table II.1). We used the script vcf-tab-to-fasta (Bergey 2012) to convert the .vcf files in fasta alignments.

Table II.2 – The total number of sites, the number of invariable and variables sites incorporated within the distinct dataset matrices built during the study. For the six matrices including the whole data set of 120 individuals from nine species belonging to the *Ectocarpus* subgroup *siliculosi* (MXX-120), the number of sites included varies depending on the percentage of missing data (i.e. sites not present in the alignment) allowed. The percentage of missing data, number of invariable and variable sites were recalculated for M20-75 after removing the 45 individuals characterized by a low number of reads and/or a low number of loci that were successfully aligned to the reference genome (see Table II.1).

Name	% Missing data	Sites	Sites invariable	Site variables
M0-120	0	0	0	0
M10-120	10	93	61	32
M30-120	30	5538	2945	2593
M50-120	50	27861	13361	14500
M70-120	70	96253	44703	51550
M90-120	90	640317	285248	355069
M20-75*	20	27907	14048	13859

*The M20-75 matrix did not include individuals from *Ectocarpus 8*

- *Phylogenetic reconstructions*

To assess phylogenetic relationships between species of the *Ectocarpus* subgroup *siliculosi*, we used maximum likelihood (ML) as implemented in RAxML v8 (Stamakis et al. 2014). Analyses were conducted using the GTRGAMMA general time reversible

model of nucleotide evolution, with branch support assessed using 1000 bootstrap replicates. First unrooted trees were built using both the M50-120 and M20-75 matrices. Moreover, two ML rooted trees were built in order to have a closer look at biogeographical relationships between samples within each clade using the M20-75 matrix. The first tree included the species *E. siliculosus*, *Ectocarpus* 1, *Ectocarpus* 6, *Ectocarpus* 7, *Ectocarpus* 8, *Ectocarpus* 9 and was rooted using *E. crouaniorum* as outgroup. The second tree included the species *E. crouaniorum, Ectocarpus* 12 and *Ectocarpus* 13 and was rooted using *E. siliculosus* as outgroup.

IV. RESULTS

- *ddRAD sequencing*

We obtained a total of 276.633.510 base pair reads over the whole 120 individuals sequenced in our study. The total number of reads per individual in the data set varied from 6391 (in *Ectocarpus* 6) to more than 20 millions of reads (in *Ectocarpus* 9). The mean number of read per species was variable ranging from 313.475 in *Ectocarpus* 8 to 6.385.741 in *Ectocarpus* 12 (Figure II.1).

Following the pipeline described previously, those reads produced a raw .vcf file containing a total of 196.804.589 sites for the 120 individuals (including variable and invariable sites). After quality filtering a total of 34.467.721 sites remained and were used to build the dataset matrices. The number of sites retained varied widely depending on the percentage of missing data allowed (Table II.2). When all 120 individuals from the nine species belonging to the *Ectocarpus* subgroup *siliculosi* were considered, none of the 34.467.721 sites was sequenced in all samples (M0-120, no missing data allowed, total number of site retained = 0, Table II.2). The number of sites retained varied from 93 (i.e. only one ddRAD locus), when only 10% of missing data were allowed, to 640.317 sites (i.e. > 7000 loci) when 90% of missing data were allowed (Table II.2). Removing the individuals showing a low number of reads (45 individuals, Table II.1, Figure II.1) from the M50-120 matrix greatly decreased the percentage of missing data (from 50% in M50-120 to only 20% in M20-75, Table II.2) while it had little effect on the number of variable sites included (from 14500 variable sites in M50-120 to 13859 variable sites in M20-75, Table II.2).

- *Phylogenetic reconstructions*

Except for *Ectocarpus* 12, none of the *Ectocarpus* species was recovered as monophyletic branche in the ML tree reconstruction using the M50-120 dataset matrix (Figure II.2). Individuals that did not gather at all with the bulk of the samples from their species (e.g. the *Ectocarpus* 7 sample BIN_220 or the *E. crouaniorum* sample CNA_641), as determined by COI-5P and ITS1 (Montecinos et al. 2016), were all characterized by a low number of reads and/or a low number of loci that were successfully aligned to the reference genome (Table II.1). Basal branches were very short and only five clades showed statistical support values of more than 80 (Figure II.2).

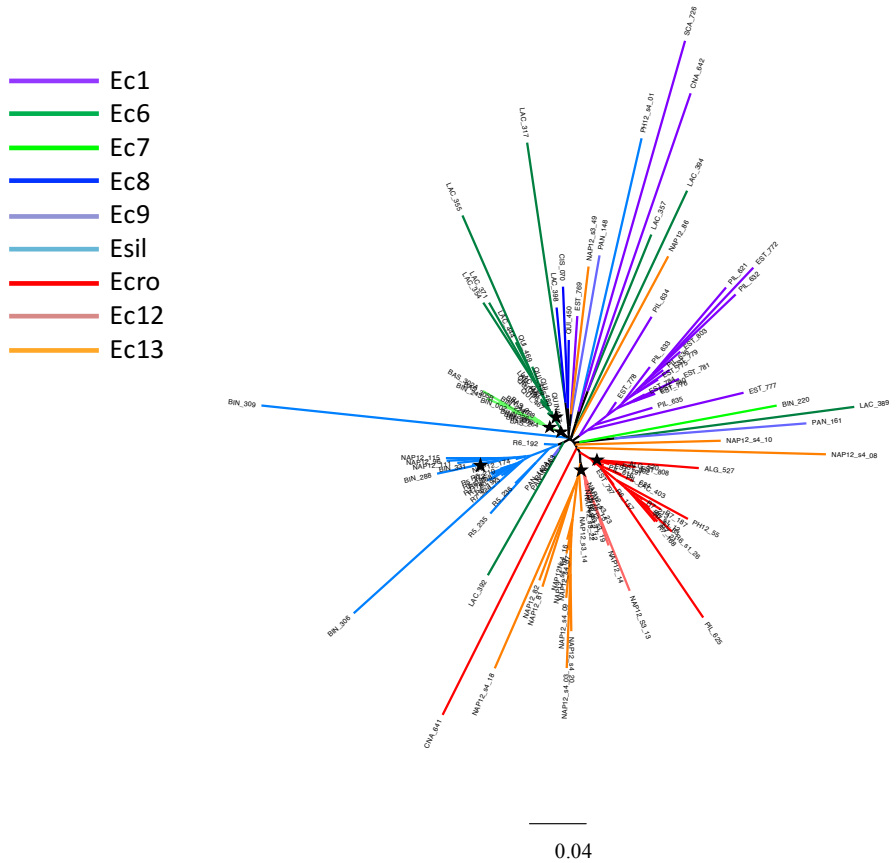

Figure II.2 – Unrooted tree reconstructed using a ML phylogenetics reconstruction using the M50-120 dataset matrix. All 120 individuals, belonging to nine species of the subgroup *siliculosi,* sampled are included in this tree. M50-120 is characterized by 50% of missing data and a total of 27.861 sites (including invariable sites). Statistical support values >80 are shown as black stars along the branches. Individuals characterized by a low number of reads and/or a low number of loci that were successfully aligned to the reference genome (i.e. the ones removed in the M20-75 dataset matrix) are indicated by grey shading. Ec1- Ec13 = *Ectocarpus* 1 – *Ectocarpus* 13, Esil= *E. siliculosus*, Ecro = *E. crouaniorum*.

Highlighting the impact of missing data on phylogenetic reconstructions, the ML tree reconstructed using the M20-75 data matrix on the other hand 1) showed clearly the existence of two divergent clades (support values =100) one including *E. crouaniorum, Ectocarpus* 12 and *Ectocarpus* 13 and the other one the rest of the five species present in M20-75 and 2) recovered all species, except *Ectocarpus* 13, as monophyletic groups with support values ranging from 94 to 100 (Figure II.3).

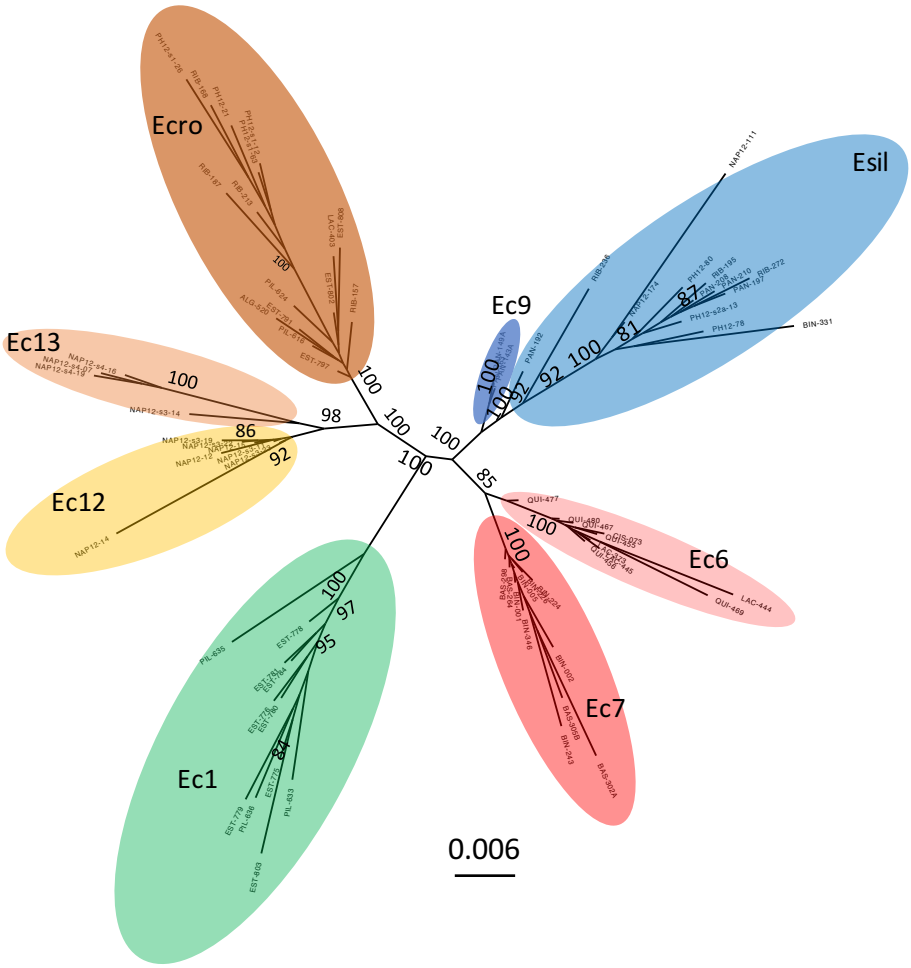

Figure II.3 – Unrooted tree reconstructed using a ML methodology using the M20-75 dataset matrix. Only 75 individuals are included in this tree and the species *Ectocarpus* 8 is not represented in the data set. M20-75 is characterized by 20% of missing data and a total of 27.907 sites (including invariable sites). Statistical support values >80 are shown on branches. Ec1- Ec13 = *Ectocarpus* 1 – *Ectocarpus* 13, Esil= *E. siliculosus*, Ecro = *E. crouaniorum*.

There was a high concordance between the species determination using the markers published in Montecinos et al. (2016) and the groups recovered using the ddRAD sequencing data matrix for all the 75 samples studied (Figure II.3).

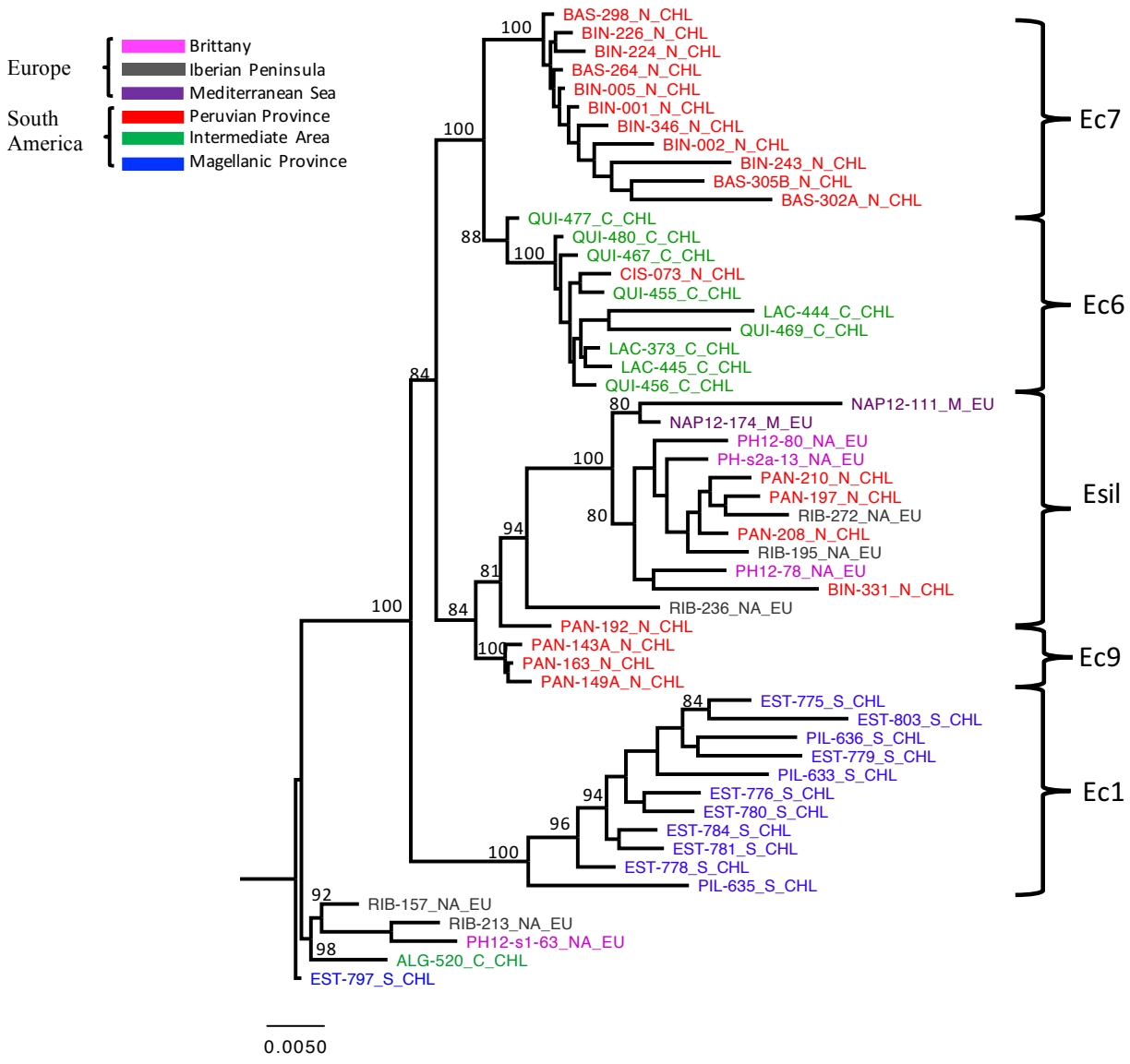

Figure II.4 – ML rooted tree reconstructed using the M20-75 data matrix. Samples of the species *E. siliculosus*, *Ectocarpus* 1, *Ectocarpus* 6, *Ectocarpus* 7, *Ectocarpus* 9 are included and the tree was rooted using *E. crouaniorum* as outgroup. Statistical support values >80 are shown on branches. The geographic origin (i.e. biographical region) of each strain is indicated by a distinct colour.

The rooted ML tree including samples of *E. siliculosus*, *Ectocarpus* 1, *Ectocarpus* 6, *Ectocarpus* 7, *Ectocarpus* 9 and presented in Figure II.4 produced a topology where three clear clades were retrieved (support value > 84): (1) *Ectocarpus* 1, (2) *Ectocarpus* 6 and *Ectocarpus* 7 and (3) *E. siliculosus* and *Ectocarpus* 9. *Ectocarpus* 1, a species encountered only in the southern part of the Intermediate area in Chile, was retrieved as the oldest diverging species (Figure II.4). *Ectocarpus* 6 and *Ectocarpus* 7 are encountered only along the East Pacific coast (Montecinos et al. 2016). *Ectocarpus* 7 is restricted to the Peruvian Province while *Ectocarpus* 6 was mostly encountered in the Northern part of the Intermediate Area (except for the strain CIS-073_N_CHL sampled in Caldera) (Figure II.4). The three samples from *Ectocarpus* 9 were all collected in Pan de Azúcar (i.e. Peruvian Province), even if the species was also reported in the Intermediate Area in Chile (Montecinos et al. 2016), and form a group tightly related genetically (Figure II.4). No clear biogeographic structure was observed within the species *E. siliculosus* with strains collected from Europe and South America that did not cluster by their geographic origin except for the two strains from Naples that group together forming a separated and well sustain branch (support = 80, Figure II.4). One individual identified as *E. siliculosus* (PAN_192_N_CHL) in Montecinos et al. (2016) appeared as an intermediate between *E. siliculosus* and *Ectocarpus* 9 in this study (Figure II.4). This individual was sampled in Pan de Azúcar (i.e. Peruvian Province), a site where both *E. siliculosus* and *Ectocarpus* 9 are encountered (Table II.1).

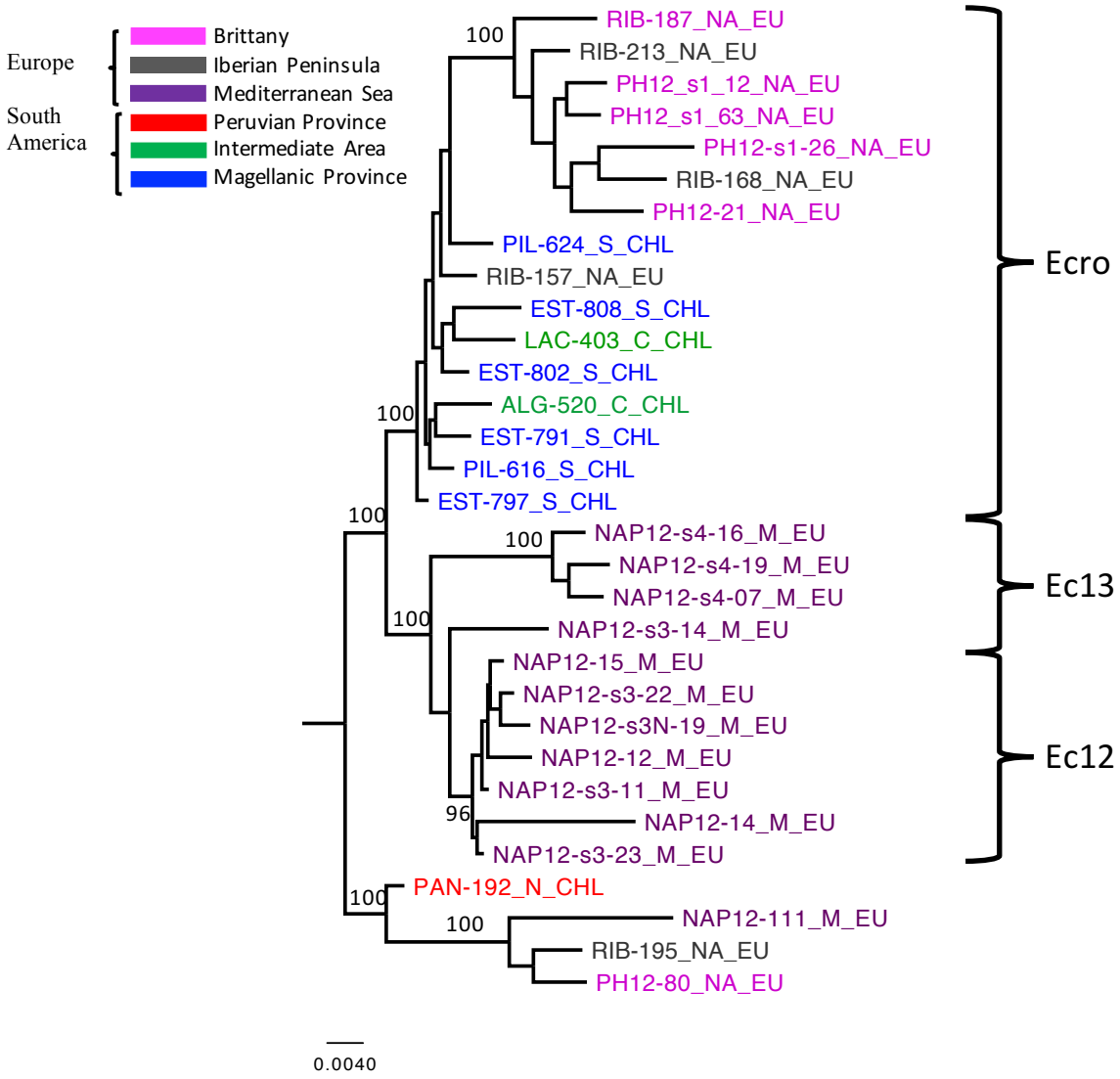

Figure II.5 –ML rooted tree reconstructed using the M20-75 data matrix. Samples from the species *Ectocarpus* 12, 13 and *E. crouaniorum* are included and the tree was rooted using *E. siliculosus* as outgroup. Statistical support values >80 are shown on branches. The geographic origin (i.e. biographical region) of each strain is indicated by a distinct colour.

Two clades were observed (support value = 100) within the rooted ML tree presented in Figure II.5: (1) *E. crouaniorum* and (2) *Ectocarpus* 12 and *Ectocarpus* 13. Contrarily with what was observed for the cosmopolitan species *E. siliculosus,* a geographic structure was detected for the widely distributed *E. crouaniorum*. Indeed, all strains collected in the European North Atlantic, except for one (i.e. RIB-157_NA_EU) formed a clade (support value = 100) embedded within a paraphyletic group formed by eight samples collected in Chile (Intermediate Area) and the remaining RIB-157_NA_EU sample collected in Ribadeo-Spain (Figure II.5). One strain identified as *Ectocarpus* 13 (NAP12-s3-14_M_EU) in Montecinos et al. (2016) appeared as an intermediate between *Ectocarpus* 12 and *Ectocarpus* 13, preventing the retrieval of *Ectocarpus* 13 as a monophyletic group (Figure II.5). Again, the samples from both *Ectocarpus* 12 and *Ectocarpus* 13 and the intermediate NAP12-s3-14_M_EU were encountered within the same locality in the Mediterranean Sea (Naples, Figure II.5).

Note that when strains from various sites from the same biogeographic region were sampled (i.e. *E. siliculosus*, *E. crouaniorum*, *Ectocarpus* 1 and *Ectocarpus* 6), clustering by site origin was never observed (Figure II.4 and II.5).

V. DISCUSSION

- A better resolution of the phylogenetic relationships among cryptic species

Inferring phylogenetic relationships between taxa can be problematic because of: (1) the lack of informative molecular variation at short evolutionary timescale; (2) the lack of established markers in poorly studied taxa; and (3) the potential phylogenetic conflicts among different genomic regions due to incomplete lineage sorting or introgression. In this context, RAD-sequencing seems promising since this technique can generate sequence data from numerous DNA fragments scattered throughout the genome, from a large number of samples, and without preliminary knowledge on the taxa under study. The use of RAD-seq has successfully resolved phylogenetic relationships of both closely related groups of species and divergent taxa (e.g. Wagner et al. 2012, Cariou et al. 2013, Hipp et al. 2013). Phylogenetic relationships in *Ectocarpus* and species delimitation in this genus of recently diverging cryptic species have always been problematic (Peters et al. 2010a, b, Montecinos et al. 2016). Using RAD-seq, our results retrieved a branching pattern largely concordant with the one recovered in previous studies using only few molecular markers (Peters et al. 2010a, b, 2015, Montecinos et al. 2016), showing two major monophyletic clades (with *E. crouaniorum, Ectocarpus* 12 and 13 highly separated from all the other species) in the *Ectocarpus siliculosi* group. Even if the sequencing results of all the samples of *Ectocarpus* 8 were of low quality and this species was not included in the tree reconstructions, our RAD-seq data set was able to retrieved all eight species - *Ectocarpus* 1, *Ectocarpus* 6, *Ectocarpus* 7, *Ectocarpus* 9, *Ectocarpus* 12, *Ectocarpus* 13, *E. siliculosus* and *E. crouaniorum* - defined by Montecinos et al. (2016) as well-separated genetic groups. We were also able to precise the close relationship between *Ectocarpus* 6 and *Ectocarpus* 7 and *E. siliculosus* and *Ectocarpus* 9. We detected two

intermediate individuals between *E. siliculosus* and *Ectocarpus* 9 and between *Ectocarpus* 12 and 13 at sites where they were encountered in sympatry, a results that we interpret as potential hybridization or introgression between closely related species pairs. It could be confirmed using adequate molecular and statistical tools as showed in the chapter 3 of this PhD Thesis.

The pipeline Stacks (Catchen et al. 2011, 2013) has been used extensively to generate RAD-seq data matrices for phylogenetic studies (e.g. Jones et al. 2013, Lexer et al. 2013, Reitzel et al. 2013, Wagner et al. 2013, Cruaud et al. 2014, Herrera et al. 2014, Leaché et al. 2014, Viricel et al. 2014, Pante et al. 2015). Originally, this pipeline was designed for genetic mapping but it has been extended to include features necessary to develop population genetic and phylogenetic studies. The software is self-sufficient and implements an off-by-N clustering strategy and may therefore be best suited to very fine-scale phylogenetic questions (Eaton 2014). Using the M20-75 data set matrix, we clearly recovered eight genetic groups (*Ectocarpus* 1, *Ectocarpus* 6, *Ectocarpus* 7, *Ectocarpus* 9, *Ectocarpus* 12, *Ectocarpus* 13, *E. siliculosus*, *E. crouaniorum*; *Ectocarpus* 8 samples are included in M20-75) showing a perfect concordance with the species determination based on mitochondrial COI-5P and the nuclear ITS1 (Montecinos et al. 2016) (Chapter 1). Moreover, the use of numerous loci scattered across the genome have allowed us to clarify some previously uncertain phylogenetic relationships within the clade including *Ectocarpus* 1, *Ectocarpus* 6, *Ectocarpus* 7, *Ectocarpus* 9 and *E. siliculosus* (Peters et al. 2010a,b, Montecinos et al. 2016). First, *E. siliculosus* and *Ectocarpus* 9 were retrieved as two closely related monophyletic groups using RAD-seq, while in Montecinos et al. (2016) *Ectocarpus* 9 was retrieved as paraphyletic to *E. siliculosus* with the only marker available, the COI-5P (the ITS1 of both species were unalignable). *Ectocarpus* 6 was retrieved as the closest species to the

model species *Ectocarpus* 7 using RAD-seq, confirming the results of previous studies (Peters et al. 2010b, Montecinos et al. 2016). *Ectocarpus* 1, *Ectocarpus* 6, *Ectocarpus* 9 and *Ectocarpus* 7 are all from the Pacific. *Ectocarpus* 1, the earliest diverging species in this clade, was found only in the Magellanic Province. Interestingly, *Ectocarpus* 7 showed a distribution limited to the Peruvian Province, while all others, except for one *Ectocarpus* 6 strain, were found in the center of Chile (Northern part of the Intermediate Area). *E. siliculosus*, the only cosmopolitan species in this clade, appeared as one of the recently diverging species with a branch embedded in the clades from the South Pacific. The strong geographical pattern of species distribution along the South East Pacific coast observed for *Ectocarpus* 1, *Ectocarpus* 6, *Ectocarpus* 9 and *Ectocarpus* 7, questions about the role of biogeographic barriers on species divergence. Indeed, recent phylogeographic studies of seaweeds revealed the existence of different cryptic species along the South East Pacific coast and that most of the genetic breaks between them were broadly congruent with two biogeographic boundaries (the red alga *Mazzaella laminarioides*, Montecinos et al. 2012; the kelp *Lessonia spp*., Tellier et al. 2011, others see review of Guillemin et al. 2016a). Moreover, the cold temperate coasts of Chile and South Peru have been considered as possible center of origin and diversification for various algae (see for example the case of the bladed Bangiales in Guillemin et al. 2016c). It is possible that part of the recently diverging clades of *Ectocarpus* also take origin in the cold waters of the Pacific, however more samples from New Zealand, southern Australia and the NW Pacific will be needed to confirm this hypothesis.

- *Different patterns of geographic structure for the two cosmopolitan species*

A contrasted pattern of geographic structure was observed for the two cosmopolitan species *E. siliculosus* and *E. crouaniorum.* Within *E. crouaniorum*, all strains sampled in the European North Atlantic (except RIB-157_NA_EU, collected in Spain) grouped together revealing a common ancestry. The younger diverging clade formed by the European samples derives from a paraphyletic assemblage of strains sampled in different provinces of Chile. This pattern suggests a South Pacific origin of *E. crouaniorum* and a recent introduction in Europe. However, the position of the sample RIB-157_NA_EU collected in Spain but observed outside the European clade, with the paraphyletic group of Chilean samples, questions about the reality of a Pacific origin and the number of introduction events. In contrast, for *E. siliculosus*, all strains collected along the Atlantic and the Pacific formed a paraphyletic assemblage except for the two Mediterranean strains that grouped in a well-sustained clade. The Gibraltar strait represents a strong biogeographic break (Patarnello et al. 2007) and we cannot discard the potential effect of this barrier on genetic differentiation. Nevertheless, the absence of any clear genetic differentiation between the Pacific and European samples suggest the occurrence of repeated bi-directional long dispersal / introduction events reshuffling the genotypes (Zhan et al. 2010; Pineda et al. 2011). Elucidating the evolutionary history of both *E. siliculosus* and *E. crouaniorum* is thus a remaining challenge requiring worldwide comprehensive sampling.

In conclusion, the occurrence of cryptic species in brown macroalgae is a phenomenon that has been evidenced using molecular markers and has been commonly recognized during the last decades (e.g. Tellier et al. 2011, McDevit and Saunders 2009). However, in the case of the genus *Ectocarpus* - as for other filamentous brown

algae – some additional difficulties arise due to their simple morphology and the almost "blind" field sampling that has to be performed. Indeed, in the field, most *Ectocarpus* samples are microscopic and are found as settled spores or few-celled individuals growing on abiotic or biotic substrata. Pebbles, shells and macroalgae recognized as *Ectocarpus* hosts have to be sampled blindly and *Ectocarpus* individuals obtained after germling emergence (Couceiro et al. 2015, Peters et al., 2015, Montecinos et al. 2016). Even when adult individuals are visible in the field, *Ectocarpus* samples cannot be distinguished from morphologically similar filamentous brown algae. The characteristic ribbon-shaped plastids of *Ectocarpus* have to be confirmed under the microscope. However, there are at present no morphological characters for the identification of the different species of *Ectocarpus* except for the branching pattern of *E. fasciculatus*, and reliable identification requires diagnostic molecular markers. Due to these sampling limitations, it is difficult to design a priori a minimum sampling size per site for the different species or even for the genus *Ectocarpus* per se. However, such sampling effort using (1) a blind sampling of different hard substrata as well as individuals visible by eye and (2) a sampling design across the depth gradient of the shore repeated in several sites, was made during this PhD thesis to better understand the importance of hybridization and reproductive isolation between the divergent but most commonly encountered species *E. crouaniorum* and *E. siliculosus* along the European coast (Chapter 3).

Appendix II.1

Problems generating RAD-seq data during this PhD thesis.

We have encountered many technical problems when we attempted to obtain RAD-seq data. The aim of this Appendix is to advert future research using NGS on *Ectocarpus* species differing from the sequenced strain..

1) In order to get good DNA, we used the method developed in the sequenced strain and tried to obtain axenic material by cultivating our strains in a medium with antibiotics. However, we have detected a very slow growth of the strains and detected fungal contamination in more than 50% of the isolates. We have tried to redo each strain cultures more than three times but never succeed to get rid of the fungal contaminations and to increase the growth rate. We concluded that bacteria were necessary for strain growth in laboratory conditions. The solution was then to add antibiotics to the cultures only 3 days before DNA extraction.
2) The data presented in this Chapter reveal a huge variation in the number of reads for each individual. The problem was that in each PCR after the ligation of barcodes 12 individuals were pooled. This allowed reducing considerably the time and price in Rad-seq materials. However, individual PCR has been shown to give better results with more similar number of reads obtained for each individual, since the competition between samples for nucleotides in the amplification is avoided. Future work should take this issue into account.

Chapter 3

Hybridization between two cryptic filamentous brown seaweed along the shore: analyzing pre and post-zygotic barriers in populations of individuals with varying ploidy levels.[1]

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CHAPTER 3.

Hybridization between two cryptic filamentous brown seaweed along the shore: analyzing pre and post-zygotic barriers in populations of individuals with varying ploidy levels

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Keywords: reproductive barrier, meiosis, haploid-diploid life cycle, shore gradient, contact zone, *Ectocarpus*.

I. Abstract

Hybridization between two cryptic species of the brown algal genus *Ectocarpus* was studied along the European coast. Clonal cultures (568 diploid and 336 haploid) isolated from field samples (10 populations) were genotyped to detect hybrid diploid sporophytes and recombinant haploid gametophytes. We analysed haploid and diploid sub-populations separately to identify admixed individuals, using STRUCTURE and GENECLASS; but those classical assignment methods are not formalized to analyse populations of individuals with varying ploidy levels and aneuploidy. We thus developed our own Bayesian method, independent of ploidy per locus, implemented in the software *XPloidAssignment*, which provides the posterior probabilities to assess the level of admixture under various scenarios of hybridization. We show that our method is particularly relevant when studying hybridization in species with complex life cycles and for most secondary contacts where variations in ploidy levels are observed. Over all *Ectocarpus* populations, the level of hybridization was estimated at 8.7%. However, hybrids were exclusively observed in sympatric populations (where the two species come in contact along the shore gradient) with a high frequency of rare alleles. More than 98% of hybrids were diploids (40% of which showed signs of aneuploidy). These results suggested the occurrence of post-meiotic reproductive isolation and demonstrate that hybridization can be quantitatively assessed using the analysis of population genetic structure in haploid-diploid species, allowing the distinction of pre- from post-zygotic isolation barriers.

II. Introduction

Hybridization has been defined by Barton & Hewitt (1985) as reproduction between individuals from genetically different populations. Even if hybridization was first considered as preventing the process of speciation, it is now recognized that it can "act as an additional, perhaps more abundant, source of adaptive genetic variation than mutation" thereby potentially promoting speciation (Seehausen 2004; Abbott *et al.* 2013). Hybridization is a common phenomenon in many multicellular animal and plant species (Elstrand *et al.* 1996; Mallet 2005) and is more frequent between closely related than genetically highly diverged species. In particular, species within rapidly diversifying groups (e.g. adaptive radiations) may be particularly prone to hybridization (Seehausen 2004; Moyle 2004; Gourbière & Mallet 2010).

Hybridization implies sexual reproduction, which in turn entails cyclic alternation between syngamy and meiosis. However, the relative timing of syngamy and meiosis varies widely across the eukaryotic tree of life (Valero *et al.* 1992; Mable & Otto 1998; Coelho *et al.* 2007). For example, syngamy and meiosis are clearly separated in time in haploid–diploid species. In such life cycles, there is an alternation between haploid individuals (gametophytes) produced by meiosis and diploid individuals (sporophytes) resulting from syngamy. Unlike the diploid-dominant plants and animals, the haploid stage in a haploid-diploid life cycle is an independent, functional organism with somatic development (reviewed in Mable & Otto 1998). Consequently, in haploid-diploid species the two hallmarks of the sexual life cycle can be studied separately by analysing the population genetic structure of the haploid gametophytes and the diploid sporophytes. In particular, the importance of post – zygotic barriers in the wild

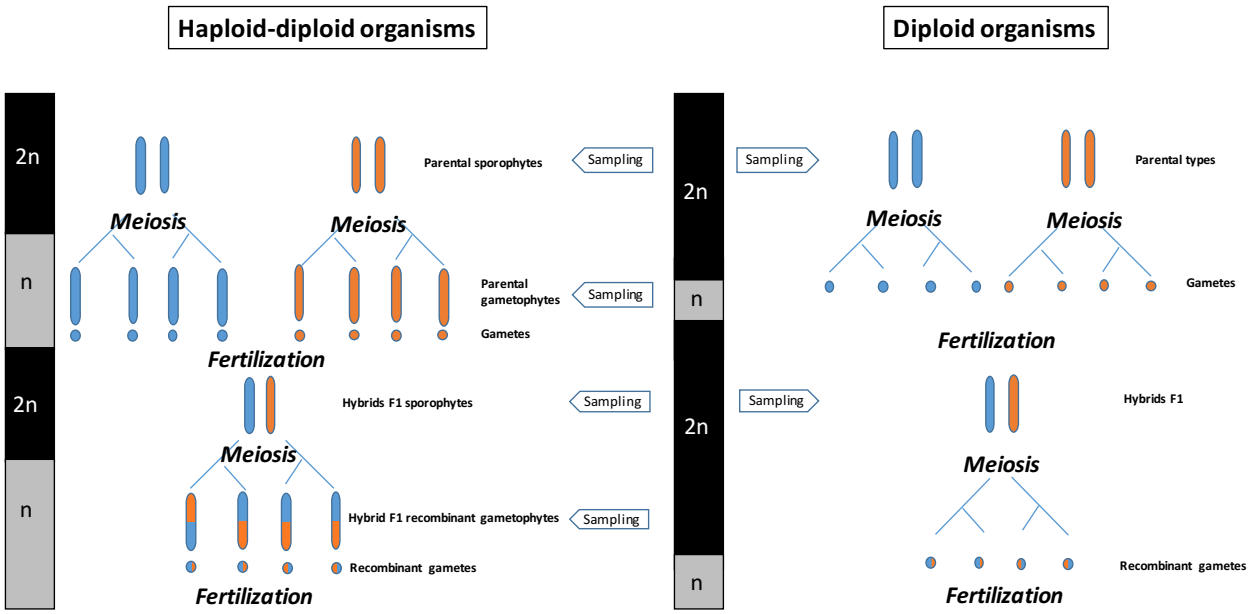

Figure III.1. The haploid-diploid life cycle model to study hybridization and reproductive barriers.
In diploid life cycles, mitotic cell division and somatic development occur entirely in the diploid phase. The haploid stage is reduced to a single-cell (i.e. gamete) produced through meiosis in the diploid phase and fertilization occurs immediately after release of gametes to recreate the diploid phase. Therefore, population genetics studies based on diploid life cycles are focused only on diploid individuals. On the other hand, in haploid-diploid life cycles, somatic development occurs in both haploid and diploid phases and there is an alternation between two types of independent functional individuals: haploid gametophytes produced by meiosis and diploid sporophytes resulting from fertilization. The direct access to the haploid part of the life cycle allows to untangle the effect of reproductive barriers preventing fertilization (i.e. an absence of diploid hybrid genotypes is expected) or preventing meiosis (i.e. an absence of recombinant haploid genotypes is expected). Conversely, in diploid species, such process cannot be distinguished directly using population genetics in the field.

may be easily estimated by comparing diploid and haploid subpopulations (i.e. by estimating the frequency of hybrid genotypes in diploid sporophytes and meiotic recombinant genotypes in haploid gametophytes) (Fig. III.1). However, published methods that estimate the level of hybridization do not consider individuals with different levels of ploidy imposing the study of haploid and diploid "sub-populations" as different data-sets (see Table 1 in Payseur & Rieseberg 2016 for examples). Investigating a large diversity of biological models is thus crucial to unravel the general importance of hybridization in evolution. However, most studies of hybridization in the wild have been carried on plants or animals. It is only recently that data are accumulating for species belonging to other phyla such as fungi (see for reviews Kohn 2005; Giraud *et al.* 2008), red algae (Zuccarello *et al.* 2005; Destombe *et al.,* 2010; Maggs *et al.* 2011; Niwa & Kobiyama 2014; Savoie & Saunders 2015; Guillemin *et al.* 2016) and brown algae (Coyer *et al.* 2002; Engel *et al.* 2005; Peters *et al.* 2010a; Geoffroy *et al.* 2015 and references therein), which differ substantially in their life cycle and life history traits from flowering plants and animals.

Since the publication of the *Ectocarpus* genome sequence by Cock *et al.* in 2010, species from this genus have become the brown algal models in various research fields and, in particular, because of their haploid-diploid life cycle (Müller 1967), in developmental genetics (Cock *et al.* 2014). *Ectocarpus* spp. are small ephemeral algae that are easy to cultivate in the laboratory but, until recently, these organisms were almost inaccessible for large population studies because of their small size. As genetics and genomics tools were developed for this algal model, several studies were undertaken in the field to better understand their ecology and diversity (Peters *et al.* 2010a, b; Couceiro *et al.* 2015; Peters *et al.* 2015; Montecinos *et al.* 2016). From these studies, it appears that the genus *Ectocarpus* forms a complex of cryptic sibling species

adapted to different hosts and/or habitats along the shore gradient. Moreover, incongruences between a nuclear (internal transcribed spacer 1 of the ribosomal DNA; ITS1) and a cytoplasmic marker (cytochrome oxidase subunit 1; COI-5P) were recently reported by Montecinos *et al.* (2016) among some species pairs suggesting the occurrence of hybridization and introgression in the field. The occurrence of putative hybrids between *E. siliculosus* and *E. crouaniorum* in the Atlantic coasts was reported by Peters *et al.* (2010a), who used a barcode approach based on ITS1 length difference. Indeed, individual species can be rapidly identified based on their ITS length using a simple PCR and the electrophoresis technique and individuals bearing both species-specific bands were regarded as putative hybrids (Peters et al. 2010a). However, although *E. siliculosus* and *E. crouaniorum* were the most frequently sampled species along the North Atlantic coast by Montecinos et al. (2016), no incongruences between the nuclear and cytoplasmic markers were reported between these two species by these authors. These results question the importance of hybridization in nature between *E. siliculosus* and *E. crouaniorum* and might indicate the occurrence of post-zygotic barriers preventing gene flow and introgression between these two species.

To address these issues, we completed a collection of more than 900 unialgal cultures derived from samples collected in ten sites along the European coast. We first combined information from cytoplasmic and nuclear markers to estimate the frequency of each parental species and putative hybrids along the European coast and the occurrence of sympatric populations. We then analysed microsatellite genetic data to conduct admixture analyses with the classical STRUCTURE and GENECLASS methods on separate diploid and haploid subpopulations. But those clustering models were not adapted to answer our question. We thus formalized a new Bayesian method to assign individuals to quantitative levels of admixture between the two cryptic species

using all individuals whatever their ploidy (ie haploid, diploid or aneuploid). The objectives of this study were (1) to test whether hybrids were restricted to sympatric zones where the two species are in contact along the shore, and (2) to determine the relative abundance of haploid and diploid hybrids and describe their genetic composition. In addition to contributing to important information concerning the biology of *Ectocarpus*, this study aimed to broaden our understanding of speciation and hybridization processes in benthic marine environments.

