Evolutionary History of the Fucus spiralis / Fucus guiryi Complex

Large-scale Analysis of the Distribution Range and Genetic Structure across the Northern Hemisphere

Master Thesis by **Amelie Prinz**; a65486@ualg.pt

Academic Year: 2019/2020

Faculdade de Ciências e Tecnologia

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Abstract

This master thesis addresses a large-scale biogeographical question, the distribution and evolutionary history of a marine species complex with a vast distribution in the northern hemisphere. This complex includes two intertidal brown macroalgae, Fucus spiralis and Fucus guiryi, whose boundaries, degree of isolation and ranges are imperfectly known. The species Fucus guiryi has been recently separated from Fucus spiralis, and several studies have been undertaken to investigate its geographical distribution and identifying the factors determining it, especially in the face of global warming and rising sea surface temperature. The essential ecosystem services that are provided by these canopy-forming macroalgae together with factors that threaten this species, highlight the need for enhanced research. In this study a genetic diversity and differentiation analysis were applied, using microsatellites and mitochondrial DNA for assessing the influence of historical and ongoing processes in explaining species distributions, range limits and gene-pools, which are driven by great climate events such as the last glacial maximum as well as, potentially, historical and ongoing hybridization. In addition to the genetic methods, species distribution models (SDM) were developed to investigate niche differences and assess potential near-future range shifts under two contrasting climate change scenarios. Genetic analyses revealed that the investigated species complex does not consist of the aforementioned species, instead we recognize a third genetic entity currently under *F. guiryi*: *Fucus macroguiryi*. Besides the distinct lower vertical position along the shore in comparison to *Fucus spiralis* and *Fucus guiryi*, when in sympatry, *Fucus macroguiryi* shows a clear genetic and morphologically separation to its relatives. Most interesting results regarding species boundaries, ranges, structure and hybridization SDMs showed that the fate of the three described entities are highly different. *Fucus spiralis* will experience a great increase in suitable habitat for the future within the “worst case” climate scenario, contrastingly to *Fucus guiryi,* which will potentially have to face extreme reduction in its population sizes in the southern margins and might even face local extinctions. *Fucus macroguiryi* showed a poleward shift and relict populations in Morocco could as well vanish in the near future.

Key words: *Fucus* spp., biogeography, intertidal, hybridization, range shifts, species distribution model, species complex

Resumo Algardo

Esta dissertação de mestrado aborda uma questão biogeográfica de larga-escala, a distribuição e a história evolutiva de um complexo espécies marinhas com uma distribuição muito vasta no hemisfério norte. Os modelos usados são duas macroalgas castanhas da zona entremarés, Fucus spiralis e Fucus guiryi. A espécie Fucus guiryi foi recentemente descrita, e vários estudos têm sido realizados para investigar sua distribuição geográfica e identificar os fatores que a influenciam, especialmente face ao aquecimento global e ao aumento da temperatura da superfície do mar. De acordo com o site AlgaeBase, Fucus spiralis foi registrada na costa africana , europa, e, de forma mais interessante, nas costas americanas (Atlântico e Pacífico). Por outro lado, a distribuição de Fucus guiryi está aparentemente restrita entre a Irlanda e a Grã-Bretanha e o seu limite sul na costa do Marrocos. Observações empíricas sugerem que a distribuição de Fucus guiryi pode ser maior do presentemente se conhece. Outro aspecto investigado neste estudo foi impulsionado por descobertas anteriores, que indicam que Fucus spiralis é composta por dois morfótipos distintos. Estes morfótipos ocorrem em níveis de maré distinctos chamados de Fucus spiralis Baixo e Fucus spiralis Alto. Existe também alguma incerteza à escala geográfica, como a diferenciação entre "Fucus guiryi alopátrico" (anteriormente "Fucus spiralis Sul") e "Fucus guiryi simpátrico”. “Fucus guiryi alopátrico” ocorre atualmente nas margens meridionais do complexo de espécies, Marrocos, Sahara Ocidental, Ilhas Canárias e no Sudoeste de Portugal, enquanto “Fucus guiryi simpátrico” é mais comum no Noroeste da Península Ibérica, França e ilhas britânicas. Neste estudo, o uso de microssatélites e DNA mitocondrial permitiu avaliar a influência de diversos processos históricos responsáveis pela diversificação e distribuição das espécies (limites de distribuição e “pools” de genes), e identificar uma terceira entidade dentro deste complexo de espécies: Fucus macroguiryi. Esta espécie apresenta tipicamente uma posição vertical mais baixa em comparação com Fucus spiralis e Fucus guiryi (quando em simpatria), e também mostra uma clara separação genética e morfológica. Apesar de ser possível a hibridização entre estas espécies, mecanismos pré-zigóticos e fatores ambientais adversos aparentemente garantem que as espécies mantenham sua integridade genética mesmo quando em simpatria. Além do mais, a hipótese de uma recente colonização de habitats marginais e de áreas antes não conhecidas, como o litoral do Noroeste da América, por *Fucus guiryi* após o último máximo glacial, se mostrou equivocada. Baixos níveis de diversidade sugerem que a América do Norte foi recentemente (re)colonizada por *Fucus spiralis.* Além disso, identificamos o Nordeste Atlântico como a principal área de persistência a longo prazo, caracterizada pela presença das

três entidades e geralmente elevada diversidade. Neste estudo destacamos o Noroeste da Península Ibérica como uma região de persistência a longo prazo e o Sudoeste de Portugal como uma zona de contato entre *Fucus spiralis* e *Fucus guiryi*. Ademais, foi confirmada a hipótese de que as populações localizadas nos Açores são geneticamente separadas das populações do continente, sendo provavelmente o resultado de um isolamento crónico devido à sua distância. Este estudo também analisou potenciais padrões de conectividade de *Fucus guiryi* ao tentar obter uma abordagem holística através da compreensão de sua dinâmica de reprodução, história de vida e nicho ecológico em relação às suas espécies irmãs, e às vezes simpátricas *Fucus spiralis* e *Fucus macroguiryi*.

Nesta tese foram igualmente desenvolvidos modelos de distribuição de espécies para investigar as possíveis migrações em direção ao pólo em dois cenários contrastantes de mudanças climáticas (RCP2.6 e RCP8.5). Mostramos que estas algas da zona entremarés, serão provavelmente altamente impactadas pelo aumento constante da temperatura da superfície do mar, tornando-as altamente vulneráveis às mudanças climáticas. À escala global, a temperatura da superfície do mar, bem como a temperatura do ar foram identificadas como as variáveis mais importantes na determinação da distribuição deste complexo de espécies. Os modelos prevêm que o destino das três espécies descritas deverá ser diferente, onde, *Fucus spiralis* no “pior cenário” considerado, sofrerá um grande aumento no habitat adequado a norte, por exemplo ao longo do Canadá, por volta de 2100. Por outro lado, *Fucus guiryi* sofrerá potencialmente uma contração extrema nas margens mais quentes da distribuição, podendo até mesmo resultar em extinções locais, como por exemplo, nos Açores. Já *Fucus macroguiryi* poderá sofrer uma expansão em direção aos polos e num futuro próximo possivelmente o desparecimento das populações relíquia de Marrocos, mas a magnitude destas migrações permanece desconhecida.