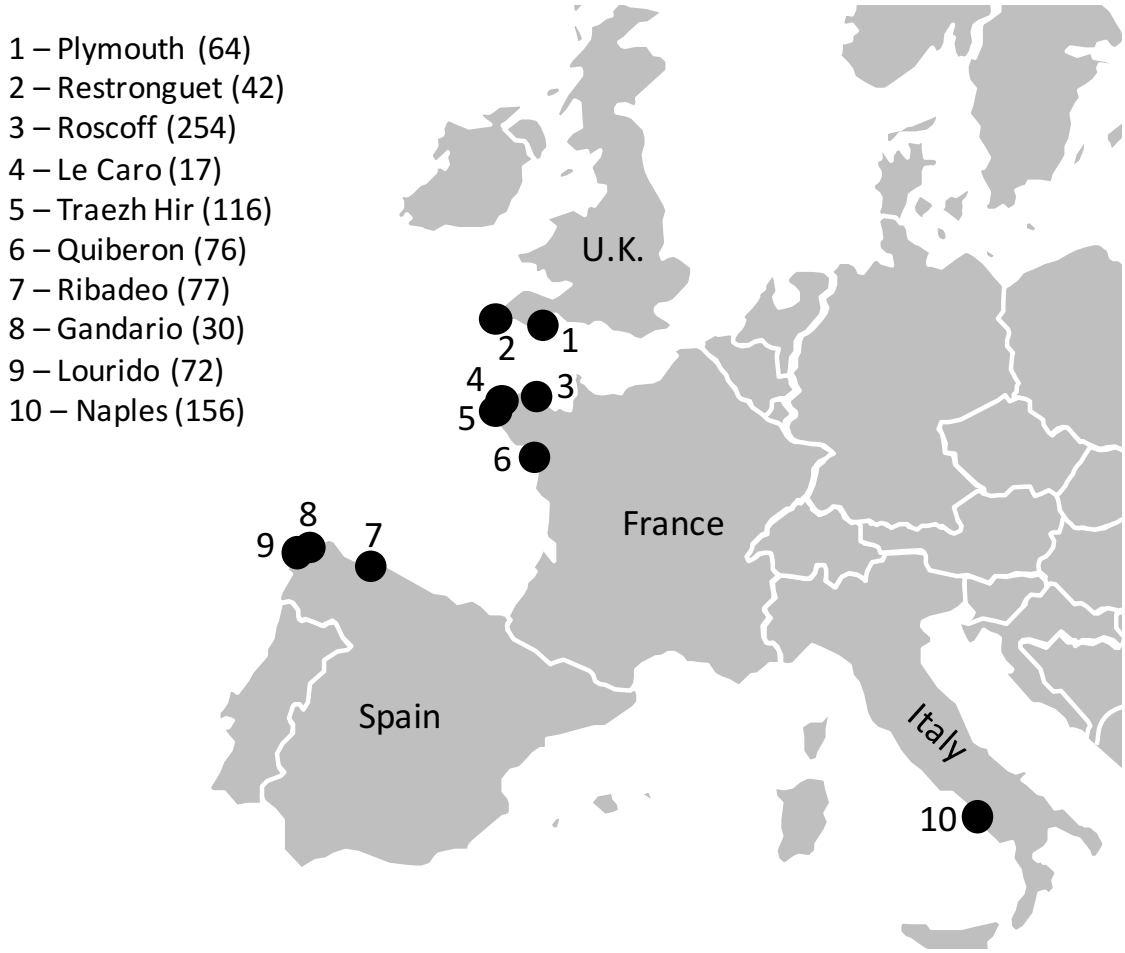

Figure III.2. Populations sampled. Names of each population and number of individuals collected (shown within brackets) are indicated in the figure top right. The sampling dates were as follow: (1) Plymouth, 2010; (2) Restronguet, 2010; (3) Roscoff, 2012; (4) Le Caro, 2012; (5) Traezh Hir, 2010; (6) Quiberon, 2010/12; (7) Ribadeo, 2013; (8) Gandario, 2013; (9) Lourido, 2013; (10) Naples, 2012.

III. Material and Methods

- *Field collections, isolation of* Ectocarpus *strains and DNA extraction*

A total of 904 *Ectocarpus* samples from the North East Atlantic (NEA) and the Mediterranean Sea were used in this study (Fig. III.2). Four hundred and ninety-four samples were collected from seven sites along the NEA in the framework of this study, while the remaining 410 samples (Roscoff and Naples sites) were previously obtained by Couceiro et al. (2015). As *Ectocarpus* species cannot be distinguished in the field (see below), sampling was blind and it was not possible to select, a priori, sites where the two species co-occurred in sympatry. Nonetheless, since *E. siliculosus* and *E. crouaniorum* have been shown to inhabit different levels of the intertidal (Peters *et al.* 2010a; Couceiro *et al.* 2015; Montecinos *et al.* 2016), whenever possible, samples were collected from high to lower tide levels on the shore. Unialgal cultures were established from all 494 newly collected samples and maintained as clonal cultures using the protocol described in Couceiro *et al.* (2015). After two months of laboratory culture, enough biomass was available for DNA extraction and total DNA was extracted from lyophilized samples using the NucleoSpinR 96 Plant Kit (Macherey-Nagel, Duren, Germany).

- *Molecular determination of ploidy and sex*

In *Ectocarpus*, sex is expressed during the haploid phase (UV system) and is determined by two different sex-determining regions (Ahmed *et al.* 2014), corresponding to the two sexes. Both the male and female sex-determining regions are present in the diploid sporophytes (heterozygous for the sex locus) whereas the haploid

gametophytes carry either the male or female allele (hemizygous for the sex locus). In order to determine the sex and the ploidy of our samples, we amplified the sex-specific regions following Couceiro *et al.* (2015). Positive/negative amplifications for male and female specific PCR primers were checked on 2% agarose gels stained with ethidium bromide.

- Preliminary sorting of samples in parental species or putative hybrids categories using the rps14-atp8 spacer, the ITS1 and diagnostic microsatellite loci and alleles

Neither the two cryptic species *E. siliculosus* (hereafter Esil) and *E. crouaniorum* (hereafter Ecro) nor their putative hybrids can be distinguished using morphological characters (Peters *et al.* 2010a, b; Montecinos *et al.* 2016). We therefore first amplified the species-specific mitochondrial *rps*14-*atp*8 spacer region developed by Couceiro *et al.* (2015) to classify each sample cytoplasm by its species of origin. Peters et al. (2004b) have demonstrated that mitochondria are maternally inherited in *Ectocarpus.* The presence/absence of PCR products corresponding to the *rps*14-*atp*8 spacers of Esil or Ecro was determined on 2% agarose gel as described in Couceiro *et al.* (2015). Second, as a preliminary method to distinguish parental species from putative hybrids, we amplified the ITS1 and tested for linkage disequilibrium between this nuclear marker and the cytoplasmic marker *rps*14-*atp*8. A fragment containing the ITS1 region together with 224 bp of the flanking 18S and 5.8S genes was amplified using the primers described by Peters *et al.* (2010a). These primers allow distinguishing both parental species based on the different length of the amplified region (850 bp in Esil but 1100 bp in Ecro). PCR conditions followed Peters *et al.* (2010a) and the number and length of the ITS1 fragments were determined by electrophoretic size separation in 2% agarose gels. Individuals were considered as putative hybrids if they bore the two ITS1

fragments (850bp and 1100bp) or showed incongruence between the mitochondrial and nuclear markers.

Finally, nine nuclear microsatellite loci were amplified using the primers and PCR conditions described by Couceiro *et al.* (2015). Microsatellite alleles were analysed in an ABI 3130 automated sequencer and scored manually using GeneMapper ® version 4 software (Life Technologies Corporation). Amplification success and allele frequency were computed with GENALEX (Peakall & Smouse 2012) for each locus. The occurrence of species-diagnostic loci (i.e. loci showing no cross-amplification between both parental species), as well as species-diagnostic alleles (i.e. alleles at loci showing cross-amplification between both parental species but otherwise exhibiting non-overlapping allele size ranges), was evaluated by determining the amplification success and allele size range of the nine microsatellite loci in the individuals previously classified as Esil or Ecro based on the cytoplasmic information. All individuals showing amplification products for at least one diagnostic locus size inconsistent with the expected species based on the cytoplasmic markers were added to a list of putative hybrids. Because diagnostic loci do not amplify in both species they generate missing data for one of the parental species. This non-amplification corresponds to a phylogenetic signal but should be distinguished from a null allele. In order to distinguish missing data caused by species-diagnostic loci from missing data caused by null alleles, the first kind of data were replaced by an artificial allele of an arbitrary size of 800bp in the individuals classified as parental species. Indeed, in the parental species, we expect complete linkage disequilibrium between these diagnostic loci (i.e. none of the loci diagnostic for Esil should amplify in Ecro and vice versa for the loci diagnostic of Ecro). In contrast, for putative hybrid individuals, recombination between diagnostic loci is expected for all generations after the F1. Thus, it was necessary to distinguish if

missing data at these loci indicated recombination or null alleles. In order to do this, we first estimated the frequency of null alleles within each parental species by assessing the frequency of non-amplification at their diagnostic loci (i.e. the frequency of missing data in species A for the loci that amplify in species A but not species B and, vice versa, the frequency of missing data in species B for the loci that amplify in species B but not in species A). Second, we estimated the frequency of null alleles within the individuals classified as putative hybrids at all diagnostic loci. Finally, we compared the frequency of null alleles between parental species and putative hybrids using a Fisher exact test implemented in GENEPOP'007 (Rousset 2008). If the frequency of null alleles was not significantly different between parental species and putative hybrids, the missing data were recoded as null alleles in the hybrids; however, if the frequency of missing data was higher in hybrids than in parental species, the missing data were recoded using the 800 bp artificial allele denoting non-amplification due to a species-specific locus. The previous comparisons between putative hybrids and parental species for all diagnostic loci were made independently on the haploid and diploid sub-populations. In addition, to ensure the reliability of the null allele estimation, each sample showing missing data was re-amplified up to three times.

We also recorded some genotypes with 3 alleles per locus at one or more loci (23 sporophytes, see results below). In all these cases, their genotypes at one given locus involved two alleles diagnostic of one species and one allele diagnostic of the second species. Because aneuploidy cannot be taken into account for most of the classical analyses, these individuals were re-categorised as diploid heterozygotes and, one allele diagnostic of each species was selected in order to retain information indicating admixture between species. The most frequent allele in each species gene pool was conserved in the re-categorised diploids.

- Statistical analyses of admixture levels

To gain insight into hybridization between Esil and Ecro, the nuclear microsatellite dataset was analyzed following four complementary methods. First, a Principal Component Analyses (PCA) of the multilocus genotypes was carried out using the R-package ADEGENET (Jombart 2008). Second, the Bayesian clustering method STRUCTURE v2.3.4 (Pritchard *et al.* 2000) was used to identify the number of genetically distinct clusters that maximize the likelihood of the data, and to assign the individuals to the clusters Esil or Ecro using only genetic information. It is important to note that, unlike multivariate analyses (such as PCA), Bayesian clustering methods rely on explicit models and assumptions such as random mating and absence of linkage disequilibrium, which are often difficult to verify and can restrict their applicability. In STRUCTURE, the number K of populations was estimated using a burn-in period of 10.000 and 100.000 MCMC replicates, applying the admixture model and correlated allele frequencies. Each run was replicated 10 times and a range of clusters (K) from 1 to 10 was tested. To verify that the K value that best fitted our data was K = 2 (two species), the ΔK statistic developed by Evanno *et al.* (2005) was calculated. Combined results from the independent runs were obtained using the greedy algorithm with 100.000 random input orders in CLUMPP (Jakobsson & Rosenberg 2007) before exporting the results to DISTRUCT (Rosenberg 2004) for viewing. The estimated membership coefficients Q for each individual in each cluster were calculated using the results obtained by CLUMPP and those individuals for which the secondary cluster represented more than 10% of the genome were regarded as putative hybrids (Vähä & Primmer 2006). Since PCA multivariate analyses and STRUCTURE allow neither different ploidy levels within the same dataset or aneuploidy, both analyses were run on

the haploid and diploid sub-populations separately using the recoded aneuploid genotypes.

The third procedure employed to analyse our microsatellite dataset involved a new Bayesian method specifically developed for this study, implemented in the software XPLOIDASSIGNMENT (Supplementary File 1, Supporting information), which assigns genotyped individuals with different ploidy levels, both between individuals and between markers in a same individual, to quantitative levels of admixture between two genetic groups a priori defined as two previously-isolated pure cryptic species. The method is described in the Supplementary File 2 (Supporting information). Briefly, this method uses the genotyped individuals a priori considered as pure breed and belonging to historically-isolated groups of populations to compute, for each marker, two differentiated gene pools (the two cryptic species in our study). The likelihood that an allele belongs to one of the differentiated gene pool, for example to species *A* or *B* is its frequency within this differentiated gene pool. The method then computes the posterior probabilities that putative hybrids were obtained from different quantitative admixture scenarios. In our case, five distinct scenarios were tested: Scenario 1 and 2, a putative hybrid belongs to one of the two parental species; Scenario 3, a putative hybrid genotype is formed by 50% of each parental species as expected in F1 hybrids; Scenario 4 and 5, a putative hybrid genotype results from an introgressive hybridization (25% of alleles from one parental species and 75% from the other) as expected in backcrosses or in ancient secondary contacts.

Here, we used XPLOIDASSIGNMENT to compute the posterior probabilities that each putative hybrid belonged to one of the two parental species (ie Esil and Ecro) or one of the three hybrid classes defined by the quantitative scenarios of admixture (i.e. ½Esil-½Ecro, ¾Ecro-¼Esil, ¾Esil-¼Ecro). In order to define the genetic pools of the

two parental species, Esil and Ecro, we constructed a data file combining both haploid and diploid samples but excluding the putative hybrids (as defined using the combination of *rps*14-*atp*8 spacer, ITS1 and microsatellite diagnostic loci); as XPLOIDASSIGNMENT allows the use of samples with varying ploidy levels, aneuploid genotypes were included with no recoding.

In our fourth method, we used the function "HYBRIDIZE" included in the R-package ADEGENET (Jombart 2008) to simulate 500 artificial genotypes for the three hybrid scenarios previously mentioned (i.e. F1, backcrosses with Esil and backcrosses with Ecro) using the parental species dataset as input (i.e. after excluding putative hybrids as defined using the *rps*14-*atp*8, ITS1 markers and diagnostic loci). Thereafter, we used GENECLASS v2.0 (Piry & Cornuet 1999) to assign all putative hybrids to one of these five groups (i.e. the two parental species and the three simulated hybrid classes). The Rannala & Mountain (1997) assignment algorithm was used as criterion for computation following the methods outlined in Paetkau et al. (2004) and the assignment probabilities of all putative hybrids to each group were tested using the ''assign or exclude individuals'' option (p=0.05). Since both ADEGENET and GENECLASS analyses are limited to diploid samples, only the diploid dataset with recoded aneuploid genotypes could be employed in our fourth approach.

Results from STRUCTURE, XPLOIDASSIGNMENT and GENECLASS were compared and combined to classify each putative hybrid into five different genetic classes corresponding to five different scenarios of hybridisation between Esil and Ecro: (1) hybrids with an equal proportion of Esil and Ecro genomes, parsimoniously interpreted as potential F1; (2) hybrids with ≥ 0.75 Esil genome, interpreted as admixed individuals derived from backcrosses or repeated interbreeding with Esil; (3) hybrids with ≥ 0.75 Ecro genome, interpreted as admixed individuals derived from backcrosses

or repeated interbreeding with Ecro; (4) individuals with ≥ 0.90 Esil genome, interpreted as part of the parental species Esil and (5) individuals with ≥ 0.90 Ecro genome, interpreted as part of the parental species Ecro. When using GENECLASS or XPLOIDASSIGNMENT methods, individuals were classified into a single hybrid class category (i.e. the one showing the maximum posterior probability) if the probability of assignment to this category was at least two times higher than to any other category; otherwise, individuals were assigned to the two or three equally probable categories.

- *Population structure and mating system*

For both species, the degree of clonality was assessed at each site in both haploid and diploid subpopulations. The number of repeated multilocus genotypes was calculated and the genotypic diversity R was computed by dividing the number of distinct genotypes (G) by the number of individuals, corrected for sample size (i.e. R = G-1/N-1, Dorken & Eckert 2001) using GENECLONE 2.0 v6.41 (Arnaud-Haond & Belkhir 2007). In addition, to explore the departure from random mating in each diploid sub-population, F*IS* values were calculated using the software GENETIX v4.05 (Belkhir *et al.* 2004). Significance of departure from random mating was tested by running 1000 permutations of alleles among individuals within samples. Finally, in order to study the importance of genetic differentiation among sites within each parental species (i.e. excluding potential hybrids), the global *FST* statistic (as defined by Weir & Cockerham 1984) was calculated independently for the haploid and diploid subpopulations of each species using GENETIX v4.05 (Belkhir *et al.* 2004). The level of significance was estimated using 1000 permutations.

IV. Results

- Species identification and detection of putative hybrids

Out of a total of 904 individuals, the mitochondrial species-specific marker *rps*14-*atp*8 identified 505 individuals as Esil and 340 as Ecro. The taxonomic identity of these 845 samples was confirmed by the amplification of their ITS1 nuclear marker (Table III.1). The sex-specific markers indicated that diploid sporophytes (347) were more frequently sampled than haploid gametophytes (158) in Esil while the frequencies of both kinds of individuals were similar in Ecro (162 sporophytes and 178 gametophytes, Table III.1). The remaining 59 samples exhibited incongruences between the nuclear and the cytoplasmic markers as all amplified both Esil (850bp) and Ecro (1100bp) ITS1 (Table III.1). These 59 sporophytes were thus classified as putative hybrids while none of the 336 haploid individuals fell into this category (Table III.1).

Table III.1. Linkage disequilibrium among molecular markers used for species identification: *rps*14-*atp*8 spacer (mtDNA) and the ITS1 (nrDNA) for diploid sporophytes (*SP*) and haploid gametophytes (*GA*) identified using the sex-specific markers.

ITS1	**Ploidy/phase**	**mt-*Esil***	**mt-*Ecro***	**Total**
Esil	*SP*	347	0	347
	GA	158	0	158
	Total SP+GA	505	0	505
Ecro	*SP*	0	162	162
	GA	0	178	178
	Total SP+GA	0	340	340
Esil+Ecro	*SP*	14*	45*	59*
	GA	0	0	0
	Total SP+GA	14*	45*	59*
Total	***SP***			**568**
	GA			**336**
	Total SP+GA			**904**

Esil = *E. siliculosus*, *Ecro* = *E. crouaniorum*, *Esil+Ecro*: individuals bearing both ITS1 bands, *: putative hybrids

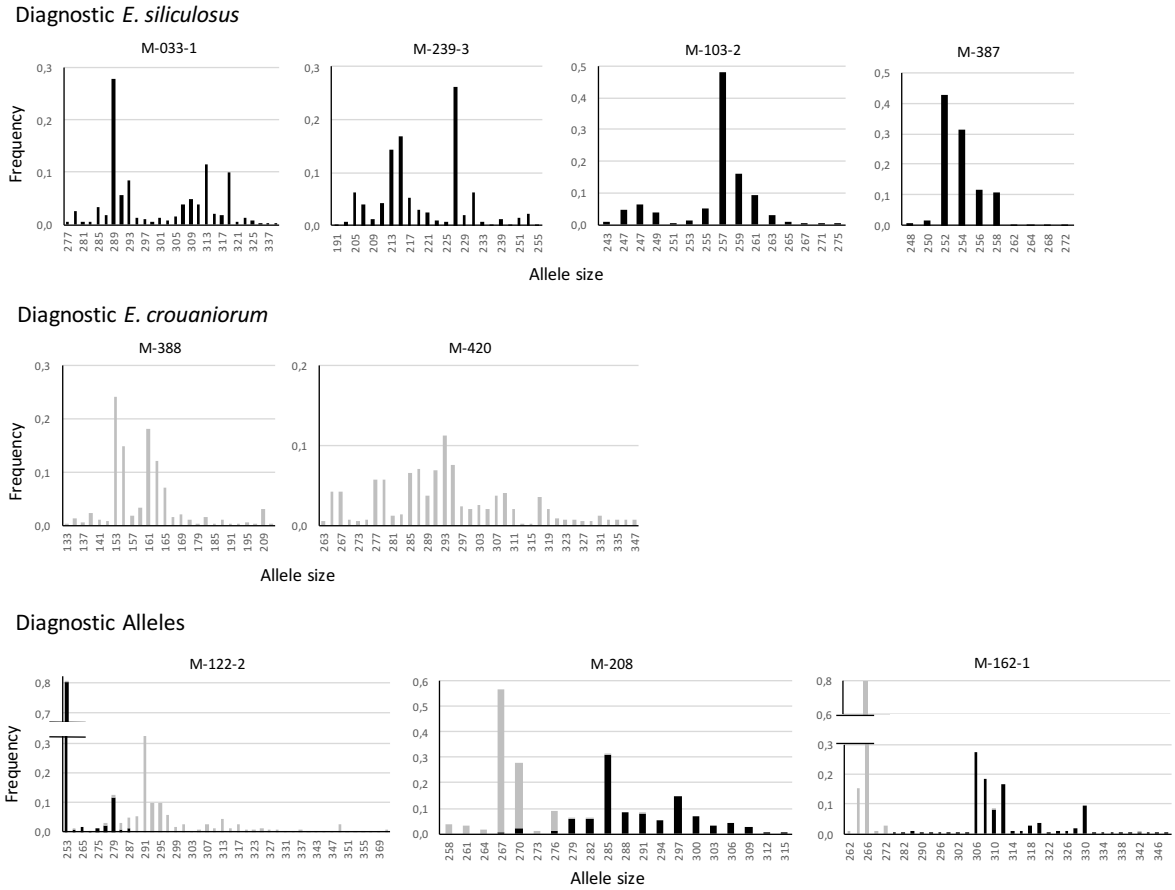

Figure III.3. Allele frequencies at 9 microsatellite loci for the parental species. *E. siliculosus* (dark bars) and *E. crouaniorum* (grey bars) identified based on the linkage disequilibrium among species-specific marker (mtDNA) and ITS1 (nrDNA) species-specific loci. Sample size for each locus and each species are given in Table III.2.

Amplification success and allele size range for the nine microsatellite loci varied according to the cytoplasmic species identification. Four microsatellite loci were found to be diagnostic for Esil. For these four loci the frequency of amplification was 0.94-0.99 when considering only individuals bearing an Esil cytoplasm, while it was only 0.12-0.13 for individuals bearing an Ecro cytoplasm (Table III.2, Fig. III.3). Similarly, two loci were found to be diagnostic for Ecro. These loci showed a frequency of amplification of 0.88-0.97 in individuals bearing an Ecro cytoplasm but less than 0.12 in individuals with Esil cytoplasm. The remaining three loci amplified in both species (M-122-2, M-208, M-162-1; Table III.2, Fig. III.3) but presented allele size differences between Esil and Ecro.

Table III.2. Linkage disequilibrium among the mitochondrial species-specific markers (*rps*14-*atp*8 spacer) and the nine nuclear microsatellite loci. The table shows the frequency of amplification in each locus for the two groups of individuals (mt-Esil and mt-Ecro) identified based on the cytoplasmic information. Microsatellite loci were classified as diagnostic for *E. siliculosus*, diagnostic for *E. crouaniorum* or showing diagnostic alleles. The frequency of amplification and allele size were used to classify the nine microsatellite loci.

Locus category	Locus name	**mt-*Esil***			**mt-*Ecro***		
		N	Amplified		N	Amplified	
Diagnostic loci *Esil*	M-033-1	519	489	0.94	385	51*	0.13
	M-239-3	519	501	0.97	385	48*	0.12
	M-103-2	519	513	0.99	385	47*	0.12
	M-387	519	508	0.98	385	48*	0.12
Diagnostic loci *Ecro*	M-388	519	23*	0.04	385	374	0.97
	M-420	519	14*	0.03	385	338	0.88
Diagnostic alleles	M-122-2	519	518	1.00	385	376	0.98
	M-208	519	515	0.99	385	384	1.00
	M-162-1	519	513	0.99	385	385	1.00

mt-*Esil*: cytoplasmic identification as *E. siliculosus*, mt-*Ecro*: cytoplasmic identification as *E. crouaniorum*, diagnostic loci: loci that amplify only or mostly in one cytoplasmic group, diagnostic alleles = loci that amplify in both cytoplasmic groups but but for which alleles differ in size between mt-*Esil* and mt-*Ecro* (see Figure III.3).

* = putative hybrid individuals showing amplification of the species-specific loci of both species

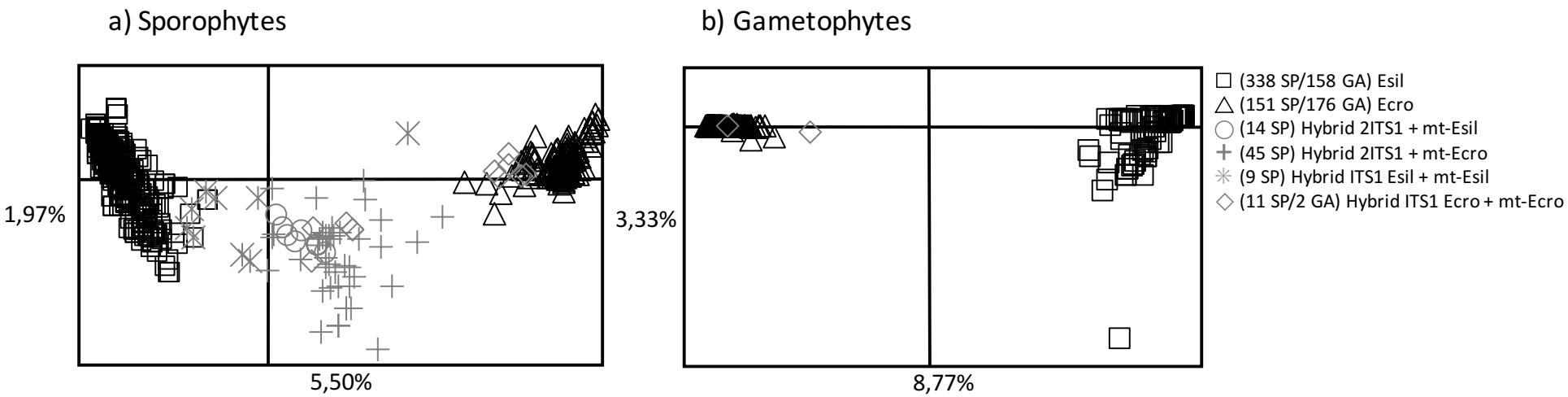

Figure III.4. Principal component analysis of *E. siliculosus*, *E. crouaniorum* and putative hybrid individuals presented for a) all sporophytes and b) all gametophytes using the nine microsatellite loci. First and second axes represent the first two factorial components.
The legend of the different symbols refers to the two parental species *E. siliculosus* (Esil) and *E. crouaniorum* (Ecro) and the four classes of hybrids (2ITS1: presence of the two ITS1 species-specific bands; ITS1 Esil: presence of the ITS1 band specific of *E. siliculosus*; ITS1 Ecro: presence of the ITS1 band specific of *E. crouaniorum*; mt-Esil: mitochondrial specific marker of *E. siliculosus*, mt-Ecro: mitochondrial specific marker of *E. crouaniorum*. The number of sporophyes (SP) and gametophytes (GA) observed in each of the six categories is given into brackets.

For the loci M-208 and M-162-1, alleles were shorter in Ecro than in Esil (Table III.2, Fig. III.3) while for the locus M-122-2 shorter alleles were observed in Esil (Table III.2, Figure III.3). Species identification combining the data from the three kind markers (i.e. mitochondrial rps14-atp8, nuclear ITS1, and nuclear microsatellite loci) increased the number of putative hybrid individuals to 81 (the genotypes of each putative hybrid for all the markers used is given in Table S1a, Appendix III.3). All these 81 putative hybrids showed amplification products for at least one diagnostic locus that was not congruent with the cytoplasmic species identification (see Table S1a, Appendix III.3). Most putative hybrids were diploids (79 out of a total of 568 sporophytes); only two putative hybrids were identified among the 336 gametophytes.

- Multivariate clustering of individuals

The results of the PCA analyses illustrate the genetic structure between the parental species and the putative hybrids for both diploid sporophytes (Fig. III.4a) and haploid gametophytes (Fig. III.4b). The first two axes of the PCA explained 7.47% and 12.10% of the genetic variability of sporophyte and gametophyte individuals, respectively. Whatever the phase under study (*i.e.* diploid sporophytes or haploid gametophytes), the PCA revealed a clear genetic differentiation between Esil and Ecro. Among the 568 studied sporophytes, 69 out of the 79 putative hybrids were clearly intermediate, being located between the Esil and Ecro groups, while the remaining 10 were not detected with this method (Table S1b, Appendix III.3). The 69 intermediate individuals included not only all the 59 putative hybrids that were characterized by an ITS1 double band pattern (14 with an Esil and 45 with an Ecro cytoplasm,

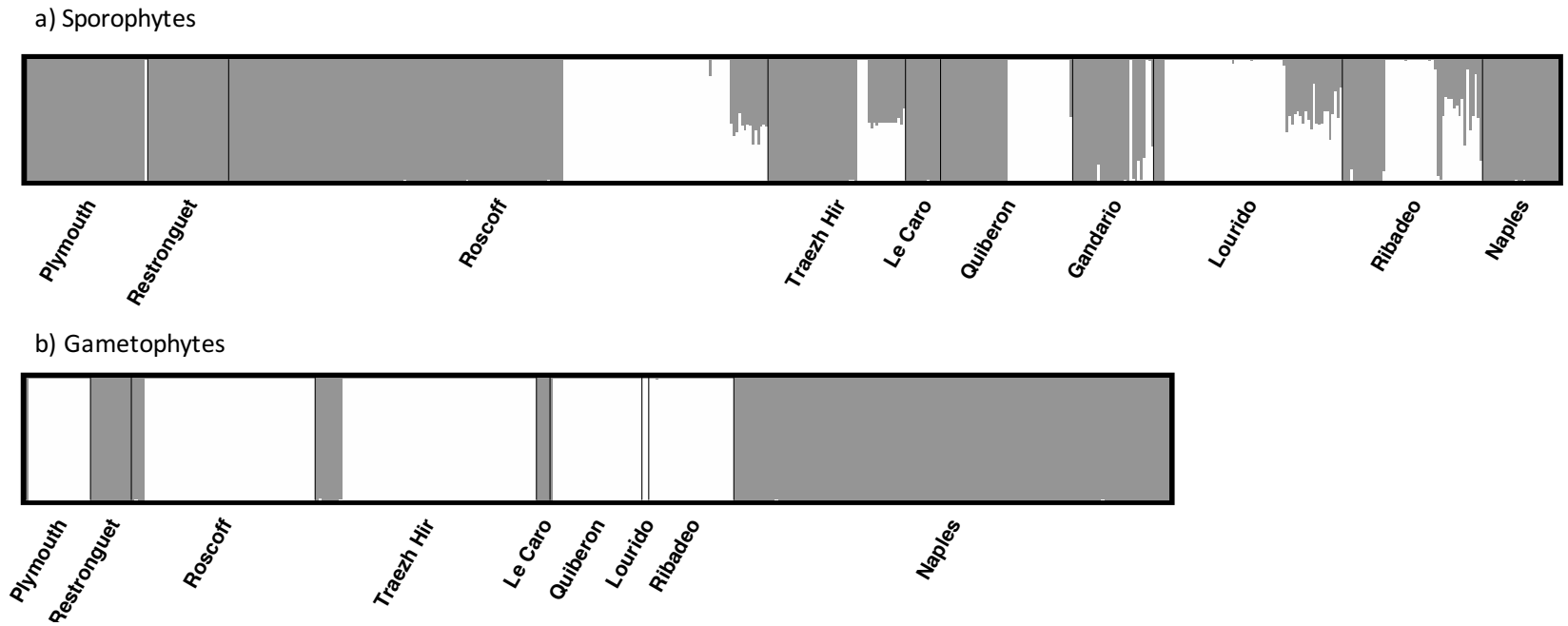

Figure III.5. Clustering analysis performed with STRUCTURE using the nine microsatellite loci. Results are shown for K2 for a) all sporophytes and b) all gametophytes. Each vertical bar represents a different individual. The shading represents the proportion of individual genome assigned to each genetic group (grey *E. siliculosus* and white *E. crouaniorum*). Sampling sites are noted below and sites are ordered from North to South.

Table III.1) but also 10 additional individuals identified as admixed only on the basis of the microsatellite locus information (5 individuals bearing mitochondrial and ITS1 specific markers of Esil and 5 individuals bearing mitochondrial and ITS1 specific markers of Ecro, Table S1b, Appendix III.3). Among the 336 haploid gametophytes, only one of the two putative hybrids could be distinguished from the two parental clusters (Fig. III.4b). This individual (Gal 159, Table S1b, Appendix III.3), which bore mitochondrial and I TS1 specific markers o f Ecro, was located near the Ecro cluster.

- *Admixture analysis and assignation of putative hybrids to genetic categories*

The clustering analysis performed with STRUCTURE confirmed that two was the optimal number of clusters for both haploid and diploid datasets (ΔK method of Evanno *et al.* 2005; Appendix III.4). The level of admixture for each individual within each population is given in Fig. III.5a for diploid sporophytes and Fig. III.5b for haploid gametophytes. The results of this Bayesian approach were very similar to those obtained with the multivariate PCA analysis: 67 of the 79 diploid putative hybrids were assigned as admixed with this method (Fig. III.5a). These 67 admixed individuals were also all classified as intermediate based on the PCA analysis (Table S1b, Appendix III.3). Most admixed individuals (61/67, Fig. III.5a) were allocated into the genetic category of potential F1 hybrids with equal proportions of Esil and Ecro genomes (15 with an Esil and 46 with an Ecro cytoplasm, Table S1b, Appendix III.3). The six remaining admixed diploid individuals were classified as coming from backcrosses or repeated interbreeding with Ecro (3 individuals) or Esil (3 individuals). However, in contrast to the previous methods, this Bayesian approach was not able to identify any putative hybrids among the haploid gametophyte individuals (Fig. III.5b). Finally,

STRUCTURE results clearly showed that all putative hybrids were only observed in sympatric populations (Fig. III.5a).

The results of the PCA considering both the diploid individuals observed in our study (489 parental individuals and 79 putative hybrids) and the 1500 diploid genotypes simulated by ADEGENET HYBRIDIZE (Jombart 2008) are shown in Figure III.6.

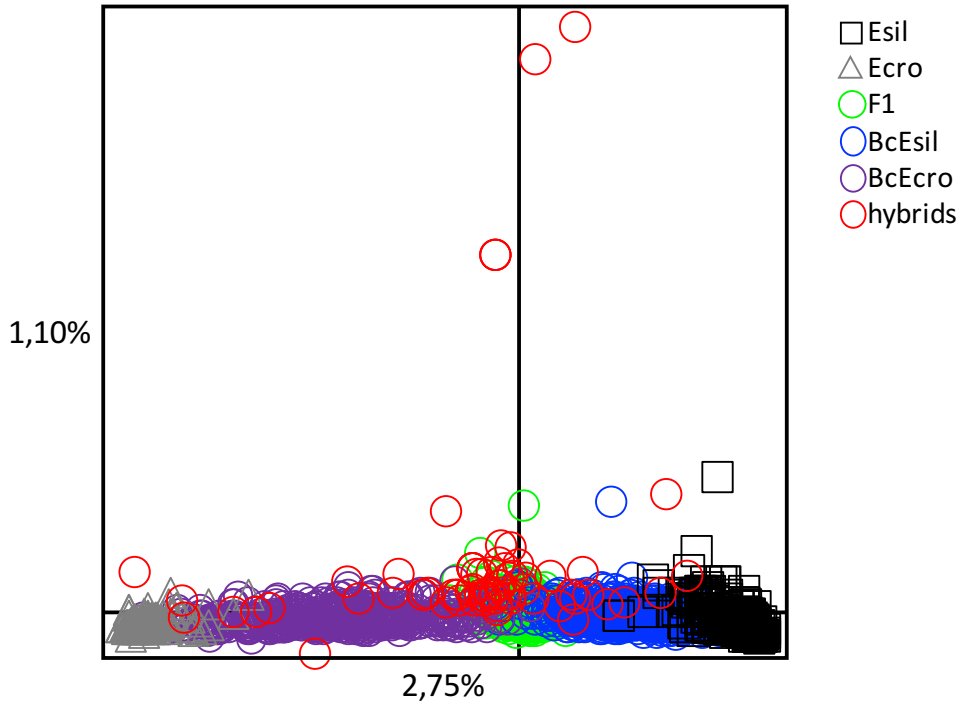

Figure III.6. Principal component analysis including all sporophytes observed in our data set and diploid artificial genotypes simulated using ADEGENET HYBRIDIZE (Jombart 2008). The parental species *E. siliculosus* and *E. crouaniorum* and the 79 putative hybrids are represented as black squares, grey triangles and red circles, respectively. The 500 artificial genotypes of each hybrid class are represented as circles: F1 (green), backcrosses with *E. siliculosus* (blue) and backcrosses with *E. crouaniorum* (purple). First and second axes represent the first two factorial components.

The first two axes of the PCA explained 3.85% of the total genetic variability and, again, a clear distinction between Esil and Ecro parental species was retrieved. The simulated F1 were easily recognisable by their intermediate position between the two

parental species while simulated backcrosses overlapped partly with the simulated F1 hybrids and their respective parental species (Fig. III.6). Again, most of the 79 putative hybrids sporophytes substantially overlapped the simulated F1 while fewer could be identified as older generations of hybrids closely related to parental species (details about assignment to the different simulated hybrid classes are given below). Finally, three outliers were detected on the Y axis (Fig.III.6). These outliers corresponded to five individuals with three different multi-locus genotypes from the Ribadeo population (GAL 176,177, 217, 237 and 239, Table S1a, Appendix III.3) that share the same rare diagnostic allele (allele 298 at locus M-162-1).

GENECLASS assigned 76 of the 79 putative hybrid sporophytes to one of the three simulated hybrid classes with only 3 individuals classified as either the parental species Esil (GAL541) or Ecro (GAL101 and EcPly10-8) (Table S1b, Appendix III.3). Again, we found that most putative hybrids (54/76) were classified as simulated F1 (15 with an Esil and 39 with an Ecro cytoplasm, Table S1b, Appendix III.3). Five hybrid individuals were assigned as simulated backcrosses with Esil and 14 as backcrosses with Ecro (Table S1b, Appendix III.3). Only three individuals could not be assigned to a single hybrid class (EcPH12-s#4-04, GAL217 and GAL200, Table S1b, Appendix III.3).

Our new method XPLOIDASSIGNMENT allowed a simultaneous analysis of all the 81 putative hybrids regardless of their ploidy and without the needs to recode aneuploid genotypes. It identified the highest number of hybrids (79/81) as compared to the other analyses described above. Note that two individuals (GAL541 and GAL101), yet identified as putative hybrid using our preliminary method based on detecting conflicting results with the mitochondrial markers, on one hand, with both the ITS1 and the species-specific microsatellite loci on the other, were assigned by all the different

admixture analyses used in this study (Table S1b, Appendix III.3) to only one of the parental species Esil (GAL541) and Ecro (GAL101). Only half of the 79 putative hybrids (43 diploids and 2 haploids) could be assigned to a single hybrid class with confidence (Table S1b, Appendix III.3). Sixty per cent of diploid sporophytes (26/43) were assigned to F1 hybrids, 11 were assigned as backcrosses with Esil and six as backcrosses with Ecro (Table S1b, Appendix III.3). The two haploid hybrids were classified as backcrosses (Table S1b, Appendix III.3). The 36 remaining putative diploid hybrids were classified in two equally possible hybrid classes, with most of them (31/36) being classified as F1 or backcrosses with Esil (Table S1b, Appendix III.3).

- Comparison among methods and frequency of hybridization in populations

The four methods were mostly congruent to distinguish admixed individuals from parental species. However, the classification of individuals into the different hybrid classes was challenging. The information of the different methods was thus combined to classify hybrids in one or more than one hybrid categories when methods were not congruent (Table S1b, Appendix III.3). The objective was to roughly distinguish hybrids recently formed from those resulting from subsequent generations of backcrosses. As two individuals (GAL541, GAL101) were consistently classified as parental species by STRUCTURE, GENECLASS and XPLOIDASSIGNMENT, a total of 79 hybrids (77 diploids and 2 haploids) out of the 81 putative hybrids detected previously were finally retained (Table S1b, Appendix III.3). 35% of the alleles genotyped in the 79 putative hybrids were rare alleles (i.e. observed at a frequency less than 5% in the parental species, Table S1a, Appendix III.3). A total of 96 rare alleles

were observed, 79 were shared between parental species and hybrids while 17 were only present in hybrids. Moreover, of the 79 shared rare alleles, two alleles (allele 307 of locus M-420 and allele 207 of locus M-239-3, Table S1a, Appendix III.3) raised above 24% in hybrids. More than 70% of the hybrids (58/79) possessed Ecro cytoplasmic markers (Table S1b, Appendix III.3). When we considered the 20 hybrids that were classified in a single category, most of them (19) could be classified as recently formed (F1) while only one was classified as backcrosses (Tables S1b, Appendix III.3).

Table III.3. Populations sampled and number of individuals collected for each species and their hybrids. Hybrids were determinate by the combination of the three methods used in this study (i.e. XploidAssignment, GeneClass and Structure; the two individuals that were first identified as hybrids but not retained using the combination of the three methods are shown within brackets). In the table, both phases (i.e. sporophytes and gametophytes) are represented in parental species and hybrids.

		Esil		***Ecro***		**Hybrids**			
Regions	**Populations**	**SP**	**GA**	**SP**	**GA**	**SP**	**GA**	**SP+GA**	**Frequency of hybrids**
U.K.	Plymouth*	44	1	0	18	1	0	1	0.015
	Restronguet	30	12	0	0	0	0	0	0.000
Brittany	Roscoff*	124	4	62	50	14	0	14	0.055
	Le Caro	13	4	0	0	0	0	0	0.000
	Traezh Hir*	33	8	4	57	14	0	14	0.121
	Quiberon*	25	1	23	26	1	0	1	0.013
NW Iberia	Ribadeo*	16	0	18	23	18	0	18	0.240
	Gandario*	22	0	1	0	7 (8)	0	7 (8)	0.133
	Lourido*	4	0	44	2	22 (23)	2	24 (25)	0.333
Mediterranean	Naples	28	128	0	0	0	0	0	0.000
	TOTAL	**339**	**158**	**152**	**176**	**77 (79)**	**2**	**79 (81)**	

**: sympatric populations*
Esil = *E. siliculosus*, *Ecro* = *E. crouaniorum*, SP= sporophytes, GA= Gametophytes

The percentage of admixed individuals over the whole data set including haploid and diploid subpopulations was estimated at 8.7% (79/904). The percentage of hybrids was much higher in diploid (77/568 =13.6%) than in haploid (2/336 = 0.6%) subpopulations. The occurrence of hybrids varied among populations and regions (from

0 to 0.33) and hybrids were only observed in sympatric populations (Table III.3). The percentage of admixed haploid and diploid individuals, when calculated as a proportion of all sympatric populations, was 11.5% (79/689).

Table III.4. Genotypic diversity and deviation from random mating in *E. siliculosus* (7 loci) and *E. crouaniorum* (5 loci).

Species	Ploidy	Populations	N	MLG	R	F_{is}	
E. siliculosus	2n	Plymouth	44	17	0.37	0,023	NS
		Restronguet	30	23	0.76	-0,045	NS
		Roscoff	104	65	0.62	0,134	***
		Traezh Hir	27	13	0.46	0,095	*
		Le Caro	11	10	0.90	-0,056	NS
		Quiberon	25	15	0.58	-0,003	NS
		Gandario	19	19	1.00	0,093	*
		Ribadeo	15	12	0.79	0,076	NS
		Naples	26	17	0.64	0,047	NS
	n	Restronguet	12	11	0.91	---	
		Traezh Hir	7	5	0.67	---	
		Naples	117	47	0.40	---	
E. crouaniorum	2n	Roscoff	54	51	0.94	0,176	***
		Quiberon	21	12	0.55	-0,024	NS
		Lourido	45	34	0.75	0,229	***
		Ribadeo	15	14	0.93	0,255	***
	n	Plymouth	12	12	1.00	---	
		Roscoff	43	41	0.95	---	
		Quiberon	18	16	0.88	---	
		Ribadeo	19	18	0.94	---	
		Traezh Hir	37	34	0.92	---	

N= number of individuals genotyped, MLG= number of different multilocus genotypes, R= clonal diversity, F_{IS}= inbreeding coefficient and test for deviation from random mating: NS: non significant, *: $p<0.05$, ***: $p<0.01$.

- Population structure and mating system

The efficiency of the nine microsatellite loci to discriminate individuals varied between species and among populations. Less than 10% of the sites harboured samples that consisted only of unique multilocus genotypes (i.e. Gandario for Esil and Plymouth for Ecro, Table III.4), all the other sites showed repeated multilocus genotypes. Clonal diversity (R values, Table III.4) ranged from 0.37 to 1 in Esil and from 0.55 to 1 in Ecro, suggesting that clonal propagation might be important in some sites. However, we did not find any significant relationship between the pattern of clonal diversity and the frequency of hybrids in our data. Moreover, we never observed significant excesses of heterozygotes as expected under clonal reproduction (Table III.4). Instead, *FIS* values were either not significantly different from zero or significantly positive depending on the species and populations (Table III.4). All but one Ecro population exhibited significantly high heterozygote deficiencies, while most Esil populations (6 over 9, Table III.4) did not show any significant departure from random mating. Differentiation among populations (global *FST* value, Weir & Cockerham, 1984) calculated for each species and each haploid and diploid subpopulations was higher for Esil than Ecro (Esil: *FST* sporophytes = 0.15, *FST* gametophytes = 0.45; Ecro: *FST* sporophytes = 0.11, *FST* gametophytes = 0.08). All these *FST* values were significantly higher than 0.

V. Discussion

- Species-diagnostic markers to detect hybridization in cryptic species

The lack of any obvious phenotypic divergence between *Ectocarpus* species of the *siliculosi* group made the study of hybridization in the field a challenge (but see Peters *et al.* 2010a; Couceiro *et al.* 2015 and Montecinos *et al.* 2016) since neither the cryptic parental species nor their intermediate hybrid forms can be recognised in the field or even in the laboratory. The use of the cytoplasmic species-specific marker rps14-atp8 and the nuclear marker ITS1 in combination with 9 microsatellite loci (6 being diagnostic of one of the parental species and 3 showing diagnostic alleles), however, allowed us to identify them and to detect signals of genetic admixture between *E. siliculosus* and *E. crouaniorum*. The use of microsatellites to estimate hybridization has been criticised because of the possible homoplasy that can arise when alleles are shared between related species (Balloux & Goudet 2002). However, if homoplasy may decrease identifiability when classifying hybrids in different hybrid scenarios, our results rather argue that, even from a handful of microsatellites, we can harvest relevant information with adapted methods. Moreover, this argument cannot hold when diagnostic alleles and diagnostic loci are used. Admixture and assignment analyses showed a high discriminatory power of the microsatellite species-diagnostic markers to distinguish at least parental from admixed individuals. The Bayesian method we developed showed congruent results with the classical STRUCTURE approaches (e.g. Ito et al. 2015; Turchetto et al. 2015) but has the advantage that it mathematically formalizes and automates the computation of the posterior probabilities of individuals to distinct scenarios of hybridization. As secondary contacts are often associated with changes in ploidy that either affect the entire genome or only some chromosomes

(aneuploidy) and as clonal reproduction may favour the emergence of duplicated genes, our method can be applied to raw genetic data without correcting them for ploidy. Although our method still requires the provision of the genotypes of the individuals identified as belonging exclusively (as far as possible) to the parental species involved, it can assign individuals to hybrid categories without clustering them into populations and thus doesn't rely on biological assumptions like Hardy-Weinberg equilibrium, sexual reproduction or maximal divergence between populations. Indeed, a higher number of true hybrids could be identified with XPLOIDASSIGNMENT and in particular the method allowed the identification of hybrids in the haploid subpopulation. Only half of the hybrids could be assigned to a single hybrid class with confidence but the distribution of posterior probabilities between admixture scenarios help to reconstruct more complex hybridization histories than only assess with our 5 scenarios. Finally, combining the four admixture methods and the results obtained for different marker types, we were able to detect 79 (8.7%) admixed individuals between *E. siliculosus* and *E. crouaniorum*.

The species-specific ITS1 marker was generally consistent with the species-diagnostic microsatellite markers and all the individuals bearing both ITS1 lengths were identified as admixed. However, of the 79 admixed individuals revealed by our study, 8 presented only the ITS1 band specific of *E. siliculosus* and 12 presented only the ITS1 band specific of *E. crouaniorum* (Table S1b, Appendix III.3). The process of concerted evolution (Arnheim *et al.* 1980) is possibly the mechanism that could explain why these hybrid individuals lost the ITS1 sequence of one parental species. This process causes the homogenization of tandem repeat sequences through genomic mechanisms of turnover like gene conversion and unequal crossing over (Dover 1994) and has been invoked as the mechanism responsible for ITS homogenization after hybridization

events in plants. Five hybrids identified as F1 in our study showed only one parental ITS1 band, which suggests that the process of concerted evolution may happen in the first hybrid generation. Moreover, three different outcomes have been reported for the ribosomal repeat following hybridization in higher plants: (i) both parental ITS sequences are retained (Kim & Jansen 1994; O'Kane *et al.* 1996; Franzke & Mummenhoff 1999) (ii) only one parental type is retained after homogenisation (Wendel *et al.* 1995, Franzke & Mummenhoff 1999) (iii) the ribosomal repeat is homogenized but contains scattered elements of both parents (Wendel *et al.* 1995; van Houten *et al.* 1993). In marine seaweeds this process is less studied but intraspecific and intra-individual polymorphisms of ITS are well documented and have been related presumably to incomplete homogenization under concerted evolution after events of recent speciation, hybridization, shift to asexual reproduction or polyploidization (Pillmann *et al.* 1997; Serrão *et al.* 1999; Famá *et al.* 2000; Coyer *et al.* 2001). In practice, our results confirm that barcoding methods based on single markers should be used with caution.

- Low level of hybridization are probably associated with reproductive barriers

The frequency of hybridization between *E. siliculosus* and *E. crouaniorum* was estimated to 8.7% based on the whole data set (haploid and diploid individuals). In addition, hybrids showed positive amplification of the cytoplasmic marker of both species suggesting that inter-specific crosses occurred in both directions. Interestingly, hybridization was only detected in sympatric/parapatric populations and almost exclusively in diploid sporophytes. In addition, most hybrids seemed to be recently formed. Together these results suggest that postzygotic mechanisms limit sexual

fertility in admixed individuals. Indeed, the near absence of admixture in haploid subpopulations suggests that meiosis probably acts as a strong post-zygotic barrier in the first hybrid generation (Fig. III.1). However, the detection of a small number of hybrids that correspond to subsequent generations of backcrosses in haploids and diploid hybrids on the one hand and the occurrence of aneuploidy on the other, suggest that the reproductive barrier, although strong, is probably not complete. Below, we discuss the importance of hybridization between these closely related species in the light of their biological and ecological characteristics and compare with the small number of other documented studies of hybridization in seaweeds. In particular, we consider the possibility of occurrence of post-zygotic barriers in these haploid-diploid species and question which mechanisms might be involved in reproductive isolation.

The environmental gradient of the shore is a classic example of a tension zone in which parental types are favoured at the opposite ends with hybrids occurring in between (see for example in marine invertebrates: Johannesson 2009; Bierne *et al.* 2003; Bouchemousse *et al.* 2016). Among seaweeds, the best-studied examples of hybridization come from various species of the genus *Fucus* that dominate the intertidal rocky shores of the North Atlantic (Coyer *et al.* 2002; Wallace *et al.* 2004; Billard *et al.* 2005; Engel et al. 2005; Coyer *et al.* 2007; Neiva *et al.* 2010; Coyer *et al.* 2011, Monteiro *et al.* 2012). The occurrence of hybridization between these species was first explained by their recent and rapid radiation within the last 3.8 million years, which resulted in a complex of highly related sister species (Serrão *et al.* 1999; Coyer *et al.* 2006). In addition, habitat-driven speciation has been invoked to explain divergence despite the occurrence of gene flow in this genus (in *F. vesiculosus/F. radicans*: Bergström *et al.* 2005; Pereyra *et al.* 2009; and in *F. spiralis/F. vesiculosus/ F. guyrii*: Zardi *et al.* 2011). Indeed, the different species are often found in sympatry/parapatry

along the European coast while distributed along the vertical selective gradient of the intertidal zone (i.e. in different abiotic and biotic micro-environmental habitats). In such a complex context, Hoarau *et al.* (2015) showed that the level of hybridization and interspecific fertilization success decreased with increasing time of sympatry between *F. serratus* and *F. distichus* suggesting reinforcement of isolation mechanisms.

In filamentous Ectocarpales, the lack of obvious phenotypic divergences between cryptic species (see Peters *et al.* 2015) explains that relatively little is known about their distribution, the importance of species co-occurrence and their level of hybridization in the wild. These brown filamentous algae were described as short-lived annuals, found on abiotic substrata or epiphytic on macrophytes, along the shore gradient from subtidal up to high intertidal pools (Russell 1967a, b, 1983). However, due to the development of species-specific molecular markers, recent studies have reported that cryptic species may be distinguished on the basis of their spatio-temporal ecological niches related to different tide levels and/or host specificity (in *Ectocarpus spp.*, see references below; in *Pylaiella* spp., Christophe Destombe com. pers.). In particular, Peters *et al.* (2010a), Couceiro *et al.* (2015) and Montecinos *et al.* (2016) showed that *E. crouaniorum* was located higher on the shore than *E. siliculosus* in several sites of the North Atlantic. In addition, in a detailed spatio-temporal study of North-Western French population, Couceiro *et al.* (2015), reported differences in host specificity between these two species with *E. crouaniorum* being exclusively found on a single species while *E. siliculosus* could be found on more than five species. However, all three studies mentioned that along the shore gradient the two cryptic species could co-occur in a contact zone where their distributions overlap. In the same way, the study of Couceiro *et al.* (2015) reported that the two species could be found on the same host, since *E. siliculosus* was able to grow on *Scytosiphon lomentaria*, the only host species for *E.*

crouaniorum. This situation resembles that of the *Fucus* hybridization zone along the steep gradient of the shore.

At sites where the two *Ectocarpus* species are found in sympatry, ecological barriers such as habitat preference combined with host specificity may limit hybridization and explain why only 11.2% of hybrids are observed. However, unlike *Fucus* species which have a classical diploid life cycle, the distinguishing feature of the haploid-diploid life cycle of *Ectocarpus* species makes it possible to distinguish the results of fertilization and meiosis by comparing diploid and haploid sub-populations. Consequently, it is possible to directly assess the importance of prezygotic compared to postzygotic reproductive barriers. In this study, we observed very few recombinant haploid individuals suggesting the presence of strong post-zygotic barriers, which confirmed previous results from crossing experiments between few individuals (Peters et al. 2010a), in which most hybrid zygotes did not develop beyond an early germination stage.

- *Presence of rare alleles in hybrids.*

Our results revealed that all 96 rare alleles were present in hybrids and two displayed a considerably higher frequency in hybrids compared to parental species. This observation may explain some of the discrepancies between the three assignment methods used to detect hybridization depending on the weight that is given to rare alleles. Interestingly, the presence of rare alleles in hybrid individuals (the rare allele phenomenon) has been observed in hybrid zones of land plants and animals using a wide range of markers, including intron haplotypes (Schilthuizen *et al.* 1999), rDNA spacer variants (Liao *et al.* 2010), microsatellites (Lexer *et al.* 2007) and SNPs (Lammers *et al.* 2013). The rare allele phenomenon refers to the observation of certain

alleles that are normally rare or virtually non-existent in both parental species rise to high frequencies in the centre of the hybrid zone. Coyne & Orr (2004) have hypothesized that, in the centre of a tension zone, the continuous generation of low-fitness recombinants will favour any allele that decreases hybrid disadvantage. Since this selective environment is uniquely restricted to the hybrid zone itself, the centre of the zone is expected to show not only clinal transition for alleles fixed in one of the two species but also a sharp increase in those gene variants (and any linked genetic markers) that help to increase the fitness of hybrids. In this study with *Ectocarpus* species, it remains unclear if the zone in which the two species are in contact in sympatric populations can be considered as a tension zone. Whether or not the presence of rare alleles in hybrids is a product of the rare allele phenomena will require a further sampling specifically designed to address this question.

- Rarity of haploid hybrids and the existence of reproductive isolation

Crossing experiments carried out by Peters *et al.* (2010a) showed that, occasionally, *E. crouaniorum* may form viable sporophyte hybrids with *E. siliculosus.* However, the presence of abortive unilocular sporangia (i.e. the site where spores are formed by meiosis on diploid sporophytes) was observed in crosses among *Ectocarpus* strains from geographically distant origins (Müller 1988; Stache 1990). Müller (1988) attributed the abortive unilocular sporangia to severe problems in chromosome pairing probably linked to differences in genome sizes (Peters *et al.* 2004a). Interestingly, in this study, we observed that 40% of hybrid sporophytes possessed three alleles for at least one microsatellite locus. In all these cases, heterozygotes with 3 alleles were composed of two alleles diagnostic of one species with the third allele diagnostic of the second species. These triallelic heterozygotes might reflect the occurrence of aneuploid gametes. Such patterns of abnormal segregation during meiosis could be a consequence of karyotypic rearrangements in chromosomes between *E. siliculosu*s and *E. crouaniorum* creating chromatids with duplications and deficiencies in gene content (recombination suppressors as described in the review of Livingstone & Rieseberg 2004). Such karyotypic rearrangements could explain the very low frequency of recombinant hybrid haploids detected in our study. In plant species, reproductive isolation has been largely linked to translocations that rearrange the genome of one species relative to another (Stebbins 1971, Quillet *et al.* 1995, Spirito 1998, Rieseberg 2001). Moreover, in angiosperms, hybrid fertility is often restored by experimentally inducing tetraploidy, suggesting that meiotic recombination between different karyotypes led to F1 hybrid sterility (Stebbins 1958). Such chromosomal rearrangements also have been linked to reduced fertility in hybrids produced in haploid-diploid species (e. g. mosses: Anderson & Snider 1982, yeasts: Liti *et al.* 2006,

ferns: Wagner 1987). However, whether such rearrangements contribute to the speciation process is a matter of debate (Sites & Moritz 1987; Coyne & Orr 2004). We observed that some of the identified diploid sporophyte hybrids shared the same multi-locus genotypes suggesting the occurrence of clonal multiplication of these hybrids. However, as asexual reproduction does also occur in the parental populations, it is difficult to ascertain that the observed clonal multiplication of diploid sporophytes is favoured because of post-zygotic barriers. Finally, despite the extreme rarity of recombinant genotypes in the haploid dataset, analyses of simulated backcrosses suggested the occurrence of subsequent generations after F1 sporophyte hybrids in our dataset. This result suggests that post-zygotic incompatibility might not be total between both species. However, several studies have argued that identifying individuals with hybrid ancestry could become increasingly difficult after the first three generations using the classical methods we employed in this paper (e.g. Lavrestky *et al.* 2016). Thus we cannot reject the hypothesis that these later hybrid generations were misidentified. In order to verify the importance of post-zygotic isolation, several further experiments can be suggested. First, as all genotyped individuals are kept as uni-algal culture in the lab, the possible occurrence of regular meiosis can be tested experimentally for each of the 77 identified hybrid sporophyte strains. Second, using next-generation sequencing, it is now possible to examine genome-wide patterns of introgression and identify genomic regions that show signatures of selection.

- *Conclusion*

In conclusion, our study demonstrates that brown algae of the genus *Ectocarpus* can be used to study hybridisation in haploid-diploid species. The diversity of taxa with various levels of divergence in *Ectocarpus* (Stache-Crain *et al.* 1997; Peters *et al.* 2010b; Peters *et al.* 2015, Montecinos *et al.* 2016) makes this genus a good model for studies of reproductive isolation. Crossing experiments between *E. siliculosus* and the genetically more distant species *E. fasciculatus* have shown that plasmogamy was only possible between male gametes of *E. fasciculatus* and females of *E. siliculosus*, however the hybrids died after germination (Müller & Gassmann 1980). In contrast, cytoplasmic introgression among closely related species belonging to the *E. siliculosi* group was suggested by Montecinos *et al.* (2016). Together, these results suggest a probable relationship between genetic distance and cross-compatibility in this genus. Follow-up studies on this system could provide further insights into the evolutionary consequences of hybridization and introgression for the maintenance or breakdown of species.

VI. Acknowledgments

We would like to thank Chloé Jollivet, Laurence Dartevelle and Jérôme Coudret for help in maintaining strains in the Station Biologique de Roscoff. We also thank Stéphane Mauger and Murray Brown for assistance in the molecular laboratory and the field, respectively. Declan Schroeder (MBA, Plymouth) and Ignacio Bárbara (Universidade da Coruña) kindly welcomed us for isolation work in their laboratories. We particularly appreciated insightful and constructive comments from Thomas Broquet, Christophe Destombe and Mark Cock. Special thanks to Christophe Destombe who conceived and drew Figure III.1. Principal funding came from a doctoral grant to Alejandro Montecinos (Becas-Chile, CONICYT, advanced human resources program), additional support from the projects MARINEXUS (EU INTERREG programme France (Channel)-UK), Bi-Cycle (France: ANR10-BLAN-1727), Clonix (France:ANR11-BSV7-00704), IDEALG (France: ANR-10-BTBR-04), "Bibliothèque du vivant" (France: INRA-MNHN-INEE-CNRS) and from the international research network "Diversity, Evolution and Biotechnology of Marine Algae" (GDRI No. 0803). The authors have declared that no competing interests exist.

Appendix III.1

XPloidAssignment

version 1.0, July 2016

A software to assign genotyped individuals to populations or genetic pools when ploidy varies between individuals or loci.

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How to cite ClonEstiMate:

Montecinos AE, Guillemin ML, Couceiro L, Peters AF, Stoeckel S, Valero M; (submitted). Hybridization between two cryptic filamentous brown seaweeds along the shore: Analysing pre- and post-zygotic barriers in populations of individuals with varying ploidy levels.

I). Purpose of this software

This program aims at assigning individuals to populations or sub-species when individuals and populations were genotyped using multiple loci. The specificity of this software is that it allows haploid,-diploid or-polyploid loci or individuals. It takes into account different numbers of alleles per locus and per individual.
The likelihood of an allele is its frequency within the population or subspecies considered. All likelihoods are combined in a Bayes formula to compute the posterior likelihood that the considered individual belong to the reference populations. This method computes three scenarios of genetic admixture between the references populations: one scenario of new secondary contact or interbreeding, and two scenarios of older secondary contact or interbreeding (such as backcrosses) where one population would constitute 75% of the genome of the studied individuals (and the complementary hybrid class 25% of the genome)
This software can be used to assign individuals to populations, migrants, admixed and hybrids, individuals lost in collection into previously genetically identified subspecies or (geographical, phenotypic, etc.) populations.

II). How to get and use XploidAssignment

1) On GNU/Linux
(tested on Lubuntu 15.10 –wily werewolf- and 16.04 –xenial xerus)
1- Download XploidAssignment1.0.tar.gz at
https://www6.rennes.inra.fr/igepp_eng/Productions/Software
2- Unpack the downloaded archive XploidAssignment1.0.tar.gz. In a terminal, enter *tar xzvf XploidAssignment1.0.tar.gz*. Through a file manager, right click on the archive and use “extract” contextual menu.
The archive contains:

- A **User Manual.pdf**,
- A binary file named **XploidAssignment1.0** in ELF (Executable and Linkable Format, used in most unix OS based excepted Mac Os),
- two *.txt files : **Genotype Data Example.txt** and **Plan Example.txt,** examples to learn how to format and use the software below.

2- Open a terminal in the folder where the GNU/Linux binary file was extracted.
Remark: cd path-to-the-folder or use your file manager application (for example, use "Tools" menu and "open a terminal here")
3- You need to run the binaries as root because the program will have to write files and even create a new folder in that path. Thus, enter: sudo ./XploidAssignment1.0
The console will ask for your root password, enter it and hit enter.
4- A first window will open. You will have to select your FREQUENCY FILE then to validate by clicking on "open" button. Then, a second window will open (sometimes so

fast that you can think that you omitted to click on the open button from the previous selection, so please take care). Select the file containing your Individual to be assigned FILE.
5- The program will run and will provide some information about what it is computing.
6- To get your results, go into the new folder the program created, named "results". In the terminal, cd results. Here, you will find **one *.txt file** whose name begins with a date. See Ouput section for help with reading this result file.

2) On Windows

(tested on windows 7 and windows 10)
1- Download XploidAssignment1.0.zip at
https://www6.rennes.inra.fr/igepp_eng/Productions/Software
2- Unzip the downloaded archive XploidAssignment1.0.zip. We recommend 7zip or the windows-integrated zip archive manager. Through the Explorer file manager, right click on the archive and use "extract" contextual menu.
The archive contains:

- A **User Guide.pdf**,
- A binary file named **XploidAssignment1.0.exe**,
- two *.txt files : **Genotype Data Example.txt** and **Plan Example.txt,** examples to learn how to format and use the software below.

3- Run the binaries as root because the program will have to write files and even create a new folder in that path. Double-click on XploidAssignment1.0.exe to run it.
4- A first window will open. You will have to select your FREQUENCY FILE then to validate by clicking on "open" button. Then, a second window will open (sometimes so fast that you can think that you omitted to click on the open button from the previous selection, so please take care). Select the file containing your Individual to be assigned FILE.
5- The program will run by opening a terminal in which it will provide some information about what it is computing.
6- To get your results, go in the new folder the program created, named "results". In the terminal, cd results. Here, you will find **one *.txt file** whose name begins with a date. See Ouput section section for help with reading this result file.

III). How to format your data

We provided 2 example files you can refer to. The software will ask for 2 files: First, one containing the allele frequencies in each reference populations, Then, one file containing the genotyped individuals you want to assign to reference populations.

1). FREQUENCY FILE

This file should be formatted in *.txt (tabulation-separated values which can be achieved using most text editor or LibreOffice or Excell). In line, we expect alleles and in column reference populations or subspecies. First line should contain the names of the populations (coded in utf-8) separated by tabulations, after a first "locus" word. From the second line on, write the data. In each column, never use space character or tabulation. One line of data should contain at least 4 columns.

- on the first column, the name of the locus (string in utf-8)
- on the second column, the number (integer) of the allele
- on the third column, the allele frequency in the first reference population or sub-species
- on the fourth column, the allele frequency in the second reference population or sub-species
- etc.

Remark: of course, all the allele frequencies at one locus should sum to 1!
Example:
first line: Locus Population_1 Population_2 F1_Population Bullfrog_Populus
second line: Locus1 182 0.2 0.3 0.13 0.08
third line: Locus1 184 0.3 0.5 0.02 0.72
fourth line: Locus1 186 0.5 0.2 0.85 0.2
fifth line: Locus2 253 0.3 0.58 0.45 0.1
etc.

2). Individual to be assigned FILE

This file should be formatted in *.txt (tabulation-separated values). In this file, you should provide the genotypes of the individuals you want to assign. Take care to provide the locus information in the same order than the one provided in the frequency file. On the first line, you should indicate the locus names, after a first word "individuals".
In this file, each line should contain the genotype of one individual to be assigned.

- in the first column, the name of the individual
- in the second column, the alleles separated by a comma (no space) of the first locus
- on the third column, the alleles separated by a comma (no space) of the second locus
- etc.

first line: Individuals Locus1 Locus2 Locus42
second line: individual1 182,184,186 253 320,322

third line: individual2 186 253,255 318,322

Remark: The first individual in this example is triploid for its first locus, haploid for its second locus and diploid for its last locus while the second individual is haploid then diploid. All ploidy level can be taken into account in our method and software.

IV). Output

Now, you would like to read the output, but that's a mess and you're just lost? Just read the next section.

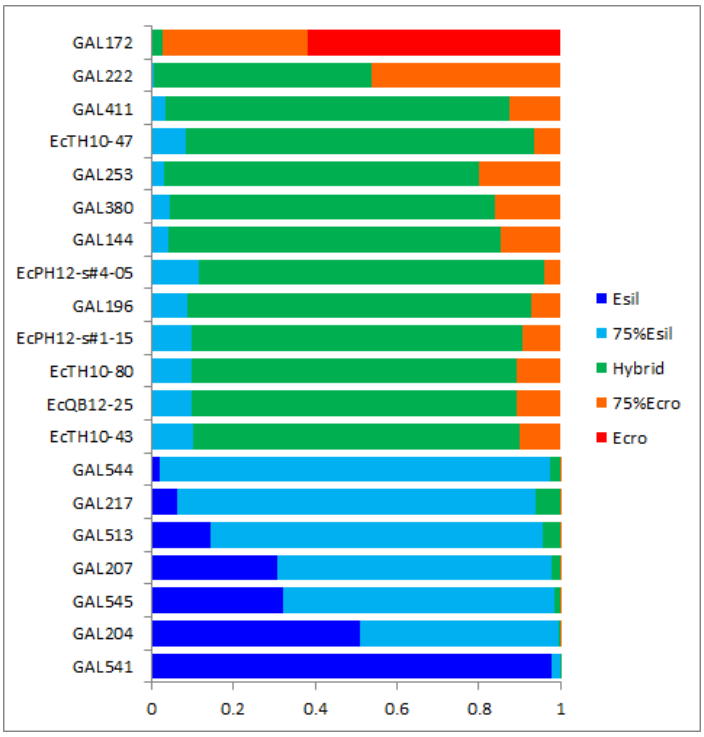
Synthetic output file of posterior probabilities

*year-month-day-hour min*AssignmentProbabilities.txt

This file should be the one you are looking for to assess the rates of clonality within the population(s) you study as analysed in the paper Montecinos *et al.* (submitted).

The file is structured so that each line contains the result of assignment of each studied individual to populations and admixed scenarios. Each line has 7 columns of results.

- 1st column: contains the DATA and summarizes the genetic data of the assigned individual, (header: *DATA*)
- 2nd column: the name of the individual, (header: *Individual*)
- 3rd to 7th columns: the posterior probabilities to belong to one of the populations and the admixed scenarios
- 3rd column: posterior probabilities to belong to the first population (header: *name_of_the_1st_population_as_entered_in_the_FREQUENCY_FILE*)
- 4th column: posterior probabilities to belong to an admixed gene pool made of 75% of the the 1st population and 25% of the 2nd population (header: *75%name_of_the_1st_population_as_entered_in_the_FREQUENCY_FILE*)
- 5th column: posterior probabilities to belong to an admixed gene pool made of half of each population, corresponding to hybrid-F1-like individuals (header: *Hybrid*)
- 6th column: posterior probabilities to belong to an admixed gene pool made of 75% of the the 2nd population and 25% of the 1st population (header: *75%name_of_the_2nd_population_as_entered_in_the_FREQUENCY_FILE*)
- 7th column: posterior probabilities to belong to the second population (header: *name_of_the_2nd_population_as_entered_in_the_FREQUENCY_FILE*)



To obtain a nice plot as above with those results use Structure-like http://pritchardlab.stanford.edu/structure.html as in Montecinos *et al.* (submitted):

Microsoft Excel: You can also open the file with excel, select all cases of the dataset, insert a plot and select "stacked bars". You can lay out the order of your individuals to match questions and hypotheses for better visualising.

Open Office: You can also open the file with calc. Verify to check the box for separator tabulations (do not check the box for comma or other separator), allow for decimal visualisation and select your data. Click the Insert Chart icon on the Standard toolbar which will open the Chart Wizard. Select "stacked bars".

V). Debugging, troubleshooting and new feature request

We carefully debugged the code and tested it on simulated and real datasets. If you suspect the presence of a bug, please feel free to contact the author, Solenn Stoeckel, and detail the suspected bug. For special purpose and interesting questions, I can develop code versions including options that you may need. In this case, please feel free to contact me.

If contacting me by email, put in the email subject line in square brackets **[XPloidAssignment1.0 request]**. Without such an email subject header, your email may sink into the oblivion of some spam or garbage folder.

Contact: solenn.stoeckel@rennes.inra.fr

Appendix III.2

Assigning genotyped individuals to gene pools and admixed gene pools using multiple genetic markers with varying ploidy levels

Let's consider γ different geographical or phenotypical populations or subspecies characterized by their specific genetic pools, denoted G, encoded at each of its λ loci by η_λ allelic frequencies. Let's consider that the likelihood of an hth allele of a locus l denoted $A_{l,h}$ to belong to a population g is its frequency in the gene pool G_g, *i.e.* $f(A_{l,h})_g$. The posterior probability of an allele h at locus l to come from a population g^* would thus be

$$P(A_{l,h} \in G_{g^*} | G_1, \dots, G_g, \dots, G_\gamma) = \frac{f(A_{l,h})_{g^*}}{\sum_{g=1}^{\gamma} f(A_{l,h})_g} \quad \text{(eq.1)}$$

We sampled N studied individuals named I_i with $i \in [1, \dots, i, \dots, N]$ to estimate their probabilities to come from each single population or from admixed gene pools between those populations. Those individuals are genotyped and we denote the DNA fingerprinting of one individual I_i as $\varphi_i = [(A_{1,1}, \dots, A_{1,\beta_1}), \dots, (\dots, A_{l,b_l}, \dots), \dots, (A_{\lambda,1}, \dots, A_{\lambda,\beta_\lambda})]$. In φ_i, one locus can have β alleles where $\beta \in [1, \infty[$ (from haplo- to polyploidy) and β can vary along loci (β_l) and between individuals. The posterior probability of one individual I_i to come from one population g^* knowing its DNA fingerprinting φ_i over all genotyped markers and the genetic composition of populations and admixed scenarios is

$$P(I_i \in G_{g^*} | G_1, \dots, G_g, \dots, G_h, \varphi_i) = \frac{\prod_{l=1}^{\lambda} \prod_{b=1}^{\beta_l} f(A_{l,b_l})_{g^*}}{\sum_{g=1}^{h} \left(\prod_{j=1}^{k} \prod_{\alpha=1}^{\beta} a_{\alpha_j}\right)} \quad \text{(eq.2)}$$

In this equation we consider a flat prior of assignation between gene pools.

If studied individuals can be assumed to descend from at least one sexual reproduction with massive recombination between markers and independent segregation/fusion of alleles at the studied loci, we expect that genetic pool of hybrid F1 between two populations, *e.g.* G_1 and G_2, should exhibit allele j at a locus l at a frequencies following

$$f(A_{l,j})^{hybrid} = \frac{f(A_{l,j})^{G_1} + f(A_{l,j})^{G_2}}{2}$$

Admixed individuals coming from backcrosses or multiple interbreedings between populations should be assigned to admixed genetic pools in which allele frequencies can be assessed as

$$f(A_{l,j})^{admixed} = p_{G_1} * f(A_{l,j})^{G_1} + p_{G_2} * f(A_{l,j})^{G_2}$$

With p_{G_1} the proportion of genetic pool coming from G_1 and p_{G_2}, the proportion coming from G_2, with $G_1 + G_2 = 1$.

The posterior probabilities provide a direct quantitative estimate of the probability that an individual belongs to an identified population and scenarios of admixture. Absolute assignment can be done considering the maximum a posteriori of those values only in cases where one posterior probability of one scenario dominates all the others.

XPloidAssignment: a software to compute the posterior probabilities of genotyped individuals with varying ploidies to come from previously identified gene pools and admixed scenarios between those gene pools

We implemented this method in a software named XPloidAssignment that allows users to compute the posterior probabilities of belonging to identified populations and scenarios of genetic admixture between those populations. The software can be downloaded for Linux and Windows at https://www6.rennes.inra.fr/igepp_eng/Productions/Software. See User Guide for further explanations.

XPloidAssignment: a software to compute the posterior probabilities of genotyped individuals with varying ploidies to come from previously identified gene pools and admixed scenarios between those gene pools

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Appendix III.3:

Table S1. Genotypes of the 81 "putative" hybrid for the markers used and their assignation in the different class of hybrids or parental species by combining the result of the different admixture analyses

Table S1a: Genotypes of the 81 "putative" hybrids for all markers used

Individuals	population	PCA ID	mitochondrial species specific marker	ITS1 species specific marker	Sex (and ploidy) specific marker	detected aneuploidy at at least one microsatellite markers	microsatellite loci with diagnostic alleles									species diagnostic microsatellite loci (Blue: Esil, yellow: Ecro, grey: rare alleles)											
							locus M-122-2			locus M-208			locus M-162-1			locu M-033-1		locus M-239-3		locus M-103-2		locus M-387		M-388		M-420	
EcPH12-s#1-46	Roscoff	490	Esil	Esil+Ecro	SP	no	253	255		267	303		266	306		281	315	215	217	257	257	256	256	153	153	305	307
EcPH12-s#1-86	Roscoff	491	Esil	Esil+Ecro	SP	yes	253	255	289	267	303	315	266	306		281	317	215	217	261	261	252	252	155	155	307	307
EcPH12-s#1-155	Roscoff	492	Esil	Esil+Ecro	SP	yes	253	255	289	267	303	315	266	306		281	319	215	217	261	261	252	252	153	153	307	307
EcPH12-s#1-174	Roscoff	493	Esil	Esil+Ecro	SP	yes	253	255	289	267	303	315	266	346		281	317	215	219	261	261	252	252	153	153	309	309
EcPH12-s#1-189	Roscoff	494	Esil	Esil+Ecro	SP	yes	253	255	289	267	303	315	266	306		281	317	215	217	261	261	252	252	153	153	307	307
EcTH10-23	Traezh Hir	495	Esil	Esil+Ecro	SP	yes	253	255	291	267	303	315	266	352		279	317	213	219	259	259	252	252	153	153	307	307
EcTH10-88	Traezh Hir	496	Esil	Esil+Ecro	SP	yes	253	255	291	267	303	315	266	352		279	317	213	219	259	259	252	252	153	153	307	307
EcTH10-90	Traezh Hir	497	Esil	Esil+Ecro	SP	yes	253	255	291	267	303	315	266	352		279	317	213	219	259	259	252	252	153	153	307	307
EcTH10-91	Traezh Hir	498	Esil	Esil+Ecro	SP	yes	253	255	291	267	303	315	266	352		279	317	213	219	259	259	252	252	153	153	307	307
EcTH10-93	Traezh Hir	499	Esil	Esil+Ecro	SP	yes	253	255	291	267	303	315	266	352		279	317	213	219	259	259	252	252	153	153	307	307
EcTH10-97	Traezh Hir	500	Esil	Esil+Ecro	SP	yes	253	255	291	267	303	315	266	352		279	317	213	219	259	259	252	252	153	153	307	307
EcTH10-116	Traezh Hir	501	Esil	Esil+Ecro	SP	yes	253	255	291	267	303	315	266	352		279	317	213	219	259	259	252	252	153	153	307	307
EcTH10-118	Traezh Hir	502	Esil	Esil+Ecro	SP	yes	253	255	291	267	303	315	266	352		279	317	213	219	259	259	252	252	153	153	307	307
GAL524	Gandario	503	Esil	Esil+Ecro	SP	no	275	275		270	297		310	338		277	277	199	231	257	269	252	258	155	161	273	291
EcPH12-s#1-29	Roscoff	504	Ecro	Esil+Ecro	SP	yes	275	291	293	267	303	315	266	348		285	285	223	223	257	257	250	250	153	153	307	307
EcPH12-s#1-67	Roscoff	505	Ecro	Esil+Ecro	SP	no	275	291		267	285		266	306		295	295	209	209	257	257	252	252	155	161	293	319
EcPH12-s#1-68	Roscoff	506	Ecro	Esil+Ecro	SP	yes	277	289		267	306		264	266	304	317	317	223	223	249	257	252	258	153	153	291	303
EcPH12-s#4-04	Roscoff	507	Ecro	Esil+Ecro	SP	no	253	279		267	285		266	326		297	297	219	219	245	245	252	252	155	173	273	307
EcPH12-s#4-05	Roscoff	508	Ecro	Esil+Ecro	SP	yes	277	291		267	270	294	264	266	306	285	285	219	219	267	267	252	252	153	165	293	293
EcPH12-s#4-13	Roscoff	509	Ecro	Esil+Ecro	SP	no	253	279		267	285		264	326		305	311	219	219	245	245	252	252	155	173	273	307
EcPH12-s#6-20	Roscoff	510	Ecro	Esil+Ecro	SP	no	253	301		267	291		266	330		285	285	207	207	257	257	258	258	159	191	277	277
EcTH10-28	Traezh Hir	511	Ecro	Esil+Ecro	SP	yes	253	255		267	303	315	266	352		279	317	213	219	259	259	252	252	153	153	307	307
EcTH10-48	Traezh Hir	512	Ecro	Esil+Ecro	SP	yes	253	255	291	267	303	315	266	352		279	317	213	219	259	259	252	252	153	153	307	307
EcTH10-55	Traezh Hir	513	Ecro	Esil+Ecro	SP	yes	279	291	309	267	306		264	266	332	317	317	221	227	259	259	252	252	153	153	291	315
GAL132	Ribadeo	514	Ecro	Esil+Ecro	SP	yes	253	289		267	270	282	266	272	310	319	319	245	245	253	253	258	258	137	157	267	267
GAL133	Ribadeo	515	Ecro	Esil+Ecro	SP	yes	253	289		267	270	282	266	272	310	317	317	245	245	255	255	258	258	137	157	267	267
GAL176	Ribadeo	516	Ecro	Esil+Ecro	SP	no	255	289		267	291		264	298		293	293	207	207	255	255	258	258	161	161	283	283
GAL177	Ribadeo	517	Ecro	Esil+Ecro	SP	no	255	289		267	291		264	298		293	293	207	207	255	255	258	258	161	161	283	283
GAL196	Ribadeo	518	Ecro	Esil+Ecro	SP	no	253	291		267	267		266	266		283	311	215	227	247	261	258	258	187	211	293	293
GAL216	Ribadeo	519	Ecro	Esil+Ecro	SP	yes	253	289		267	270	279	266	272	310	319	319	245	245	255	255	258	258	137	157	267	267
GAL237	Ribadeo	520	Ecro	Esil+Ecro	SP	no	253	309		267	291		264	298		293	293	207	207	255	255	258	258	161	161	283	283
GAL239	Ribadeo	521	Ecro	Esil+Ecro	SP	no	255	289		267	291		264	298		293	293	207	207	255	255	258	258	161	161	283	283
GAL77	Lourido	522	Ecro	Esil+Ecro	SP	no	279	289		267	291		266	312		283	283	207	207	257	257	252	252	153	169	293	311
GAL461	Lourido	523	Ecro	Esil+Ecro	SP	no	277	277		267	288		266	310		281	281	227	227	261	261	258	258	153	163	291	311
GAL473	Lourido	524	Ecro	Esil+Ecro	SP	no	253	277		267	267		266	310		337	337	245	245	259	259	252	252	155	169	293	309
GAL62	Lourido	525	Ecro	Esil+Ecro	SP	no	277	289		267	291		266	312		283	283	207	207	261	261	252	252	153	169	293	311
GAL82	Lourido	526	Ecro	Esil+Ecro	SP	no	279	289		267	291		266	312		283	283	207	207	259	259	252	252	153	169	293	309
GAL84	Lourido	527	Ecro	Esil+Ecro	SP	no	279	295		267	300		266	306		315	315	207	207	255	255	252	252	151	151	291	291
GAL92	Lourido	528	Ecro	Esil+Ecro	SP	no	277	289		267	291		266	312		283	283	207	207	261	261	252	252	153	169	293	311
GAL94	Lourido	529	Ecro	Esil+Ecro	SP	no	277	295		267	300		266	306		315	315	207	207	255	255	252	252	151	151	291	291
GAL104	Lourido	530	Ecro	Esil+Ecro	SP	yes	279	295	309	267	300		266	306		315	315	207	207	257	257	252	252	151	151	291	337
GAL358	Lourido	531	Ecro	Esil+Ecro	SP	no	277	289		267	291		266	312		283	283	207	207	255	255	245	245	153	169	293	311
GAL431	Lourido	532	Ecro	Esil+Ecro	SP	no	279	279		267	267		266	310		281	281	227	227	261	261	258	258	153	163	291	309
GAL460	Lourido	533	Ecro	Esil+Ecro	SP	no	279	291		267	288		266	310		281	281	227	227	261	261	258	258	153	163	291	311
GAL475	Lourido	534	Ecro	Esil+Ecro	SP	no	279	279		267	291		266	312		283	283	207	207	261	261	252	252	153	169	293	311

Individuals	population	PCA ID	mitochondrial species specific marker	ITS1 species specific marker	Sex (and ploidy) specific marker	detected aneuploidy at at least one microsatellite markers	microsatellite loci with diagnostic alleles									species diagnostic microsatellite loci (Blue: Esil, yellow: Ecro, grey: rare alleles)											
							locus M-122-2			locus M-208			locus M-162-1			locu M-033-1		locus M-239-3		locus M-103-2		locus M-387		M-388		M-420	
GAL476	Lourido	535	Ecro	Esil+Ecro	SP	no	277	289		267	291		266	312		283	283	207	207	261	261	252	252	153	169	293	311
GAL477	Lourido	536	Ecro	Esil+Ecro	SP	no	277	289		267	291		266	312		283	283	207	207	261	261	252	252	153	169	293	311
EcTH10-43	Traezh Hir	537	Ecro	Esil+Ecro	SP	yes	271	291	309	267	306		264	266	332	0	0	221	221	259	259	252	252	153	153	291	315
EcTH10-47	Traezh Hir	538	Ecro	Esil+Ecro	SP	yes	253	255	291	267	303	315	266	352		279	317	213	219	800	800	252	252	153	153	307	307
GAL130	Ribadeo	539	Ecro	Esil+Ecro	SP	yes	277	295		267	267		264	266	310	305	305	209	209	265	265	252	252	159	159	0	0
GAL144	Ribadeo	540	Ecro	Esil+Ecro	SP	yes	277	289		267	270	285	266	310		309	309	219	219	257	257	800	800	161	211	265	269
GAL222	Ribadeo	541	Ecro	Esil+Ecro	SP	no	291	349		267	282		266	266		317	317	800	800	255	255	258	258	155	159	265	265
GAL253	Ribadeo	542	Ecro	Esil+Ecro	SP	yes	277	293		267	291		264	266	310	305	305	209	209	257	257	800	800	159	163	269	333
GAL217	Ribadeo	543	Ecro	Esil+Ecro	SP	yes	251	287		270	279	285	264	298		315	315	213	213	257	257	256	256	149	149	0	0
GAL107	Lourido	544	Ecro	Esil+Ecro	SP	no	253	291		267	294		266	306		309	309	227	227	255	255	252	252	155	161	0	0
GAL83	Lourido	545	Ecro	Esil+Ecro	SP	no	279	289		267	291		312	312		283	283	800	800	259	259	252	252	153	169	293	311
GAL380	Lourido	546	Ecro	Esil+Ecro	SP	no	277	289		267	291		266	312		283	283	207	207	249	249	800	800	153	169	293	309
GAL411	Lourido	547	Ecro	Esil+Ecro	SP	yes	253	289	307	267	288		266	312		315	315	227	227	800	800	250	250	139	153	279	291
GAL432	Lourido	548	Ecro	Esil+Ecro	SP	no	277	277		267	288		266	310		281	281	800	800	800	800	258	258	153	163	291	309
GAL204	Ribadeo	549	Esil	Esil	SP	no	253	253		291	294		312	318		311	315	211	215	247	257	250	252	149	155	0	0
GAL207	Ribadeo	550	Esil	Esil	SP	no	253	265		270	309		328	338		309	309	207	215	247	257	252	258	141	141	0	0
GAL513	Gandario	551	Esil	Esil	SP	no	253	253		270	297		310	338		289	311	199	231	257	269	252	258	139	141	0	0
GAL541	Gandario	552	Esil	Esil	SP	no	253	253		312	312		334	334		293	311	199	211	257	257	252	258	143	143	0	0
GAL544	Gandario	553	Esil	Esil	SP	no	277	277		288	300		320	342		283	311	211	211	249	257	252	256	163	163	0	0
GAL545	Gandario	554	Esil	Esil	SP	no	253	253		291	300		304	310		287	287	221	237	247	247	252	252	139	139	0	0
GAL160	Ribadeo	555	Esil	Esil	SP	no	253	253		294	306		288	318		0	0	800	800	245	257	246	250	137	139	0	0
GAL200	Ribadeo	556	Esil	Esil	SP	no	317	317		270	270		0	0		291	291	800	800	241	241	270	270	155	155	0	0
GAL514	Gandario	557	Esil	Esil	SP	no	253	253		291	291		290	304		281	311	207	231	800	800	252	256	139	143	0	0
EcPLY10-8	Plymouth	558	Ecro	Ecro	SP	no	293	293		273	273		270	270		317	317	800	800	800	800	800	800	155	155	299	299
GAL232	Ribadeo	559	Ecro	Ecro	SP	no	275	301		267	267		264	268		293	293	800	800	800	800	800	800	165	183	265	265
GAL515	Gandario	560	Ecro	Ecro	SP	no	291	291		267	267		266	266		311	311	800	800	800	800	800	800	153	161	305	305
GAL516	Gandario	561	Ecro	Ecro	SP	no	293	311		267	279		264	272		311	311	800	800	800	800	800	800	155	161	273	291
GAL101	Lourido	562	Ecro	Ecro	SP	no	291	307		267	267		266	266		0	0	191	191	800	800	800	800	153	155	279	301
GAL405	Lourido	563	Ecro	Ecro	SP	no	291	323		267	267		264	266		0	0	800	800	800	800	250	252	155	211	285	289
GAL454	Lourido	564	Ecro	Ecro	SP	yes	253	303		267	276	291	264	266	304	323	323	199	199	257	257	252	252	153	153	287	287
EcPH12-s#1-15	Roscoff	565	Ecro	Ecro	SP	yes	279	291	295	267	291		266	308		0	0	199	199	243	243	252	252	165	165	293	293
EcPH12-s#2A-16	Roscoff	566	Ecro	Ecro	SP	yes	253	253		267	270	291	264	266	296	0	0	207	207	255	255	252	252	155	191	293	313
EcTH10-80	Traezh Hir	567	Ecro	Ecro	SP	no	279	291		267	306		264	266		0	0	223	223	259	259	252	252	153	153	291	315
EcQB12-25	Quiberon	568	Ecro	Ecro	SP	no	279	291		267	306		264	266		0	0	223	223	259	259	252	252	153	153	291	315
GAL159	Ribadeo	335	Ecro	Ecro	F	no	277			0			266			309		800		800		800		145		0	
GAL172	Ribadeo	336	Ecro	Ecro	M	no	295			267			266			301		800		800		800		159		311	

Esil: *E. siliculosus*; Ecro: *E. crouaniorum*, SP= sporophytes bearing male and female sex specific markers, F= female gametophytes, M= male gametophytes, color used: (Blue: Esil alleles, yellow: Ecro alleles, grey: rare alleles)

Table S1b: Assignation of the 81 "putative" hybrids in the different class of hybrids or parental species by combining the result of the different admixture analyses (XploidAssignment, STRUCTURE and Geneclass).