A investigação sobre este complexo de espécies da zona entremarés permitiu clarificar aspectos sobre sua dinâmica evolutiva e o papel de uma série de mecanismos, como expansões na distribuição, incluindo o papel de climas passados, efeitos de gargalo (bottlenecks), história de vida e modos de reprodução, bem como o contributo da hibridização, ajudando a explicar os padrões biogeográficos e genéticos que vemos hoje.

Palavras-chave: *Fucus* spp., biogeografia, zona entremarés, hibridização, alterações de distribuição, modelo de distribuição de espécies, complexo de espécies

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List of Abbreviations

BIC	Bayesian Information Criterion
DAPC	Discriminant analysis of principal components
DA	Discriminant analysis
dNTPs	desoxyribonucleoside triphosphates
LGM	Last glacial maximum
maxAir	maximum air temperature
maxSST	maximum sea surface temperature
MCMC	Markoc chain monte carlo
MgCl2	Magnesion chloride
minAir	minimum air temperature
minSST	minimum sea surface temperature
MLG	Multi locus genotype
mtIGS	Mitochondrial inter genetic spacer
mt*	entity in the context of mitochondrial DNA
msat*	entity in the context of multi locus genotype data
NW	North West
PAC	Principal component analysis
PCR	Polymerase chain reaction
RCP	representative concentration pathways
SW	South West
SDM	Species distribution modelling

*will be used in combination with entity names in order for the reader to understand in which context the term for each entity used

The abbreviations for the sample locations can be found in the Appendix

1. Introduction

The key questions of population and community ecology are referring to the size of the populations, the structure and the rules determining their assembly. Physiological capacities and resources, as well as habitat quality and configuration, are responsible for the abundance on an individual level. Predation, competition dynamics and mutualistic relationships are regulating the occurrences and abundances of communities. Finally, the accessibility of new areas, colonisation and dispersion possibilities and the earth climate history eventually establish the distribution of species on earth in a distinct pattern. Especially the latter mentioned plays a major role in biogeography and evolution. Biogeographical studies had been of special interest in science history since the 18th century. Numerous factors of the biotic and abiotic surroundings, as well as historical processes, which stand in complex relation to an intrinsic advantageous setting of genetics, allow organisms to exist and explain patterns we see today.

1.1 The Genus Fucus

The genus *Fucus* is among the most ecologically relevant and characteristic genus of brown algae in the northern hemisphere. As Neiva et al., (2016) expressed it, members of *Fucus* and related genera (family Fucaceae) showed to be a “particularly fruitful research model” for evolutionary investigations. This seaweed genus has undergone recent radiation (Leclerc et al,, 1998; Serrão et al., 1999) which is still ongoing. This fast divergence between *Fucus* species, which is likely enhanced in response to stress, allows the examination of the genetic structures and provides insight into the biogeographical histories, diversification and population dynamics of species. Advanced molecular technologies are nowadays available to expand phylogeographic research on fucoid populations, which allow estimations about possible connections or refuge areas in the past (Hoarau et al., 2007; Maggs et al., 2008; Muhlin & Brawley, 2009). Neiva et al., (2016) summarized, that the distribution of Atlantic fucoid seaweeds is assumed to consist of edge populations, which are often located in the most southern ranges and characterized by higher genetic diversity because of long-term persistence leading to the accumulation of mutations that resultingly lead to differences in the gene pools. Additionally, populations that colonized newly available habitats after the last glacial maximum (LGM, 20 000 – 18 000 years ago), normally at higher latitudes, tend to have lower genetic diversity because of their recent age and genetic bottlenecks during the colonization process. Expansions of species was found to produce homogenous landscapes of genetic structure due to the spread of few alleles at the front. The fact that fucoid species tend to have a relatively low dispersal potential (Ladah et al., 2008), a high genetic diversity and uniqueness can be used as an estimator for long time persistence and isolation in respect to other populations. Areas that were serving as refugia at unsuitable climatic history events are expected to display higher diversities within refugia and high genetic dissimilarity between refugia due to reduced geographical ranges that are associated with isolation (Hewitt, 1996, 2004), which was confirmed by prior studies (Hoarau et al., 2007).

Fucus species are externally fertilizing perennial brown macroalgae which are highly abundant on intertidal semi-exposed and sheltered rocky shores in temperate latitudes (in the sense of Spalding et al., 2007) where they show a patchy to continuous distribution pattern or form characteristic belts depending on the environmental conditions. Regarding marine macroalgae temperature is one of the most crucial factors determining their distributions and its effects on survival, reproduction and setting range limits has been largely studied (Assis et al., 2018; Bartsch et al., 2012; Breeman, 1988, 1990; Hoek et al., 1990; Lüning, 1984; Martínez et al., 2012; Mathieson & Niemeck, 1978; Riera et al,. 2015; Saada et al., 2016;). Especially in respect to continuously increasing sea surface temperatures, only populations that are by chance inhabiting a suitable environment, or that can acclimatize to new conditions, that is not as much affected by global warming, can withstand these stressors. Upwelling areas, for example, have shown to buffer the effects of climate change and can therefore serve as a refuge for a representative of the brown macroalgae *Fucus* (Lourenço et al., 2016). This is important because *Fucus* are strictly intertidal and cannot migrate to deeper cooler waters as kelps and many invertebrates.

Intertidal rocky shores represent a unique ecosystem that show a gradient of selective pressures which are terrestrial and marine related. Organisms occurring in these areas are constantly confronted with harsh environmental conditions including daily cyclic fluctuations of elevated exposure to heat, high levels of wave energy, excess irradiance, nutrient limitation, and desiccation stress induced by emersion at low tides. However, fucoid species seem to have the ability to persist under such challenging conditions and multiple species can segregate on the same shore according to species-specific tide-levels (Billard et al., 2010; Zardi et al., 2011). Development of hyaline hairs for elevated phosphate uptake (Hurd et al., 1993), growth in dense mats (Bewley, 1995; Hunt & Denny, 2008), rapid changes in turgor pressure as a result of acclimatisation after hyper- or hyposaline stress (Karsten, 2012), antioxidant enzymes and low reactive oxygen species production in response to desiccation stress (Flores-Molina et al., 2014), proteins associated with extreme efficient desiccation tolerance (López-Cristoffanini et al., 2015), heat shock proteins (Mota et al., 2018) as well as thick cell walls, and the accumulation of compatible solutes are just a few examples of the adaptational responses and strategies to greatly changing habitat conditions within even very small distances. Particularly marine intertidal species are highly sensitive to raising temperatures because they often exist at their upper temperature tolerance limits (Tomanek, 2010).

The definition of a species of the genus *Fucus* might not be as easy as it seems. As already mentioned, this genus has undergone recent radiation which is still in process (Leclerc et al., 1998), but species delineation remains challenging (Coyer et al. 2006). Because this genus is still in such an early stage of speciation, many species have diverged only relatively recently and many are still able to hybridize (Engel et al., 2005; Moalic et al., 2011; Neiva et al., 2010). The role of hybridization in the evolutionary history of *Fucus* is still insufficiently documented and understood. In addition to incomplete reproductive barriers, *Fucus* also showed high intraspecific morphological variation (Anderson & Scott, 1998; Jordan & Vadas, 1972) depending on its habitat quality and exposure to stressors among others. Sufficient genetic exchange may temper or even mask speciation dynamics.