							Posterior probability of XPloidAssignment method					Classifica-tion using the Xploid-assignment method	STRUCTURE method of assignment to the parental species		Classifica-tion using the STRUC-TURE method	Assignment (rank 1 and 2) to the different hybrid classes using GeneClass method									Classifica-tion using the Gene-Class method	Combination of the methods
Individuals	populations	mt species specific marker	ITS1 species specific marker	Sex and ploidy specific marker	detected aneuploidy	PCA	to the scenarios concidering only the two parental		to the secnarios considering different levels of admixtures							rank	score	rank	score	F1	BcEsil	BcEcro	Esil	Ecro		
							Ecro	Esil	50% Ecro-50% Esil	75%Ecro-75%Esil	75% Esil-25% Ecro	categories	Ecro	Esil	categories	1	%	2	%	'-log(L)	'-log(L)	'-log(L)	'-log(L)	'-log(L)	categories	categories
EcPH12-s#1-46	Roscoff	Esil	Esil+Ecro	SP	no	Intermediate	0,000	0,000	0,391	0,003	0,607	1 or 2	0,373	0,628	1	F1	93.994	BcEsil	6.005	18.028	19.222	23.273	34.349	44.735	1	1 or 2
EcPH12-s#1-86	Roscoff	Esil	Esil+Ecro	SP	yes	Intermediate	0,000	0,000	0,481	0,003	0,516	1 or 2	0,452	0,548	1	F1	95.944	BcEsil	3.972	17.597	18.980	20.653	34.969	44.606	1	1 or 2
cPH12-s#1-155	Roscoff	Esil	Esil+Ecro	SP	yes	Intermediate	0,000	0,000	0,477	0,003	0,520	1 or 2	0,415	0,585	1	F1	98.229	BcEsil	1.687	16.466	18.231	19.535	34.493	44.042	1	1 or 2
cPH12-s#1-174	Roscoff	Esil	Esil+Ecro	SP	yes	Intermediate	0,000	0,000	0,510	0,004	0,486	1 or 2	0,460	0,540	1	F1	94.841	BcEsil	4.692	19.433	20.738	21.740	36.758	44.042	1	1 or 2
cPH12-s#1-189	Roscoff	Esil	Esil+Ecro	SP	yes	Intermediate	0,000	0,000	0,483	0,003	0,514	1 or 2	0,454	0,546	1	F1	97.190	BcEsil	2.694	17.088	18.645	20.011	34.969	44.042	1	1 or 2
EcTH10-23	Traezh Hir	Esil	Esil+Ecro	SP	yes	Intermediate	0,000	0,000	0,527	0,005	0,469	1 or 2	0,476	0,524	1	F1	92.509	BcEsil	6.806	17.899	19.032	20.030	36.208	43.155	1	1 or 2
EcTH10-88	Traezh Hir	Esil	Esil+Ecro	SP	yes	Intermediate	0,000	0,000	0,527	0,005	0,469	1 or 2	0,476	0,524	1	F1	92.509	BcEsil	6.806	17.899	19.032	20.030	36.208	43.155	1	1 or 2
EcTH10-90	Traezh Hir	Esil	Esil+Ecro	SP	yes	Intermediate	0,000	0,000	0,527	0,005	0,469	1 or 2	0,476	0,524	1	F1	92.509	BcEsil	6.806	17.899	19.032	20.030	36.208	43.155	1	1 or 2
EcTH10-91	Traezh Hir	Esil	Esil+Ecro	SP	yes	Intermediate	0,000	0,000	0,527	0,005	0,469	1 or 2	0,476	0,524	1	F1	92.509	BcEsil	6.806	17.899	19.032	20.030	36.208	43.155	1	1 or 2
EcTH10-93	Traezh Hir	Esil	Esil+Ecro	SP	yes	Intermediate	0,000	0,000	0,527	0,005	0,469	1 or 2	0,476	0,525	1	F1	92.509	BcEsil	6.806	17.899	19.032	20.030	36.208	43.155	1	1 or 2
EcTH10-97	Traezh Hir	Esil	Esil+Ecro	SP	yes	Intermediate	0,000	0,000	0,527	0,005	0,469	1 or 2	0,475	0,525	1	F1	92.509	BcEsil	6.806	17.899	19.032	20.030	36.208	43.155	1	1 or 2
EcTH10-116	Traezh Hir	Esil	Esil+Ecro	SP	yes	Intermediate	0,000	0,000	0,527	0,005	0,469	1 or 2	0,475	0,525	1	F1	92.509	BcEsil	6.806	17.899	19.032	20.030	36.208	43.155	1	1 or 2
EcTH10-118	Traezh Hir	Esil	Esil+Ecro	SP	yes	Intermediate	0,000	0,000	0,527	0,005	0,469	1 or 2	0,476	0,524	1	F1	92.509	BcEsil	6.806	17.899	19.032	20.030	36.208	43.155	1	1 or 2
GAL524	Gandario	Esil	Esil+Ecro	SP	no	Intermediate	0,000	0,000	0,202	0,001	0,797	2	0,279	0,721	1	F1	87.455	BcEsil	12.072	26.765	27.625	29.032	37.751	49.050	1	1 or 2
EcPH12-s#1-29	Roscoff	Ecro	Esil+Ecro	SP	yes	Intermediate	0,000	0,000	0,765	0,023	0,212	1	0,474	0,526	1	F1	98.483	BcEsil	1.517	24.112	25.924	30.570	42.871	40.865	1	1
EcPH12-s#1-67	Roscoff	Ecro	Esil+Ecro	SP	no	Intermediate	0,000	0,000	0,609	0,008	0,382	1 or 2	0,401	0,599	1	F1	99.405	BcEsil	0.479	16.598	18.914	19.531	38.622	38.925	1	1 or 2
EcPH12-s#1-68	Roscoff	Ecro	Esil+Ecro	SP	yes	Intermediate	0,000	0,000	0,784	0,032	0,184	1	0,557	0,443	1	F1	93.668	BcEsil	6.332	21.874	23.044	28.728	41.574	40.820	1	1
EcPH12-s#4-04	Roscoff	Ecro	Esil+Ecro	SP	no	Intermediate	0,000	0,000	0,190	0,001	0,809	2	0,295	0,705	1	BcEsil	51.326	F1	48.600	23.561	23.538	26.381	36.416	44.948	1 or 2	1 or 2
EcPH12-s#4-05	Roscoff	Ecro	Esil+Ecro	SP	yes	Intermediate	0,000	0,000	0,843	0,042	0,116	1	0,419	0,581	1	F1	99.585	BcEsil	0.406	18.890	21.279	22.917	38.890	38.369	1	1
EcPH12-s#4-13	Roscoff	Ecro	Esil+Ecro	SP	no	Intermediate	0,000	0,000	0,183	0,001	0,816	2	0,300	0,700	1	BcEsil	68.721	F1	31.210	23.854	23.511	26.509	35.494	46.949	2	1 or 2
EcPH12-s#6-20	Roscoff	Ecro	Esil+Ecro	SP	no	Intermediate	0,000	0,000	0,517	0,005	0,478	1 or 2	0,448	0,552	1	F1	98.160	BcEsil	1.523	19.712	21.522	22.203	35.880	43.411	1	1 or 2
EcTH10-28	Traezh Hir	Ecro	Esil+Ecro	SP	yes	Intermediate	0,000	0,000	0,360	0,002	0,638	1 or 2	0,434	0,566	1	F1	82.159	BcEsil	17.451	19.288	19.960	21.611	33.793	44.882	1	1 or 2
EcTH10-48	Traezh Hir	Ecro	Esil+Ecro	SP	yes	Intermediate	0,000	0,000	0,527	0,005	0,469	1 or 2	0,476	0,524	1	F1	92.509	BcEsil	6.806	17.899	19.032	20.030	36.208	43.155	1	1 or 2
EcTH10-55	Traezh Hir	Ecro	Esil+Ecro	SP	yes	Intermediate	0,000	0,000	0,704	0,014	0,282	1	0,458	0,542	1	F1	88.382	BcEsil	11.413	20.699	21.588	23.332	37.662	41.988	1	1
GAL132	Ribadeo	Ecro	Esil+Ecro	SP	yes	Intermediate	0,000	0,000	0,816	0,047	0,137	1	0,533	0,468	1	F1	98.953	BcEsil	1.036	25.262	27.242	29.225	41.700	42.573	1	1
GAL133	Ribadeo	Ecro	Esil+Ecro	SP	yes	Intermediate	0,000	0,000	0,816	0,045	0,140	1	0,688	0,312	1	F1	95.704	BcEsil	4.286	25.647	26.996	29.643	41.362	42.573	1	1
GAL176	Ribadeo	Ecro	Esil+Ecro	SP	no	Intermediate	0,000	0,000	0,656	0,017	0,326	1	0,675	0,325	1	F1	94.078	BcEsil	5.894	27.351	28.554	30.886	39.442	43.125	1	1
GAL177	Ribadeo	Ecro	Esil+Ecro	SP	no	Intermediate	0,000	0,000	0,656	0,017	0,326	1	0,675	0,325	1	F1	94.078	BcEsil	5.894	27.351	28.554	30.886	39.442	43.125	1	1
GAL196	Ribadeo	Ecro	Esil+Ecro	SP	no	Intermediate	0,000	0,000	0,841	0,073	0,086	1	0,597	0,403	1	F1	99.374	BcEcro	0.514	16.631	19.578	18.918	39.391	38.940	1	1
GAL216	Ribadeo	Ecro	Esil+Ecro	SP	yes	Intermediate	0,000	0,000	0,815	0,040	0,145	1	0,618	0,382	1	F1	97.901	BcEsil	2.094	24.422	26.092	28.713	40.470	41.251	1	1
GAL237	Ribadeo	Ecro	Esil+Ecro	SP	no	Intermediate	0,000	0,000	0,439	0,005	0,556	1 or 2	0,533	0,467	1	F1	87.117	BcEsil	12.874	28.583	29.414	32.543	37.570	47.715	1	1 or 2
GAL239	Ribadeo	Ecro	Esil+Ecro	SP	no	Intermediate	0,000	0,000	0,656	0,017	0,326	1	0,675	0,325	1	F1	94.078	BcEsil	5.894	27.351	28.554	30.886	39.442	43.125	1	1
GAL77	Lourido	Ecro	Esil+Ecro	SP	no	Intermediate	0,000	0,000	0,602	0,009	0,389	1 or 2	0,531	0,469	1	F1	97.022	BcEsil	2.106	16.829	18.492	18.875	37.515	38.597	1	1 or 2
GAL461	Lourido	Ecro	Esil+Ecro	SP	no	Intermediate	0,000	0,000	0,446	0,005	0,549	1 or 2	0,459	0,541	1	F1	98.053	BcEsil	1.677	18.845	20.612	21.405	37.105	38.302	1	1 or 2
GAL473	Lourido	Ecro	Esil+Ecro	SP	no	Intermediate	0,000	0,000	0,726	0,046	0,229	1	0,549	0,451	1	F1	98.526	BcEsil	1.443	21.106	22.941	24.613	39.690	37.690	1	1
GAL62	Lourido	Ecro	Esil+Ecro	SP	no	Intermediate	0,000	0,000	0,640	0,014	0,347	1 or 2	0,573	0,427	1	F1	97.701	BcEcro	1.198	18.528	20.476	20.439	39.483	38.299	1	1 or 2
GAL82	Lourido	Ecro	Esil+Ecro	SP	no	Intermediate	0,000	0,000	0,609	0,009	0,381	1 or 2	0,533	0,467	1	F1	97.625	BcEcro	1.188	17.519	19.434	19.433	38.164	38.489	1	1 or 2
GAL84	Lourido	Ecro	Esil+Ecro	SP	no	Intermediate	0,000	0,000	0,543	0,006	0,451	1 or 2	0,467	0,533	1	F1	78.487	BcEsil	21.511	21.777	22.339	26.347	36.318	41.331	1	1 or 2
GAL92	Lourido	Ecro	Esil+Ecro	SP	no	Intermediate	0,000	0,000	0,640	0,014	0,347	1 or 2	0,574	0,426	1	F1	97.701	BcEcro	1.198	18.528	20.476	20.439	39.483	38.299	1	1 or 2
GAL94	Lourido	Ecro	Esil+Ecro	SP	no	Intermediate	0,000	0,000	0,582	0,009	0,409	1 or 2	0,501	0,500	1	F1	82.315	BcEsil	17.682	22.303	22.971	26.715	37.026	41.033	1	1 or 2
GAL104	Lourido	Ecro	Esil+Ecro	SP	yes	Intermediate	0,000	0,000	0,504	0,005	0,491	1 or 2	0,427	0,573	1	F1	88.260	BcEsil	11.739	19.996	20.872	24.839	35.942	41.930	1	1 or 2
GAL358	Lourido	Ecro	Esil+Ecro	SP	no	Intermediate	0,000	0,000	0,754	0,066	0,181	1	0,804	0,196	3	F1	90.235	BcEcro	6.878	26.021	27.516	27.139	46.039	38.299	1	1 or 3
GAL431	Lourido	Ecro	Esil+Ecro	SP	no	Intermediate	0,000	0,000	0,629	0,011	0,360	1 or 2	0,468	0,532	1	F1	99.198	BcEcro	0.438	17.153	19.589	19.507	37.132	35.469	1	1 or 2
GAL460	Lourido	Ecro	Esil+Ecro	SP	no	Intermediate	0,000	0,000	0,616	0,009	0,375	1 or 2	0,466	0,534	1	F1	98.666	BcEsil	0.979	17.144	19.147	19.588	38.944	36.967	1	1 or 2
GAL475	Lourido	Ecro	Esil+Ecro	SP	no	Intermediate	0,000	0,000	0,359	0,002	0,639	1 or 2	0,470	0,530	1	F1	95.346	BcEsil	4.312	17.826	19.170	20.271	35.539	39.564	1	1 or 2

Individuals	populations	mt species specific marker	ITS1 species specific marker	Sex and ploidy specific marker	detected aneuploidy	PCA	Posterior probability of XPloidAssignment method: to the scenarios concidering only the two parental		to the secnarios considering different levels of admixtures			Classifica-tion using the Xploid-assignment method	STRUCTURE method of assignment to the parental species		Classifica-tion using the STRUC-TURE method	Assignment (rank 1 and 2) to the different hybrid classes using GeneClass method									Classifica-tion using the Gene-Class method	Combination of the methods
							Ecro	Esil	50% Ecro-50% Esil	75%Ecro-75%Esil	75% Esil-25% Ecro	categories	Ecro	Esil	categories	rank 1	score %	rank 2	score %	F1 '-log(L)	BcEsil '-log(L)	BcEcro '-log(L)	Esil '-log(L)	Ecro '-log(L)	categories	categories
GAL476	Lourido	Ecro	Esil+Ecro	SP	no	Intermediate	0,000	0,000	0,640	0,014	0,347	1 or 2	0,574	0,426	1	F1	97.701	BcEcro	1.198	18.528	20.476	20.439	39.483	38.299	1	1 or 2
GAL477	Lourido	Ecro	Esil+Ecro	SP	no	Intermediate	0,000	0,000	0,640	0,014	0,347	1 or 2	0,574	0,426	1	F1	97.701	BcEcro	1.198	18.528	20.476	20.439	39.483	38.299	1	1 or 2
EcTH10-43	Traezh Hir	Ecro	Esil+Ecro	SP	yes	Intermediate	0,000	0,000	0,797	0,100	0,103	1	0,466	0,534	1	F1	96.378	BcEsil	2.524	21.425	23.007	23.369	38.758	36.337	1	1
EcTH10-47	Traezh Hir	Ecro	Esil+Ecro	SP	yes	Intermediate	0,000	0,000	0,850	0,065	0,085	1	0,598	0,402	1	BcEcro	99.999	F1	0.001	23.663	24.837	18.529	41.685	36.962	3	1 or 3
GAL130	Ribadeo	Ecro	Esil+Ecro	SP	yes	Intermediate	0,000	0,000	0,728	0,040	0,232	1	0,419	0,581	1	F1	78.338	BcEcro	21.640	20.196	23.741	20.755	33.831	33.722	1	1
GAL144	Ribadeo	Ecro	Esil+Ecro	SP	yes	Intermediate	0,000	0,000	0,812	0,146	0,042	1	0,535	0,466	1	BcEcro	99.998	F1	0.002	25.361	28.566	20.708	42.987	32.561	3	1 or 3
GAL222	Ribadeo	Ecro	Esil+Ecro	SP	no	Intermediate	0,000	0,000	0,533	0,462	0,005	1 or 3	0,880	0,120	3	BcEcro	######	F1	0.000	26.150	28.500	20.047	49.459	29.368	3	1 or 3
GAL253	Ribadeo	Ecro	Esil+Ecro	SP	yes	Intermediate	0,000	0,000	0,771	0,200	0,030	1	0,521	0,479	1	BcEcro	######	F1	0.000	29.885	32.220	24.312	44.960	34.703	3	1 or 3
GAL217	Ribadeo	Ecro	Esil+Ecro	SP	yes	Intermediate	0,000	0,061	0,061	0,000	0,878	2	0,163	0,837	2	F1	53.397	BcEsil	46.569	28.143	28.202	35.054	31.348	43.969	1 or 2	1 or 2
GAL107	Lourido	Ecro	Esil+Ecro	SP	no	Intermediate	0,000	0,000	0,273	0,001	0,726	2	0,338	0,662	1	F1	78.070	BcEsil	21.754	12.041	12.596	14.689	26.861	38.005	1	1 or 2
GAL83	Lourido	Ecro	Esil+Ecro	SP	no	Intermediate	0,000	0,000	0,792	0,034	0,174	1	0,546	0,454	1	BcEcro	99.997	F1	0.003	23.484	24.520	18.980	39.175	34.949	3	1 or 3
GAL380	Lourido	Ecro	Esil+Ecro	SP	no	Intermediate	0,000	0,000	0,794	0,160	0,046	1	0,734	0,266	1	BcEcro	######	F1	0.000	25.984	27.942	20.331	46.285	32.113	3	1 or 3
GAL411	Lourido	Ecro	Esil+Ecro	SP	yes	Intermediate	0,000	0,000	0,840	0,125	0,035	1	0,521	0,479	1	BcEcro	93.723	F1	6.149	24.899	26.579	23.716	43.519	35.300	3	1 or 3
GAL432	Lourido	Ecro	Esil+Ecro	SP	no	Intermediate	0,000	0,000	0,529	0,463	0,008	1 or 3	0,772	0,228	3	BcEcro	######	Ecro	0.000	30.581	32.389	18.337	47.986	25.658	3	1 or 3
GAL204	Ribadeo	Esil	Esil	SP	no		0,000	0,510	0,004	0,000	0,486	2 or 4	0,037	0,963	4	BcEsil	81.058	F1	17.712	18.814	18.153	26.230	19.972	53.854	2	2 or 4
GAL207	Ribadeo	Esil	Esil	SP	no		0,000	0,309	0,023	0,000	0,668	2	0,009	0,991	4	BcEsil	80.998	F1	18.525	20.055	19.415	22.769	21.679	51.585	2	2 or 4
GAL513	Gandario	Esil	Esil	SP	no	Intermediate	0,000	0,145	0,045	0,000	0,810	2	0,013	0,987	4	F1	74.127	BcEsil	25.725	19.760	20.220	22.568	23.121	49.251	1	1 or 2 or 4
GAL541	Gandario	Esil	Esil	SP	no		0,000	0,977	0,000	0,000	0,023	4	0,003	0,997	4	Esil	99.970	BcEsil	0.017	27.243	27.108	30.941	23.343	53.795	4	4
GAL544	Gandario	Esil	Esil	SP	no	Intermediate	0,000	0,021	0,028	0,000	0,951	2	0,161	0,839	2	BcEsil	69.460	F1	30.530	19.961	19.604	23.729	23.757	47.138	2	2
GAL545	Gandario	Esil	Esil	SP	no		0,000	0,320	0,016	0,000	0,664	2	0,010	0,990	4	BcEsil	67.981	F1	31.503	22.342	22.008	27.093	24.128	47.803	2	2 or 4
GAL160	Ribadeo	Esil	Esil	SP	no	Intermediate	0,000	0,000	0,273	0,004	0,723	2	0,292	0,708	1	BcEcro	99.753	BcEsil	0.152	25.473	25.270	22.453	27.922	39.081	3	1 or 2 or 3
GAL200	Ribadeo	Esil	Esil	SP	no	Intermediate	0,020	0,000	0,143	0,835	0,002	3	0,921	0,079	5	BcEcro	63.788	Ecro	36.212	31.413	32.857	24.669	40.449	24.915	3 or 5	3 or 5
GAL514	Gandario	Esil	Esil	SP	no	Intermediate	0,000	0,000	0,079	0,000	0,920	2	0,192	0,808	2	BcEcro	95.765	Esil	2.468	29.626	29.116	27.266	28.854	46.028	3	2 or 3
EcPLY10-8	Plymouth	Ecro	Ecro	SP	no		0,517	0,000	0,007	0,476	0,000	3 or 5	0,997	0,003	5	Ecro	99.222	BcEcro	0.778	47.993	49.242	26.994	60.053	24.889	5	3 or 5
GAL232	Ribadeo	Ecro	Ecro	SP	no	Intermediate	0,009	0,000	0,037	0,954	0,000	3	0,915	0,085	5	BcEcro	99.656	Ecro	0.344	42.240	43.791	22.000	56.849	24.462	3	3 or 5
GAL515	Gandario	Ecro	Ecro	SP	no		0,330	0,000	0,004	0,666	0,000	3	0,995	0,005	5	BcEcro	77.614	Ecro	22.386	31.944	35.030	11.505	57.497	12.045	3	3 or 5
GAL516	Gandario	Ecro	Ecro	SP	no		0,054	0,000	0,017	0,928	0,000	3	0,984	0,016	5	BcEcro	98.103	Ecro	1.897	36.795	38.919	16.128	60.085	17.841	3	3 or 5
GAL101	Lourido	Ecro	Ecro	SP	no		0,942	0,000	0,000	0,057	0,000	5	0,997	0,003	5	Ecro	99.896	BcEcro	0.104	29.484	31.030	15.134	55.232	12.151	5	5
GAL405	Lourido	Ecro	Ecro	SP	no		0,057	0,000	0,014	0,929	0,000	3	0,952	0,048	5	BcEcro	98.470	Ecro	1.530	26.807	29.323	13.373	53.674	15.181	3	3 or 5
GAL454	Lourido	Ecro	Ecro	SP	yes	Intermediate	0,000	0,000	0,788	0,028	0,184	1	0,401	0,599	1	F1	99.162	BcEsil	0.837	19.965	22.039	25.228	37.359	40.707	1	1
EcPH12-s#1-15	Roscoff	Ecro	Ecro	SP	yes	Intermediate	0,000	0,000	0,809	0,093	0,098	1	0,465	0,535	1	F1	99.240	BcEsil	0.383	17.137	19.551	19.557	35.731	31.330	1	1
cPH12-s#2A-16	Roscoff	Ecro	Ecro	SP	yes	Intermediate	0,000	0,000	0,694	0,019	0,287	1	0,450	0,550	1	F1	99.281	BcEsil	0.714	17.159	19.302	21.449	31.745	37.505	1	1
EcTH10-80	Traezh Hir	Ecro	Ecro	SP	no	Intermediate	0,000	0,000	0,792	0,109	0,100	1	0,522	0,478	1	F1	98.415	BcEsil	1.583	17.297	19.090	22.231	38.228	31.314	1	1
EcQB12-25	Quiberon	Ecro	Ecro	SP	no	Intermediate	0,000	0,000	0,792	0,109	0,100	1	0,522	0,478	1	F1	98.415	BcEsil	1.583	17.297	19.090	22.231	38.228	31.314	1	1
GAL159	Ribadeo	Ecro	Ecro	F	no	Intermediate	0,083	0,000	0,288	0,597	0,032	3	0,993	0,007	5	---	---	---	---	---	---	---	---	---	---	3 or 5
GAL172	Ribadeo	Ecro	Ecro	M	no		0,618	0,000	0,027	0,355	0,000	3 or 5	0,997	0,003	5	---	---	---	---	---	---	---	---	---	---	3 or 5

Codes of individuals classification to parental species or to the different classes of hybrids:

1 = F1 or few generation after F1 (equal proportion of Esil and Ecro genomes)

2 = subsequent generation after F1, more Esil genome

3 = subsequent generation after F1, more Ecro genome

4 = parental species Esil

5= parental species Ecro

When using XPloidAssigment or GeneClass methods, individuals were classified in a single category when its probability of assignment to this category was at least two times higher than to another category; when not, individuals were assigned to the two or the three equally probable categories. When using Structure method, individuals were assigned to the different categories according to the respective frequency of Esil and Ecro genome (1: 0.25 to 0.75 of Esil genome; 2: 0.75 to 0.95 Esil genome; 3: 0.75 to 0.95 Ecro genome; 4: > 0.95 Esil genome; 5: > 0.95 Ecro genome).

Appendix III.4

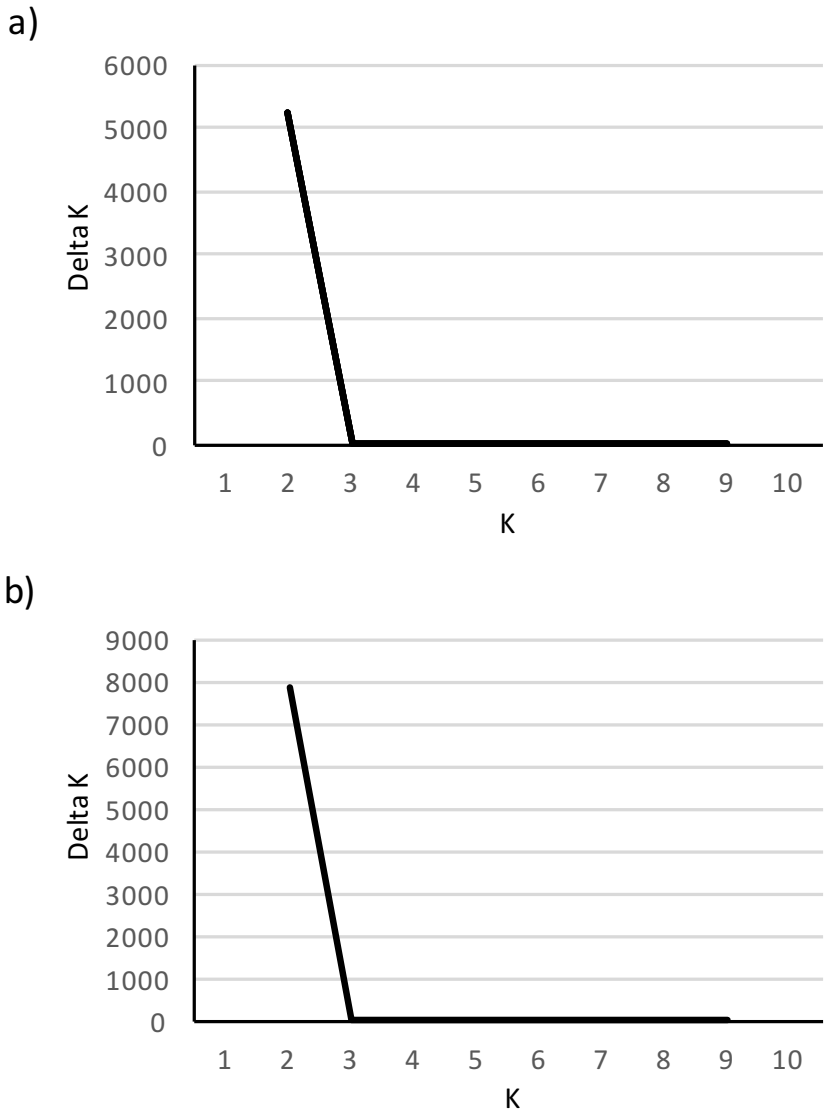

Figure S1. Graphical plot of ΔK values for: a) Sporophytes and b) Gametophytes. The maximum value of ΔK was obtained for K=2 for both Sporophytes and Gametophytes.

Conclusions and Perspectives

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CONCLUSIONS AND PERSPECTIVE

I. Species divergence, diversity and distribution within the genus *Ectocarpus* section siliculosi

- *A high number of cryptic species with various levels of divergence*

We have doubled the number of cryptic species defined within the genus *Ectocarpus* section *siliculosi* (15 species) compared with what was previously published (two species were formally recognized jointly with 6 different "lineages" by Stache-Crain et al. 1997, Peters et al. 2010a, b, 2015). The higher number of identified species can be explained by the increased sampling effort (we have used the most extensive sampling done so far in this genus to the date) and the increased power of the markers and the methods used. In addition, we have shown good congruence between the different markers tested, ITS1, COI-5P and SNPs and between the different analyses. It is clear that, in the genus *Ectocarpus*, morphology alone is inadequate to recognize species and its use have led to gross underestimation of the species diversity in this group. Our study thus supports the value of molecular identification in filamentous brown algae (such as barcoding, Hebert et al. 2003, Hajibabaei et al. 2007). Future research on these algae will have to take into account that (1) it is impossible to distinguish species morphologically and (2) that they could form groups of cryptic species characterized by various levels of divergence. For example, in our phylogenomics study (Chapter 2), the total number of sites available for the whole data set was low, due to the level of species divergence in *Ectocarpus* and the difficulty in

Table S1 – Individuals sequenced by RAD-seq. The species, region, population and phase of each strain are show.

		Ec 1			Ec 6			Ec 7			Ec 8			Ec 9			Ec 12			Ec 13			Esil			Ecro			Esil-Ecro			Ecro-Ec12			Esil-Ec12			Esil-Ec13			
Region	**Population**	**f**	**m**	**sp**	**f**	**m**	**sp**	**f**	**m**	**sp**	**f**	**m**	**sp**	**f**	**m**	**sp**	**f**	**m**	**sp**	**f**	**m**	**sp**	**f**	**m**	**sp**	**f**	**m**	**sp**	**f**	**m**	**sp**	**f**	**m**	**sp**	**f**	**m**	**sp**	**f**	**m**	**sp**	**total**
NA Europe	Roscoff	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	3	10	3	3	7	0	0	10	0	0	0	0	0	0	0	0	0	41
NA Europe	Ribadeo	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	2	2	5	0	0	10	0	0	0	0	0	0	0	0	0	38
NA Europe	Gandario	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
MED Europe	Naples	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	0	4	11	5	5	5	0	0	0	0	0	0	0	0	0	0	0	8	0	0	5	55
N Chile	Pan de Azucar	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	2	0	0	0	15	0	0	5	0	0	0	0	0	15	0	0	0	0	0	0	44
N Chile	Caldera	0	0	0	0	0	2	5	5	5	0	0	2	0	0	0	0	0	0	0	0	0	2	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	34
C Chile	Quintay	0	0	0	3	0	6	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	9	0	0	0	0	0	0	24
C Chile	Las Cruces	0	0	0	1	0	15	0	0	0	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	25
S Valdivia	Valdivia	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	1	13	0	0	0	0	0	0	0	0	0	0	0	0	27
S Valdivia	Estaquilla	0	0	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	12	0	0	0	0	0	0	0	0	0	0	0	0	29
	Total	**0**	**0**	**24**	**4**	**0**	**23**	**5**	**5**	**5**	**0**	**3**	**3**	**0**	**0**	**7**	**0**	**0**	**15**	**0**	**6**	**11**	**12**	**8**	**67**	**6**	**8**	**53**	**0**	**0**	**20**	**0**	**0**	**24**	**0**	**0**	**8**	**0**	**0**	**5**	**322**

Ec1- Ec13 = *Ectocarpus* 1 – *Ectocarpus* 13, Esil= *E. siliculosus*, Ecro = *E. crouaniorum*, f = female, m = male.

aligning reads to the reference genome of *Ectocarpus* 7 using the Stacks pipeline. Taking into account the various levels of divergence among these cryptic species, a de novo approach should have been preferred. It would have allowed, to raise the number of markers available in order to analyze the 322 strains that were sequenced using RAD-seq during my PhD including nine cryptic species and four types of hybrids (Table 1). However, we did not succeed to get the matrices using the stack de novo analysis because the time taken for the pipeline to run the clustering between reads was too long. Thus we had to select the reads using the reference genome, and only 75 of the 332 strains sequenced were used for the phylogenomics study.

In further analyses, we are thinking of re-analyzing the data with another pipeline, PyRAD (Eaton 2014). Eaton (2014) showed through a simulation that these clustering and alignment algorithms were less prone to locus splitting than Stacks. Indeed, Pante et al. (2015) compared PyRAD and Stacks in an empirical analysis of octocorals and found that PyRAD returned matrices with a greater number of loci that resolved more nodes of the phylogeny. PyRAD has been used in a relatively small but growing number of studies (e.g. Escudero et al. 2014, Herrera et al. 2014, Hipp et al. 2014, Pante et al. 2015). In addition, in PyRad, it is possible for each individual, to generate a consensus sequence based on each locus information and then to investigate the possible function and cellular localization of RAD-seq loci based on their homology with expressed sequences tags (ESTs). Note that in this genus, datasets of expressed sequences can be tested on a database of ESTs from the *Ectocarpus* genome project (Cock et al. 2010). These analyses could help to link variable and / or fixed SNPs to genes sustaining genetic differentiation and speciation process in this group.

The phylogenomics analyses performed on the reduced data set of 75 individuals, nevertheless, allowed to reconstruct phylogenetic relationships among and within the

two clades of the *Ectocarpus siliculosi* group that were unresolved in earlier studies. Within the two clades "Esil" (including *E. siliculosus*) and "Ecro" (including *E. crouaniorum*), our results indicated potential hybridization or introgression between closely related species pairs. Interestingly, a high diversity of taxa with various levels of divergence was revealed within the clade Esil. Follow-up studies on this system should now include experimental crossing design and genomic analyses of sympatric and allopatric populations to provide further insights into the evolutionary consequences of hybridization for the maintenance or breakdown of species in these brown algae. The strain collection that was established during this PhD thesis opens new opportunities to study the relationship between genetic distance and cross compatibility in this genus. In addition, as mentioned above, the RAD-seq data set build during this PhD provide short-term prospects to study the genomics of speciation within the genus *Ectocarpus*.

- *Species geographic distribution, phylogeography and scenario of introduction*

The different *Ectocarpus* species showed highly contrasted geographic distribution patterns. Even if the sampling needs to be increased since large geographic areas were not sampled in the present study, we have evidenced patterns ranging from species restricted to one biogeographic region (*Ectocarpus* 7) to cosmopolitan species (*E. siliculosus* and *E. crouaniorum*). Moreover, the high number of cryptic species present in sympatry within the same locality raised the question of which evolutionary mechanisms could reduce interspecific competition and promote such patterns. In this context, the intertidal rocky shores, have been an important object of ecological study for over a century (Jonsson et al. 2006) investigating how the sharp gradients of physical and biotic selective pressures shapes species distribution. More recently, genetics and evolutionary population genetics approaches were used to examine how

the habitat-driven divergence is sufficiently strong to drive sympatric speciation (in the marine snail *Littorina saxatilis* Johannesson, 2016) or to maintain species integrity (between *Fucus sp.*, Billard et al. 2010 and Zardi et al. 2011) despite existing gene flow. The blind population sampling performed in this PhD was designed to survey the species distribution along the shore gradient following Peters et al. (2010a) and Couceiro et al. (2015). Indeed, these previous studies showed that *E. crouaniorum* was located higher on the shore than *E. siliculosus* in the North Atlantic. The new sites from the Iberian Peninsula and from Chile analyzed during this PhD confirmed this pattern (Montecinos et al. 2016, Chapter 1). Moreover, *Ectocarpus* 6, which is present only along the southern most localities sampled in Chile, was more restricted to mid intertidal pools (Montecinos et al. 2016).

In contrast, the closely related *Ectocarpus* 6 and *Ectocarpus* 7 showed contrasting distribution patterns that could be the result of allopatric or parapatric speciation across the 30-33°S major biogeographic boundary of the Chilean coast. Indeed, the congruence of genetic divergence with these biogeographic breaks has been demonstrated for various species of marine invertebrates (Haye et al. 2014) and of brown and red seaweed (Guillemin et al. 2016a) suggesting a major role of this barrier in allopatric or parapatric speciation events. While *Ectocarpus* 6 is encountered in the cold waters of the northern limit of the Magellan biogeographic province, *Ectocarpus* 7 is restricted to the Peruvian Province which is characterized by continuous upwelling of cold water (16°C–20°C at the sea surface) but 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). Future research and adequate tools based on population genomic data sets could help to address these questions and elucidate the mechanisms underlying such patterns.

Finally, signals of recent introduction were hypothesized for the two cosmopolitan species *E. siliculosus* and *E. crouaniorum*. While in *E. siliculosus* no clear separation between European and Chilean strains could be observed, within *E. crouaniorum*, all European strains except one were grouped in a clade deriving from the Pacific. These results suggested recurrent numerous introductions for *E. siliculosus* and a recent introduction in Europe from the South East Pacific for *E. crouaniorum.* However, the sampling was not sufficient to confirm these hypotheses, worldwide sampling is requested and further analyses testing different scenarios of colonization and expansion could help to clarify the recent evolutionary history of these two species.

II. Reproductive Isolation

- *New opportunities to study hybridization in different evolutionary contexts*

We have already pointed out in the previous paragraph, how the diversity of taxa with various levels of divergence revealed in this PhD makes the *Ectocarpus* genus a good model for the study of reproductive isolation. The existence of porous reproductive barriers within this group suggests that speciation is still ongoing and the integration of several findings suggests a probable relationship between genetic distance and cross compatibility in this genus. Moreover, the results obtained in this PhD should now allow to study hybridization in nature but in different evolutionary contexts by selecting the species of interest. Coyne and Orr (2004) have distinguished primary hybrid zones, where divergence is taking place in situ as natural selection alters allele frequencies between populations, from secondary hybrid zones, where hybridization arise in turn from a secondary contact between two divergent species that were previously allopatric. The results of this PhD project suggest that we have encountered situations of primary contact zone where speciation is still in progress. This may be the case for the three closely related species of the Ecro clade for which patterns of incongruence between nuclear and cytoplasmic patterns were observed and in agreement with the recognition of an individual occupying an intermediate position between sympatric *Ectocarpus* 12 and *Ectocarpu*s 13 evidenced using the SNPs data set.

In contrast, the results of the chapter 3 suggest that hybridization between *E. siliculosus* and *E. crouaniorum* could be a consequence of a secondary contact zone. Cytoplasmic introgression was not detected between these species belonging to the two

highly divergent clades Esil and Ecro and we detected a strong reproductive barrier acting at the F1 hybrid generation between both species. With the new possibilities to use whole genome sequencing data, it is now possible to identify the actual genomic regions being homogenized by gene flow or those that resist introgression using a reference genome or a genetic map (reviewed in Payseur and Rieseberg et al. 2016). Thus, using the RAD-seq data produced during this PhD, it will be possible to contrast the situations of primary and secondary contact zones to produce key results for understanding the evolution of reproductive isolation.

The most popular strategy used to find genes associated with speciation processes has been to scan genomes for genomic signatures of selection (reviewed in Feder et al. 2012). These narrow regions containing genes putatively involved in speciation processes have been termed "speciation islands" implying a probable link between the observed pattern of high differentiation and reproductive isolation (Noor and Bennet, 2009). Furthermore, several empirical studies and theoretical models of speciation have argued that loci involved in reproductive isolation will preferentially accumulate in regions with little recombination such as the regions near the centromeres or located in the sex chromosome (Butlin 2005, Noor et al. 2001, Rieseberg 2001, Navarro and Barton 2003). Indeed, this is explained by the fact that recombination counteracts the effect of genetic divergence. Because of the existence of a reference genome in *Ectocarpus*, it will be possible to explore patterns of divergence in non-recombining regions in comparison to the recombining autosomal regions. In particular, as sex is expressed during the haploid phase of the life cycle, in diploid individuals both the female (U) and the male (V) sex chromosomes contain non-recombining regions (SDR). The idea is to separate the reads that are located in the SDR region from the others in order to perform genome scans using the two sets of SNPs. Moreover, the idea

will be to complete this RAD-seq approach by sequencing the male and female markers that were developed for all the *Ectocarpu*s species (markers used in chapter 3 to determine sex/phase by simple amplification, Ahmed et al. 2014). These sequences will be used to build phylogenetic reconstructions and will be compared to the phylogenetics reconstructions obtained in this PhD. If true, the hypothesis of accumulation of loci associated to speciation in the sex-determining region, implies that we will obtain deeper separation between clades for phylogenetic reconstructions using the sex markers than for the rest of the nuclear loci studied.