Seaweeds and especially canopy forming species are considered to be ecosystem-engineers providing essential ecosystem services such as food source, habitats, protection and breeding grounds for other organisms (Dayton, 1985; Hagerman, 1966). The shift of populations of the *Fucus spiralis* species complex, being a primary producer and therefore representing the basic group within the marine coastal food chain, may have severe impacts in a broad sense. *Fucus* serves for example as food source for *Littorina obtusata* (Estep & Dabrowski, 1980; Dongen, 1956; Young, 1975), a grazing snail that is again eaten by many predator species living in the intertidal zones. Particularly, crustacean species such as Amphipoda, Isopoda, Mysidacea and Decapoda can be found within the "Fucus-zone". These species are consequently the basic food source for littoral fish species like perch and pike. In addition, *Fucus* patches also offer protection against desiccation and heat during low tide, as species can hide under the moist canopy. Seaweeds also play an important role as carbon sinks, sequestering globally up to 0.46 – 2.55 pg of carbon per year (rewiewed by Mineur et al., 2015). Coastal reclamation and destruction induce habitat loss for intertidal species that are bound to rocky substrates. Pollution and the change in water quality e.g. eutrophication processes also contribute to threaten the species habitats. Moreover, increasing sea surface temperatures especially in the coastal areas have influence on the physiological capacities and stress exposure (e.g. a negative effect on photosynthesis; see Graiff et al., 2015; Hunt & Denny, 2008; Karsten, 2012; Mathieson & Niemeck, 1978; Schagerl & Möstl, 2011) or reproductive failure (Wethey & Woodin, 2008) and potentially drive these organisms to extinction if the temperatures will elevate in a time too short for the species to adapt or "seek" refuge in colder regions. The resulting effects of a loss or replacement of these species will have impacts on an ecosystem level, will influence biogeochemical cycling and also will have negative effects on the economy and social society (Pecl et al., 2017).

1.2 Current Knowledge on Genetics, Phylogeny and Taxonomy

Since the 1990ies there had been an enormous increase regarding the development of advanced molecular technologies such as next generation sequencing. These technologies are becoming more affordable and researchers are now able to sequence whole genomes of research organisms, design primers and amplify sets of microsatellites at a relatively low cost. Microsatellites can be used to investigate the degree and intensity of geneflow, and detect historical events such as bottlenecks, vicariance and expansions, thus helping to explore the evolutionary history and biogeography of species. The mtDNA spacer has been widely used in the past in both intra-generic and intra-specific studies to distinguish species and populations. Especially the mtDNA spacer was much more informative to explore the relationship on a genus level than other loci such as the nuclear ribosomal DNA internal transcribed spacer or the mtDNA23S (Coyer et al., 2006). Prior studies showed that that the *Fucus*/Pelvetiopsis *lineage* diverged from *Ascophyllum* probably 10 – 16 MYA and further started diverging 2.3 – 5.5 MYA into two distinct lineages (Cánovas et al., 2011; Hoarau et al., 2007) – lineage I (*Fucus serratus* and *Fucus distichus*) and lineage II (*Fucus ceranoides*, *Fucus vesiculosus* and the hermaphrodite complex here studied). Both include dioecious and hermaphroditic representatives (Figure 1). Furthermore, in 2010, there was evidence presented that *Fucus spiralis* consists of two (Billard et al., 2010) or three (Coyer et al., 2011) different entities: *Fucus spiralis High*, *Fucus spiralis Low* and *Fucus spiralis South*. The designation of *Fucus spiralis High* and *Low* is related to the distinct vertical distributional patterns along the intertidal gradient of selective pressures. These *Fucus* species share the same distributional range as *Fucus vesiculosus*. On the other hand, Coyer et al., (2011) suggested that “the southern entity diverged early in allopatry and hybridized with *Fucus vesiculosus* in sympatry to produce *Fucus spiralis Low*”. These findings eventually led to the final conclusion that previously named *Fucus spiralis High* and *Fucus spiralis Low* are in fact not two different ecotypes but two different species (Zardi et al., 2011). Hereinafter, *Fucus spiralis Low* and *Fucus var.platycapus* are in the literature referred to as *Fucus guiryi*, a taxa currently encompassing as well the entity *Fucus spiralis South*. Interestingly, there is the tendency that as the evolution of the genus Fucus preceded the more representatives of this genus were adapted to higher temperatures (Figure 1). This can be explained through its evolutionary history that had been investigated previously (Billard et al., 2010; Cánovas et al., 2011; Hoarau et al., 2007).