- *Consequences of the haploid-diploid life cycle on reproductive isolation*

Our results exemplified the relative ease to detect at which level reproductive barriers occur in a haploid-diploid life cycle (Chapter 3). In commonly studied organism characterized by diploid life cycles, the haploid stage is reduced to a single-cell (i.e. gamete), produced through meiosis, and fertilization occurs immediately after release of gametes to re-establish the diploid phase. In haploid-diploid life cycles, somatic development occurs in both haploid and diploid phases and there is an alternation between two types of independent functional individuals: haploid gametophytes produced by meiosis and diploid sporophytes resulting from fertilization. This direct access to the haploid part of the life cycle allows untangling the effect of reproductive barriers preventing fertilization (i.e. an absence of diploid hybrid genotypes is expected) or preventing meiosis (i.e. an absence of recombinant haploid genotypes is expected). Conversely, the two kinds of reproductive barriers cannot be distinguished directly using population genetics in the field and more complex garden experiments are needed.

Recently, Rescan (2016) discussed the possible consequences various ploidy levels on the dynamics of reproductive isolation. In particular, she demonstrated that

reproductive isolation evolved more rapidly in the haploid than in the diploid life cycle using an experimental evolution approach in the yeast *Saccharomyces cerevisiae*. She explained that, while reproductive incompatibilities are fully expressed in the first generation (F1) in haploid hybrids, diploid hybrid F1 may benefit from heterosis and thus reproductive incompatibilities will evolve slower. Thus in haploid-diploid species (as in the case of *Ectocarpus*), we may expect reproductive isolation to evolve faster than in diploid species since, as in haploid species, reproductive incompatibilities should be fully expressed in the F1. We might question if the high diversity of species revealed in this PhD thesis could not be the consequence of the haploid-diploid life cycle on the rate of genetic divergence. Interestingly, many biogeographic and phylogenetic studies have shown a surprisingly high level of species diversity in genera composed of haploid-diploid organisms such as in red (e.g., Payo et al. 2012, Pardo et al. 2014, 2015, Guillemin et al. 2016b, Robuchon et al. 2015) or in brown algae (Kogame et al. 2015, Leliaert et al. 2014, Montecinos et al. 2016). In this context, it would be interesting to compare the speciation rate in groups, in which various life cycles co-exist, such as brown algae. This comparative approach could be realized within the context of the "Phaeoexplorer" project that is currently in progress in Roscoff. The aim of this project is to generate annotated genome assemblies for a broad range of brown algal species at different phylogenetic distances from the model brown alga *Ectocarpus*. For the comparative approach, we will select within different clades of the brown algal species that differ by the extent of their haploid phase and test if the divergence rate is repeatedly higher in species characterized by a more prolonged haploid phase.

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Appendix: SECTION AP

Appendix A.1

Table S1. COI-5P sequences used in the study carried out in the chapter 1. Details of the date, geographic origin, Genbank accession number and reference of each COI-5P sequence used in this study.

N°	Name of sample	Date	Country	Locality	Genbank Accession	Reference
1	SAM08-3	2008	United Kingdom	Dunstaffnage	LM995017	Peters et al. 2015
2	GOS08-02	2008	United Kingdom	Gosport Marina	LM995009	Peters et al. 2015
3	Ec318	2006	France	Cherbourg	LM995014	Peters et al. 2015
4	BLZ11-27	2011	France	Roscoff	LM995041	Peters et al. 2015
5	BLZ11-78	2011	France	Roscoff	LM995086	Peters et al. 2015
6	BLZ11-47	2011	France	Roscoff	LM995058	Peters et al. 2015
7	EcVAZ11-10	2011	France	Roscoff	LM995005	Peters et al. 2015
8	EcNAP12-64	2012	Italy	Naples	LM995012	Peters et al. 2015
9	GR11-33	2011	Greece	Athens, Kavouri	LM995375	Peters et al. 2015
10	GR11-38B	2011	Greece	Athens, Kavouri	LM995379	Peters et al. 2015
11	GR11-12A	2011	Greece	Athens, Agios Kosmas	LM995368	Peters et al. 2015
12	Ec294	2006	Peru	San Juan de Marcona	LM995008	Peters et al. 2015
13	Ec32(genome strain)	---	Peru	San Juan de Marcona	FP885846	Peters et al. 2015
14	Ec156	2006	Chile	Pisagua	LM995007	Peters et al. 2015
15	Ec157	2006	Chile	Pisagua	LM995010	Peters et al. 2015
16	Ec666	2009	USA	Falmouth, Massachusetts	LM995016	Peters et al. 2015
17	Ec705	2006	Korea	Jejudo, Kimnjung	LM995011	Peters et al. 2015

18	Ec002	1988	New Zeland	Kaikoura	LM995018	Peters et al. 2015
19	Ec006	1988	Australia	Victoria	LM995019	Peters et al. 2015
20	WIC08-01	2008	United Kingdom	Wick	KT983131	This paper
21	WIC08-02	2008	United Kingdom	Wick	KT983132	This paper
22	WIC08-03	2008	United Kingdom	Wick	KT983133	This paper
23	WIC08-04	2008	United Kingdom	Wick	KT983134	This paper
24	WIC08-05	2008	United Kingdom	Wick	KT983135	This paper
25	WIC08-07	2008	United Kingdom	Wick	KT983136	This paper
26	WIC08-08	2008	United Kingdom	Wick	KT983137	This paper
27	WIC08-17	2008	United Kingdom	Wick	KT983138	This paper
28	WIC08-18	2008	United Kingdom	Wick	KT983139	This paper
29	WIC08-19	2008	United Kingdom	Wick	KT983140	This paper
30	RAT08-6	2008	United Kingdom	Rattray Head	KT983384	This paper
31	BUT08-05	2008	United Kingdom	Berwick	KT983155	This paper
32	BUT08-11	2008	United Kingdom	Berwick	KT983156	This paper
33	BUT08-13	2008	United Kingdom	Berwick	KT983157	This paper
34	BUT08-24	2008	United Kingdom	Berwick	KT983158	This paper
35	BUT08-25	2008	United Kingdom	Berwick	KT983159	This paper
36	BUT08-26	2008	United Kingdom	Berwick	KT983160	This paper
37	BUT08-27	2008	United Kingdom	Berwick	KT983161	This paper
38	BUT08-30	2008	United Kingdom	Berwick	KT983162	This paper
39	BUT08-31	2008	United Kingdom	Berwick	KT983163	This paper
40	BUT08-35	2008	United Kingdom	Berwick	KT983164	This paper
41	BUT08-40	2008	United Kingdom	Berwick	KT983165	This paper
42	BUT08-41	2008	United Kingdom	Berwick	KT983166	This paper

43	OBA07-108	2007	United Kingdom	Dunstaffnage	KT982922	This paper
44	OBA07-15	2007	United Kingdom	Dunstaffnage	KT982923	This paper
45	SAM08-04	2008	United Kingdom	Dunstaffnage	KT982924	This paper
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47	SAM08-09	2008	United Kingdom	Dunstaffnage	KT982926	This paper
48	SAM08-11	2008	United Kingdom	Dunstaffnage	KT983383	This paper
49	GWY08-05	2008	United Kingdom	Mull of Galloway	KT983141	This paper
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55	GWY08-15	2008	United Kingdom	Mull of Galloway	KT983147	This paper
56	GWY08-16	2008	United Kingdom	Mull of Galloway	KT983148	This paper
57	GWY08-17	2008	United Kingdom	Mull of Galloway	KT983149	This paper
58	GWY08-21	2008	United Kingdom	Mull of Galloway	KT983150	This paper
59	GWY08-22	2008	United Kingdom	Mull of Galloway	KT983151	This paper
60	GWY08-23	2008	United Kingdom	Mull of Galloway	KT983152	This paper
61	GWY08-24	2008	United Kingdom	Mull of Galloway	KT983153	This paper
62	GWY08-26	2008	United Kingdom	Mull of Galloway	KT983154	This paper
63	HAS08-06	2008	United Kingdom	Pett level	KT982927	This paper
64	HAS08-07	2008	United Kingdom	Pett level	KT982928	This paper
65	HAS08-09	2008	United Kingdom	Pett level	KT982929	This paper
66	HAS08-12	2008	United Kingdom	Pett level	KT982930	This paper
67	HAS08-13	2008	United Kingdom	Pett level	KT982931	This paper

68	HAS08-14	2008	United Kingdom	Pett level	KT982932	This paper
69	HAS08-16	2008	United Kingdom	Pett level	KT982933	This paper
70	HAS08-17	2008	United Kingdom	Pett level	KT982934	This paper
71	HAS08-18	2008	United Kingdom	Pett level	KT982935	This paper
72	HAS08-19	2008	United Kingdom	Pett level	KT982936	This paper
73	HAS08-20	2008	United Kingdom	Pett level	KT982937	This paper
74	EcQAB10-001	2010	United Kingdom	Plymouth	KT982764	This paper
75	EcQAB10-02	2010	United Kingdom	Plymouth	KT982765	This paper
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78	EcQAB10-05	2010	United Kingdom	Plymouth	KT982768	This paper
79	EcQAB10-06	2010	United Kingdom	Plymouth	KT982769	This paper
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93	EcQAB10-22	2010	United Kingdom	Plymouth	KT982783	This paper
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125	Esil.Ros.h	1970	France	Roscoff	KT982830	This paper
126	Ec393	2003	France	Roscoff	KT982817	This paper
127	Ec332	2006	France	Cherbourg	KT983057	This paper
128	Ec334	2007	France	Roscoff	KT983081	This paper
129	EcPH10-17	2010	France	Roscoff	KT982832	This paper
130	EcPH10-10	2010	France	Roscoff	KT983385	This paper
131	EcPH10-136	2010	France	Roscoff	KT983386	This paper
132	EcPH10-72	2010	France	Roscoff	KT983076	This paper
133	EcPH10-7	2010	France	Roscoff	KT983387	This paper
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136	L162	2010	France	Roscoff	KT983407	This paper
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138	EcPH10-22	2010	France	Roscoff	KT983082	This paper
139	EcPH10-38	2010	France	Roscoff	KT983083	This paper
140	EcPH11-s5-13	2011	France	Roscoff	KT982816	This paper
141	EcPH11-s5-14	2011	France	Roscoff	KT982818	This paper
142	EcPH11-s5-15	2011	France	Roscoff	KT982819	This paper

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145	EcPH11-s5-19	2011	France	Roscoff	KT982822	This paper
146	EcPH11-s5-20	2011	France	Roscoff	KT982823	This paper
147	EcPH11-s2B-41	2011	France	Roscoff	KT982824	This paper
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157	EcPH11-s2A-14	2011	France	Roscoff	KT983075	This paper
158	EcPH11-21	2011	France	Roscoff	KT983077	This paper
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160	EcPH11-09	2011	France	Roscoff	KT983079	This paper
161	BLZ11-44	2011	France	Roscoff	KT983080	This paper
162	EcPH11-s5-38	2011	France	Roscoff	KT983084	This paper
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166	EcPH11-28	2011	France	Roscoff	KT983088	This paper
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186	L205	2010	France	Saint Malo	KT983403	This paper
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188	L183	2010	France	Saint Malo	KT983405	This paper
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190	L216	2010	France	Saint Malo	KT983409	This paper
191	L168	2010	France	Saint Malo	KT982920	This paper
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195	EcTH10-06	2010	France	Traezh Hir	KT982953	This paper
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203	EcTH10-15	2010	France	Traezh Hir	KT982961	This paper
204	EcTH10-16	2010	France	Traezh Hir	KT982962	This paper
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208	EcTH10-21	2010	France	Traezh Hir	KT983167	This paper
209	EcTH10-26	2010	France	Traezh Hir	KT983168	This paper
210	EcTH10-31	2010	France	Traezh Hir	KT983169	This paper
211	EcTH10-32	2010	France	Traezh Hir	KT983170	This paper
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216	EcTH10-37	2010	France	Traezh Hir	KT983175	This paper
217	EcTH10-38	2010	France	Traezh Hir	KT983176	This paper

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220	EcTH10-42	2010	France	Traezh Hir	KT983179	This paper
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222	EcQB10-02	2010	France	Quiberon	KT982787	This paper
223	EcQB10-03	2010	France	Quiberon	KT982788	This paper
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236	EcQB12-05	2012	France	Quiberon	KT983058	This paper
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254	EcRIB-162	2013	Spain	Ribadeo	KT982856	This paper
255	EcRIB-179	2013	Spain	Ribadeo	KT982857	This paper
256	EcRIB-186	2013	Spain	Ribadeo	KT982858	This paper
257	EcRIB-195	2013	Spain	Ribadeo	KT982859	This paper
258	EcRIB-203	2013	Spain	Ribadeo	KT982860	This paper
259	EcRIB-204	2013	Spain	Ribadeo	KT982861	This paper
260	EcRIB-207	2013	Spain	Ribadeo	KT982862	This paper
261	EcRIB-208	2013	Spain	Ribadeo	KT982863	This paper
262	EcRIB-231	2013	Spain	Ribadeo	KT982864	This paper
263	EcRIB-234	2013	Spain	Ribadeo	KT982865	This paper
264	EcRIB-236	2013	Spain	Ribadeo	KT982866	This paper
265	EcRIB-148	2013	Spain	Ribadeo	KT983105	This paper
266	EcRIB-157	2013	Spain	Ribadeo	KT983106	This paper
267	EcRIB-158	2013	Spain	Ribadeo	KT983107	This paper

268	EcRIB-183	2013	Spain	Ribadeo	KT983108	This paper
269	EcRIB-185	2013	Spain	Ribadeo	KT983109	This paper
270	EcRIB-187	2013	Spain	Ribadeo	KT983110	This paper
271	EcRIB-190	2013	Spain	Ribadeo	KT983111	This paper
272	EcRIB-191	2013	Spain	Ribadeo	KT983112	This paper
273	EcRIB-194	2013	Spain	Ribadeo	KT983113	This paper
274	EcRIB-209	2013	Spain	Ribadeo	KT983114	This paper
275	EcRIB-213	2013	Spain	Ribadeo	KT983115	This paper
276	EcRIB-214	2013	Spain	Ribadeo	KT983116	This paper
277	EcRIB-215	2013	Spain	Ribadeo	KT983117	This paper
278	EcRIB-129	2013	Spain	Ribadeo	KT983118	This paper
279	EcGAN-509	2013	Spain	Coruña	KT982843	This paper
280	EcGAN-512	2013	Spain	Coruña	KT982844	This paper
281	EcGAN-528	2013	Spain	Coruña	KT982845	This paper
282	EcGAN-529	2013	Spain	Coruña	KT982846	This paper
283	EcGAN-532	2013	Spain	Coruña	KT982847	This paper
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287	EcGAN-544	2013	Spain	Coruña	KT982851	This paper
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290	EcGAN-515	2013	Spain	Coruña	KT983127	This paper
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292	EcGAN-525	2013	Spain	Coruña	KT983129	This paper

293	EcLOU-57	2013	Spain	Coruña	KT982840	This paper
294	EcLOU-71	2013	Spain	Coruña	KT982841	This paper
295	EcLOU-98	2013	Spain	Coruña	KT982842	This paper
296	EcLOU-102	2013	Spain	Coruña	KT983119	This paper
297	EcLOU-371	2013	Spain	Coruña	KT983120	This paper
298	EcLOU-391	2013	Spain	Coruña	KT983121	This paper
299	EcLOU-393	2013	Spain	Coruña	KT983122	This paper
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303	EcLOU-418	2013	Spain	Coruña	KT983126	This paper
304	EcLOU-454	2013	Spain	Coruña	KT983130	This paper
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313	EcPOV-03	2013	Portugal	Viana do Castelo	KT982835	This paper
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341	EcNAP12-12	2012	Italy	Naples	KU134121	This paper
342	EcNAP12-94	2012	Italy	Naples	KU134122	This paper

343	EcNAP12-s3-17	2012	Italy	Naples	KU134123	This paper
344	EcNAP12-s3-11	2012	Italy	Naples	KU134124	This paper
345	EcNAP12-s3-13	2012	Italy	Naples	KU134125	This paper
346	EcNAP12-s3-19	2012	Italy	Naples	KU134126	This paper
347	EcNAP12-s3-22	2012	Italy	Naples	KU134127	This paper
348	EcNAP12-s3-23	2012	Italy	Naples	KU134128	This paper
349	EcNAP12-s4-16	2012	Italy	Naples	KU134141	This paper
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354	EcNAP12-92	2012	Italy	Naples	KU134146	This paper
355	EcNAP12-45	2012	Italy	Naples	KU134147	This paper
356	EcNAP12-s3-51	2012	Italy	Naples	KU134148	This paper
357	EcNAP12-s4-10	2012	Italy	Naples	KU134149	This paper
358	EcNAP12-s3-35	2012	Italy	Naples	KU134150	This paper
359	EcNAP12-s3-08	2012	Italy	Naples	KU134151	This paper
360	EcNAP12-s3-03	2012	Italy	Naples	KU134152	This paper
361	EcNAP12-s3-50	2012	Italy	Naples	KU134153	This paper
362	EcNAP12-s4-19	2012	Italy	Naples	KU134154	This paper
363	EcNAP12-s4-09	2012	Italy	Naples	KU134155	This paper
364	EcNAP12-82	2012	Italy	Naples	KU134156	This paper
365	GR11-28	2011	Greece	Korinthos	KU134129	This paper
366	GR11-36	2011	Greece	Korinthos	KU134130	This paper
367	GR11-38A	2011	Greece	Korinthos	KU134131	This paper

368	GR11-40	2011	Greece	Korinthos	KU134132	This paper
369	GR11-45	2011	Greece	Korinthos	KU134133	This paper
370	GR11-46	2011	Greece	Korinthos	KU134134	This paper
371	GR11-48	2011	Greece	Korinthos	KU134135	This paper
372	LES08	2009	Greece	Lesbos	KT982913	This paper
373	LES15	2009	Greece	Lesbos	KT982914	This paper
374	LES11	2009	Greece	Lesbos	KU134116	This paper
375	LES4	2009	Greece	Lesbos	KU134117	This paper
376	LES1	2009	Greece	Lesbos	KU134136	This paper
377	LES2	2009	Greece	Lesbos	KU134137	This paper
378	LES3	2009	Greece	Lesbos	KU134138	This paper
379	LES7	2009	Greece	Lesbos	KU134139	This paper
380	LES10	2009	Greece	Lesbos	KU134140	This paper
381	EcPAN-84	2013	Spain	Pan de Azúcar	KT982867	This paper
382	EcPAN-90	2013	Spain	Pan de Azúcar	KT982868	This paper
383	EcPAN-95	2013	Spain	Pan de Azúcar	KT982869	This paper
384	EcPAN-128	2013	Spain	Pan de Azúcar	KT982870	This paper
385	EcPAN-138	2013	Spain	Pan de Azúcar	KT982871	This paper
386	EcPAN-140	2013	Spain	Pan de Azúcar	KT982872	This paper
387	EcPAN-174	2013	Spain	Pan de Azúcar	KT982873	This paper
388	EcPAN-175	2013	Spain	Pan de Azúcar	KT982874	This paper
389	EcPAN-192	2013	Spain	Pan de Azúcar	KT982875	This paper
390	EcPAN-197	2013	Spain	Pan de Azúcar	KT982876	This paper
391	EcPAN-161A	2013	Chile	Pan de Azúcar	KT982966	This paper
392	EcPAN-117	2013	Chile	Pan de Azúcar	KT982967	This paper

393	EcPAN-119	2013	Chile	Pan de Azúcar	KT982968	This paper
394	EcPAN-177	2013	Chile	Pan de Azúcar	KT982969	This paper
395	EcPAN-183	2013	Chile	Pan de Azúcar	KT982970	This paper
396	EcPAN-185	2013	Chile	Pan de Azúcar	KT982971	This paper
397	EcPAN-233	2013	Chile	Pan de Azúcar	KT982972	This paper
398	EcPAN-083	2013	Chile	Pan de Azúcar	KT983194	This paper
399	EcPAN-087A	2013	Chile	Pan de Azúcar	KT983195	This paper
400	EcPAN-091A	2013	Chile	Pan de Azúcar	KT983196	This paper
401	EcPAN-097A	2013	Chile	Pan de Azúcar	KT983197	This paper
402	EcPAN-085	2013	Chile	Pan de Azúcar	KT983198	This paper
403	EcPAN-086	2013	Chile	Pan de Azúcar	KT983199	This paper
404	EcPAN-087	2013	Chile	Pan de Azúcar	KT983200	This paper
405	EcPAN-114A	2013	Chile	Pan de Azúcar	KT983201	This paper
406	EcPAN-139A	2013	Chile	Pan de Azúcar	KT983202	This paper
407	EcPAN-139B	2013	Chile	Pan de Azúcar	KT983203	This paper
408	EcPAN-144A	2013	Chile	Pan de Azúcar	KT983204	This paper
409	EcPAN-174A	2013	Chile	Pan de Azúcar	KT983205	This paper
410	EcPAN-109	2013	Chile	Pan de Azúcar	KT983206	This paper
411	EcPAN-110	2013	Chile	Pan de Azúcar	KT983207	This paper
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414	EcPAN-122	2013	Chile	Pan de Azúcar	KT983210	This paper
415	EcPAN-160	2013	Chile	Pan de Azúcar	KT983211	This paper
416	EcPAN-181	2013	Chile	Pan de Azúcar	KT983212	This paper
417	EcPAN-182	2013	Chile	Pan de Azúcar	KT983213	This paper

418	EcPAN-089	2013	Chile	Pan de Azúcar	KU134058	This paper
419	EcPAN-104	2013	Chile	Pan de Azúcar	KU134059	This paper
420	EcPAN-148	2013	Chile	Pan de Azúcar	KU134060	This paper
421	EcPAN-161	2013	Chile	Pan de Azúcar	KU134061	This paper
422	EcPAN-163	2013	Chile	Pan de Azúcar	KU134062	This paper
423	EcPAN-143A	2013	Chile	Pan de Azúcar	KU134066	This paper
424	EcPAN-149A	2013	Chile	Pan de Azúcar	KU134067	This paper
425	EcPAN-79	2013	Chile	Pan de Azúcar	KU134157	This paper
426	EcPAN-82	2013	Chile	Pan de Azúcar	KU134158	This paper
427	EcBIN-272	2013	Chile	Bahía Inglesa, Caldera	KT982877	This paper
428	EcBIN-273	2013	Chile	Bahía Inglesa, Caldera	KT982878	This paper
429	EcBIN-277	2013	Chile	Bahía Inglesa, Caldera	KT982879	This paper
430	EcBIN-279	2013	Chile	Bahía Inglesa, Caldera	KT982880	This paper
431	EcBIN-282	2013	Chile	Bahía Inglesa, Caldera	KT982881	This paper
432	EcBIN-283	2013	Chile	Bahía Inglesa, Caldera	KT982882	This paper
433	EcBIN-285	2013	Chile	Bahía Inglesa, Caldera	KT982883	This paper
434	EcBIN-284	2013	Chile	Bahía Inglesa, Caldera	KT982884	This paper
435	EcBIN-288	2013	Chile	Bahía Inglesa, Caldera	KT982885	This paper
436	EcBIN-291	2013	Chile	Bahía Inglesa, Caldera	KT982886	This paper
437	EcBIN-292	2013	Chile	Bahía Inglesa, Caldera	KT982887	This paper
438	EcBIN-293	2013	Chile	Bahía Inglesa, Caldera	KT982888	This paper
439	EcBIN-294	2013	Chile	Bahía Inglesa, Caldera	KT982889	This paper
440	EcBIN-295	2013	Chile	Bahía Inglesa, Caldera	KT982890	This paper
441	EcBIN-306	2013	Chile	Bahía Inglesa, Caldera	KT982891	This paper
442	EcBIN-307	2013	Chile	Bahía Inglesa, Caldera	KT982892	This paper

443	EcBIN-308	2013	Chile	Bahía Inglesa, Caldera	KT982893	This paper
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448	EcBIN-330	2013	Chile	Bahía Inglesa, Caldera	KT982898	This paper
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450	EcBIN-332	2013	Chile	Bahía Inglesa, Caldera	KT982900	This paper
451	EcBIN-333	2013	Chile	Bahía Inglesa, Caldera	KT982901	This paper
452	EcBIN-337	2013	Chile	Bahía Inglesa, Caldera	KT982902	This paper
453	EcBIN-338	2013	Chile	Bahía Inglesa, Caldera	KT982903	This paper
454	EcBIN-339	2013	Chile	Bahía Inglesa, Caldera	KT982904	This paper
455	EcBIN-341	2013	Chile	Bahía Inglesa, Caldera	KT982905	This paper
456	EcBIN-343	2013	Chile	Bahía Inglesa, Caldera	KT982906	This paper
457	EcBIN-227	2013	Chile	Bahía Inglesa, Caldera	KU134013	This paper
458	EcBIN-01	2013	Chile	Bahía Inglesa, Caldera	KU134014	This paper
459	EcBIN-02	2013	Chile	Bahía Inglesa, Caldera	KU134015	This paper
460	EcBIN-04	2013	Chile	Bahía Inglesa, Caldera	KU134016	This paper
461	EcBIN-05	2013	Chile	Bahía Inglesa, Caldera	KU134017	This paper
462	EcBIN-06	2013	Chile	Bahía Inglesa, Caldera	KU134018	This paper
463	EcBIN-07	2013	Chile	Bahía Inglesa, Caldera	KU134019	This paper
464	EcBIN-08	2013	Chile	Bahía Inglesa, Caldera	KU134020	This paper
465	EcBIN-09	2013	Chile	Bahía Inglesa, Caldera	KU134021	This paper
466	EcBIN-10	2013	Chile	Bahía Inglesa, Caldera	KU134022	This paper
467	EcBIN-220	2013	Chile	Bahía Inglesa, Caldera	KU134024	This paper

468	EcBIN-221	2013	Chile	Bahía Inglesa, Caldera	KU134025	This paper
469	EcBIN-224	2013	Chile	Bahía Inglesa, Caldera	KU134026	This paper
470	EcBIN-226	2013	Chile	Bahía Inglesa, Caldera	KU134027	This paper
471	EcBIN-243	2013	Chile	Bahía Inglesa, Caldera	KU134028	This paper
472	EcBIN-246	2013	Chile	Bahía Inglesa, Caldera	KU134029	This paper
473	EcBIN-247	2013	Chile	Bahía Inglesa, Caldera	KU134030	This paper
474	EcBIN-276	2013	Chile	Bahía Inglesa, Caldera	KU134032	This paper
475	EcBIN-346	2013	Chile	Bahía Inglesa, Caldera	KU134037	This paper
476	EcBIN-348	2013	Chile	Bahía Inglesa, Caldera	KU134038	This paper
477	EcBIN-350	2013	Chile	Bahía Inglesa, Caldera	KU134039	This paper
478	EcBIN-351	2013	Chile	Bahía Inglesa, Caldera	KU134040	This paper
479	EcBAS-252A	2013	Chile	Bahía Salada, Caldera	KT982907	This paper
480	EcBAS-264	2013	Chile	Bahía Salada, Caldera	KU134031	This paper
481	EcBAS-298	2013	Chile	Bahía Salada, Caldera	KU134033	This paper
482	EcBAS-303	2013	Chile	Bahía Salada, Caldera	KU134034	This paper
483	EcBAS-304	2013	Chile	Bahía Salada, Caldera	KU134035	This paper
484	EcBAS-305	2013	Chile	Bahía Salada, Caldera	KU134036	This paper
485	EcBAS-255A	2013	Chile	Bahía Salada, Caldera	KU134041	This paper
486	EcBAS-255D	2013	Chile	Bahía Salada, Caldera	KU134042	This paper
487	EcBAS-302A	2013	Chile	Bahía Salada, Caldera	KU134043	This paper
488	EcBAS-305B	2013	Chile	Bahía Salada, Caldera	KU134044	This paper
489	EcCIS-071	2013	Chile	Caleta Cisne, Caldera	KU134051	This paper
490	EcCIS-70	2013	Chile	Caleta Cisne, Caldera	KU134053	This paper
491	EcCIS-073	2013	Chile	Caleta Cisne, Caldera	KT987931	This paper
492	EcPVI-36	2013	Chile	Puerto Viejo, Caldera	KU134023	This paper

493	EcPVI-035	2013	Chile	Puerto Viejo, Caldera	KT987930	This paper
494	Ec454	---	Chile	Caldera	KU134105	This paper
495	EcALG-520	2013	Chile	Algarrobo	KT982995	This paper
496	EcALG-521	2013	Chile	Algarrobo	KT982996	This paper
497	EcALG-522	2013	Chile	Algarrobo	KT982997	This paper
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501	EcALG-521A	2013	Chile	Algarrobo	KT983001	This paper
502	EcALG-524	2013	Chile	Algarrobo	KU134108	This paper
503	EcQUI-482	2013	Chile	Quintay	KT982974	This paper
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511	EcQUI-497	2013	Chile	Quintay	KT982982	This paper
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515	EcQUI-507	2013	Chile	Quintay	KT982986	This paper
516	EcQUI-508	2013	Chile	Quintay	KT982987	This paper
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524	EcQUI-455	2013	Chile	Quintay	KT987960	This paper
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533	EcQUI-484	2013	Chile	Quintay	KT983214	This paper
534	EcQUI-490	2013	Chile	Quintay	KT983215	This paper
535	EcQUI-499	2013	Chile	Quintay	KT983216	This paper
536	EcQUI-500	2013	Chile	Quintay	KT983217	This paper
537	EcQUI-501	2013	Chile	Quintay	KT983218	This paper
538	EcQUI-504	2013	Chile	Quintay	KT983219	This paper
539	EcQUI-506	2013	Chile	Quintay	KT983220	This paper
540	EcQUI-519	2013	Chile	Quintay	KT983221	This paper
541	EcQUI-513	2013	Chile	Quintay	KT983222	This paper
542	EcQUI-450	2013	Chile	Quintay	KU134052	This paper

543	EcLAC-403	2013	Chile	Las Cruces	KT982973	This paper
544	EcLAC-397	2013	Chile	Las Cruces	KU134054	This paper
545	EcLAC-412	2013	Chile	Las Cruces	KU134055	This paper
546	EcLAC-398	2013	Chile	Las Cruces	KU134056	This paper
547	EcLAC-424	2013	Chile	Las Cruces	KU134106	This paper
548	EcLAC-384	2013	Chile	Las Cruces	KU134107	This paper
549	EcLAC-354	2013	Chile	Las Cruces	KT987932	This paper
550	EcLAC-355	2013	Chile	Las Cruces	KT987933	This paper
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553	EcLAC-363	2013	Chile	Las Cruces	KT987936	This paper
554	EcLAC-365	2013	Chile	Las Cruces	KT987937	This paper
555	EcLAC-366	2013	Chile	Las Cruces	KT987938	This paper
556	EcLAC-369	2013	Chile	Las Cruces	KT987939	This paper
557	EcLAC-371	2013	Chile	Las Cruces	KT987940	This paper
558	EcLAC-373	2013	Chile	Las Cruces	KT987941	This paper
559	EcLAC-374	2013	Chile	Las Cruces	KT987942	This paper
560	EcLAC-386	2013	Chile	Las Cruces	KT987943	This paper
561	EcLAC-387	2013	Chile	Las Cruces	KT987944	This paper
562	EcLAC-388	2013	Chile	Las Cruces	KT987945	This paper
563	EcLAC-389	2013	Chile	Las Cruces	KT987946	This paper
564	EcLAC-390	2013	Chile	Las Cruces	KT987947	This paper
565	EcLAC-392	2013	Chile	Las Cruces	KT987948	This paper
566	EcLAC-394	2013	Chile	Las Cruces	KT987949	This paper
567	EcLAC-407	2013	Chile	Las Cruces	KT987950	This paper

568	EcLAC-411	2013	Chile	Las Cruces	KT987951	This paper
569	EcLAC-415	2013	Chile	Las Cruces	KT987952	This paper
570	EcLAC-425	2013	Chile	Las Cruces	KT987953	This paper
571	EcLAC-430	2013	Chile	Las Cruces	KT987954	This paper
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575	EcLAC-444	2013	Chile	Las Cruces	KT987958	This paper
576	EcLAC-445	2013	Chile	Las Cruces	KT987959	This paper
577	EcLAC-402A	2013	Chile	Las Cruces	KT987974	This paper
578	EcLAC-379	2013	Chile	Las Cruces	KT987977	This paper
579	EcDIC-605	2013	Chile	Dichato	KT983009	This paper
580	EcDIC-577	2013	Chile	Dichato	KU134069	This paper
581	EcDIC-578	2013	Chile	Dichato	KU134070	This paper
582	EcDIC-579	2013	Chile	Dichato	KU134071	This paper
583	EcDIC-581	2013	Chile	Dichato	KU134072	This paper
584	EcDIC-583	2013	Chile	Dichato	KU134073	This paper
585	EcDIC-584	2013	Chile	Dichato	KU134074	This paper
586	EcDIC-586	2013	Chile	Dichato	KU134075	This paper
587	EcDIC-587	2013	Chile	Dichato	KU134076	This paper
588	EcDIC-594	2013	Chile	Dichato	KU134077	This paper
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592	EcDIC-600	2013	Chile	Dichato	KU134081	This paper

593	EcDIC-601	2013	Chile	Dichato	KU134082	This paper
594	EcDIC-602	2013	Chile	Dichato	KU134083	This paper
595	EcDIC-603	2013	Chile	Dichato	KU134084	This paper
596	EcDIC-604	2013	Chile	Dichato	KU134085	This paper
597	EcDIC-609	2013	Chile	Dichato	KU134086	This paper
598	EcDIC-610	2013	Chile	Dichato	KU134087	This paper
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603	EcDIC-656	2013	Chile	Dichato	KU134093	This paper
604	EcDIC-657	2013	Chile	Dichato	KU134094	This paper
605	EcDIC-658	2013	Chile	Dichato	KU134095	This paper
606	EcCOL-572	2013	Chile	Cocholgüe, Tomé	KT982908	This paper
607	EcCOL-529	2013	Chile	Cocholgüe, Tomé	KT983002	This paper
608	EcCOL-530	2013	Chile	Cocholgüe, Tomé	KT983003	This paper
609	EcCOL-531	2013	Chile	Cocholgüe, Tomé	KT983004	This paper
610	EcCOL-544	2013	Chile	Cocholgüe, Tomé	KT983005	This paper
611	EcCOL-573	2013	Chile	Cocholgüe, Tomé	KT983006	This paper
612	EcCOL-574	2013	Chile	Cocholgüe, Tomé	KT983007	This paper
613	EcCOL-576	2013	Chile	Cocholgüe, Tomé	KT983008	This paper
614	EcCOL-566	2013	Chile	Cocholgüe, Tomé	KT983223	This paper
615	EcCOL_568	2013	Chile	Cocholgüe, Tomé	KU134068	This paper
616	EcCOL-558A	2013	Chile	Cocholgüe, Tomé	KU134104	This paper
617	EcCOL-532	2013	Chile	Cocholgüe, Tomé	KU134109	This paper

618	EcCOL-558	2013	Chile	Cocholgüe, Tomé	KU134110	This paper
619	EcCOL-560	2013	Chile	Cocholgüe, Tomé	KU134111	This paper
620	EcCOL-569	2013	Chile	Cocholgüe, Tomé	KU134112	This paper
621	EcCOL-559	2013	Chile	Cocholgüe, Tomé	KT987969	This paper
622	EcCOL-567	2013	Chile	Cocholgüe, Tomé	KT987970	This paper
623	EcCOL-568A	2013	Chile	Cocholgüe, Tomé	KT987975	This paper
624	EcPIL-611	2013	Chile	Pilolcura, Valdivia	KT983010	This paper
625	EcPIL-616	2013	Chile	Pilolcura, Valdivia	KT983011	This paper
626	EcPIL-618	2013	Chile	Pilolcura, Valdivia	KT983012	This paper
627	EcPIL-624	2013	Chile	Pilolcura, Valdivia	KT983013	This paper
628	EcPIL-625	2013	Chile	Pilolcura, Valdivia	KT983014	This paper
629	EcPIL-628	2013	Chile	Pilolcura, Valdivia	KT983015	This paper
630	EcPIL-650	2013	Chile	Pilolcura, Valdivia	KT983016	This paper
631	EcPIL-621	2013	Chile	Pilolcura, Valdivia	KT983224	This paper
632	EcPIL-631	2013	Chile	Pilolcura, Valdivia	KT983225	This paper
633	EcPIL-632	2013	Chile	Pilolcura, Valdivia	KT983226	This paper
634	EcPIL-633	2013	Chile	Pilolcura, Valdivia	KT983227	This paper
635	EcPIL-634	2013	Chile	Pilolcura, Valdivia	KT983228	This paper
636	EcPIL-635	2013	Chile	Pilolcura, Valdivia	KT983231	This paper
637	EcPIL-636	2013	Chile	Pilolcura, Valdivia	KT983232	This paper
638	EcPIL-651	2013	Chile	Pilolcura, Valdivia	KT983233	This paper
639	EcPIL-648	2013	Chile	Pilolcura, Valdivia	KU134090	This paper
640	EcPIL-639	2013	Chile	Pilolcura, Valdivia	KT987971	This paper
641	EcCNA-638	2013	Chile	Curiñanco, Valdivia	KT983017	This paper
642	EcCNA-640	2013	Chile	Curiñanco, Valdivia	KT983018	This paper

643	EcCNA-641	2013	Chile	Curiñanco, Valdivia	KT983019	This paper
644	EcCNA-644	2013	Chile	Curiñanco, Valdivia	KT983020	This paper
645	EcCNA-645	2013	Chile	Curiñanco, Valdivia	KT983021	This paper
646	EcCNA-660	2013	Chile	Curiñanco, Valdivia	KT983022	This paper
647	EcCNA-659	2013	Chile	Curiñanco, Valdivia	KT983023	This paper
648	EcCNA-642	2013	Chile	Curiñanco, Valdivia	KT983229	This paper
649	EcSCA-726	2013	Chile	San Carlos, Corral, Valdivia	KT983230	This paper
650	EcSCA-721	2013	Chile	San Carlos, Corral, Valdivia	KU134063	This paper
651	EcSCA-722	2013	Chile	San Carlos, Corral, Valdivia	KU134064	This paper
652	EcSCA-723	2013	Chile	San Carlos, Corral, Valdivia	KU134065	This paper
653	EcPLG-708	2013	Chile	Niebla, Valdivia	KU134057	This paper
654	EcPLG-712	2013	Chile	Niebla, Valdivia	KU134096	This paper
655	EcPLG-716	2013	Chile	Niebla, Valdivia	KU134097	This paper
656	EcPLG-717	2013	Chile	Niebla, Valdivia	KU134098	This paper
657	EcPLG-718	2013	Chile	Niebla, Valdivia	KU134099	This paper
658	EcPLG-682A	2013	Chile	Niebla, Valdivia	KU134113	This paper
659	EcPLG-686	2013	Chile	Niebla, Valdivia	KU134114	This paper
660	EcPLG-688	2013	Chile	Niebla, Valdivia	KU134115	This paper
661	EcPLG-713	2013	Chile	Niebla, Valdivia	KT987972	This paper
662	EcPLG-714	2013	Chile	Niebla, Valdivia	KT987973	This paper
663	EcEST-782	2013	Chile	Estaquilla	KT983028	This paper
664	EcEST-785	2013	Chile	Estaquilla	KT983029	This paper
665	EcEST-786	2013	Chile	Estaquilla	KT983030	This paper
666	EcEST-788	2013	Chile	Estaquilla	KT983031	This paper
667	EcEST-789	2013	Chile	Estaquilla	KT983032	This paper

668	EcEST-790	2013	Chile	Estaquilla	KT983033	This paper
669	EcEST-791	2013	Chile	Estaquilla	KT983034	This paper
670	EcEST-792	2013	Chile	Estaquilla	KT983035	This paper
671	EcEST-793	2013	Chile	Estaquilla	KT983036	This paper
672	EcEST-794	2013	Chile	Estaquilla	KT983037	This paper
673	EcEST-795	2013	Chile	Estaquilla	KT983038	This paper
674	EcEST-796	2013	Chile	Estaquilla	KT983039	This paper
675	EcEST-797	2013	Chile	Estaquilla	KT983040	This paper
676	EcEST-798	2013	Chile	Estaquilla	KT983041	This paper
677	EcEST-800	2013	Chile	Estaquilla	KT983042	This paper
678	EcEST-801	2013	Chile	Estaquilla	KT983043	This paper
679	EcEST-802	2013	Chile	Estaquilla	KT983044	This paper
680	EcEST-804	2013	Chile	Estaquilla	KT983045	This paper
681	EcEST-805	2013	Chile	Estaquilla	KT983046	This paper
682	EcEST-806	2013	Chile	Estaquilla	KT983047	This paper
683	EcEST-807	2013	Chile	Estaquilla	KT983048	This paper
684	EcEST-808	2013	Chile	Estaquilla	KT983049	This paper
685	EcEST-809	2013	Chile	Estaquilla	KT983050	This paper
686	EcEST-810	2013	Chile	Estaquilla	KT983051	This paper
687	EcEST-811	2013	Chile	Estaquilla	KT983052	This paper
688	EcEST-813	2013	Chile	Estaquilla	KT983053	This paper
689	EcEST-815	2013	Chile	Estaquilla	KT983054	This paper
690	EcEST-816	2013	Chile	Estaquilla	KT983055	This paper
691	EcEST-769	2013	Chile	Estaquilla	KT983237	This paper
692	EcEST-771	2013	Chile	Estaquilla	KT983238	This paper

693	EcEST-772	2013	Chile	Estaquilla	KT983239	This paper
694	EcEST-775	2013	Chile	Estaquilla	KT983240	This paper
695	EcEST-776	2013	Chile	Estaquilla	KT983241	This paper
696	EcEST-777	2013	Chile	Estaquilla	KT983242	This paper
697	EcEST-778	2013	Chile	Estaquilla	KT983243	This paper
698	EcEST-779	2013	Chile	Estaquilla	KT983244	This paper
699	EcEST-780	2013	Chile	Estaquilla	KT983245	This paper
700	EcEST-781	2013	Chile	Estaquilla	KT983246	This paper
701	EcEST-783	2013	Chile	Estaquilla	KT983247	This paper
702	EcEST-784	2013	Chile	Estaquilla	KT983248	This paper
703	EcEST-787	2013	Chile	Estaquilla	KT983249	This paper
704	EcEST-803	2013	Chile	Estaquilla	KT983250	This paper
705	EcACH-745	2013	Chile	Achao	KT982909	This paper
706	EcACH-748	2013	Chile	Achao	KT982910	This paper
707	EcACH-757	2013	Chile	Achao	KT982911	This paper
708	EcACH-761	2013	Chile	Achao	KT982912	This paper
709	EcACH-751	2013	Chile	Achao	KT983024	This paper
710	EcACH-752	2013	Chile	Achao	KT983025	This paper
711	EcACH-753	2013	Chile	Achao	KT983026	This paper
712	EcACH-754	2013	Chile	Achao	KT983027	This paper
713	EcACH-749	2013	Chile	Achao	KT983234	This paper
714	EcACH-755	2013	Chile	Achao	KT983235	This paper
715	EcACH-762	2013	Chile	Achao	KT983236	This paper
716	EcACH-760	2013	Chile	Achao	KU134045	This paper
717	EcACH-765	2013	Chile	Achao	KU134046	This paper

718	EcACH-758	2013	Chile	Achao	KU134047	This paper
719	EcACH-763	2013	Chile	Achao	KU134048	This paper
720	EcACH-746A	2013	Chile	Achao	KU134049	This paper
721	EcACH-759	2013	Chile	Achao	KU134050	This paper
722	EcACH-746	2013	Chile	Achao	KU134100	This paper
723	EcACH-747	2013	Chile	Achao	KU134101	This paper
724	EcACH-750	2013	Chile	Achao	KU134102	This paper
725	EcACH-756	2013	Chile	Achao	KU134103	This paper
726	Ec642	2009	USA	Oregon	KT983251	This paper
727	Ec641	2009	USA	Oregon	KT987976	This paper
728	Ec707	2006	Korea	Hanrim	KT982915	This paper
729	Ec717	2005	Korea	Hanrim	KT983056	This paper

Appendix A.2

Table S2. ITS1 sequences used in the study carried out in the chapter 1. Details of the date, geographic origin, Genbank accession number and reference of each ITS1 sequence used in this study.

N°	Name of sample	Date	Country	Locality	Genbank Accession	Reference
1	Esil.Man.f	1976	United Kingdom	Isle of Man	U38771	Stache-Crain et al. 1997
2	W009	2004	United Kingdom	Devon	FR668929	Unpublished
3	Esil.Ros.h	1970	France	Roscoff	U38760	Stache-Crain et al. 1997
4	Ec393	2003	France	Roscoff	FN564440	Peters et al. 2010a
5	Ec496	2005	France	Roscoff	FN564451	Peters et al. 2010a
6	Ec537	2005	France	Traezh Hir	FN564450	Peters et al. 2010a
7	Ec540	2005	France	Traezh Hir	FN564448	Peters et al. 2010a
8	Ec318	2006	France	Cherbourg	FN564443	Peters et al. 2010a
9	Esilnap.m	no data	Italy	Naples	U38754	Stache-Crain et al. 1997
10	EcsilNa70m	1975	Italy	Naples	FR668809	Unpublished
11	Ec_sil_Nap_EA1f	1965	Italy	Naples	FR668820	Unpublished
12	EcsilNa84f	1975	Italy	Naples	FR668810	Unpublished
13	EcNa112fNa	1975	Italy	Naples	FR668811	Unpublished
14	Esil.Nap.f	no data	Italy	Naples	U38755	Stache-Crain et al. 1997
15	EcCI200-2	1994	Spain	Canary Islands	FR668887	Unpublished
16	EcCI193-2	1994	Spain	Canary Islands	FR668885	Unpublished
17	CI146-1	no data	Spain	Canary Islands	U38775	Stache-Crain et al. 1997
18	CI171-1	1994	Spain	Canary Islands	FR668884	Unpublished
19	EcsilNFL31E3f	no data	Canada	Newfoundland	FR668807	Unpublished

20	Esil.wil.f	1978	USA	Wilmington, North Carolina	U38756	Stache-Crain et al. 1997
21	E.SB	1977	USA	Santa Barbara, California	U38759	Stache-Crain et al. 1997
22	EcSBML7-1	1977	USA	Santa Barbara, California	FR668855	Unpublished
23	E.SF	no data	USA	Santa Francisco, California	U38758	Stache-Crain et al. 1997
24	Wil1a	no data	USA	Wilmington, North Carolina	U38776	Stache-Crain et al. 1997
25	Ec286	2006	Peru	Bahía Mendieta	FN564454	Peters et al. 2010b
26	Ec298	2006	Peru	San Juan de Marcona	FN564456	Peters et al. 2010b
27	Esil.Peru.h	1988	Peru	San Juan de Marcona	AJ550048	Unpublished
28	Ec721	2006	Chile	Arica	FN564446	Peters et al. 2010b
29	Ec156	2006	Chile	Pisagua	FN564457	Peters et al. 2010b
30	Ec157	2006	Chile	Pisagua	FN564453	Peters et al. 2010b
31	Ec524	2006	Chile	Chañaral	FN564444	Peters et al. 2010b
32	Ec608	2004-2005	Chile	Chañaral	FN564459	Peters et al. 2010b
33	Ec454	no data	Chile	Caldera	FR668740	Unpublished
34	Ec456	1990	Chile	Coquimbo, Caldera	FN564455	Unpublished
35	Esil.Ch-1.f	1985	Chile	Puerto Puyuhuapi	U38767	Stache-Crain et al. 1997
36	Efas.Ch-2.f	1992	Chile	Valdivia	U38772	Stache-Crain et al. 1997
37	RC32-4	no data	Chile	Juan Fernandez	U38763	Stache-Crain et al. 1997
38	EcSAM-117	1989	Argentina	Puerto Deseado	FR668866	Unpublished
39	Esil.NZ-1.f	1988	New Zealand	Kaikoura	U38766	Stache-Crain et al. 1997
40	EcsilNZ15d2m	1988	New Zealand	Kaikoura	FR668748	Unpublished
41	EcsilchapNZ613m	no data	New Zealand	Kaikoura	FR668744	Unpublished
42	Chap615f	no data	New Zealand	Kaikoura	U38761	Stache-Crain et al. 1997
43	Esil.Vic.f	no data	Australia	Sorrento, Victoria	U38764	Stache-Crain et al. 1997
44	Ec006	1988	Australia	Sorrento, Victoria	FR668837	Unpublished

45	EcsilVic88-12-15f	1988	Australia	Sorrento, Victoria	FR668838	Unpublished
46	Esil.Saf.f	no data	South Africa	Port Elizabeth	U38757	Stache-Crain et al. 1997
47	Ec718	2005	Korea	Hupo	FN564461	Unpublished
48	Ec714	2005	Korea	Sangjokam	FN564464	Unpublished
49	Ec705	2006	Korea	Kimnyung	FN564445	Unpublished
50	EcJAP91-5	1991	Japan	Namikata, Ehime	FR668897	Unpublished
51	WIC08-1	2008	United Kingdom	Wick	KU134398	This paper
52	WIC08-2	2008	United Kingdom	Wick	KU134399	This paper
53	WIC08-3	2008	United Kingdom	Wick	KU134400	This paper
54	WIC08-4	2008	United Kingdom	Wick	KU134401	This paper
55	WIC08-5	2008	United Kingdom	Wick	KU134402	This paper
56	WIC08-7	2008	United Kingdom	Wick	KU134403	This paper
57	WIC08-8	2008	United Kingdom	Wick	KU134404	This paper
58	WIC08-9	2008	United Kingdom	Wick	KU134405	This paper
59	WIC08-16	2008	United Kingdom	Wick	KU134406	This paper
60	WIC08-18	2008	United Kingdom	Wick	KU134407	This paper
61	OBA07-4	2007	United Kingdom	Dunstaffnage	KU134162	This paper
62	OBA07-15	2007	United Kingdom	Dunstaffnage	KU134163	This paper
63	OBA07-48	2007	United Kingdom	Dunstaffnage	KU134164	This paper
64	OBA07-10	2007	United Kingdom	Dunstaffnage	KU134165	This paper
65	SAM08-4	2008	United Kingdom	Dunstaffnage	KU134166	This paper
66	SAM08-5	2008	United Kingdom	Dunstaffnage	KU134167	This paper
67	SAM08-9	2008	United Kingdom	Dunstaffnage	KU134168	This paper
68	SAM08-03	2008	United Kingdom	Dunstaffnage	KU134545	This paper
69	BUT08-30	2008	United Kingdom	Berwick	KU134417	This paper

70	BUT08-31	2008	United Kingdom	Berwick	KU134418	This paper
71	BUT08-32	2008	United Kingdom	Berwick	KU134419	This paper
72	BUT08-24	2008	United Kingdom	Berwick	KU134420	This paper
73	BUT08-35	2008	United Kingdom	Berwick	KU134421	This paper
74	BUT08-40	2008	United Kingdom	Berwick	KU134422	This paper
75	BUT08-41	2008	United Kingdom	Berwick	KU134423	This paper
76	BUT08-11	2008	United Kingdom	Berwick	KU134424	This paper
77	BUT08-13	2008	United Kingdom	Berwick	KU134425	This paper
78	BUT08-25	2008	United Kingdom	Berwick	KU134426	This paper
79	BUT08-26	2008	United Kingdom	Berwick	KU134427	This paper
80	BUT08-27	2008	United Kingdom	Berwick	KU134428	This paper
81	GWY08-16	2008	United Kingdom	Mull of Galloway	KU134429	This paper
82	GWY08-17	2008	United Kingdom	Mull of Galloway	KU134430	This paper
83	GWY08-21	2008	United Kingdom	Mull of Galloway	KU134431	This paper
84	GWY08-24	2008	United Kingdom	Mull of Galloway	KU134432	This paper
85	GWY08-23	2008	United Kingdom	Mull of Galloway	KU134433	This paper
86	GWY08-26	2008	United Kingdom	Mull of Galloway	KU134434	This paper
87	GWY08-05	2008	United Kingdom	Mull of Galloway	KU134435	This paper
88	GWY08-06	2008	United Kingdom	Mull of Galloway	KU134436	This paper
89	GWY08-07	2008	United Kingdom	Mull of Galloway	KU134437	This paper
90	GWY08-08	2008	United Kingdom	Mull of Galloway	KU134438	This paper
91	GWY08-09	2008	United Kingdom	Mull of Galloway	KU134439	This paper
92	GWY08-13	2008	United Kingdom	Mull of Galloway	KU134440	This paper
93	GWY08-14	2008	United Kingdom	Mull of Galloway	KU134441	This paper
94	GWY08-15	2008	United Kingdom	Mull of Galloway	KU134442	This paper

95	HAS08-5	2008	United Kingdom	Pett level	KU134159	This paper
96	HAS08-9	2008	United Kingdom	Pett level	KU134160	This paper
97	HAS08-18	2008	United Kingdom	Pett level	KU134161	This paper
98	HAS08-7	2008	United Kingdom	Pett level	KU134305	This paper
99	HAS08-8	2008	United Kingdom	Pett level	KU134306	This paper
100	HAS08-11	2008	United Kingdom	Pett level	KU134307	This paper
101	HAS08-12	2008	United Kingdom	Pett level	KU134308	This paper
102	HAS08-13	2008	United Kingdom	Pett level	KU134309	This paper
103	HAS08-14	2008	United Kingdom	Pett level	KU134310	This paper
104	HAS08-16	2008	United Kingdom	Pett level	KU134311	This paper
105	HAS08-17	2008	United Kingdom	Pett level	KU134312	This paper
106	HAS08-19	2008	United Kingdom	Pett level	KU134313	This paper
107	HAS08-20	2008	United Kingdom	Pett level	KU134314	This paper
108	LH14	2008	United Kingdom	Gosport marina	KU134542	This paper
109	LH7	2008	United Kingdom	Gosport marina	KU134543	This paper
110	LH13	2008	United Kingdom	Gosport marina	KU134544	This paper
111	EcQAB10-1	2010	United Kingdom	Plymouth	KU134213	This paper
112	EcQAB10-2	2010	United Kingdom	Plymouth	KU134214	This paper
113	EcQAB10-3	2010	United Kingdom	Plymouth	KU134215	This paper
114	EcQAB10-4	2010	United Kingdom	Plymouth	KU134216	This paper
115	EcQAB10-5	2010	United Kingdom	Plymouth	KU134217	This paper
116	EcQAB10-6	2010	United Kingdom	Plymouth	KU134218	This paper
117	EcQAB10-7	2010	United Kingdom	Plymouth	KU134219	This paper
118	EcQAB10-8	2010	United Kingdom	Plymouth	KU134220	This paper
119	EcQAB10-9	2010	United Kingdom	Plymouth	KU134221	This paper

120	EcQAB10-10	2010	United Kingdom	Plymouth	KU134222	This paper
121	EcQAB10-11	2010	United Kingdom	Plymouth	KU134223	This paper
122	EcQAB10-12	2010	United Kingdom	Plymouth	KU134224	This paper
123	EcQAB10-13	2010	United Kingdom	Plymouth	KU134225	This paper
124	EcQAB10-14	2010	United Kingdom	Plymouth	KU134226	This paper
125	EcQAB10-15	2010	United Kingdom	Plymouth	KU134227	This paper
126	EcQAB10-16	2010	United Kingdom	Plymouth	KU134228	This paper
127	EcQAB10-17	2010	United Kingdom	Plymouth	KU134229	This paper
128	EcQAB10-18	2010	United Kingdom	Plymouth	KU134230	This paper
129	EcQAB10-19	2010	United Kingdom	Plymouth	KU134231	This paper
130	EcQAB10-20	2010	United Kingdom	Plymouth	KU134232	This paper
131	EcQAB10-21	2010	United Kingdom	Plymouth	KU134233	This paper
132	EcQAB10-22	2010	United Kingdom	Plymouth	KU134234	This paper
133	EcQAB10-23	2010	United Kingdom	Plymouth	KU134235	This paper
134	EcQAB10-24	2010	United Kingdom	Plymouth	KU134236	This paper
135	EcPLY10-31	2010	United Kingdom	Plymouth	KU134472	This paper
136	EcPLY10-19	2010	United Kingdom	Plymouth	KU134473	This paper
137	EcPLY10-20	2010	United Kingdom	Plymouth	KU134474	This paper
138	EcPLY10-21	2010	United Kingdom	Plymouth	KU134475	This paper
139	EcPLY10-23	2010	United Kingdom	Plymouth	KU134476	This paper
140	EcPLY10-26	2010	United Kingdom	Plymouth	KU134477	This paper
141	EcPLY10-27	2010	United Kingdom	Plymouth	KU134478	This paper
142	EcPLY10-28	2010	United Kingdom	Plymouth	KU134479	This paper
143	EcPLY10-29	2010	United Kingdom	Plymouth	KU134480	This paper
144	EcPLY10-30	2010	United Kingdom	Plymouth	KU134481	This paper

145	EcPLY10-34	2010	United Kingdom	Plymouth	KU134482	This paper
146	EcPLY10-35	2010	United Kingdom	Plymouth	KU134483	This paper
147	Ec242	2011	United Kingdom	Plymouth	KU134540	This paper
148	EcREP10-38	2010	United Kingdom	Restronguet	KU134315	This paper
149	EcREP10-39	2010	United Kingdom	Restronguet	KU134316	This paper
150	EcREP10-42	2010	United Kingdom	Restronguet	KU134317	This paper
151	EcREP10-43	2010	United Kingdom	Restronguet	KU134318	This paper
152	EcREP10-44	2010	United Kingdom	Restronguet	KU134319	This paper
153	EcREP10-45	2010	United Kingdom	Restronguet	KU134320	This paper
154	EcREP10-48	2010	United Kingdom	Restronguet	KU134321	This paper
155	EcREP10-49	2010	United Kingdom	Restronguet	KU134322	This paper
156	EcREP10-50	2010	United Kingdom	Restronguet	KU134323	This paper
157	EcREP10-51	2010	United Kingdom	Restronguet	KU134324	This paper
158	EcREP10-52	2010	United Kingdom	Restronguet	KU134325	This paper
159	EcREP10-54	2010	United Kingdom	Restronguet	KU134326	This paper
160	Ec334	2007	France	Roscoff	KU134470	This paper
161	EcPH10-22	2010	France	Roscoff	KU134456	This paper
162	EcPH10-38	2010	France	Roscoff	KU134455	This paper
163	EcPH10-72	2010	France	Roscoff	KU134471	This paper
164	EcPH11-s5-15	2011	France	Roscoff	KU134261	This paper
165	EcPH11-s2B-45	2011	France	Roscoff	KU134262	This paper
166	EcPH11-s5-13	2011	France	Roscoff	KU134263	This paper
167	EcPH11-s2B-43	2011	France	Roscoff	KU134264	This paper
168	EcPH11-s5-19	2011	France	Roscoff	KU134265	This paper
169	EcPH11-s2B-41	2011	France	Roscoff	KU134266	This paper

170	EcPH11-s5-18	2011	France	Roscoff	KU134267	This paper
171	EcPH11-s5-17	2011	France	Roscoff	KU134268	This paper
172	EcPH11-s2A-04	2011	France	Roscoff	KU134269	This paper
173	EcPH11-s2A-06	2011	France	Roscoff	KU134270	This paper
174	EcPH11-s2A-09	2011	France	Roscoff	KU134271	This paper
175	EcPH11-s2A-16	2011	France	Roscoff	KU134272	This paper
176	BLZ11-27	2011	France	Roscoff	KU134273	This paper
177	BLZ11-51	2011	France	Roscoff	KU134274	This paper
178	EcPH11-28	2011	France	Roscoff	KU134457	This paper
179	EcPH11-27	2011	France	Roscoff	KU134458	This paper
180	EcPH11-21	2011	France	Roscoff	KU134459	This paper
181	EcPH11-16	2011	France	Roscoff	KU134460	This paper
182	EcPH11-9	2011	France	Roscoff	KU134461	This paper
183	EcPH11-6	2011	France	Roscoff	KU134462	This paper
184	EcPH11-47	2011	France	Roscoff	KU134463	This paper
185	EcPH11-1	2011	France	Roscoff	KU134464	This paper
186	EcPH11-38	2011	France	Roscoff	KU134465	This paper
187	EcPH11-33	2011	France	Roscoff	KU134466	This paper
188	EcPH11-2A-01	2011	France	Roscoff	KU134467	This paper
189	EcPH11-2A-14	2011	France	Roscoff	KU134468	This paper
190	EcPH11-s5-38	2011	France	Roscoff	KU134469	This paper
191	EcTH10-04	2010	France	Traezh Hir	KU134327	This paper
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229	EcQB10-12	2010	France	Quiberon	KU134251	This paper
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240	EcQB12-16	2012	France	Quiberon	KU134453	This paper
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292	EcRIA-298	2013	Spain	Ría de Arousa	KU134506	This paper
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305	EcNAP12-114	2012	Italy	Naples	KU134255	This paper
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310	EcNAP12-113	2012	Italy	Naples	KU134260	This paper
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331	GR11-45	2009	Greece	Korinthos	KU134684	This paper
332	GR11-46	2009	Greece	Korinthos	KU134685	This paper
333	GR11-48	2009	Greece	Korinthos	KU134686	This paper
334	LES10	2009	Greece	Lesbos	KU134681	This paper
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338	LES7	2009	Greece	Lesbos	KU134690	This paper
339	Les4	2009	Greece	Lesbos	KU134728	This paper
340	Ec287	2006	Peru	Mendieta	KU134591	This paper
341	Ec279	2006	Peru	Mendieta	KU134623	This paper
342	Ec521	2004-2005	Chile	Pan de Azúcar	KU134592	This paper
343	Ec606	2005-2005	Chile	Pan de Azúcar	KU134345	This paper
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482	EcLAC-444	2013	Chile	Las Cruces	KU134569	This paper
483	EcLAC-357	2013	Chile	Las Cruces	KU134574	This paper
484	EcLAC-365	2013	Chile	Las Cruces	KU134575	This paper
485	EcLAC-407	2013	Chile	Las Cruces	KU134576	This paper
486	EcLAC-373	2013	Chile	Las Cruces	KU134577	This paper
487	EcLAC-374	2013	Chile	Las Cruces	KU134578	This paper
488	EcLAC-388	2013	Chile	Las Cruces	KU134579	This paper
489	EcLAC-387	2013	Chile	Las Cruces	KU134580	This paper
490	EcLAC-389	2013	Chile	Las Cruces	KU134581	This paper
491	EcLAC-390	2013	Chile	Las Cruces	KU134582	This paper
492	EcLAC-411	2013	Chile	Las Cruces	KU134583	This paper
493	EcLAC-415	2013	Chile	Las Cruces	KU134584	This paper
494	EcLAC-433	2013	Chile	Las Cruces	KU134585	This paper

495	EcLAC-435	2013	Chile	Las Cruces	KU134587	This paper
496	EcLAC-443	2013	Chile	Las Cruces	KU134588	This paper
497	EcLAC-356	2013	Chile	Las Cruces	KU134589	This paper
498	EcLAC-394	2013	Chile	Las Cruces	KU134590	This paper
499	EcLAC-397	2013	Chile	Las Cruces	KU134626	This paper
500	EcLAC-398	2013	Chile	Las Cruces	KU134627	This paper
501	EcLAC-412	2013	Chile	Las Cruces	KU134628	This paper
502	EcLAC-424	2013	Chile	Las Cruces	KU134680	This paper
503	EcDIC-605	2013	Chile	Dichato	KU134358	This paper
504	EcDIC-577	2013	Chile	Dichato	KU134651	This paper
505	EcDIC-578	2013	Chile	Dichato	KU134652	This paper
506	EcDIC-579	2013	Chile	Dichato	KU134653	This paper
507	EcDIC-583	2013	Chile	Dichato	KU134654	This paper
508	EcDIC-584	2013	Chile	Dichato	KU134655	This paper
509	EcDIC-586	2013	Chile	Dichato	KU134656	This paper
510	EcDIC-587	2013	Chile	Dichato	KU134657	This paper
511	EcDIC-594	2013	Chile	Dichato	KU134658	This paper
512	EcDIC-595	2013	Chile	Dichato	KU134659	This paper
513	EcDIC-596	2013	Chile	Dichato	KU134660	This paper
514	EcDIC-598	2013	Chile	Dichato	KU134661	This paper
515	EcDIC-600	2013	Chile	Dichato	KU134662	This paper
516	EcDIC-601	2013	Chile	Dichato	KU134663	This paper
517	EcDIC-602	2013	Chile	Dichato	KU134664	This paper
518	EcDIC-603	2013	Chile	Dichato	KU134665	This paper
519	EcDIC-604	2013	Chile	Dichato	KU134666	This paper

520	EcDIC-609	2013	Chile	Dichato	KU134667	This paper
521	EcDIC-610	2013	Chile	Dichato	KU134668	This paper
522	EcDIC-646	2013	Chile	Dichato	KU134669	This paper
523	EcDIC-647	2013	Chile	Dichato	KU134670	This paper
524	EcDIC-653	2013	Chile	Dichato	KU134671	This paper
525	EcDIC-654	2013	Chile	Dichato	KU134672	This paper
526	EcDIC-657	2013	Chile	Dichato	KU134673	This paper
527	EcDIC-658	2013	Chile	Dichato	KU134674	This paper
528	EcCOL-572	2013	Chile	Cocholgüe, Tomé	KU134208	This paper
529	EcCOL-529	2013	Chile	Cocholgüe, Tomé	KU134352	This paper
530	EcCOL-530	2013	Chile	Cocholgüe, Tomé	KU134353	This paper
531	EcCOL-531	2013	Chile	Cocholgüe, Tomé	KU134354	This paper
532	EcCOL-573	2013	Chile	Cocholgüe, Tomé	KU134355	This paper
533	EcCOL-574	2013	Chile	Cocholgüe, Tomé	KU134356	This paper
534	EcCOL-576	2013	Chile	Cocholgüe, Tomé	KU134357	This paper
535	EcCOL-566	2013	Chile	Cocholgüe, Tomé	KU134513	This paper
536	EcCOL-568A	2013	Chile	Cocholgüe, Tomé	KU134549	This paper
537	EcCOL-559	2013	Chile	Cocholgüe, Tomé	KU134564	This paper
538	EcCOL-569	2013	Chile	Cocholgüe, Tomé	KU134678	This paper
539	EcCOL-568	2013	Chile	Cocholgüe, Tomé	KU134679	This paper
540	EcPLG-713	2013	Chile	Nieba, Valdivia	KU134572	This paper
541	EcPLG-708	2013	Chile	Nieba, Valdivia	KU134630	This paper
542	EcPLG-714	2013	Chile	Nieba, Valdivia	KU134586	This paper
543	EcPLG-712	2013	Chile	Nieba, Valdivia	KU134648	This paper
544	EcPLG-716	2013	Chile	Nieba, Valdivia	KU134675	This paper

545	EcPLG-717	2013	Chile	Nieba, Valdivia	KU134676	This paper
546	EcPIL-616	2013	Chile	Pilolcura, Valdivia	KU134359	This paper
547	EcPIL-618	2013	Chile	Pilolcura, Valdivia	KU134360	This paper
548	EcPIL-624	2013	Chile	Pilolcura, Valdivia	KU134361	This paper
549	EcPIL-625	2013	Chile	Pilolcura, Valdivia	KU134362	This paper
550	EcPIL-628	2013	Chile	Pilolcura, Valdivia	KU134363	This paper
551	EcPIL-650	2013	Chile	Pilolcura, Valdivia	KU134367	This paper
552	EcPIL-621	2013	Chile	Pilolcura, Valdivia	KU134514	This paper
553	EcPIL-631	2013	Chile	Pilolcura, Valdivia	KU134515	This paper
554	EcPIL-632	2013	Chile	Pilolcura, Valdivia	KU134516	This paper
555	EcPIL-633	2013	Chile	Pilolcura, Valdivia	KU134517	This paper
556	EcPIL-635	2013	Chile	Pilolcura, Valdivia	KU134518	This paper
557	EcPIL-651	2013	Chile	Pilolcura, Valdivia	KU134535	This paper
558	EcPIL-634	2013	Chile	Pilolcura, Valdivia	KU134538	This paper
559	EcPIL-636	2013	Chile	Pilolcura, Valdivia	KU134539	This paper
560	EcPIL-639	2013	Chile	Pilolcura, Valdivia	KU134558	This paper
561	EcPIL-648	2013	Chile	Pilolcura, Valdivia	KU134647	This paper
562	EcCNA-659	2013	Chile	Curiñanco, Valdivia	KU134368	This paper
563	EcCNA-660	2013	Chile	Curiñanco, Valdivia	KU134369	This paper
564	EcCNA-642	2013	Chile	Curiñanco, Valdivia	KU134519	This paper
565	EcCNA-640	2013	Chile	Curiñanco, Valdivia	KU134364	This paper
566	EcCNA-641	2013	Chile	Curiñanco, Valdivia	KU134365	This paper
567	EcCNA-645	2013	Chile	Curiñanco, Valdivia	KU134366	This paper
568	EcSCA-726	2013	Chile	San Carlos, Corral, Valdivia	KU134536	This paper
569	EcSCA-722	2013	Chile	San Carlos, Corral, Valdivia	KU134637	This paper

570	EcSCA-723	2013	Chile	San Carlos, Corral, Valdivia	KU134638	This paper
571	EcSCA-721	2013	Chile	San Carlos, Corral, Valdivia	KU134639	This paper
572	EcEST-786	2013	Chile	Estaquilla	KU134374	This paper
573	EcEST-788	2013	Chile	Estaquilla	KU134375	This paper
574	EcEST-789	2013	Chile	Estaquilla	KU134376	This paper
575	EcEST-790	2013	Chile	Estaquilla	KU134377	This paper
576	EcEST-791	2013	Chile	Estaquilla	KU134378	This paper
577	EcEST-792	2013	Chile	Estaquilla	KU134379	This paper
578	EcEST-793	2013	Chile	Estaquilla	KU134380	This paper
579	EcEST-794	2013	Chile	Estaquilla	KU134381	This paper
580	EcEST-795	2013	Chile	Estaquilla	KU134382	This paper
581	EcEST-796	2013	Chile	Estaquilla	KU134383	This paper
582	EcEST-797	2013	Chile	Estaquilla	KU134384	This paper
583	EcEST-798	2013	Chile	Estaquilla	KU134385	This paper
584	EcEST-800	2013	Chile	Estaquilla	KU134386	This paper
585	EcEST-801	2013	Chile	Estaquilla	KU134387	This paper
586	EcEST-802	2013	Chile	Estaquilla	KU134388	This paper
587	EcEST-804	2013	Chile	Estaquilla	KU134389	This paper
588	EcEST-805	2013	Chile	Estaquilla	KU134390	This paper
589	EcEST-808	2013	Chile	Estaquilla	KU134391	This paper
590	EcEST-809	2013	Chile	Estaquilla	KU134392	This paper
591	EcEST-810	2013	Chile	Estaquilla	KU134393	This paper
592	EcEST-811	2013	Chile	Estaquilla	KU134394	This paper
593	EcEST-813	2013	Chile	Estaquilla	KU134395	This paper
594	EcEST-815	2013	Chile	Estaquilla	KU134396	This paper

595	EcEST-816	2013	Chile	Estaquilla	KU134397	This paper
596	EcEST-803	2013	Chile	Estaquilla	KU134512	This paper
597	EcEST-771	2013	Chile	Estaquilla	KU134523	This paper
598	EcEST-772	2013	Chile	Estaquilla	KU134524	This paper
599	EcEST-775	2013	Chile	Estaquilla	KU134525	This paper
600	EcEST-776	2013	Chile	Estaquilla	KU134526	This paper
601	EcEST-777	2013	Chile	Estaquilla	KU134527	This paper
602	EcEST-778	2013	Chile	Estaquilla	KU134528	This paper
603	EcEST-779	2013	Chile	Estaquilla	KU134529	This paper
604	EcEST-780	2013	Chile	Estaquilla	KU134530	This paper
605	EcEST-781	2013	Chile	Estaquilla	KU134531	This paper
606	EcEST-783	2013	Chile	Estaquilla	KU134532	This paper
607	EcEST-784	2013	Chile	Estaquilla	KU134533	This paper
608	EcEST-787	2013	Chile	Estaquilla	KU134534	This paper
609	EcEST-769	2013	Chile	Estaquilla	KU134537	This paper
610	EcACH-745	2013	Chile	Achao	KU134209	This paper
611	EcACH-748	2013	Chile	Achao	KU134210	This paper
612	EcACH-757	2013	Chile	Achao	KU134211	This paper
613	EcACH-761	2013	Chile	Achao	KU134212	This paper
614	EcACH-751	2013	Chile	Achao	KU134370	This paper
615	EcACH-752	2013	Chile	Achao	KU134371	This paper
616	EcACH-753	2013	Chile	Achao	KU134372	This paper
617	EcACH-754	2013	Chile	Achao	KU134373	This paper
618	EcACH-749	2013	Chile	Achao	KU134520	This paper
619	EcACH-755	2013	Chile	Achao	KU134521	This paper

620	EcACH-762	2013	Chile	Achao	KU134522	This paper
621	EcACH-746A	2013	Chile	Achao	KU134631	This paper
622	EcACH-758	2013	Chile	Achao	KU134632	This paper
623	EcACH-759	2013	Chile	Achao	KU134633	This paper
624	EcACH-760	2013	Chile	Achao	KU134634	This paper
625	EcACH-763	2013	Chile	Achao	KU134635	This paper
626	EcACH-765	2013	Chile	Achao	KU134636	This paper
627	EcACH-756	2013	Chile	Achao	KU134649	This paper
628	EcACH-747	2013	Chile	Achao	KU134650	This paper
629	Ec666	2009	USA	Falmouth, Massachusetts	KU134541	This paper
630	Ec717	2005	Korea	Hanrim	KU134511	This paper

Appendix B.1

Table S1. Genotypes of sporophytes individuals identified (based on diagnostic microsatellite, ITS1 and species-specific cytoplasmic marker) as *E. siliculosus* (n = 338) and *E. crouaniorum* (n = 151) in the chapter 3. This identification was used previous to the admixture analyses. The number of samples in each population, name of the strain, ITS1 length, and positive amplification in the cytoplasmic marker (Esil = positive amplification Esil, Ecro = positive amplification Ecro) are shown. In diagnostic locus, the non-amplification in one of the parental species corresponds to a phylogenetic signal but should be distinguished from a null allele. To solve this problem, these missing data points were replaced by an artificial allele of an arbitrary size of (800bp). In contrast, missing data or null alleles were noted as 0.

number	Population	Strains	ITS1 length	mtDNA	M-122-2	M-208	M-162-1	M-033-1	M-239-3	M-103-2	M-387	M-388	M-420
1	Plymouth	EcQAB10-1	850	Esil	253253	276279	328330	293301	217227	257257	254254	800800	800800
2	Plymouth	EcQAB10-2	850	Esil	253253	294303	308310	289307	227231	257257	250254	800800	800800
3	Plymouth	EcQAB10-3	850	Esil	253253	276279	328330	293301	217227	257257	254254	800800	800800
4	Plymouth	EcQAB10-4	850	Esil	253253	300300	306312	289289	227227	257257	254254	800800	800800
5	Plymouth	EcQAB10-5	850	Esil	253253	270297	312330	289313	227253	257257	252254	800800	800800
6	Plymouth	EcQAB10-6	850	Esil	253253	288300	306308	289289	217227	257265	252258	800800	800800
7	Plymouth	EcQAB10-7	850	Esil	253253	297297	308308	289289	227231	257261	254254	800800	800800
8	Plymouth	EcQAB10-8	850	Esil	253253	306306	308308	289289	227227	257257	254254	800800	800800
9	Plymouth	EcQAB10-9	850	Esil	253253	306306	308308	289289	227227	257257	254254	800800	800800
10	Plymouth	EcQAB10-10	850	Esil	253253	294303	308310	289307	227231	257257	250254	800800	800800
11	Plymouth	EcQAB10-11	850	Esil	253253	276279	328330	293301	217227	257257	254254	800800	800800
12	Plymouth	EcQAB10-12	850	Esil	253253	300300	312326	289289	231231	243257	252258	800800	800800
13	Plymouth	EcQAB10-13	850	Esil	253253	288300	306308	289289	217227	257265	252258	800800	800800
14	Plymouth	EcQAB10-14	850	Esil	253253	294303	308310	289307	227231	257257	250254	800800	800800
15	Plymouth	EcQAB10-15	850	Esil	253253	297300	308310	277285	227227	257257	254254	800800	800800
16	Plymouth	EcQAB10-16	850	Esil	253253	297300	308312	289313	227227	257257	254254	800800	800800

17	Plymouth	EcQAB10-17	850	Esil	253253	297300	312330	293313	227227	259261	254254	800800	800800
18	Plymouth	EcQAB10-18	850	Esil	253253	294303	308310	289307	227231	257257	250254	800800	800800
19	Plymouth	EcQAB10-19	850	Esil	253253	300300	312330	289289	231231	243257	252258	800800	800800
20	Plymouth	EcQAB10-20	850	Esil	253253	294303	308310	289307	227231	257257	250254	800800	800800
21	Plymouth	EcQAB10-22	850	Esil	253253	276279	328330	293301	217227	257257	254254	800800	800800
22	Plymouth	EcQAB10-23	850	Esil	253253	276279	328330	293301	217227	257257	254254	800800	800800
23	Plymouth	EcQAB10-24	850	Esil	253253	288300	306308	289289	217227	257265	252258	800800	800800
24	Plymouth	EcQAB10-25	850	Esil	253253	288300	308310	289319	227231	257261	254254	800800	800800
25	Plymouth	EcQAB10-27	850	Esil	253253	300303	306312	289313	227229	257259	252254	800800	800800
26	Plymouth	EcQAB10-28	850	Esil	253253	288300	306308	289289	217227	257265	252258	800800	800800
27	Plymouth	EcQAB10-29	850	Esil	253253	294303	308310	289307	227231	257257	250254	800800	800800
28	Plymouth	EcQAB10-30	850	Esil	253253	306306	308308	289289	227227	257257	254254	800800	800800
29	Plymouth	EcQAB10-32	850	Esil	253253	294303	308310	289307	227231	257257	250254	800800	800800
30	Plymouth	EcQAB10-33	850	Esil	253253	294303	308310	289307	227231	257257	250254	800800	800800
31	Plymouth	EcQAB10-34	850	Esil	253253	294303	308310	289307	227231	257257	250254	800800	800800
32	Plymouth	EcQAB10-35	850	Esil	253253	294303	308310	289307	227231	257257	250254	800800	800800
33	Plymouth	EcQAB10-36	850	Esil	253253	276279	328330	293301	217227	257257	254254	800800	800800
34	Plymouth	EcQAB10-37	850	Esil	253253	279297	330330	293299	215215	257257	252252	800800	800800
35	Plymouth	EcQAB10-38	850	Esil	253253	300300	306312	289289	227227	257257	254254	800800	800800
36	Plymouth	EcQAB10-39	850	Esil	253253	300300	306312	289289	227227	257257	254254	800800	800800
37	Plymouth	EcQAB10-40	850	Esil	253253	297300	308310	277285	227227	257257	254254	800800	800800
38	Plymouth	EcQAB10-41	850	Esil	253253	276279	328330	293301	217227	257257	254254	800800	800800
39	Plymouth	EcQAB10-43	850	Esil	253253	297297	308308	289289	227231	257261	254254	800800	800800
40	Plymouth	EcQAB10-44	850	Esil	253253	297300	310312	289289	217227	257257	252254	800800	800800
41	Plymouth	EcQAB10-45	850	Esil	253253	294303	308310	289307	227231	257257	250254	800800	800800
42	Plymouth	EcQAB10-46	850	Esil	253253	297300	308310	277285	227227	257257	254254	800800	800800
43	Plymouth	EcQAB10-47	850	Esil	253253	276279	326330	293301	217227	257257	254254	800800	800800

44	Plymouth	EcQAB10-48	850	Esil	253253	306306	308308	289289	227227	257257	254254	800800	800800
1	Restronguet	EcREP10-11	850	Esil	253253	288303	330330	293293	217217	247257	252252	800800	800800
2	Restronguet	EcREP10-12	850	Esil	253253	300303	312330	293293	215227	257259	252254	800800	800800
3	Restronguet	EcREP10-14	850	Esil	279279	297300	308330	289289	213227	257257	252254	800800	800800
4	Restronguet	EcREP10-16	850	Esil	279279	297297	310310	293293	217227	247257	252252	800800	800800
5	Restronguet	EcREP10-17	850	Esil	253279	297303	308330	293313	215227	247259	252254	800800	800800
6	Restronguet	EcREP10-18	850	Esil	253253	291297	330330	289293	213227	257259	254254	800800	800800
7	Restronguet	EcREP10-19	850	Esil	253279	297297	310330	293293	213217	247247	252254	800800	800800
8	Restronguet	EcREP10-21	850	Esil	253253	297300	306330	293309	215227	247257	252254	800800	800800
9	Restronguet	EcREP10-22	850	Esil	253253	300303	306312	289313	205227	257257	252254	800800	800800
10	Restronguet	EcREP10-23	850	Esil	253279	288297	330330	289289	215217	247257	252254	800800	800800
11	Restronguet	EcREP10-24	850	Esil	253253	291297	330330	289293	213227	257259	254254	800800	800800
12	Restronguet	EcREP10-26	850	Esil	253253	288297	308330	289293	215227	247257	252252	800800	800800
13	Restronguet	EcREP10-27	850	Esil	253253	288297	330330	289293	217221	257259	252252	800800	800800
14	Restronguet	EcREP10-28	850	Esil	253279	279297	310330	289293	213229	257257	254254	800800	800800
15	Restronguet	EcREP10-30	850	Esil	253279	285288	330330	289293	217227	247247	252252	800800	800800
16	Restronguet	EcREP10-34	850	Esil	253253	297300	310312	293319	213231	247257	252254	800800	800800
17	Restronguet	EcREP10-35	850	Esil	253253	288297	330330	293293	217229	257257	252252	800800	800800
18	Restronguet	EcREP10-36	850	Esil	253279	288288	310330	289293	213217	247257	252254	800800	800800
19	Restronguet	EcREP10-42	850	Esil	253253	288300	308330	289289	217227	247257	252254	800800	800800
20	Restronguet	EcREP10-43	850	Esil	253253	288300	308330	289289	217227	247257	252254	800800	800800
21	Restronguet	EcREP10-44	850	Esil	253253	297300	306330	293309	215227	247257	252254	800800	800800
22	Restronguet	EcREP10-45	850	Esil	253253	279285	330330	289293	213229	247257	252254	800800	800800
23	Restronguet	EcREP10-46	850	Esil	253253	288300	308310	309313	217227	257257	252254	800800	800800
24	Restronguet	EcREP10-48	850	Esil	253253	300309	308308	289289	215227	257257	252252	800800	800800
25	Restronguet	EcREP10-49	850	Esil	253253	288297	330330	293293	217229	257257	252252	800800	800800
26	Restronguet	EcREP10-50	850	Esil	253253	303306	330334	289293	217217	247257	252252	800800	800800

27	Restronguet	EcREP10-51	850	Esil	253253	288297	308330	289293	215227	247257	252252	800800	800800
28	Restronguet	EcREP10-52	850	Esil	253279	279297	310330	289293	213229	257257	254254	800800	800800
29	Restronguet	EcREP10-54	850	Esil	253279	297303	310330	289293	213215	247247	252252	800800	800800
30	Restronguet	REP10-40	850	Esil	279279	297300	308330	289289	213227	257257	252254	800800	800800
1	Roscoff	EcPH12-s#1-14	850	Esil	265279	285291	306312	305317	199205	257257	252254	800800	800800
2	Roscoff	EcPH12-s#1-19	850	Esil	277277	285294	310334	285285	223227	255257	252258	800800	800800
3	Roscoff	EcPH12-s#1-32	850	Esil	253281	309315	308346	317321	217217	245245	252252	800800	800800
4	Roscoff	EcPH12-s#1-173	850	Esil	253253	279285	308308	283289	219251	249261	252254	800800	800800
5	Roscoff	EcPH12-s#1-182	850	Esil	253253	279282	310312	289289	215251	249257	252254	800800	800800
6	Roscoff	EcPH12-s#2a-04	850	Esil	253253	297297	306330	291323	227227	261261	252254	800800	800800
7	Roscoff	EcPH12-s#2a-08	850	Esil	253253	270297	306318	291291	205205	255259	254256	800800	800800
8	Roscoff	EcPH12-s#2a-18	850	Esil	253253	270297	306318	291311	205227	255259	254256	800800	800800
9	Roscoff	EcPH12-s#2a-20	850	Esil	253253	297297	306330	291323	227231	261261	252254	800800	800800
10	Roscoff	EcPH12-s#2a-21	850	Esil	253253	285297	306310	285323	227231	259263	252254	800800	800800
11	Roscoff	EcPH12-s#2a-22	850	Esil	253253	279282	310312	289289	215215	249257	252254	800800	800800
12	Roscoff	EcPH12-s#2a-28	850	Esil	253253	270297	306318	291291	205227	255259	254256	800800	800800
13	Roscoff	EcPH12-s#2a-32	850	Esil	253253	285294	306308	289289	231231	259259	252254	800800	800800
14	Roscoff	EcPH12-s#2a-35	850	Esil	253253	279282	308312	289289	227229	249257	252254	800800	800800
15	Roscoff	EcPH12-s#2a-36	850	Esil	253253	270297	306318	291307	205227	255259	254256	800800	800800
16	Roscoff	EcPH12-s#2a-	850	Esil	253253	270297	306318	291291	227227	255259	254256	800800	800800