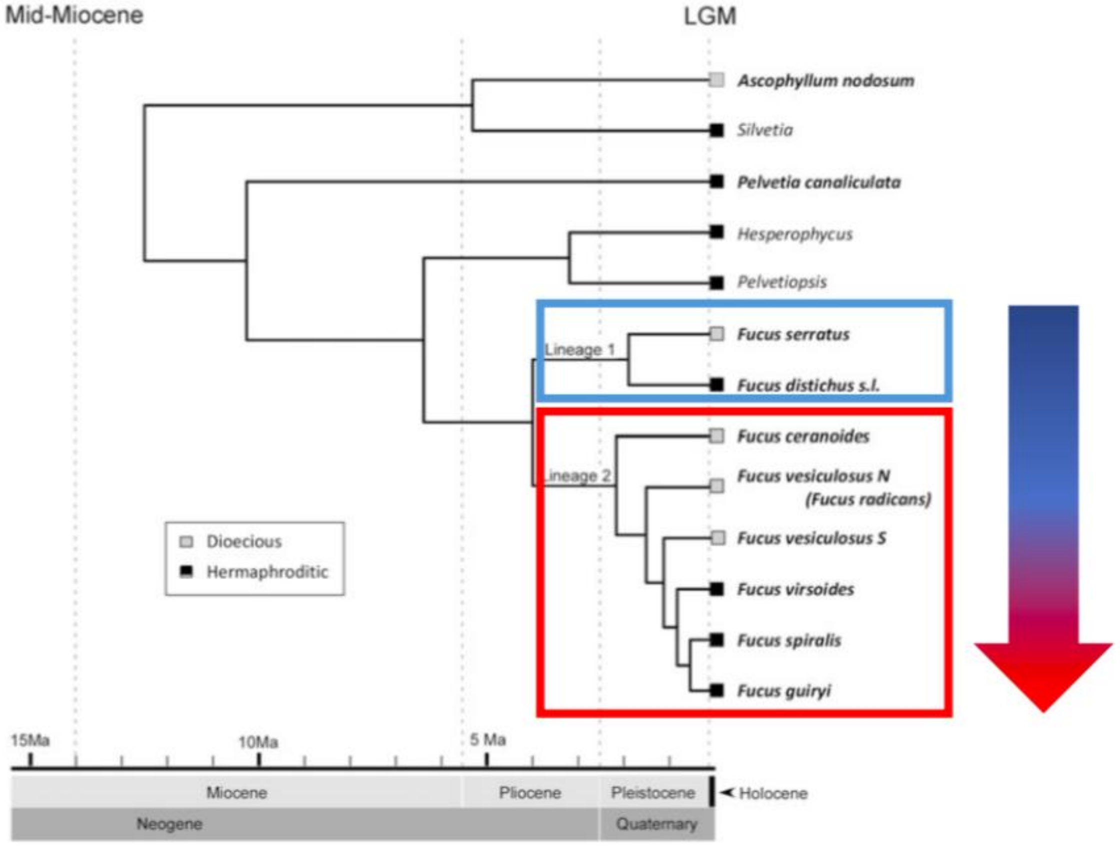


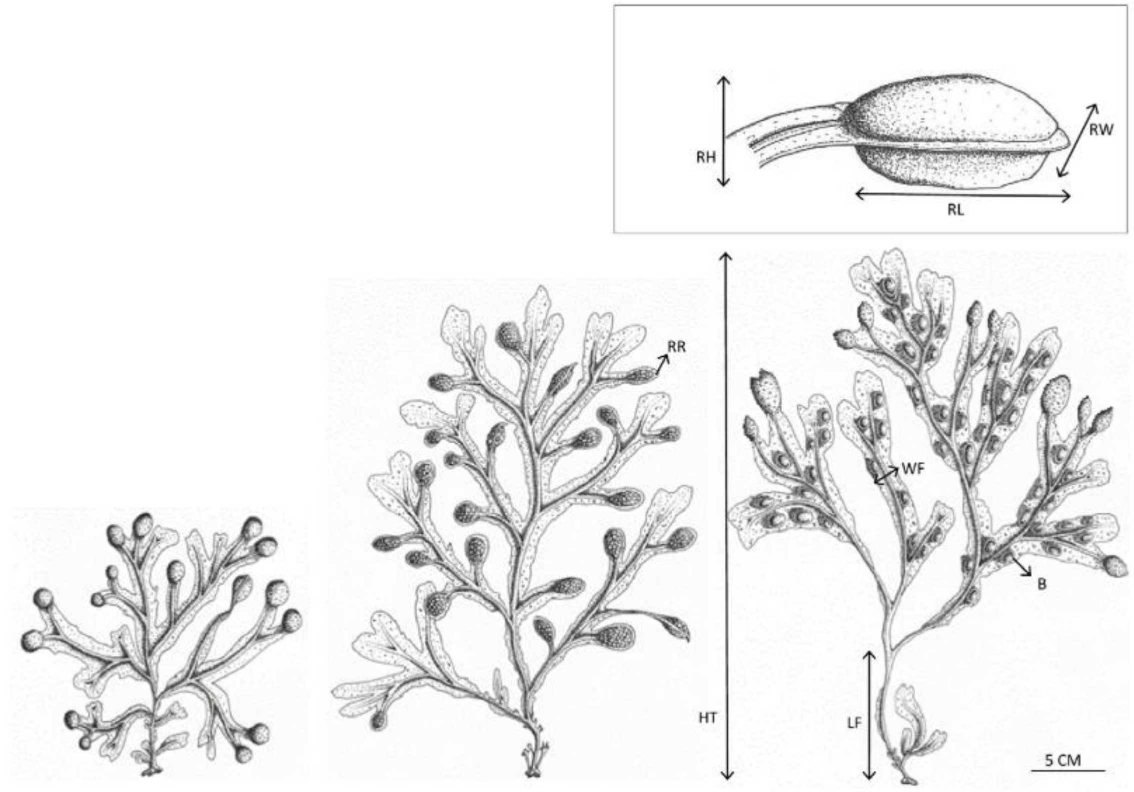
Figure 1: Simplified phylogenetic tree based on 13 coding loci of Fucaceae extracted from Neiva et al., 2016 (adapted from Cánovas et al., 2011). The reproduction mode is indicated (dioecious/hermaphroditic) and branches are proportional to the divergence time with Atlantic taxa. The first lineage, consisting of F.serratus and F. distichus, is known to inhabit more northern cold-water environments. Lineage 2 (F. ceranoides, F. vesiculosus, F. radicans, F. virsoides, F. spiralis and F. guiryi) however represent a more southern extension and have supposedly higher stress-tolerance.

5.5 MYA the Bering Strait opened and enabled 4 trans-arctic colonization events from the Pacific into the Atlantic Ocean. The first crossing is estimated to be 16.4 – 5.4 MYA and involves a splitting of the Atlantic lineage of Fucaceae leading to *Pelvetia canaliculate*, with the second crossing event that lead to the Atlantic genus *Ascophyllum*, which split from its Pacific sister genus *Silvetia*. The third trans-Arctic crossing, which is according to Cánovas et al. (2011) the most successful in terms of continuous speciation was the divergence of *Hesperophycus-Pelvetiopsis* in the Pacific and the lineage leading to *Fucus* – the genus which arose in the Pliocene approximately 5 – 2 MYA (Cánovas et al., 2011).

The most recent evolved representatives of the genus Fucus, *Fucus guiryi* and *Fucus spiralis*, both can apparently occur in sympatry on the coasts of Great Britain, Ireland and the Atlantic coasts of France and Spain. Additionally, *Fucus spiralis* is also present on both coasts of the USA, Iceland and Scandinavia implying a lower temperature tolerance whereas *Fucus guiryi* (in alloparty) is occupying more southern ranges such as South Portugal, Macaronesia and the Moroccan and West-Saharan coast. *Fucus guiryi* is presumed to have a potentially greater thermal tolerance given its southern population limits and warmer climates. Representatives of *Fucus* can have overlapping geographical ranges. One should take into account that *Fucus vesiculosus*, *Fucus spiralis* and *Fucus guiryi* can be sympatric, whereas in more southern areas *Fucus guiryi* and *Fucus vesiculosus* occur allopatrically. If sympatric species assemblages are present, these show a specific vertical zonation along intertidal shores depending on the species-specific emersion tolerances (Billard et al., 2010; Dring & Brown, 1982; Zardi et al., 2011). *Fucus serratus* and *Fucus vesiculosus*, for example are present on lower intertidal levels, contrasting to *Fucus guiryi* and *Fucus spiralis*. Scientists hypothesised that on the one hand biotic factors such as competition, grazing etc., are predominantly determining the lower shore limit and on the other hand abiotic stressors influence more the upper shore limits (Connell, 1972). Yet, these oversimplified postulates involve in reality of course more complex variables. Experimental findings showed that interspecific competition might be directly responsible for setting the vertical limits of intertidal canopy algae (in this case for *Fucus vesiculosus* and *serratus* and other fucoid algae) (Hawkins & Hartnoll, 1985; Schonbeck & Norton, 1980) and intraspecific competition is known to affect the early stages of development in certain *Fucus* species (Creed et al., 1997).