		39											
17	Roscoff	EcPH12-s#2a-40	850	Esil	253253	270297	306318	291311	205227	255259	254256	800800	800800
18	Roscoff	EcPH12-92	850	Esil	253253	270297	306318	291291	205227	255259	254256	800800	800800
19	Roscoff	EcPH12-93	850	Esil	253253	270297	306318	285311	205227	255259	254256	800800	800800
20	Roscoff	EcPH12-96	850	Esil	253253	282309	312312	289289	227227	259259	252254	800800	800800
21	Roscoff	EcPH12-98	850	Esil	253253	294297	308308	291317	227227	261263	252254	800800	800800
22	Roscoff	EcPH12-100	850	Esil	253253	279282	310312	289289	215251	249257	252254	800800	800800
23	Roscoff	EcPH12-103	850	Esil	253253	285297	312312	289309	227227	259259	252254	800800	800800
24	Roscoff	EcPH12-104	850	Esil	253253	285294	306308	289289	231231	259259	252254	800800	800800
25	Roscoff	EcPH12-109	850	Esil	253253	282309	312312	289313	215227	259259	252254	800800	800800
26	Roscoff	EcPH12-110	850	Esil	253253	279282	310312	289289	215251	249257	252254	800800	800800
27	Roscoff	EcPH12-111	850	Esil	253253	279282	308312	289289	227227	249257	252254	800800	800800
28	Roscoff	EcPH12-113	850	Esil	253253	285297	312312	289309	227227	259259	252256	800800	800800
29	Roscoff	EcPH12-114	850	Esil	253253	285297	312312	289289	227227	259259	252254	800800	800800
30	Roscoff	EcPH12-120	850	Esil	253253	288294	306312	289289	219227	255259	252254	800800	800800
31	Roscoff	EcPH12-148	850	Esil	253279	297300	330330	291307	217255	255259	252252	800800	800800
32	Roscoff	EcPH12-149	850	Esil	253253	294297	306308	291317	227231	261263	252254	800800	800800
33	Roscoff	EcPH12-s#2A-15	850	Esil	253253	270297	306318	291311	205227	255259	254256	800800	800800
34	Roscoff	EcPH12-s#2A-23	850	Esil	253253	285297	306308	287293	213227	247259	252254	800800	800800
35	Roscoff	EcPH12-s#2A-33	850	Esil	253253	270297	306318	291311	205227	255259	254256	800800	800800
36	Roscoff	EcPH12-s#2B-03	850	Esil	253253	285297	312312	289309	227227	259259	252254	800800	800800
37	Roscoff	EcPH12-s#2B-04	850	Esil	253253	279282	310312	289289	215251	249257	252254	800800	800800
38	Roscoff	EcPH12-s#2B-05	850	Esil	253253	282309	312312	289313	215227	257259	252254	800800	800800

39	Roscoff	EcPH12-s#2B-06	850	Esil	253253	297297	306330	291323	227233	261261	252254	800800	800800
40	Roscoff	EcPH12-s#2B-09	850	Esil	253253	282309	312312	289313	215227	257259	252254	800800	800800
41	Roscoff	EcPH12-s#2B-15	850	Esil	253253	291291	306312	289293	227253	259259	252252	800800	800800
42	Roscoff	EcPH12-s#2B-20	850	Esil	253253	282309	312312	289313	215227	257259	252254	800800	800800
43	Roscoff	EcPH12-s#2B-21	850	Esil	253253	279282	310312	289289	215251	249257	252254	800800	800800
44	Roscoff	EcPH12-s#2B-22	850	Esil	253253	285297	312312	289309	227227	259259	252254	800800	800800
45	Roscoff	EcPH12-s#2B-12	850	Esil	253253	282306	308312	289289	215229	249257	252258	800800	800800
46	Roscoff	EcPH12-s#2B-18	850	Esil	253253	285297	312312	289289	227227	259259	252254	800800	800800
47	Roscoff	EcPH12-58	850	Esil	253253	285297	306310	285323	227231	259263	252254	800800	800800
48	Roscoff	EcPH12-59	850	Esil	253253	297297	306330	291323	227231	261261	252254	800800	800800
49	Roscoff	EcPH12-71	850	Esil	253253	279282	308312	289289	227229	249257	252254	800800	800800
50	Roscoff	EcPH12-74	850	Esil	253253	282285	312312	289289	227227	257259	252252	800800	800800
51	Roscoff	EcPH12-80	850	Esil	253253	285297	306310	285323	227231	259263	252254	800800	800800
52	Roscoff	EcPH12-84	850	Esil	253253	285285	306308	289293	219227	259261	252252	800800	800800
53	Roscoff	EcPH12-86	850	Esil	253253	285285	308308	289293	219227	259261	252252	800800	800800
54	Roscoff	EcPH12-88	850	Esil	253253	294297	330330	291291	219227	259263	252252	800800	800800
55	Roscoff	EcPH12-89	850	Esil	253253	285285	306308	289293	219227	259261	252252	800800	800800
56	Roscoff	EcPH12-s#3-06	850	Esil	253279	291291	312338	295297	207223	257257	254258	800800	800800
57	Roscoff	EcPH12-s#3-15	850	Esil	279279	285291	306308	313325	205227	255267	254262	800800	800800
58	Roscoff	EcPH12-s#3-17	850	Esil	253279	285306	308314	285297	207207	255255	252252	800800	800800
59	Roscoff	EcPH12-s#3-20	850	Esil	277279	285291	310312	297297	223225	257261	248252	800800	800800
60	Roscoff	EcPH12-s#3-24	850	Esil	253279	285306	308314	285297	207207	255255	252252	800800	800800

61	Roscoff	EcPH12-s#3-38	850	Esil	253279	291291	312338	295297	207223	255257	254258	800800	800800
62	Roscoff	EcPH12-s#4-02	850	Esil	253279	291297	306308	303309	205233	249257	252258	800800	800800
63	Roscoff	EcPH12-s#4-06	850	Esil	279281	288291	308310	297321	227227	255255	252254	800800	800800
64	Roscoff	EcPH12-s#4-07	850	Esil	279279	291297	306310	285313	207207	247263	252252	800800	800800
65	Roscoff	EcPH12-s#4-10	850	Esil	253253	279282	310312	289289	215251	249257	252254	800800	800800
66	Roscoff	EcPH12-s#4-11	850	Esil	253279	291294	298314	317317	199211	255257	252252	800800	800800
67	Roscoff	EcPH12-s#4-17	850	Esil	253253	303303	318320	289313	227227	259261	252254	800800	800800
68	Roscoff	EcPH12-s#4-25	850	Esil	279279	294294	308310	285305	205205	257259	248252	800800	800800
69	Roscoff	EcPH12-s#4-31	850	Esil	253279	291294	308312	309309	209209	257257	252252	800800	800800
70	Roscoff	EcPH12-s#4-34	850	Esil	279279	291306	308318	285297	205205	257267	258258	800800	800800
71	Roscoff	EcPH12-s#4-35	850	Esil	279279	291291	306308	303303	211211	255257	252252	800800	800800
72	Roscoff	EcPH12-s#4-49	850	Esil	253253	285294	306308	289289	231231	259259	252254	800800	800800
73	Roscoff	EcPH12-s#4-50	850	Esil	253265	285291	308316	305305	209209	255257	256258	800800	800800
74	Roscoff	EcPH12-s#5-03	850	Esil	253253	282291	308312	289289	227227	259259	252254	800800	800800
75	Roscoff	EcPH12-s#5-08	850	Esil	253253	285297	312312	289309	227227	259271	252256	800800	800800
76	Roscoff	EcPH12-s#5-09	850	Esil	279281	291294	306308	285305	221221	259261	248252	800800	800800
77	Roscoff	EcPH12-s#5-10	850	Esil	253253	279282	310312	289289	215251	249257	252254	800800	800800
78	Roscoff	EcPH12-s#5-11	850	Esil	279279	285291	296310	283313	207209	261263	252268	800800	800800
79	Roscoff	EcPH12-s#5-12	850	Esil	253253	282291	308312	289289	227227	259259	252254	800800	800800
80	Roscoff	EcPH12-s#5-14	850	Esil	279279	291294	314340	285285	205207	243257	252252	800800	800800
81	Roscoff	EcPH12-s#5-16	850	Esil	253253	285297	312312	289309	227227	259259	252254	800800	800800
82	Roscoff	EcPH12-s#5-17	850	Esil	253253	282306	308312	289289	215229	249257	252258	800800	800800
83	Roscoff	EcPH12-s#5-19	850	Esil	253253	282285	312312	289289	227227	257259	252252	800800	800800
84	Roscoff	EcPH12-s#5-20	850	Esil	253253	282285	312312	289289	227227	257259	252252	800800	800800
85	Roscoff	EcPH12-s#5-21	850	Esil	253253	282309	312312	289313	215227	257259	252254	800800	800800
86	Roscoff	EcPH12-s#5-23	850	Esil	253253	285291	312334	305313	207209	243259	252256	800800	800800
87	Roscoff	EcPH12-s#5-28	850	Esil	253253	285297	312312	289309	227227	259259	252254	800800	800800

88	Roscoff	EcPH12-s#5-29	850	Esil	253277	291294	310312	285305	207207	249257	252258	800800	800800
89	Roscoff	EcPH12-s#5-30	850	Esil	255279	285291	320338	305305	191223	255257	250252	800800	800800
90	Roscoff	EcPH12-s#5-33	850	Esil	279279	291297	296310	303303	221225	243257	258272	800800	800800
91	Roscoff	EcPH12-s#5-35	850	Esil	253253	282309	312312	289313	215215	257259	252254	800800	800800
92	Roscoff	EcPH12-s#6-14	850	Esil	253253	282291	308312	289289	227227	259259	252254	800800	800800
93	Roscoff	EcPH12-s#6-38	850	Esil	279281	294294	314336	305305	207207	255259	248268	800800	800800
94	Roscoff	EcPH12-128	850	Esil	253253	294297	306308	291317	231231	261263	252254	800800	800800
95	Roscoff	EcPH12-131	850	Esil	253253	282309	312312	289313	215227	257259	252254	800800	800800
96	Roscoff	EcPH12-133	850	Esil	253253	282309	312312	289313	215227	257259	252254	800800	800800
97	Roscoff	EcPH12-135	850	Esil	253253	270297	306318	291311	205227	255259	254256	800800	800800
98	Roscoff	EcPH12-138	850	Esil	253253	282309	312312	289313	215215	259259	252254	800800	800800
99	Roscoff	EcPH12-139	850	Esil	253253	285297	312312	289309	227227	259271	252256	800800	800800
100	Roscoff	EcPH12-141	850	Esil	253253	285297	312312	289309	227227	259259	252254	800800	800800
101	Roscoff	EcPH12-142	850	Esil	253253	285297	312312	289309	227227	259259	252254	800800	800800
102	Roscoff	EcPH12-143	850	Esil	253253	282282	312312	289289	215227	257257	252252	800800	800800
103	Roscoff	EcPH12-144	850	Esil	253253	285297	312312	289289	227227	259259	252254	800800	800800
104	Roscoff	EcPH12-145	850	Esil	253253	297297	306330	291323	227231	261261	252254	800800	800800
105	Roscoff	EcPH12-s#2a-13	850	Esil	275279	291291	314316	0	223223	257257	252254	800800	800800
106	Roscoff	EcPH12-s#2a-29	850	Esil	253253	297297	306330	0	227233	261261	252254	800800	800800
107	Roscoff	EcPH12-95	850	Esil	253253	282291	308312	289289	0	259259	252254	800800	800800
108	Roscoff	EcPH12-101	850	Esil	253253	279282	310312	289289	215215	249257	0	800800	800800
109	Roscoff	EcPH12-102	850	Esil	253253	282306	308312	289289	215215	249257	0	800800	800800
110	Roscoff	EcPH12-105	850	Esil	253253	282285	312312	0	227227	257259	252252	800800	800800
111	Roscoff	EcPH12-106	850	Esil	253253	282309	312312	0	215227	257259	252254	800800	800800
112	Roscoff	EcPH12-108	850	Esil	253253	282285	312312	0	0	257259	252252	800800	800800
113	Roscoff	EcPH12-123	850	Esil	253253	282309	312312	0	215215	257257	252252	800800	800800

114	Roscoff	EcPH12-94	850	Esil	253253	297297	306330	291323	227231	261261	0	800800	800800
115	Roscoff	EcPH12-s#3-12	850	Esil	253279	291306	306308	285297	205227	255275	0	800800	800800
116	Roscoff	EcPH12-s#4-28	850	Esil	253253	282306	308312	0	0	249257	252258	800800	800800
117	Roscoff	EcPH12-s#4-33	850	Esil	253253	291300	306314	279279	207207	255259	0	800800	800800
118	Roscoff	EcPH12-s#4-47	850	Esil	279279	291297	306310	285313	207207	247263	0	800800	800800
119	Roscoff	EcPH12-s#5-02	850	Esil	253253	270297	306318	291311	205227	255259	0	800800	800800
120	Roscoff	EcPH12-s#5-22	850	Esil	275279	291291	314316	0	0	257257	252254	800800	800800
121	Roscoff	EcPH12-129	850	Esil	253253	294297	306308	291317	227231	261263	0	800800	800800
122	Roscoff	EcPH12-136	850	Esil	253253	282309	312312	289313	227227	259259	0	800800	800800
123	Roscoff	EcPH12-137	850	Esil	253253	282285	312318	289289	227227	259259	0	800800	800800
124	Roscoff	EcPH12-140	850	Esil	253253	0	0	289313	215227	257259	252254	800800	800800
125	Roscoff	EcPH12-s#1-01	>1000	Ecro	293293	267270	266266	800800	800800	800800	800800	165165	287287
126	Roscoff	EcPH12-s#1-04	>1000	Ecro	291291	267270	266266	800800	800800	800800	800800	155155	279281
127	Roscoff	EcPH12-s#1-12	>1000	Ecro	293357	261270	266266	800800	800800	800800	800800	157163	319319
128	Roscoff	EcPH12-s#1-13	>1000	Ecro	289349	261267	266266	800800	800800	800800	800800	155161	293329
129	Roscoff	EcPH12-s#1-16	>1000	Ecro	295349	267270	266266	800800	800800	800800	800800	153169	279291
130	Roscoff	EcPH12-s#1-17	>1000	Ecro	313313	270270	266266	800800	800800	800800	800800	153163	293311
131	Roscoff	EcPH12-s#1-18	>1000	Ecro	291311	270276	264266	800800	800800	800800	800800	153153	293301
132	Roscoff	EcPH12-s#1-20	>1000	Ecro	281291	270270	266266	800800	800800	800800	800800	153163	293293
133	Roscoff	EcPH12-s#1-24	>1000	Ecro	293313	270270	264266	800800	800800	800800	800800	153153	265291
134	Roscoff	EcPH12-s#1-26	>1000	Ecro	291291	273276	266266	800800	800800	800800	800800	155155	267289
135	Roscoff	EcPH12-s#1-27	>1000	Ecro	291293	261267	266266	800800	800800	800800	800800	163165	267307
136	Roscoff	EcPH12-s#1-30	>1000	Ecro	291313	267270	266266	800800	800800	800800	800800	153167	267287
137	Roscoff	EcPH12-s#1-40	>1000	Ecro	299349	267270	266266	800800	800800	800800	800800	161165	287287
138	Roscoff	EcPH12-s#1-42	>1000	Ecro	291291	267270	266266	800800	800800	800800	800800	137167	293293
139	Roscoff	EcPH12-s#1-47	>1000	Ecro	293313	270270	266266	800800	800800	800800	800800	161161	305305
140	Roscoff	EcPH12-s#1-48	>1000	Ecro	291291	267270	264266	800800	800800	800800	800800	163171	287305

141	Roscoff	EcPH12-s#1-55	>1000	Ecro	291291	261267	266266	800800	800800	800800	800800	155163	267307
142	Roscoff	EcPH12-s#1-56	>1000	Ecro	291295	267270	266266	800800	800800	800800	800800	153157	297297
143	Roscoff	EcPH12-s#1-57	>1000	Ecro	291291	267276	266266	800800	800800	800800	800800	161163	293311
144	Roscoff	EcPH12-s#1-60	>1000	Ecro	293313	270270	266266	800800	800800	800800	800800	161163	311311
145	Roscoff	EcPH12-s#1-61	>1000	Ecro	299349	267270	266266	800800	800800	800800	800800	161165	287287
146	Roscoff	EcPH12-s#1-62	>1000	Ecro	293293	270270	266266	800800	800800	800800	800800	161169	297297
147	Roscoff	EcPH12-s#1-63	>1000	Ecro	295295	261267	266266	800800	800800	800800	800800	163167	311317
148	Roscoff	EcPH12-s#1-69	>1000	Ecro	293293	261267	266266	800800	800800	800800	800800	153169	293333
149	Roscoff	EcPH12-s#1-70	>1000	Ecro	291291	270270	266266	800800	800800	800800	800800	155161	293309
150	Roscoff	EcPH12-s#1-88	>1000	Ecro	295295	270270	266266	800800	800800	800800	800800	153161	287331
151	Roscoff	EcPH12-s#1-145	>1000	Ecro	291291	267270	266266	800800	800800	800800	800800	153163	281309
152	Roscoff	EcPH12-s#1-150	>1000	Ecro	291291	267270	266266	800800	800800	800800	800800	153161	267307
153	Roscoff	EcPH12-s#1-156	>1000	Ecro	291295	267270	266266	800800	800800	800800	800800	155155	287307
154	Roscoff	EcPH12-s#1-188	>1000	Ecro	291291	267267	264266	800800	800800	800800	800800	161163	277285
155	Roscoff	EcPH12-s#1-190	>1000	Ecro	291291	267270	264266	800800	800800	800800	800800	169171	287305
156	Roscoff	EcPH12-s#1-191	>1000	Ecro	291291	261270	266266	800800	800800	800800	800800	153163	307307
157	Roscoff	EcPH12-s#1-192	>1000	Ecro	293293	261267	266266	800800	800800	800800	800800	163169	287309
158	Roscoff	EcPH12-s#1-079	>1000	Ecro	291313	267270	266266	800800	800800	800800	800800	161163	311319
159	Roscoff	EcPH12-s#1-103	>1000	Ecro	291313	267267	264266	800800	800800	800800	800800	161163	277295
160	Roscoff	EcPH12-s#1-107	>1000	Ecro	291295	270270	266266	800800	800800	800800	800800	153163	307307
161	Roscoff	EcPH12-s#1-	>1000	Ecro	293313	267270	264264	800800	800800	800800	800800	153153	291309

		115											
162	Roscoff	EcPH12-s#1-124	>1000	Ecro	299349	267270	266266	800800	800800	800800	800800	161165	287287
163	Roscoff	EcPH12-s#1-126	>1000	Ecro	291313	261270	264266	800800	800800	800800	800800	161161	321321
164	Roscoff	EcPH12-s#1-166	>1000	Ecro	291295	267270	266266	800800	800800	800800	800800	153163	287307
165	Roscoff	EcPH12-s#1-171	>1000	Ecro	291291	270276	266266	800800	800800	800800	800800	153161	309319
166	Roscoff	EcPH12-s#1-175	>1000	Ecro	293313	267270	266266	800800	800800	800800	800800	153163	287329
167	Roscoff	EcPH12-s#1-177	>1000	Ecro	293293	267276	266266	800800	800800	800800	800800	153153	301309
168	Roscoff	EcPH12-s#1-183	>1000	Ecro	293349	270270	266266	800800	800800	800800	800800	153167	309309
169	Roscoff	EcPH12-s#1-185	>1000	Ecro	293293	267270	264264	800800	800800	800800	800800	157171	267305
170	Roscoff	EcPH12-s#1-144	>1000	Ecro	287291	270270	266266	800800	800800	800800	800800	169171	287291
171	Roscoff	EcPH12-s#2a-37	>1000	Ecro	291291	267270	264266	800800	800800	800800	800800	153165	293293
172	Roscoff	EcPH12-s#2A-11	>1000	Ecro	295311	267267	266266	800800	800800	800800	800800	165191	277335
173	Roscoff	EcPH12-s#2A-14	>1000	Ecro	297297	267276	264266	800800	800800	800800	800800	159169	279293
174	Roscoff	EcPH12-s#2B-10	>1000	Ecro	281281	267270	264266	800800	800800	800800	800800	153153	273273
175	Roscoff	EcPH12-s#2B-02	>1000	Ecro	281311	267270	266266	800800	800800	800800	800800	153165	291291
176	Roscoff	EcPH12-s#2B-23	>1000	Ecro	281311	267270	266266	800800	800800	800800	800800	153165	291291
177	Roscoff	EcPH12-s#2B-33	>1000	Ecro	291351	267267	266266	800800	800800	800800	800800	153155	293293

178	Roscoff	EcPH12-77	>1000	Ecro	293295	267270	266266	800800	800800	800800	800800	163163	279279
179	Roscoff	EcPH12-s#5-18	>1000	Ecro	255255	267285	266310	800800	800800	800800	800800	135161	317317
180	Roscoff	EcPH12-s#1-49	>1000	Ecro	0	270276	264266	800800	800800	800800	800800	155161	295325
181	Roscoff	EcPH12-s#1-65	>1000	Ecro	291291	267270	266266	800800	800800	800800	800800	161165	0
182	Roscoff	EcPH12-s#1-71	>1000	Ecro	0	270270	266266	800800	800800	800800	800800	157163	305305
183	Roscoff	EcPH12-s#1-153	>1000	Ecro	0	261276	266266	800800	800800	800800	800800	155163	267309
184	Roscoff	EcPH12-s#1-154	>1000	Ecro	0	270276	266266	800800	800800	800800	800800	153155	267331
185	Roscoff	EcPH12-s#1-184	>1000	Ecro	0	270270	266266	800800	800800	800800	800800	153153	267293
186	Roscoff	EcPH12-s#1-81	>1000	Ecro	293293	267270	264264	800800	800800	800800	800800	157163	0
1	Le Caro	EcLC12-02	850	Esil	279279	297300	296330	291293	215217	257259	252252	800800	800800
2	Le Caro	EcLC12-05	850	Esil	253267	294297	306330	289295	227231	253257	252252	800800	800800
3	Le Caro	EcLC12-09	850	Esil	253267	294297	306330	289295	227231	253257	252252	800800	800800
4	Le Caro	EcLC12-10	850	Esil	253253	285297	306326	287293	215227	257259	252252	800800	800800
5	Le Caro	EcLC12-11	850	Esil	279279	285297	308328	289293	215217	257257	252252	800800	800800
6	Le Caro	EcLC12-12	850	Esil	253265	297306	308326	285289	217251	255261	252254	800800	800800
7	Le Caro	EcLC12-13	850	Esil	253279	279285	282326	289331	211251	251257	252254	800800	800800
8	Le Caro	EcLC12-14	850	Esil	253253	285285	312330	285293	215219	243257	252252	800800	800800
9	Le Caro	EcLC12-15	850	Esil	265279	297297	330352	289293	215219	257257	252252	800800	800800
10	Le Caro	EcLC12-18	850	Esil	253279	285306	328330	289293	215217	257257	252252	800800	800800
11	Le Caro	EcLC12-19	850	Esil	253253	297297	330330	289289	215215	247257	252252	800800	800800
12	Le Caro	EcLC12-04	850	Esil	253267	294297	0	289295	227231	253257	252252	800800	800800
13	Le Caro	EcLC12-16	850	Esil	279279	288288	282326	0	207207	257257	252252	800800	800800
1	Traezh Hir	EcTH10-01	850	Esil	279279	279288	308308	307309	221253	245261	252254	800800	800800
2	Traezh Hir	EcTH10-02	850	Esil	279279	279288	308308	307309	221253	245261	252254	800800	800800
3	Traezh Hir	EcTH10-04	850	Esil	279279	279288	308308	307309	221253	245261	252254	800800	800800

4	Traezh Hir	EcTH10-05	850	Esil	279279	279288	308308	307309	221253	245261	252254	800800	800800
5	Traezh Hir	EcTH10-06	850	Esil	253253	297297	326328	289289	207211	253257	252252	800800	800800
6	Traezh Hir	EcTH10-07	850	Esil	253253	279297	292310	289293	219227	257257	252254	800800	800800
7	Traezh Hir	EcTH10-08	850	Esil	279279	279288	308308	307309	221253	245261	252254	800800	800800
8	Traezh Hir	EcTH10-09	850	Esil	279279	279288	308308	307309	221253	245261	252254	800800	800800
9	Traezh Hir	EcTH10-10	850	Esil	279279	279288	308308	307309	221253	245261	252254	800800	800800
10	Traezh Hir	EcTH10-12	850	Esil	279279	279288	308308	307309	221253	245261	252254	800800	800800
11	Traezh Hir	EcTH10-13	850	Esil	253253	291309	306312	293301	231253	259259	252252	800800	800800
12	Traezh Hir	EcTH10-14	850	Esil	279279	279288	308308	307309	221253	245261	252254	800800	800800
13	Traezh Hir	EcTH10-15	850	Esil	253253	279285	310312	285289	227227	257257	254268	800800	800800
14	Traezh Hir	EcTH10-16	850	Esil	253253	291309	306312	293301	231253	259259	252252	800800	800800
15	Traezh Hir	EcTH10-17	850	Esil	279279	279288	308308	307309	221253	245261	252254	800800	800800
16	Traezh Hir	EcTH10-18	850	Esil	279279	279288	308308	307309	221253	245261	252254	800800	800800
17	Traezh Hir	EcTH10-53	850	Esil	253253	291309	306312	293301	231253	259259	252252	800800	800800
18	Traezh Hir	EcTH10-170	850	Esil	253253	285291	306308	291305	227231	259263	252258	800800	800800
19	Traezh Hir	EcTH10-172	850	Esil	253279	285285	308308	289289	227227	257263	252254	800800	800800
20	Traezh Hir	EcTH10-174	850	Esil	253253	303303	320320	279279	225225	251251	252252	800800	800800
21	Traezh Hir	EcTH10-175	850	Esil	253253	285285	308308	289289	227231	257263	252252	800800	800800
22	Traezh Hir	EcTH10-176	850	Esil	253253	279285	308312	307309	205213	257257	252252	800800	800800
23	Traezh Hir	EcTH10-177	850	Esil	253265	282285	308310	285293	219219	259261	252254	800800	800800
24	Traezh Hir	EcTH10-178	850	Esil	253279	285285	306308	291307	227229	257261	252252	800800	800800
25	Traezh Hir	EcTH10-198	850	Esil	253279	288291	308310	291307	227229	261261	252252	800800	800800
26	Traezh Hir	EcTH10-202	850	Esil	253265	282285	308310	285293	219219	259261	252254	800800	800800
27	Traezh Hir	EcTH10-203	850	Esil	253265	282285	308310	285293	219219	259261	252254	800800	800800
28	Traezh Hir	EcTH10-03	850	Esil	279279	279288	308308	307309	221253	0	252254	800800	800800
29	Traezh Hir	EcTH10-20	850	Esil	253253	303306	332332	0	0	259259	252252	800800	800800
30	Traezh Hir	EcTH10-22	850	Esil	253253	303306	332332	0	0	259259	252252	800800	800800

31	Traezh Hir	EcTH10-57	850	Esil	277281	270315	308346	0	0	245257	252258	800800	800800
32	Traezh Hir	EcTH10-58	850	Esil	277281	270315	308346	0	0	245257	252258	800800	800800
33	Traezh Hir	EcTH10-64	850	Esil	253253	297297	328328	0	0	253257	252252	800800	800800
34	Traezh Hir	EcTH10-79	>1000	Ecro	299299	267267	264266	800800	800800	800800	800800	153153	293313
35	Traezh Hir	EcTH10-94	>1000	Ecro	281295	267270	264266	800800	800800	800800	800800	161181	287293
36	Traezh Hir	EcTH10-49	>1000	Ecro	297297	267267	266266	800800	800800	800800	800800	153153	0
37	Traezh Hir	EcTH10-82	>1000	Ecro	301301	267267	266266	800800	800800	800800	800800	135135	0
1	Quiberon	EcQB10-01	850	Esil	253253	285300	308308	289313	227233	257259	252258	800800	800800
2	Quiberon	EcQB10-08	850	Esil	253253	279291	308324	295309	205227	257263	252254	800800	800800
3	Quiberon	EcQB10-19	850	Esil	253253	285285	306310	289291	227227	257263	252252	800800	800800
4	Quiberon	EcQB10-20	850	Esil	253253	285288	308308	307313	211227	257261	252254	800800	800800
5	Quiberon	EcQB10-21	850	Esil	253253	285285	308308	291291	227229	257259	252252	800800	800800
6	Quiberon	EcQB10-22	850	Esil	253253	279285	308308	289309	213213	257257	252252	800800	800800
7	Quiberon	EcQB10-23	850	Esil	253279	282288	312312	289311	205213	257257	252252	800800	800800
8	Quiberon	EcQB10-24	850	Esil	253279	282288	312312	289311	205213	257257	252252	800800	800800
9	Quiberon	EcQB10-25	850	Esil	253253	285291	312312	291295	227227	257263	252252	800800	800800
10	Quiberon	EcQB10-26	850	Esil	253279	282288	312312	289311	205213	257257	252252	800800	800800
11	Quiberon	EcQB10-02	850	Esil	253253	282285	296310	289289	205205	257267	252252	800800	800800
12	Quiberon	EcQB10-03	850	Esil	253253	282285	296310	289289	205205	257267	252252	800800	800800
13	Quiberon	EcQB10-04	850	Esil	253253	285300	308308	289313	227233	257259	252258	800800	800800
14	Quiberon	EcQB10-05	850	Esil	253279	285285	308312	309315	213227	257257	252252	800800	800800
15	Quiberon	EcQB10-06	850	Esil	253253	279291	308324	295309	205227	257263	252254	800800	800800
16	Quiberon	EcQB10-07	850	Esil	253253	279285	308308	289309	213213	257257	252252	800800	800800
17	Quiberon	EcQB10-09	850	Esil	253253	279291	308324	295309	205227	257263	252254	800800	800800
18	Quiberon	EcQB10-10	850	Esil	253253	279291	308324	295309	205227	257263	252254	800800	800800
19	Quiberon	EcQB10-11	850	Esil	253253	288291	308308	289289	205211	255257	252254	800800	800800
20	Quiberon	EcQB10-12	850	Esil	253253	288291	308308	289289	205211	255257	252254	800800	800800

21	Quiberon	EcQB10-13	850	Esil	253253	285285	308308	289289	207227	257267	252264	800800	800800
22	Quiberon	EcQB10-15	850	Esil	253279	282288	312312	289311	205213	257257	252252	800800	800800
23	Quiberon	EcQB10-16	850	Esil	253253	285291	306308	289291	213227	257257	252252	800800	800800
24	Quiberon	EcQB10-17	850	Esil	253253	285291	308312	289295	213227	257257	252252	800800	800800
25	Quiberon	EcQB10-18	850	Esil	253253	285300	308330	289313	227233	257259	252258	800800	800800
26	Quiberon	EcQB12-05	>1000	Ecro	287291	267276	266266	800800	800800	800800	800800	153153	291317
27	Quiberon	EcQB12-09	>1000	Ecro	293295	267276	266266	800800	800800	800800	800800	153161	293293
28	Quiberon	EcQB12-17	>1000	Ecro	287291	267276	266266	800800	800800	800800	800800	153153	291303
29	Quiberon	EcQB12-18	>1000	Ecro	297305	267267	266266	800800	800800	800800	800800	153153	303337
30	Quiberon	EcQB12-19	>1000	Ecro	287301	267267	266266	800800	800800	800800	800800	153153	287291
31	Quiberon	EcQB12-11	>1000	Ecro	293293	267267	266266	800800	800800	800800	800800	137163	281293
32	Quiberon	EcQB12-20	>1000	Ecro	287301	267267	266266	800800	800800	800800	800800	153153	287291
33	Quiberon	EcQB12-29	>1000	Ecro	287291	267276	266266	800800	800800	800800	800800	153153	291303
34	Quiberon	EcQB12-34	>1000	Ecro	291297	270276	266266	800800	800800	800800	800800	153153	303303
35	Quiberon	EcQB12-35	>1000	Ecro	287291	267276	266266	800800	800800	800800	800800	153153	291317
36	Quiberon	EcQB12-36	>1000	Ecro	289293	267276	266266	800800	800800	800800	800800	153161	323337
37	Quiberon	EcQB12-37	>1000	Ecro	287291	267276	266266	800800	800800	800800	800800	153153	291303
38	Quiberon	EcQB12-38	>1000	Ecro	297299	267267	266266	800800	800800	800800	800800	153165	293323
39	Quiberon	EcQB12-39	>1000	Ecro	291291	270270	266266	800800	800800	800800	800800	153161	319319
40	Quiberon	EcQB12-40	>1000	Ecro	281291	267267	266266	800800	800800	800800	800800	139153	269287
41	Quiberon	EcQB12-48	>1000	Ecro	287291	267276	266266	800800	800800	800800	800800	153153	291317
42	Quiberon	EcQB12-49	>1000	Ecro	287291	267276	266266	800800	800800	800800	800800	153153	291303
43	Quiberon	EcQB12-50	>1000	Ecro	287291	267276	266266	800800	800800	800800	800800	153153	291317
44	Quiberon	EcQB12-53	>1000	Ecro	297305	267267	266266	800800	800800	800800	800800	153153	303337
45	Quiberon	EcQB12-56	>1000	Ecro	293295	267276	266266	800800	800800	800800	800800	161165	335335
46	Quiberon	EcQB12-57	>1000	Ecro	287291	267276	266266	800800	800800	800800	800800	153153	291317
47	Quiberon	EcQB12-08	>1000	Ecro	293299	267267	266266	800800	800800	800800	800800	0	287297

48	Quiberon	EcQB12-12	>1000	Ecro	293295	267276	266266	800800	800800	800800	800800	161165	0
1	Ribadeo	GAL161	850	Esil	253253	294306	288318	287325	205227	247261	248252	800800	800800
2	Ribadeo	GAL162	850	Esil	253253	294306	288318	287325	205227	247261	248252	800800	800800
3	Ribadeo	GAL186	850	Esil	253253	312312	302302	287325	205227	247261	252252	800800	800800
4	Ribadeo	GAL195	850	Esil	253253	267294	310310	313325	213245	245259	252252	800800	800800
5	Ribadeo	GAL203	850	Esil	255265	288300	306306	291299	205213	253259	256256	800800	800800
6	Ribadeo	GAL208	850	Esil	275275	288300	312322	287293	219219	257265	252254	800800	800800
7	Ribadeo	GAL231	850	Esil	275275	288300	312322	287293	219219	257265	252254	800800	800800
8	Ribadeo	GAL234	850	Esil	255265	288300	306318	291299	205213	253259	256258	800800	800800
9	Ribadeo	GAL235	850	Esil	255265	288300	306316	291299	205213	253259	256258	800800	800800
10	Ribadeo	GAL236	850	Esil	255265	288300	306316	291299	205213	253259	256258	800800	800800
11	Ribadeo	GAL238	850	Esil	275275	288288	302312	305311	217235	259259	252258	800800	800800
12	Ribadeo	GAL255	850	Esil	253253	285297	306316	287325	213227	255263	252252	800800	800800
13	Ribadeo	GAL270	850	Esil	253253	294306	288318	311311	205227	247261	248252	800800	800800
14	Ribadeo	GAL179	850	Esil	253253	0	334334	287325	205227	247261	252252	800800	800800
15	Ribadeo	GAL256	850	Esil	265265	294294	320320	0	209221	245263	252252	800800	800800
16	Ribadeo	GAL272	850	Esil	253277	267291	306314	0	191209	247255	252256	800800	800800
17	Ribadeo	GAL129	>1000	Ecro	289289	267270	264264	800800	800800	800800	800800	153211	295295
18	Ribadeo	GAL136	>1000	Ecro	289347	267270	264266	800800	800800	800800	800800	159165	281281
19	Ribadeo	GAL137	>1000	Ecro	293293	267267	264266	800800	800800	800800	800800	139139	293293
20	Ribadeo	GAL140	>1000	Ecro	277303	267270	264272	800800	800800	800800	800800	155163	327327
21	Ribadeo	GAL157	>1000	Ecro	291307	267267	266266	800800	800800	800800	800800	155181	265277
22	Ribadeo	GAL158	>1000	Ecro	291307	267267	266266	800800	800800	800800	800800	155181	277277
23	Ribadeo	GAL164	>1000	Ecro	291297	267267	266266	800800	800800	800800	800800	139155	265295
24	Ribadeo	GAL174	>1000	Ecro	279291	267267	266266	800800	800800	800800	800800	139155	265293
25	Ribadeo	GAL198	>1000	Ecro	291331	267267	266266	800800	800800	800800	800800	187211	293293
26	Ribadeo	GAL202	>1000	Ecro	289307	267276	266266	800800	800800	800800	800800	141187	265265

27	Ribadeo	GAL209	>1000	Ecro	291353	264264	264264	800800	800800	800800	800800	155155	263267
28	Ribadeo	GAL218	>1000	Ecro	291307	267267	266266	800800	800800	800800	800800	155181	265277
29	Ribadeo	GAL219	>1000	Ecro	295295	267267	266266	800800	800800	800800	800800	153159	285293
30	Ribadeo	GAL230	>1000	Ecro	291343	267276	266266	800800	800800	800800	800800	139139	265265
31	Ribadeo	GAL233	>1000	Ecro	291343	267276	266266	800800	800800	800800	800800	139187	265265
32	Ribadeo	GAL170	>1000	Ecro	277289	267267	264266	800800	800800	800800	800800	141159	0
33	Ribadeo	GAL240	>1000	Ecro	289289	273279	270270	800800	800800	800800	800800	139159	0
34	Ribadeo	GAL259	>1000	Ecro	291291	267267	266266	800800	800800	800800	800800	141155	0
1	Gandario	GAL495	850	Esil	253275	288288	312342	291311	213231	247257	252252	800800	800800
2	Gandario	GAL497	850	Esil	253253	288288	312342	311311	213231	247257	252252	800800	800800
3	Gandario	GAL504	850	Esil	253253	288291	316316	309313	211219	259263	252256	800800	800800
4	Gandario	GAL507	850	Esil	253277	300300	288288	283323	207211	247249	252256	800800	800800
5	Gandario	GAL508	850	Esil	275275	270297	312338	289311	199231	257257	252258	800800	800800
6	Gandario	GAL509	850	Esil	253277	288291	290306	281311	207231	249255	252256	800800	800800
7	Gandario	GAL512	850	Esil	277277	288291	306342	311337	207213	249249	252256	800800	800800
8	Gandario	GAL518	850	Esil	253253	297300	310310	291323	199215	257257	252258	800800	800800
9	Gandario	GAL519	850	Esil	253253	288300	288342	281311	207211	257261	252256	800800	800800
10	Gandario	GAL520	850	Esil	253253	267267	276276	311311	207211	247247	252252	800800	800800
11	Gandario	GAL529	850	Esil	253277	294300	288328	281311	213213	249259	252256	800800	800800
12	Gandario	GAL530	850	Esil	253279	291300	306336	291311	211221	247257	256258	800800	800800
13	Gandario	GAL535	850	Esil	253253	297300	312336	311341	219231	257257	252258	800800	800800
14	Gandario	GAL537	850	Esil	253253	291291	290306	281311	207231	259259	252256	800800	800800
15	Gandario	GAL538	850	Esil	277277	288294	306342	287287	207211	247247	252252	800800	800800
16	Gandario	GAL552	850	Esil	253277	291300	318318	287311	207211	247263	252252	800800	800800
17	Gandario	GAL528	850	Esil	253279	291297	306342	283313	213213	0	252252	800800	800800
18	Gandario	GAL532	850	Esil	253253	288291	306306	0	207215	255259	252256	800800	800800
19	Gandario	GAL540	850	Esil	277277	294300	0	315337	211211	249249	252252	800800	800800

20	Gandario	GAL547	850	Esil	253253	276315	340344	289311	219235	249249	0	800800	800800
21	Gandario	GAL549	850	Esil	253253	291291	0	287287	0	249249	252252	800800	800800
22	Gandario	GAL525	>1000	Ecro	291295	267267	266266	800800	800800	800800	800800	161163	0
1	Lourido	GAL57	850	Esil	253277	288288	312328	277283	199217	255261	252256	800800	800800
2	Lourido	GAL71	850	Esil	255255	288300	304320	279279	211211	255255	252256	800800	800800
3	Lourido	GAL98	850	Esil	253253	285300	306306	281321	209221	247257	252254	800800	800800
4	Lourido	GAL481	850	Esil	253253	300300	310316	289289	205205	257259	252254	800800	800800
5	Lourido	GAL61	>1000	Ecro	291291	258267	266266	800800	800800	800800	800800	161169	285295
6	Lourido	GAL64	>1000	Ecro	291315	258267	266266	800800	800800	800800	800800	163163	285295
7	Lourido	GAL66	>1000	Ecro	289323	267267	266266	800800	800800	800800	800800	161161	275275
8	Lourido	GAL73	>1000	Ecro	291319	258267	266266	800800	800800	800800	800800	161161	285295
9	Lourido	GAL74	>1000	Ecro	291317	258267	266266	800800	800800	800800	800800	161161	285295
10	Lourido	GAL91	>1000	Ecro	291315	258267	266266	800800	800800	800800	800800	161161	285295
11	Lourido	GAL102	>1000	Ecro	291307	267267	266266	800800	800800	800800	800800	153155	279301
12	Lourido	GAL105	>1000	Ecro	291291	267267	266266	800800	800800	800800	800800	153153	279301
13	Lourido	GAL106	>1000	Ecro	291325	267267	264266	800800	800800	800800	800800	155209	285289
14	Lourido	GAL110	>1000	Ecro	291325	267267	266266	800800	800800	800800	800800	155155	289289
15	Lourido	GAL111	>1000	Ecro	291317	258267	266266	800800	800800	800800	800800	161161	285295
16	Lourido	GAL112	>1000	Ecro	291291	267267	266272	800800	800800	800800	800800	151155	279291
17	Lourido	GAL365	>1000	Ecro	291291	267267	264266	800800	800800	800800	800800	159161	277277
18	Lourido	GAL371	>1000	Ecro	291317	258267	266266	800800	800800	800800	800800	161161	287295
19	Lourido	GAL372	>1000	Ecro	291325	267267	264266	800800	800800	800800	800800	155155	289289
20	Lourido	GAL384	>1000	Ecro	291295	267267	266272	800800	800800	800800	800800	155163	265301
21	Lourido	GAL386	>1000	Ecro	291317	258267	266266	800800	800800	800800	800800	161161	285295
22	Lourido	GAL390	>1000	Ecro	291317	258267	266266	800800	800800	800800	800800	161161	285295
23	Lourido	GAL391	>1000	Ecro	289315	258267	266266	800800	800800	800800	800800	161161	285295
24	Lourido	GAL393	>1000	Ecro	291291	267267	264266	800800	800800	800800	800800	155155	289289