When these species occur in sympatry, hybridization is very likely to occur (Billard et al., 2005; Billard et al., 2010; Perrin et al., 2007). Clusters have been identified as intermediates between *Fucus spiralis* and *Fucus vesiculosus* (Billard et al., 2010). Studies in the past showed that intermediates are also able to successfully reproduce (Coyer et al., 2007; Engel et al., 2005).
With the application of genetic approaches such as the usage of multiple protein-coding regions (Cánovas et al., 2011), nuclear and mitochondrial marker (Coyer et al., 2011) and diagnostic microsatellites (Zardi et al., 2011) the results of these studies confirmed that allopatric *Fucus guiryi* present in Morocco and the southern parts of Iberia has a particular phylogenetic position in respect to its sister species *Fucus spiralis* and *Fucus vesiculosus.* However, this definite phylogenetic resolution only refers to the southern populations of *Fucus guiryi*. The mystery about the phylogenetic assignment of sympatric populations of *Fucus guiryi* remain unsolved. Introgression, for example, might be a reason for the blurry interspecific boundaries (Zardi et al., 2011). Species boundaries are clearly blurred due to gene flow among hybridizing species, however, in such heterogenous environments like the intertidal zone, selection and/or strong environmental gradients are counteracting this tendency (Zardi et al., 2011). Studies showed that prezygotic isolation via asynchronous spawning or gametic incompatibility also reinforces species barriers (Monteiro et al., 2012) when different *Fucus* species share a geographic area. Hybridization opportunities nevertheless exist, but if and how they result in genetic exchange among the *Fucus spiralis/guiryi* complex remains largely unassessed.

All *Fucus* species have a similar lifecycle, morphology (Figure 2) and ecological niche in a broad sense. Empirical observations indicated that the total life span of *Fucus* species is approximately five years (according to Coleman & Brawley, 2005). *Fucus* species are characterized by their dominant diploid sporophyte generation and much reduced gametophytic generation. Meiosporangia are produced in specialized cavities called conceptacles, which are situated in a receptacle. *Fucus* species are highly sensitive to water motion and only release their gametes under very calm conditions (Ladah et al., 2003). This results in an extremely high fertilization success (higher than 80% according to Pearson & Brawley, 1996) with some exceptions for example in the Baltic Sea (Serrão et al., 1999).



F. spiralis *F. spiralis* var. *platycarpus* *F. vesiculosus*

Figure 2: Drawings of morphotypes and illustration of morphological traits from Zardi et al., (2011). F.spiralis var. platycarpus is in the literature later on referred to as F. guiryi. Measurements of thallus height (HT), length of the frond between the holdfast and the first dichotomy (LF), receptacle sterile rim (RR), air bladders (B), monopodial branching (M), width of apical frond (WF), receptacle height(RH), length (RL) and width (RW) were used in this study to characterize each species.

Unlike most marine species that have planktonic larval stages, *Fucus* propagules are restricted to short-lived phototactic sperm and negatively buoyant eggs and zygotes, typically resulting in recruitment near parents and in relatively closed populations. In addition, since *Fucus spiralis* and *Fucus guiryi* lack of any floating organs, their dispersion of detached fragments via drifting is potentially low. However, *Fucus spiralis* was observed to successfully disperse long distances in mixed algal rafts with *Ascophyllum nodosum* (Coleman & Brawley, 2005). These findings can be taken into account but should not be considered to be the major dispersal mode of *Fucus spiralis* and *Fucus guiryi* respectively. Fucoid seaweeds are such interesting models in phylogeographical research because they disperse little and therefore tend to retain genetic signatures of past range shifts, including climatic refugia and poleward expansions.

1.3 Climate Oscillations, the Last Glacial Maximum, Refugia and Recolonization Dynamics

Planet earth experienced several periods of great climatic changes induced by glacial and interglacial periods. The latter ones occurred for less than 10% of time during the Pleistocene epoch of earth history (Lambeck et al., 2002). Especially the last glacial maximum (LGM) which caused a maximum cooling around 20 K – 18 KYA (Frenzel, 1992) and a maximum sea level low stand (of 120 – 130m) at 26.5 K to 19 KYA (Clark et al., 2009) played a major role regarding the distribution of marine biota on the northern hemisphere. Massive ice sheets were expanding up to the coasts of Ireland and probably even reached parts of Brittany. Therefore, populations that could not withstand such cold temperatures contracted in the north, were shifted and expanded southwards sometimes in the form of small and scattered populations.

After deglaciation, within the Holocene period (12 ka–present), species were only able to migrate northward towards not yet colonized areas, which lead to expansions in the north and presumably extinctions in the south. The absence of adequate fossil records limits the possibilities of research, nevertheless genetic modelling studies have provided valuable insights: The recolonization dynamics of many species had been examined and through the combination of paleoclimatic and genetic data the identification of marine refugia areas is possible (Maggs et al., 2008). These findings, especially for seaweeds coincided with results from other researchers (Hoarau et al., 2007), where three main refugial areas were identified that were mostly ice-free: (1) South Western Ireland, (2) Brittany / The English Channel and (3) The Iberian Peninsula (Figure 3). Concerning the North-West Atlantic, Maggs et al., (2008) also identified the areas near Florida, the Gulf of Mexico and Canadian Maritimes as refugia.

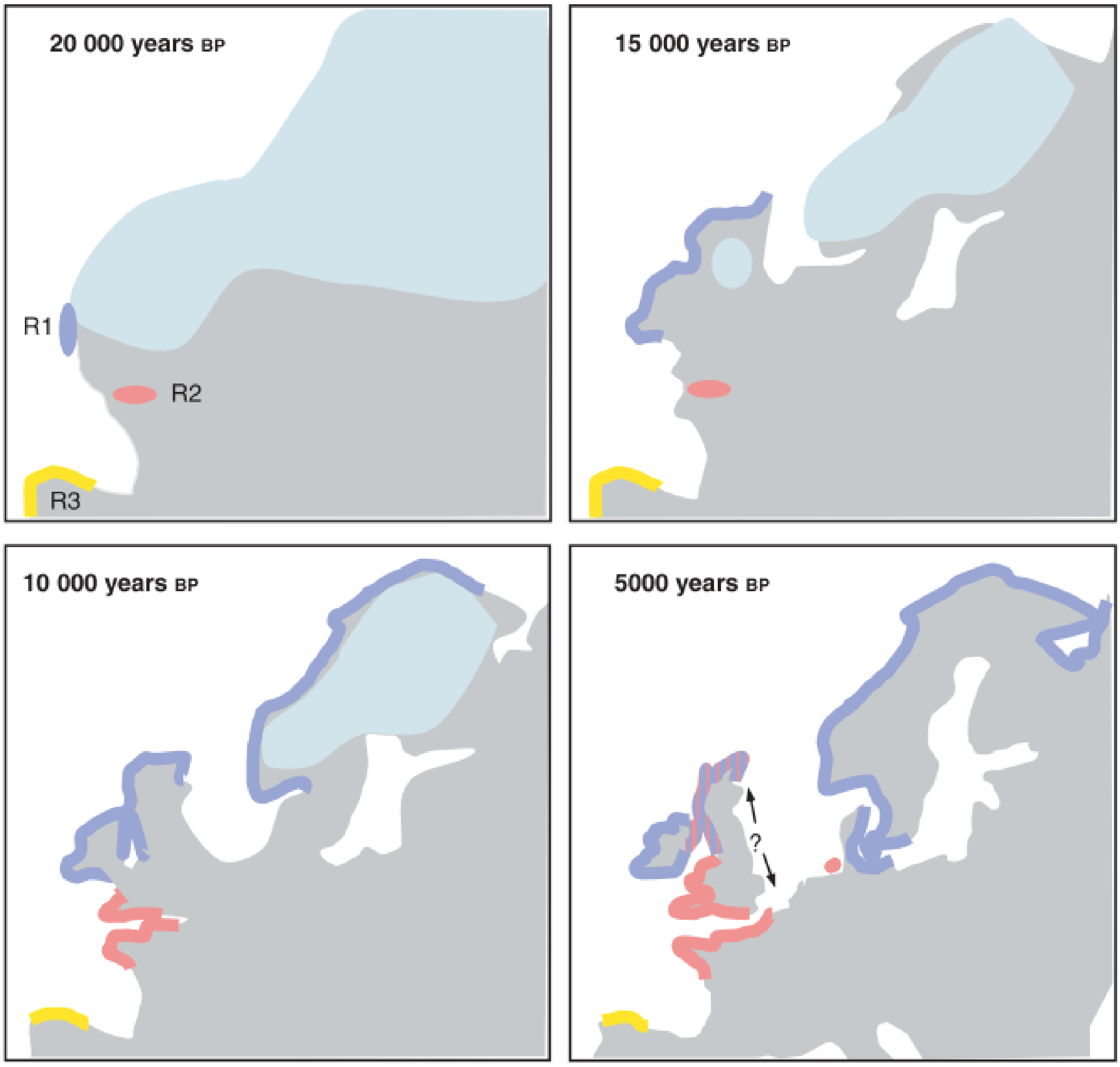


Figure 3: Identification of refugia during LGM for Fucus serratus. Hypothetical phylogeographic and putative recolonization is illustrated over time. Sampling along the east coast of the UK is still necessary to make reliable conclusions. Figure obtained from Hoarau et al. (2007).

The northern areas became for many organisms just recently accessible with suitable temperature ranges and ice-sheet retreat allowing them to expand their range. This leads to the fact that many populations of the same species show lower levels of genetic diversity, when these populations are further north in respect to other populations, which might inhabit former refuge areas (Assis et al., 2018; Neiva et al., 2012). Allelic richness for *Fucus guiryi* for example was found to be the highest in southern populations (Lourenço et al., 2016) in comparison to northern ones. In this case upwelling zones were identified as refuge areas and buffers for climate change. The diversity of mitochondrial DNA (mtDNA) haplotype data for *Fucus serratus* showed the same trend (Hoarau et al., 2007) of higher diversity in southern refugial areas. *Fucus vesiculosus* showed high mtDNA haplotype diversity in Brittany and the

English Channel, whereas the diversity was comparably low in for the Baltic Sea and the White Sea (Coyer et al., 2011). Moreover, *Pelvetia canaliculata*, a common brown algae, exhibited the highest haplotype diversity in its central distribution range and showed a consistent decrease along higher latitudes (Neiva et al., 2014). Even though there is much evidence that points towards this trend that Hewitt (1996) summarized as “southern richness and northern purity”, there are many other factors that should be considered. Firstly, surviving populations in northern regions might experience bottleneck processes and resultingly mimic newly colonized regions. Secondly, high diversity (in southern regions) could also arise from secondary contact and resultingly genetic exchange with other populations. Two facts that were mentioned within in former studies implicated that the LGM had severe impacts on the distribution of *Fucus* species: exponential demographic growth happened to correlate with interglacial periods (Hoarau et al., 2007) and major expansions of *Fucus serratus* and *Fucus vesiculosus* occurred during pre LGM interglacial periods (Coyer et al., 2011).

Other significant events in earth’s history are for example the opening of the Bering strait, which allowed marine species a trans-oceanic dispersal between the Pacific and the Atlantic. It is assumed that during the last opening of the Bering strait (5.5. – 5.4 MYA), ancestors of the Atlantic *Fucaceae* genera invaded the Atlantic through the Arctic (Coyer et al., 2006). A framework by Cánovas et al., (2011) displayed patterns of speciation for the genera *Fucus* and also showed that the timing of lineage splitting between *Ascophyllum* and *Fucus* lineages stands in relation to the shift of reproduction mode from hermaphroditic to dioecious. *Fucus distichus*, which precise phylogenetic position still needs further investigation, and *Fucus serratus* very likely diverged in the Atlantic or in the Arctic basin. It is assumed that *Fucus serratus, Fucus ceraniodes* and *Fucus vesiculosus* evolved in the East Atlantic, as well as the hermaphroditic complex. *Fucus serratus* was introduced in the West Atlantic about 150 years ago via shipping (Brawley et al., 2009). Yet, *Fucus spiralis* is hypothesized to have colonized the West Atlantic by natural means, as *Fucus vesiculosus* and *Ascohpyllum*, but is probably introduced as well at the Pacific coast (Coyer et al., 2006). So far, *Fucus guiryi* and *Fucus spiralis* also seem to have diverged in the East Atlantic.

As this extensive literature research implies, there had been done fundamental and crucial work in order to bring light on the phylogeny, the identification of the drivers of speciation and impacts of environmental factors that determine the distribution range of representatives of the genus *Fucus*.

1.4 State of the Art: Species Distribution Modelling

Species distribution models (SDMs, aka. niche models or environmental models) enable the identification of suitable environmental conditions through the incorporation of geographic data, which is two or three dimensional, and environmental data, that comprises factors in a multidimensional way (Elith & Leathwick, 2009; Raybaud et al., 2013; Rushton et al., 2004). Simultaneously with advanced geographic information systems, that were developed in the 1960ies to produce, analyse and manage geographic data in combination with advanced statistical technology, species distribution models (SDM) gained importance in the science community especially due to the need for protection of endangered species in order to implement future conservation measures. The usage of SDM also finds application in the management of undesired invasive species that have the potential to outcompete native species (Gallien et al., 2012; Václavík & Meentemeyer, 2009) and allows to effectively take actions. Instead of explanatory purposes for the current distribution of species, SDMs are nowadays more frequently used to predict the distribution of species in the future to see how climate change will structure the distribution of marine biodiversity. Regarding hindcasts, there is the prominent aspect of genetic diversity and in how far past climate changes mediated genetic diversity. In marine and freshwater environment SDMs found usage from the turn of the century on (Elith & Leathwick, 2009). Moreover, the outcome of SDMs depend on the mobility of the species of interest, the scales of space and time and the main purpose of investigation. In the case of *Fucus spiralis* and *Fucus guiryi*, the characterization of their environmental niches at large biogeographical scales will be relatively easy since they are sessile organisms although misidentifications with related species can occur.

The concept of the “ecological niche” is fundamental for understanding biogeographic distributions ranges of certain organisms in the present and past. By investigating the distribution range and abundances of a species, it already provides an idea about its optimum ecological niche. Suitable environmental factors, of both biotic and abiotic interactions have to fulfil distinct conditions to enable not only the persistence but also a successful reproduction of an organism. In macroecology a big-picture statistical approach is used that aims to uncover the relationship between complex ecological systems and the environment and therefore explains statistically patterns of abundance, distribution and diversity. Fundamental work in this area had been already done by several authors (Boltovskoy & Correa, 2016; Krug et al., 2018; Spalding et al., 2007; Longhurst, 2010) who defined biogeographic patterns of diversity and classified systems based on species composition, atmosphere-oceanographic forcing, hydrodynamics, hydrography, productivity and trophic connections or based on epipelagic waters and even Protista in the worlds ocean.

The concept of the ecological niche represents the basics of macroecology, was presented originally by Hutchinson (1957) and was redefined by Holt (2009). This ecological niche is a hypervolume that consists of multiple environmental variables where a species can survive and successfully reproduce. Key ideas had been developed in the past to analyse the relationship between species and the values of environmental gradients, where it was hypothesized that species responses to the environment are bell-shaped (Gaussian), equally spaced and of equal amplitude with their width restricted by competition or stress (Gauch & Chase, 1974; Whittaker et al., 1973).