25	Lourido	GAL394	>1000	Ecro	291307	267267	266266	800800	800800	800800	800800	153155	279301
26	Lourido	GAL395	>1000	Ecro	291307	267267	266266	800800	800800	800800	800800	153165	279279
27	Lourido	GAL397	>1000	Ecro	295307	267267	266266	800800	800800	800800	800800	153165	279279
28	Lourido	GAL398	>1000	Ecro	289289	267267	266266	800800	800800	800800	800800	165165	279279
29	Lourido	GAL402	>1000	Ecro	289313	267267	264264	800800	800800	800800	800800	161165	319319
30	Lourido	GAL406	>1000	Ecro	277277	267291	266342	800800	800800	800800	800800	181181	291291
31	Lourido	GAL408	>1000	Ecro	291323	267267	264266	800800	800800	800800	800800	155209	285289
32	Lourido	GAL409	>1000	Ecro	291323	267267	264266	800800	800800	800800	800800	155209	285289
33	Lourido	GAL410	>1000	Ecro	291291	258267	266266	800800	800800	800800	800800	161161	285295
34	Lourido	GAL413	>1000	Ecro	291291	258267	266266	800800	800800	800800	800800	163163	295295
35	Lourido	GAL418	>1000	Ecro	295295	267267	266266	800800	800800	800800	800800	153165	279279
36	Lourido	GAL421	>1000	Ecro	329329	267267	266272	800800	800800	800800	800800	155155	295295
37	Lourido	GAL424	>1000	Ecro	279301	267276	272272	800800	800800	800800	800800	151155	291311
38	Lourido	GAL425	>1000	Ecro	291291	258267	264266	800800	800800	800800	800800	161167	287287
39	Lourido	GAL426	>1000	Ecro	315315	258267	266266	800800	800800	800800	800800	161161	285285
40	Lourido	GAL430	>1000	Ecro	291291	258267	266266	800800	800800	800800	800800	161161	285295
41	Lourido	GAL435	>1000	Ecro	291317	258267	266266	800800	800800	800800	800800	161161	285295
42	Lourido	GAL444	>1000	Ecro	295307	267267	266266	800800	800800	800800	800800	153165	279279
43	Lourido	GAL446	>1000	Ecro	295307	267267	264264	800800	800800	800800	800800	155165	277277
44	Lourido	GAL447	>1000	Ecro	291317	258267	266266	800800	800800	800800	800800	161161	285295
45	Lourido	GAL448	>1000	Ecro	295307	267267	266266	800800	800800	800800	800800	151151	279301
46	Lourido	GAL452	>1000	Ecro	291325	267267	266266	800800	800800	800800	800800	155155	289289
47	Lourido	GAL470	>1000	Ecro	291291	258267	266266	800800	800800	800800	800800	161161	285293
1	Naples	EcNAP12-02	850	Esil	253253	285285	306310	279279	215215	257257	252254	800800	800800
2	Naples	EcNAP12-05	850	Esil	253253	306309	306324	279279	211211	247257	252258	800800	800800
3	Naples	EcNAP12-06	850	Esil	253253	306309	306324	279279	211211	247257	252258	800800	800800
4	Naples	EcNAP12-07	850	Esil	253253	285285	320324	313321	215215	257257	258258	800800	800800

5	Naples	EcNAP12-10	850	Esil	253253	306309	306324	279279	211211	247257	252258	800800	800800
6	Naples	EcNAP12-20	850	Esil	253253	285285	306310	315319	211213	257261	254258	800800	800800
7	Naples	EcNAP12-21	850	Esil	253253	285285	306306	313319	213215	257257	254256	800800	800800
8	Naples	EcNAP12-29	850	Esil	253253	285285	306306	279319	215215	247257	254254	800800	800800
9	Naples	EcNAP12-30	850	Esil	253253	285285	306306	279319	215215	247257	254254	800800	800800
10	Naples	EcNAP12-33	850	Esil	253253	285285	306310	315319	211213	257261	254258	800800	800800
11	Naples	EcNAP12-34	850	Esil	253253	285288	306320	313319	213213	247261	258258	800800	800800
12	Naples	EcNAP12-36	850	Esil	253253	285285	306306	313319	215239	247257	254256	800800	800800
13	Naples	EcNAP12-37	850	Esil	253287	285285	306306	315319	213213	247257	258262	800800	800800
14	Naples	EcNAP12-42	850	Esil	253253	285306	306306	313319	213239	257261	254258	800800	800800
15	Naples	EcNAP12-43	850	Esil	253253	285285	306310	315319	211213	257261	254258	800800	800800
16	Naples	EcNAP12-48	850	Esil	287287	285306	306320	315319	215219	257257	254258	800800	800800
17	Naples	EcNAP12-62	850	Esil	253253	285288	306320	313319	213213	247261	258258	800800	800800
18	Naples	EcNAP12-96	850	Esil	253253	285285	306306	313319	213215	257257	254256	800800	800800
19	Naples	EcNAP12-101	850	Esil	253253	285285	306306	313319	213215	257257	256258	800800	800800
20	Naples	EcNAP12-115	850	Esil	253253	285285	306320	319319	213215	257257	254258	800800	800800
21	Naples	EcNAP12-169	850	Esil	253253	285285	306306	319319	215239	247257	254258	800800	800800
22	Naples	EcNAP12-173	850	Esil	253253	285288	306320	313319	213213	247261	258258	800800	800800
23	Naples	EcNAP12-174	850	Esil	253253	285285	306306	319319	213213	247261	254258	800800	800800
24	Naples	EcNAP12-s#3-38	850	Esil	253253	285285	306310	315315	211213	257261	254258	800800	800800
25	Naples	EcNAP12-s#3-45	850	Esil	253253	285285	306320	315319	215239	257257	254256	800800	800800
26	Naples	EcNAP12-s#4-06	850	Esil	253253	285285	320324	313321	215215	257257	258258	800800	800800
27	Naples	EcNAP12-44	850	Esil	253253	285288	306320	0	213215	257257	254258	800800	800800
28	Naples	EcNAP12-198	850	Esil	253253	285285	306308	0	211211	247257	254258	800800	800800

Appendix B.2

Table S1. Genotypes of gametophytes individuals identified (based on diagnostic microsatellite, ITS1 and species-specific cytoplasmic marker) as *E. siliculosus* (n = 158) and *E. crouaniorum* (n = 176) in the chapter 3. This identification was used previous to the admixture analyses. The number of samples in each population, name of the strain, ITS1 length, and positive amplification in the cytoplasmic marker (Esil = positive amplification Esil, Ecro = positive amplification Ecro) are shown. In diagnostic locus, the non-amplification in one of the parental species corresponds to a phylogenetic signal but should be distinguished from a null allele. To solve this problem, these missing data points were replaced by an artificial allele of an arbitrary size of (800bp). In contrast, missing data or null alleles were noted as 0..

number	Population	strains	ITS1 length	mtDNA	M-122-2	M-208	M-162-1	M-033-1	M-239-3	M-103-2	M-387	M-388	M-420
1	Plymouth	EcQAB10-21	850	Esil	253	306	308	289	227	257	254	800	800
2	Plymouth	EcPLY10-9	>1000	Ecro	293	270	266	800	800	800	800	157	311
3	Plymouth	EcPLY10-11	>1000	Ecro	329	267	262	800	800	800	800	179	267
4	Plymouth	EcPLY10-12	>1000	Ecro	295	276	262	800	800	800	800	155	269
5	Plymouth	EcPLY10-13	>1000	Ecro	289	270	264	800	800	800	800	165	321
6	Plymouth	EcPLY10-14	>1000	Ecro	293	267	264	800	800	800	800	165	285
7	Plymouth	EcPLY10-15	>1000	Ecro	293	270	266	800	800	800	800	157	307
8	Plymouth	EcPLY10-16	>1000	Ecro	297	270	262	800	800	800	800	181	305
9	Plymouth	EcPLY10-19	>1000	Ecro	289	276	266	800	800	800	800	155	0
10	Plymouth	EcPLY10-20	>1000	Ecro	291	267	266	800	800	800	800	155	307
11	Plymouth	EcPLY10-22	>1000	Ecro	291	264	262	800	800	800	800	155	309
12	Plymouth	EcPLY10-25	>1000	Ecro	295	270	266	800	800	800	800	163	307
13	Plymouth	EcPLY10-26	>1000	Ecro	291	267	264	800	800	800	800	163	0
14	Plymouth	EcPLY10-28	>1000	Ecro	297	273	272	800	800	800	800	163	0
15	Plymouth	EcPLY10-29	>1000	Ecro	291	270	270	800	800	800	800	139	0
16	Plymouth	EcPLY10-30	>1000	Ecro	279	270	272	800	800	800	800	133	0
17	Plymouth	EcPLY10-31	>1000	Ecro	295	267	266	800	800	800	800	155	0

18	Plymouth	EcPLY10-35	>1000	Ecro	333	273	270	800	800	800	800	157	285
19	Plymouth	EcPLY10-36	>1000	Ecro	295	270	266	800	800	800	800	155	293
1	Restronguet	EcREP10-6	850	Esil	253	297	330	289	213	257	252	800	800
2	Restronguet	EcREP10-7	850	Esil	253	297	312	289	215	257	252	800	800
3	Restronguet	EcREP10-13	850	Esil	279	279	330	293	213	247	254	800	800
4	Restronguet	EcREP10-25	850	Esil	279	297	330	293	229	257	252	800	800
5	Restronguet	EcREP10-29	850	Esil	253	297	312	289	213	257	254	800	800
6	Restronguet	EcREP10-32	850	Esil	253	288	330	293	217	247	252	800	800
7	Restronguet	EcREP10-33	850	Esil	279	303	330	313	229	247	252	800	800
8	Restronguet	EcREP10-38	850	Esil	253	288	330	293	217	247	252	800	800
9	Restronguet	EcREP10-39	850	Esil	253	279	330	293	213	247	252	800	800
10	Restronguet	EcREP10-47	850	Esil	253	303	330	293	213	257	252	800	800
11	Restronguet	EcREP10-56	850	Esil	253	297	310	293	215	257	252	800	800
12	Restronguet	EcREP10-58	850	Esil	253	288	330	293	213	247	252	800	800
1	Roscoff	EcPH12-112	850	Esil	253	285	312	0	227	259	256	800	800
2	Roscoff	EcPH12-78	850	Esil	253	0	0	307	217	259	252	800	800
3	Roscoff	EcPH12-90	850	Esil	253	297	306	311	205	255	256	800	800
4	Roscoff	EcPH12-91	850	Esil	253	297	306	291	205	255	254	800	800
5	Roscoff	EcPH12-02	>1000	Ecro	289	270	266	800	800	800	800	153	293
6	Roscoff	EcPH12-03	>1000	Ecro	291	270	266	800	800	800	800	165	267
7	Roscoff	EcPH12-04	>1000	Ecro	291	267	266	800	800	800	800	153	295
8	Roscoff	EcPH12-07	>1000	Ecro	295	270	266	800	800	800	800	167	267
9	Roscoff	EcPH12-09	>1000	Ecro	293	270	266	800	800	800	800	169	293
10	Roscoff	EcPH12-11	>1000	Ecro	291	270	266	800	800	800	800	163	331
11	Roscoff	EcPH12-12	>1000	Ecro	291	261	266	800	800	800	800	137	307
12	Roscoff	EcPH12-13	>1000	Ecro	291	270	266	800	800	800	800	163	267
13	Roscoff	EcPH12-15	>1000	Ecro	291	270	266	800	800	800	800	163	307

14	Roscoff	EcPH12-18	>1000	Ecro	291	267	264	800	800	800	800	163	293
15	Roscoff	EcPH12-21	>1000	Ecro	289	270	266	800	800	800	800	153	293
16	Roscoff	EcPH12-24	>1000	Ecro	291	261	266	800	800	800	800	155	267
17	Roscoff	EcPH12-25	>1000	Ecro	313	267	266	800	800	800	800	171	309
18	Roscoff	EcPH12-38	>1000	Ecro	291	270	264	800	800	800	800	163	331
19	Roscoff	EcPH12-40	>1000	Ecro	291	270	266	800	800	800	800	153	287
20	Roscoff	EcPH12-43	>1000	Ecro	281	261	266	800	800	800	800	163	307
21	Roscoff	EcPH12-44	>1000	Ecro	0	270	266	800	800	800	800	163	309
22	Roscoff	EcPH12-45	>1000	Ecro	291	270	266	800	800	800	800	153	331
23	Roscoff	EcPH12-48	>1000	Ecro	291	267	266	800	800	800	800	165	293
24	Roscoff	EcPH12-49	>1000	Ecro	295	267	266	800	800	800	800	163	287
25	Roscoff	EcPH12-s#1-91B	>1000	Ecro	291	270	264	800	800	800	800	163	293
26	Roscoff	EcPH12-23	>1000	Ecro	291	270	264	800	800	800	800	167	269
27	Roscoff	EcPH12-26	>1000	Ecro	293	267	266	800	800	800	800	195	279
28	Roscoff	EcPH12-28	>1000	Ecro	349	267	266	800	800	800	800	165	309
29	Roscoff	EcPH12-31	>1000	Ecro	295	270	266	800	800	800	800	161	293
30	Roscoff	EcPH12-32	>1000	Ecro	295	267	266	800	800	800	800	161	293
31	Roscoff	EcPH12-34	>1000	Ecro	291	270	266	800	800	800	800	165	293
32	Roscoff	EcPH12-36	>1000	Ecro	291	270	266	800	800	800	800	195	283
33	Roscoff	EcPH12-37	>1000	Ecro	313	270	266	800	800	800	800	153	267
34	Roscoff	EcPH12-41	>1000	Ecro	313	270	266	800	800	800	800	161	267
35	Roscoff	EcPH12-46	>1000	Ecro	291	261	266	800	800	800	800	163	307
36	Roscoff	EcPH12-47	>1000	Ecro	291	270	266	800	800	800	800	161	293
37	Roscoff	EcPH12-52	>1000	Ecro	355	267	266	800	800	800	800	199	295
38	Roscoff	EcPH12-53	>1000	Ecro	349	267	272	800	800	800	800	141	0
39	Roscoff	EcPH12-54	>1000	Ecro	287	270	266	800	800	800	800	153	283
40	Roscoff	EcPH12-55	>1000	Ecro	291	270	266	800	800	800	800	155	291

41	Roscoff	EcPH12-57	>1000	Ecro	349	270	272	800	800	800	800	161	0
42	Roscoff	EcPH12-60	>1000	Ecro	313	270	266	800	800	800	800	153	291
43	Roscoff	EcPH12-63	>1000	Ecro	313	267	266	800	800	800	800	153	291
44	Roscoff	EcPH12-64	>1000	Ecro	313	267	266	800	800	800	800	153	0
45	Roscoff	EcPH12-65	>1000	Ecro	369	267	266	800	800	800	800	155	0
46	Roscoff	EcPH12-66	>1000	Ecro	287	270	266	800	800	800	800	153	283
47	Roscoff	EcPH12-67	>1000	Ecro	349	270	266	800	800	800	800	161	303
48	Roscoff	EcPH12-68	>1000	Ecro	371	270	266	800	800	800	800	159	305
49	Roscoff	EcPH12-73	>1000	Ecro	291	267	266	800	800	800	800	153	0
50	Roscoff	EcPH12-81	>1000	Ecro	295	276	264	800	800	800	800	153	267
51	Roscoff	EcPH12-82	>1000	Ecro	297	270	266	800	800	800	800	163	0
52	Roscoff	EcPH12-83	>1000	Ecro	291	267	266	800	800	800	800	153	303
53	Roscoff	EcPH12-85	>1000	Ecro	311	270	266	800	800	800	800	163	295
54	Roscoff	EcPH12-87	>1000	Ecro	291	267	264	800	800	800	800	161	303
1	Le Caro	EcLC12-07	850	Esil	253	297	330	289	215	257	252	800	800
2	Le Caro	EcLC12-08	850	Esil	253	285	326	289	215	257	252	800	800
3	Le Caro	EcLC12-17	850	Esil	253	297	330	289	215	247	252	800	800
4	Le Caro	EcLC12-21	850	Esil	253	297	330	0	215	0	252	800	800
1	Traezh Hir	EcTH10-169	850	Esil	253	285	308	293	219	0	254	800	800
2	Traezh Hir	EcTH10-171	850	Esil	253	303	318	303	225	251	252	800	800
3	Traezh Hir	EcTH10-197	850	Esil	253	306	308	289	251	257	252	800	800
4	Traezh Hir	EcTH10-199	850	Esil	253	297	308	289	217	261	252	800	800
5	Traezh Hir	EcTH10-200	850	Esil	253	297	308	289	217	261	252	800	800
6	Traezh Hir	EcTH10-201	850	Esil	253	297	308	289	217	261	252	800	800
7	Traezh Hir	EcTH10-205	850	Esil	253	285	310	285	227	261	252	800	800
8	Traezh Hir	EcTH10-206	850	Esil	253	285	330	309	227	247	252	800	800
9	Traezh Hir	EcTH10-21	>1000	Ecro	301	267	266	800	800	800	800	165	0

10	Traezh Hir	EcTH10-25	>1000	Ecro	301	270	264	800	800	800	800	163	277
11	Traezh Hir	EcTH10-26	>1000	Ecro	281	267	266	800	800	800	800	161	279
12	Traezh Hir	EcTH10-31	>1000	Ecro	0	267	264	800	800	800	800	163	277
13	Traezh Hir	EcTH10-32	>1000	Ecro	291	270	266	800	800	800	800	163	277
14	Traezh Hir	EcTH10-33	>1000	Ecro	301	270	264	800	800	800	800	153	277
15	Traezh Hir	EcTH10-34	>1000	Ecro	301	267	264	800	800	800	800	191	0
16	Traezh Hir	EcTH10-35	>1000	Ecro	291	267	266	800	800	800	800	155	0
17	Traezh Hir	EcTH10-37	>1000	Ecro	291	267	266	800	800	800	800	153	277
18	Traezh Hir	EcTH10-38	>1000	Ecro	0	267	266	800	800	800	800	153	277
19	Traezh Hir	EcTH10-39	>1000	Ecro	325	267	266	800	800	800	800	0	285
20	Traezh Hir	EcTH10-41	>1000	Ecro	289	267	264	800	800	800	800	165	291
21	Traezh Hir	EcTH10-42	>1000	Ecro	253	270	266	800	800	800	800	153	277
22	Traezh Hir	EcTH10-44	>1000	Ecro	349	264	266	800	800	800	800	159	323
23	Traezh Hir	EcTH10-46	>1000	Ecro	297	270	266	800	800	800	800	153	317
24	Traezh Hir	EcTH10-51	>1000	Ecro	337	267	266	800	800	800	800	0	287
25	Traezh Hir	EcTH10-52	>1000	Ecro	291	276	266	800	800	800	800	165	289
26	Traezh Hir	EcTH10-54	>1000	Ecro	0	267	266	800	800	800	800	155	277
27	Traezh Hir	EcTH10-56	>1000	Ecro	291	264	266	800	800	800	800	0	347
28	Traezh Hir	EcTH10-59	>1000	Ecro	301	267	266	800	800	800	800	163	289
29	Traezh Hir	EcTH10-60	>1000	Ecro	291	270	266	800	800	800	800	195	309
30	Traezh Hir	EcTH10-61	>1000	Ecro	327	267	266	800	800	800	800	155	285
31	Traezh Hir	EcTH10-62	>1000	Ecro	291	267	266	800	800	800	800	155	0
32	Traezh Hir	EcTH10-63	>1000	Ecro	289	267	266	800	800	800	800	163	0
33	Traezh Hir	EcTH10-66	>1000	Ecro	337	264	266	800	800	800	800	159	0
34	Traezh Hir	EcTH10-67	>1000	Ecro	297	270	264	800	800	800	800	153	0
35	Traezh Hir	EcTH10-68	>1000	Ecro	297	267	266	800	800	800	800	153	0
36	Traezh Hir	EcTH10-69	>1000	Ecro	291	270	266	800	800	800	800	163	277

37	Traezh Hir	EcTH10-70	>1000	Ecro	337	267	266	800	800	800	800	155	285
38	Traezh Hir	EcTH10-71	>1000	Ecro	289	270	266	800	800	800	800	135	283
39	Traezh Hir	EcTH10-72	>1000	Ecro	297	267	266	800	800	800	800	153	317
40	Traezh Hir	EcTH10-73	>1000	Ecro	297	267	264	800	800	800	800	161	0
41	Traezh Hir	EcTH10-74	>1000	Ecro	291	267	266	800	800	800	800	163	277
42	Traezh Hir	EcTH10-76	>1000	Ecro	327	267	266	800	800	800	800	187	297
43	Traezh Hir	EcTH10-77	>1000	Ecro	291	270	266	800	800	800	800	135	0
44	Traezh Hir	EcTH10-78	>1000	Ecro	319	270	266	800	800	800	800	135	283
45	Traezh Hir	EcTH10-83	>1000	Ecro	297	267	264	800	800	800	800	161	317
46	Traezh Hir	EcTH10-84	>1000	Ecro	297	267	266	800	800	800	800	161	317
47	Traezh Hir	EcTH10-85	>1000	Ecro	297	267	264	800	800	800	800	161	301
48	Traezh Hir	EcTH10-86	>1000	Ecro	291	267	266	800	800	800	800	153	0
49	Traezh Hir	EcTH10-87	>1000	Ecro	287	267	266	800	800	800	800	169	291
50	Traezh Hir	EcTH10-89	>1000	Ecro	287	276	266	800	800	800	800	155	277
51	Traezh Hir	EcTH10-92	>1000	Ecro	339	270	266	800	800	800	800	155	277
52	Traezh Hir	EcTH10-95	>1000	Ecro	319	267	266	800	800	800	800	153	0
53	Traezh Hir	EcTH10-96	>1000	Ecro	327	267	272	800	800	800	800	159	297
54	Traezh Hir	EcTH10-100	>1000	Ecro	371	267	266	800	800	800	800	163	277
55	Traezh Hir	EcTH10-101	>1000	Ecro	297	267	264	800	800	800	800	153	0
56	Traezh Hir	EcTH10-102	>1000	Ecro	293	267	264	800	800	800	800	153	283
57	Traezh Hir	EcTH10-104	>1000	Ecro	371	267	266	800	800	800	800	153	289
58	Traezh Hir	EcTH10-105	>1000	Ecro	295	267	266	800	800	800	800	155	309
59	Traezh Hir	EcTH10-106	>1000	Ecro	297	267	264	800	800	800	800	161	0
60	Traezh Hir	EcTH10-107	>1000	Ecro	297	270	264	800	800	800	800	161	317
61	Traezh Hir	EcTH10-108	>1000	Ecro	297	270	264	800	800	800	800	161	317
62	Traezh Hir	EcTH10-109	>1000	Ecro	313	267	272	800	800	800	800	159	297
63	Traezh Hir	EcTH10-110	>1000	Ecro	297	267	264	800	800	800	800	161	317

64	Traezh Hir	EcTH10-112	>1000	Ecro	305	267	266	800	800	800	800	155	291
65	Traezh Hir	EcTH10-115	>1000	Ecro	291	267	266	800	800	800	800	155	279
1	Quiberon	EcQB10-14	850	Esil	253	285	308	313	227	257	252	800	800
2	Quiberon	EcQB12-02	>1000	Ecro	289	276	266	800	800	800	800	185	297
3	Quiberon	EcQB12-03	>1000	Ecro	297	267	272	800	800	800	800	155	287
4	Quiberon	EcQB12-04	>1000	Ecro	293	267	266	800	800	800	800	153	265
5	Quiberon	EcQB12-06	>1000	Ecro	345	270	266	800	800	800	800	161	321
6	Quiberon	EcQB12-07	>1000	Ecro	345	270	266	800	800	800	800	0	333
7	Quiberon	EcQB12-13	>1000	Ecro	295	270	264	800	800	800	800	155	287
8	Quiberon	EcQB12-14	>1000	Ecro	301	267	266	800	800	800	800	0	265
9	Quiberon	EcQB12-15	>1000	Ecro	317	267	266	800	800	800	800	153	319
10	Quiberon	EcQB12-16	>1000	Ecro	293	267	264	800	800	800	800	155	265
11	Quiberon	EcQB12-21	>1000	Ecro	313	267	266	800	800	800	800	0	0
12	Quiberon	EcQB12-22	>1000	Ecro	299	267	266	800	800	800	800	0	297
13	Quiberon	EcQB12-24	>1000	Ecro	325	276	266	800	800	800	800	193	301
14	Quiberon	EcQB12-27	>1000	Ecro	293	267	266	800	800	800	800	153	295
15	Quiberon	EcQB12-01	>1000	Ecro	291	267	266	800	800	800	800	187	289
16	Quiberon	EcQB12-28	>1000	Ecro	281	270	266	800	800	800	800	155	347
17	Quiberon	EcQB12-30	>1000	Ecro	295	267	266	800	800	800	800	163	293
18	Quiberon	EcQB12-31	>1000	Ecro	293	267	266	800	800	800	800	161	295
19	Quiberon	EcQB12-32	>1000	Ecro	293	267	266	800	800	800	800	153	265
20	Quiberon	EcQB12-33	>1000	Ecro	305	270	266	800	800	800	800	165	325
21	Quiberon	EcQB12-41	>1000	Ecro	295	267	264	800	800	800	800	0	285
22	Quiberon	EcQB12-45	>1000	Ecro	313	282	266	800	800	800	800	153	0
23	Quiberon	EcQB12-51	>1000	Ecro	291	276	266	800	800	800	800	0	0
24	Quiberon	EcQB12-52	>1000	Ecro	291	276	266	800	800	800	800	0	347
25	Quiberon	EcQB12-60	>1000	Ecro	291	276	266	800	800	800	800	161	265

26	Quiberon	EcQB12-26	>1000	Ecro	293	267	266	800	800	800	800	161	295
27	Quiberon	EcQB12-47	>1000	Ecro	293	267	266	800	800	800	800	161	275
1	Ribadeo	GAL141	>1000	Ecro	331	267	264	800	800	800	800	155	293
2	Ribadeo	GAL148	>1000	Ecro	291	267	264	800	800	800	800	139	285
3	Ribadeo	GAL163	>1000	Ecro	317	267	266	800	800	800	800	159	293
4	Ribadeo	GAL167	>1000	Ecro	279	273	270	800	800	800	800	163	315
5	Ribadeo	GAL168	>1000	Ecro	319	267	266	800	800	800	800	159	293
6	Ribadeo	GAL169	>1000	Ecro	289	267	266	800	800	800	800	159	265
7	Ribadeo	GAL178	>1000	Ecro	291	267	264	800	800	800	800	155	293
8	Ribadeo	GAL183	>1000	Ecro	317	267	266	800	800	800	800	209	0
9	Ribadeo	GAL185	>1000	Ecro	295	267	266	800	800	800	800	209	0
10	Ribadeo	GAL187	>1000	Ecro	291	267	264	800	800	800	800	141	333
11	Ribadeo	GAL190	>1000	Ecro	295	267	266	800	800	800	800	209	309
12	Ribadeo	GAL191	>1000	Ecro	295	267	266	800	800	800	800	209	309
13	Ribadeo	GAL193	>1000	Ecro	295	267	264	800	800	800	800	159	293
14	Ribadeo	GAL194	>1000	Ecro	317	267	266	800	800	800	800	209	295
15	Ribadeo	GAL213	>1000	Ecro	295	270	266	800	800	800	800	209	0
16	Ribadeo	GAL214	>1000	Ecro	291	261	264	800	800	800	800	155	263
17	Ribadeo	GAL215	>1000	Ecro	291	264	264	800	800	800	800	155	295
18	Ribadeo	GAL223	>1000	Ecro	291	267	266	800	800	800	800	155	0
19	Ribadeo	GAL224	>1000	Ecro	291	270	266	800	800	800	800	209	293
20	Ribadeo	GAL226	>1000	Ecro	289	267	264	800	800	800	800	209	0
21	Ribadeo	GAL227	>1000	Ecro	289	267	264	800	800	800	800	209	325
22	Ribadeo	GAL241	>1000	Ecro	295	267	266	800	800	800	800	209	311
23	Ribadeo	GAL182	>1000	Ecro	317	267	266	800	800	800	800	209	293
1	Lourido	GAL400	>1000	Ecro	295	267	266	800	800	800	800	153	279
2	Lourido	GAL438	>1000	Ecro	291	267	266	800	800	800	800	161	295

1	Naples	EcNAP12-22	850	Esil	253	285	306	313	215	257	256	800	800
2	Naples	EcNAP12-23	850	Esil	253	285	306	319	215	261	256	800	800
3	Naples	EcNAP12-24	850	Esil	287	306	320	315	215	257	258	800	800
4	Naples	EcNAP12-25	850	Esil	253	285	306	319	215	257	256	800	800
5	Naples	EcNAP12-26	850	Esil	287	285	304	313	211	257	258	800	800
6	Naples	EcNAP12-27	850	Esil	253	285	306	313	215	257	256	800	800
7	Naples	EcNAP12-28	850	Esil	253	285	320	319	215	257	258	800	800
8	Naples	EcNAP12-31	850	Esil	287	285	306	279	215	257	254	800	800
9	Naples	EcNAP12-32	850	Esil	253	285	306	319	215	257	254	800	800
10	Naples	EcNAP12-38	850	Esil	253	285	320	0	213	257	254	800	800
11	Naples	EcNAP12-39	850	Esil	253	285	306	319	213	257	258	800	800
12	Naples	EcNAP12-40	850	Esil	253	285	306	319	215	257	258	800	800
13	Naples	EcNAP12-41	850	Esil	287	0	302	319	213	257	262	800	800
14	Naples	EcNAP12-46	850	Esil	253	285	312	313	213	257	256	800	800
15	Naples	EcNAP12-47	850	Esil	253	306	306	315	211	257	254	800	800
16	Naples	EcNAP12-49	850	Esil	253	285	306	319	239	247	254	800	800
17	Naples	EcNAP12-50	850	Esil	253	285	306	315	211	257	258	800	800
18	Naples	EcNAP12-51	850	Esil	253	285	306	313	213	257	256	800	800
19	Naples	EcNAP12-52	850	Esil	253	285	306	313	215	257	256	800	800
20	Naples	EcNAP12-54	850	Esil	253	285	306	313	213	257	254	800	800
21	Naples	EcNAP12-55	850	Esil	253	285	306	319	213	257	258	800	800
22	Naples	EcNAP12-56	850	Esil	253	285	306	319	215	257	256	800	800
23	Naples	EcNAP12-57	850	Esil	253	285	306	319	215	257	256	800	800
24	Naples	EcNAP12-59	850	Esil	253	285	306	313	213	257	256	800	800
25	Naples	EcNAP12-60	850	Esil	253	285	306	319	213	257	256	800	800
26	Naples	EcNAP12-65	850	Esil	253	285	306	319	215	247	258	800	800
27	Naples	EcNAP12-66	850	Esil	253	285	306	319	215	247	258	800	800

28	Naples	EcNAP12-68	850	Esil	253	285	306	319	215	257	256	800	800
29	Naples	EcNAP12-69	850	Esil	253	285	306	0	215	257	256	800	800
30	Naples	EcNAP12-71	850	Esil	253	285	306	313	215	257	256	800	800
31	Naples	EcNAP12-72	850	Esil	253	285	306	319	215	257	258	800	800
32	Naples	EcNAP12-73	850	Esil	253	285	306	313	213	257	256	800	800
33	Naples	EcNAP12-74	850	Esil	253	285	306	313	215	257	256	800	800
34	Naples	EcNAP12-75	850	Esil	253	285	306	313	215	261	258	800	800
35	Naples	EcNAP12-76	850	Esil	253	285	306	313	213	257	256	800	800
36	Naples	EcNAP12-78	850	Esil	253	285	306	313	215	257	254	800	800
37	Naples	EcNAP12-79	850	Esil	253	285	306	313	215	257	256	800	800
38	Naples	EcNAP12-80	850	Esil	253	285	306	313	215	257	256	800	800
39	Naples	EcNAP12-83	850	Esil	253	285	306	313	215	257	254	800	800
40	Naples	EcNAP12-85	850	Esil	253	285	306	313	213	257	256	800	800
41	Naples	EcNAP12-87	850	Esil	253	285	306	313	215	257	254	800	800
42	Naples	EcNAP12-88	850	Esil	253	285	306	313	213	257	256	800	800
43	Naples	EcNAP12-90	850	Esil	253	285	306	313	213	257	256	800	800
44	Naples	EcNAP12-91	850	Esil	253	285	306	313	213	257	256	800	800
45	Naples	EcNAP12-95	850	Esil	253	285	306	313	215	257	254	800	800
46	Naples	EcNAP12-98	850	Esil	253	285	306	313	213	257	254	800	800
47	Naples	EcNAP12-99	850	Esil	253	285	306	315	239	257	258	800	800
48	Naples	EcNAP12-102	850	Esil	253	285	306	319	213	257	256	800	800
49	Naples	EcNAP12-104	850	Esil	253	285	306	319	213	247	254	800	800
50	Naples	EcNAP12-105	850	Esil	253	285	306	313	213	257	258	800	800
51	Naples	EcNAP12-106	850	Esil	253	285	306	319	213	257	256	800	800
52	Naples	EcNAP12-107	850	Esil	253	285	306	319	213	247	254	800	800
53	Naples	EcNAP12-108	850	Esil	253	285	306	0	0	247	254	800	800
54	Naples	EcNAP12-110	850	Esil	253	285	306	313	213	257	256	800	800

55	Naples	EcNAP12-112	850	Esil	253	285	306	319	215	257	256	800	800
56	Naples	EcNAP12-113	850	Esil	253	285	306	313	215	257	256	800	800
57	Naples	EcNAP12-114	850	Esil	287	306	320	319	215	257	254	800	800
58	Naples	EcNAP12-116	850	Esil	253	285	306	313	239	243	258	800	800
59	Naples	EcNAP12-117	850	Esil	253	285	306	319	215	257	254	800	800
60	Naples	EcNAP12-118	850	Esil	253	285	306	313	215	257	256	800	800
61	Naples	EcNAP12-119	850	Esil	253	285	306	313	215	257	256	800	800
62	Naples	EcNAP12-120	850	Esil	253	285	306	313	215	257	256	800	800
63	Naples	EcNAP12-121	850	Esil	253	285	306	319	213	257	254	800	800
64	Naples	EcNAP12-122	850	Esil	253	285	306	313	213	257	256	800	800
65	Naples	EcNAP12-124	850	Esil	253	285	320	319	213	257	254	800	800
66	Naples	EcNAP12-126	850	Esil	253	285	306	279	215	257	254	800	800
67	Naples	EcNAP12-127	850	Esil	253	285	306	313	213	257	254	800	800
68	Naples	EcNAP12-128	850	Esil	287	285	306	319	215	257	254	800	800
69	Naples	EcNAP12-129	850	Esil	253	285	306	319	215	257	256	800	800
70	Naples	EcNAP12-130	850	Esil	253	285	306	313	213	257	256	800	800
71	Naples	EcNAP12-131	850	Esil	253	285	306	313	213	257	256	800	800
72	Naples	EcNAP12-132	850	Esil	253	285	306	0	0	257	258	800	800
73	Naples	EcNAP12-133	850	Esil	287	285	304	313	213	261	258	800	800
74	Naples	EcNAP12-134	850	Esil	253	285	306	319	215	257	256	800	800
75	Naples	EcNAP12-135	850	Esil	253	285	320	319	215	257	258	800	800
76	Naples	EcNAP12-136	850	Esil	253	285	306	319	213	257	254	800	800
77	Naples	EcNAP12-137	850	Esil	253	285	306	319	213	257	254	800	800
78	Naples	EcNAP12-139	850	Esil	253	285	306	0	0	257	254	800	800
79	Naples	EcNAP12-141	850	Esil	253	285	306	319	213	257	256	800	800
80	Naples	EcNAP12-142	850	Esil	253	285	306	315	215	257	254	800	800
81	Naples	EcNAP12-143	850	Esil	253	285	306	0	215	257	254	800	800

82	Naples	EcNAP12-144	850	Esil	253	285	306	319	215	257	256	800	800
83	Naples	EcNAP12-145	850	Esil	253	285	306	319	215	257	256	800	800
84	Naples	EcNAP12-146	850	Esil	253	285	306	313	213	257	256	800	800
85	Naples	EcNAP12-147	850	Esil	253	285	306	313	215	257	256	800	800
86	Naples	EcNAP12-148	850	Esil	253	285	306	319	213	257	256	800	800
87	Naples	EcNAP12-149	850	Esil	253	285	306	313	213	257	256	800	800
88	Naples	EcNAP12-150	850	Esil	253	285	306	313	215	257	256	800	800
89	Naples	EcNAP12-151	850	Esil	253	285	306	313	213	257	258	800	800
90	Naples	EcNAP12-152	850	Esil	253	285	320	319	213	257	254	800	800
91	Naples	EcNAP12-153	850	Esil	253	285	320	319	215	257	258	800	800
92	Naples	EcNAP12-156	850	Esil	253	285	320	313	0	257	254	800	800
93	Naples	EcNAP12-157	850	Esil	253	285	306	311	215	257	256	800	800
94	Naples	EcNAP12-158	850	Esil	253	285	306	313	215	257	256	800	800
95	Naples	EcNAP12-159	850	Esil	253	285	306	313	213	257	256	800	800
96	Naples	EcNAP12-160	850	Esil	253	285	306	317	213	257	254	800	800
97	Naples	EcNAP12-161	850	Esil	253	285	306	313	213	261	256	800	800
98	Naples	EcNAP12-162	850	Esil	253	285	306	319	213	257	254	800	800
99	Naples	EcNAP12-163	850	Esil	253	285	306	319	213	257	256	800	800
100	Naples	EcNAP12-164	850	Esil	253	285	306	317	239	257	256	800	800
101	Naples	EcNAP12-165	850	Esil	253	288	306	319	215	261	258	800	800
102	Naples	EcNAP12-166	850	Esil	253	306	306	319	213	257	258	800	800
103	Naples	EcNAP12-167	850	Esil	0	285	306	0	215	257	254	800	800
104	Naples	EcNAP12-170	850	Esil	253	285	306	317	213	257	254	800	800
105	Naples	EcNAP12-172	850	Esil	287	306	320	319	219	257	258	800	800
106	Naples	EcNAP12-175	850	Esil	253	285	306	319	215	257	258	800	800
107	Naples	EcNAP12-176	850	Esil	253	285	306	313	213	257	256	800	800
108	Naples	EcNAP12-177	850	Esil	253	285	320	319	215	257	258	800	800

109	Naples	EcNAP12-178	850	Esil	253	285	306	0	0	257	256	800	800
110	Naples	EcNAP12-179	850	Esil	253	285	310	313	213	257	258	800	800
111	Naples	EcNAP12-180	850	Esil	253	285	306	319	213	257	256	800	800
112	Naples	EcNAP12-181	850	Esil	253	285	306	319	215	247	254	800	800
113	Naples	EcNAP12-182	850	Esil	253	285	306	317	215	257	256	800	800
114	Naples	EcNAP12-183	850	Esil	253	285	310	315	213	261	258	800	800
115	Naples	EcNAP12-185	850	Esil	253	285	306	279	213	257	254	800	800
116	Naples	EcNAP12-186	850	Esil	253	285	306	317	239	247	254	800	800
117	Naples	EcNAP12-187	850	Esil	253	285	306	319	215	247	254	800	800
118	Naples	EcNAP12-188	850	Esil	253	285	306	317	213	257	258	800	800
119	Naples	EcNAP12-191	850	Esil	253	285	306	319	215	247	254	800	800
120	Naples	EcNAP12-192	850	Esil	253	285	320	319	215	257	258	800	800
121	Naples	EcNAP12-194	850	Esil	253	285	306	319	215	257	256	800	800
122	Naples	EcNAP12-195	850	Esil	253	285	320	319	215	257	254	800	800
123	Naples	EcNAP12-196	850	Esil	253	285	306	313	239	257	256	800	800
124	Naples	EcNAP12-199	850	Esil	253	285	306	319	213	247	254	800	800
125	Naples	EcNAP12-200	850	Esil	253	285	306	279	215	257	254	800	800
126	Naples	EcNAP12-201	850	Esil	253	285	306	319	213	247	254	800	800
127	Naples	EcNAP12-s#3-33	850	Esil	253	285	306	319	215	257	258	800	800
128	Naples	EcNAP12-s#3-31	850	Esil	253	285	306	319	0	0	254	800	800

The genus *Ectocarpus Lyngbye* (Ectocarpales, Phaeophyceae) comprises marine filamentous algae characterized by an alternation between two independent multicellular organisms of different ploidy. The general objective of the thesis was to study species delineation and speciation within this genus. We started clarifying the number of cryptic species using two unlinked loci (COI-5P and ITS1) and an integrative approach associating barcode gap detection analyses with phylogenetic reconstructions. We showed the presence of at least 15 species partitioned within a monophyletic group composed of *E. crouaniorum* (Ecro) and two closely related species and a paraphyletic assemblage composed of the remaining 12 other species including *E. siliculosus* (Esil). Second, Rad sequencing and phylogenomics analyses allowed to resolve the relationships within the paraphyletic assemblage. The different species becomes well separated into two divergent clades (Ecro and Esil). A diversity of taxa with various levels of divergence was revealed within the clade Esil and hybridization between the closest and sympatric species was suggested. Finally, the importance of reproductive isolation among the two commonest but most divergent species Esil and Ecro was studied using species-specific nuclear and cytoplasmic markers jointly with 9 microsatellites. We showed that meiosis acts as a strong reproductive barrier among these two species and demonstrates that the species of the genus *Ectocarpus* are excellent systems to study evolutionary consequences of hybridization and introgression for the maintenance or breakdown of species because of their haploid diploid life cycle.

Le genre *Ectocarpus* Lyngbye (Ectocarpales, Phaeophyceae) regroupe des algues marines filamenteuses caractérisées par un cycle haploïde-diploïde. L'objectif de la thèse était de délimiter les espèces et d'étudier la spéciation dans ce genre. Nous avons commencé par clarifier le nombre d'espèces cryptiques en utilisant deux loci indépendants et une approche intégrative associant une analyse de détection de « barcode gap » avec des reconstructions phylogénétiques. Nos résultats montrent l'existence d'au moins 15 espèces qui se répartissent en un groupe monophylétique composé *d'E. crouaniorum* (Ecro) et de deux espèces proches ainsi que d'un mélange paraphylétique composé des 12 autres espèces incluant *E. siliculosu*s (Esil). Deuxièmement, les analyses de séquençage Rad et de phylogénomique ont permis de résoudre les relations au sein du groupe paraphylétique. Les espèces se regroupent maintenant en deux clades divergents (Ecro and Esil). Des niveaux de divergence variables entre espèces sont révélés au sein du clade Esil. Des phénomènes d'hybridation entre les espèces les plus apparentées, et trouvées en sympatrie, sont suspectés. Finalement, l'importance de l'isolement reproducteur a été étudié entre les espèces Esil et Ecro, les plus communes, mais les plus divergentes, en utilisant des marqueurs spécifiques de chacune des espèces. Nos résultats indiquent que la méiose agit comme une forte barrière reproductive entre ces espèces et démontrent que les espèces du genre *Ectocarpus* sont d'excellents systèmes pour étudier les conséquences évolutives de l'hybridation et de l'introgression pour le maintien ou la divergence des espèces grâce à leur cycle haploïde-diploïde.

El género *Ectocarpus* Lyngbye (Ectocarpales, Phaeophyceae) está formado por algas marinas filamentosas con alternancia de generaciones en su ciclo de vida (haploide-diploide). Esta tesis tuvo por objetivo delimitar las especies dentro de este género y estudiar los mecanismos de especiación. Nosotros empezamos clarificando el numero de especies crípticas usando dos genes no ligados y una aproximación integrativa asociando el análisis de barcoding genético con filogenias. Los resultados mostraron la presencia de al menos 15 especies particionadas dentro un clado monofilético compuesto de *E. crouaniorum* (Ecro) y dos especies estrechamente relacionadas, y un ensamblaje parafilético incluyendo *E. siliculosus* (Esil) y las restantes 12 especies. Segundo, análisis de filogenómica permitieron resolver las relaciones dentro de este ensamblaje. Las diferentes especies fueron claramente separadas mostrando diferentes niveles de divergencia y fueron agrupadas en dos clados. Además, la posible hibridación entre especies filogenéticamente cercanas y simpátricas fue evidenciada. Finalmente, mediante la combinación de marcadores moleculares provenientes del núcleo y citoplasma, junto a 9 microsatélites, estudiamos la importancia del aislamiento reproductivo entre las especies más comunes y divergentes del género (Esil y Ecro). Así, nosotros mostramos que la meiosis actúa como una fuerte barrera reproductiva entre estas dos especies. Por último, destacamos que las especies pertenecientes al género *Ectocarpus* constituyen un modelo apropiado para estudiar los procesos de hibridación e introgresión, así como la divergencia de especies debido a su ciclo de vida haploide-diploide.