Two terms are distinguished within the term “niche”. Firstly, the fundamental niche is the response of a species to the environment in absence of biotic interactions like competition, predation or facilitation, as well as historical and environmental constrains to colonize (e.g. dispersal barriers). The realized niche on the other hand includes environmental dimensions where the species actually occurs – i.e survives and reproduces - including the effects of biotic interactions. The realized niche is in most of the cases smaller than the fundamental one due to negative interspecific interactions, barriers, etc. The observation of organisms in the field are always corresponding to the realized niche which in the model is transferred to the illustration of the “potential” fundamental niche. The produced species distribution model in the end shows the probability of occurrence which is equal to the level of habitat suitability. To sum up, the distribution of a species is determined by 3 factors: biotic and abiotic origin and movement. A species can be absent from a suitable habitat due to dispersal limitations or irreconcilable boundaries. When populations go locally extinct in response to stochasticity, dispersal determines how fast the empty suitable habitats will be recolonized. Evolutionary speaking, younger species occupy less of their potential range extents than older species.

Niche conservatism is another fundamental concept that hosts the main assumptions regarding the impact of climate change of marine communities. Diversity on planet earth is generated by evolution, yet there has been mounting evidence for the tendency of conserving ecological traits by retaining ecological ancestral ecological traits over time, which is considered to be “niche conservatism”. Niche conservatism is the tendency of species to retain ancestral ecological traits which is based on the idea that the physiological tolerance limits will remain similar over time. According to this concept, the distributional limits of the species are shaped by the constrains of dispersal, the biotic and abiotic conditions of the environment and the failure of organisms to adapt to unsuitable conditions. Hypothetically speaking, every species could potentially be distributed everywhere, and spatial patterns of distribution and diversity would be absent or random if dispersal limits would not exist. Communities would become saturated with ecological similar species over evolutionary time and no other species could invade the ecological space because of competitive exclusion (Wiens et al., 2010) . However, because organisms show highly conserved niche characteristics (Peterson, 2011) over different time scales (from individual life spans up to thousands or even millions of years according to Peterson et al., 1999; Peterson, 2011) they are bound to suitable environmental conditions.

This maintenance of ecological characteristics eventually leads to populations shifts into more suitable areas. Species have shown to be able to adapt on increased temperatures by using heat shock proteins etc. however, especially in heterogenous habitats, natural selection serves as a conservative force on fundamental niches (Holt & Gaines, 1992) because rates of adaptation in environments outside of the fundamental niche are found to be slower than the extinction process. Rear edge populations also show inherent higher stress levels, overall reduced fitness and lower adaptive capacity relative to larger central populations making populations in the south extremely vulnerable (Pearson et al., 2009). This poleward shift was observed in many prior studies (Jueterbock et al., 2016; Jueterbock et al., 2013; Müller et al., 2009; Nicastro et al., 2013a; Raybaud et al., 2013; Southward et al., 1995; Takao et al., 2015) investigating the effect of global warming on marine species, which apparently shift faster (50 km per decade according to Helmuth et al., (2006) and Wethey & Woodin, (2008)) than terrestrial species (Parmesan et al., 1999; Parmesan et al., 2000) making them even more susceptible to global warming. To sum up, global warming serves as clear evidence for niche conservatism because without niche conservatisms species would persist locally while climate conditions shift. The existence of niche conservatism can be tested by comparing phylogenetic conservatism, which assumes that closely related species tend to share similar values for a given trait (aka. phylogenetic signal) or can involve species distribution modelling (Wiens et al., 2010).

For species belonging to the genus Fucus it is remarkably challenging to predict the distribution. Coastal habitats are highly heterogenous and are influenced by both terrestrial and marine factors. Especially tidal changes in certain areas that exceed for example several meters and variations in the microclimate (Monteiro et al., 2019) expose these organisms to high magnitudes of stress and elevate selection pressure. Rocky-shore organisms especially the conspicuous structural species that often show a vertical gradient driven distribution along the shore, are regarded as early warming indicators for the impacts of climate change (Helmuth et al., 2006). Due to the fact that *Fucus guiryi* had been just recently described as an own species in (Zardi et al., 2011), only 16 papers are published yet (according to Web of Science) that involve research on this organism where is it dealt with as an own species. In addition, it has been noted that *Fucus guiryi* populations were declining during the past decades (Riera et al., 2015) highlighting the need for research especially on these threatened populations.

A variety of climate projections for the future are nowadays available and represent a range of plausible pathways that are determined by human choices, emissions, concentrations and temperature change. The selections of which scenarios to use depend on the research question and the scale of investigation (time- and space-wise). Different “representative concentration pathways” (RCP) (Vuuren et al., 2011) were used in this study that were developed by research groups and are continuously reassessed and improved (Moss et al., 2010). These models display different scenarios in greenhouse gas emissions and land-use trajectories, which resultingly change the physical and chemical properties of the oceans and therefore determine species distributions. The produced RCPs describe radiative forcing levels of 2.6, 4.5, 6.0, and 8.5 W/m^2 by the end of the 21st century (Figure 4) of which the globally averaged mole fractions of CO_2 is reaching 421, 538, 670, and 936 ppm, respectively (Vuuren et al. 2011). Significant differences within all scenarios can be found from the year 2050 onwards.

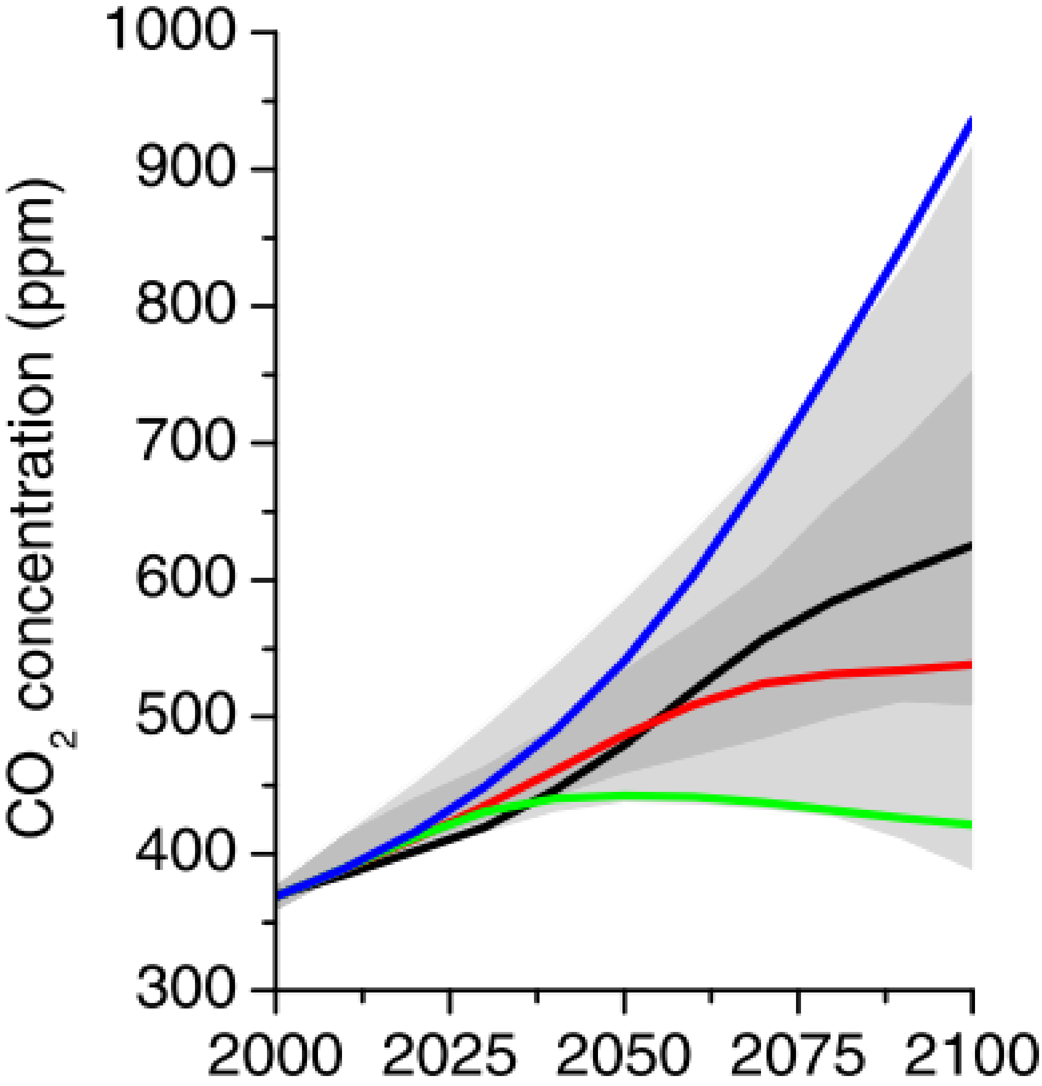


Figure 4: CO2 emission for the 21st century according to four different RCPs (RCP2.6, in green, RCP4.5 in red, RCP 6.0 in black, and RCP8.5 in blue), Graph extracted form Vuuren et al., (2011).

The most commonly used (Assis et al., 2018; Assis et al., 2016; Neiva et al., 2015; Takao et al., 2015) are the scenarios where greenhouse gas emissions are reduced substantially over time (RCP 2.6) according to Vuuren et al. (2007) or increase at an exponential rate – also described as "business-as-usual-scenario" (RCP 8.5) (Riahi et al., 2007). In the RCP 2.6, developed by IMAGE modelling team, the radiative forcing level first reaches a "peak"- value around 3.1 W/m2 mid-century, returning to 2.6 W/m2 by 2100. This peak and subsequently stabilization are represented in the two medium emission scenarios searching a peak in 2040 (RCP4.5) or later in 2080 (RCP6.0). However, to illustrate to most converse outcomes for the future in the year of 2100 the "best-case-scenario" with the lowest and the "worst-case-scenario" indicating the highest greenhouse emissions and therefore the most effects that global warming will have were chosen for this study.

In the past there had been numerous studies that investigate the biogeographical distribution of several *Fucus* species and brown macroalgae broadly speaking. Within these studies increasing temperatures as a consequence of global warming was commonly found to be the most important variable that determine the geographic occurrence of macro algae (Lourenço et al., 2016; Melero-Jiménez et al., 2017; Sanchez de Pedro et al., 2019; Takao et al., 2015; Zardi et al., 2015). This variable was found to have the most influence on poleward shifts that were observed in these studies (Assis et al., 2014; Jonsson et al., 2018; Neiva et al., 2014; Nicastro et al., 2013b; Southward et al., 1995). Lourenço et al. (2016) found a correlation between the persistence of Fucus populations in the southern range limits of Morocco and the Western Sahara and upwelling areas. This again highlights the importance and severe effect of water temperature on the occurrence and successful survival in the light of climate change of fucoid algae. Populations that were not found to be within these refuge areas had gone extinct. Maximum summer air temperature, minimum winter sea surface temperature (SST), maximum summer SST and tidal coefficients were predictors with more explanatory value for the high-intertidal seaweed *Pelvetia canaliculata* (Neiva et al., 2014). Apart from terrestrial variables such as long term average of air temperature of the hottest summer month, long term average of the air temperature of the coldest winter month and the long term average of the relative humidity of the wettest summer month also marine related variables were chosen for the model in the study of Assis et al. (2014) to predict the distribution of *Fucus vesiculosus.* They further identified the long-term average of the SST of the hottest summer month and the long-term average of the SST of the coldest winter month as distributional drivers. In addition, intertidal availability was one of the most important variables whereas tidal amplitude and ocean salinity were never chosen for the best model. Whilst Assis et al. (2014) excluded salinity from their model for *Fucus vesiculosus*, Jonsson et al. (2018) found out that "among variables that were expected to change in future scenarios, the reduced salinity in the future was the main driver of the change in distribution, while nutrients (nitrate and phosphate), water transparency (measured as Secchi depth) and other factors, like wave exposure had smaller effects".

Another study conducted in Japan (Takao et al., 2015) on the distribution of seaweeds under multiple climate change scenarios used the monthly mean SST in the coldest and warmest months from the years of 1960 to 2099 and SST-based indices to evaluate the effect of warming seawater in the spatial extent of suitable habitat versus unsuitable habitat for the temperate brown macroalgae *Ecklonia cava*. Herbivorous grazing pressure of *Siganus fuscescens* was incorporated in this research. The results differed dramatically in this study depending on the emission scenario used (RCP2.6 and RCP8.5). A poleward shift of *Ecklonia cava* was observed, where in the lowest emission scenario this seaweed would not be impacted as much by seawater warming directly but would be affected by intensified year-round grazing pressure. *Ecklonia cava* would experience both, an extreme loss of suitable habitat induced by increased temperatures and additional stress by grazing in the RCP8.5 scenario.

Jueterbock et al. (2016) investigated the impact on climate change on the arctic seaweed *Fucus distichus*, a congener of *Fucus spiralis* and *Fucus guiryi*. Their prediction was performed until the year 2200 and the main focus of this study was the investigation of possible niche overlaps with three dominant temperate macroalgae due to melting sea ice and the resulting availability of new suitable habitat for this species in higher latitudes. The authors used four uncorrelated variables: maximum SST and the concentration of calcite, nitrate and chlorophyll a. Especially the last 3 variables had not been found to be explanatory for the distribution of Fucus species in other studies and therefore were considered in the exploration of the environmental niche.

Another study by Assis et al. (2018) that used ecological niche modelling to predict the location of genetic diversity hotspots from distributional ranges during the LGM (20KYA), the Mid-Holocene (6KYA) and the present as well as the fate for the future in 2100 under two contrasting scenarios (RCP2.6 and RCP8.5.) in eight species of large brown algae that differed in their thermal tolerances (from the Arctic to warm temperate regions). Temperature – maximum winter temperature to be more specific – was the most important factor for these species. Neiva et al. (2015) applied variations of SST, air temperature, total precipitation and relative air humidity to investigate the distribution of the intertidal seaweed *Bifurcaria bifurcata*. In this case, air temperature and SST were the most crucial variables. The niche of the cold-temperate kelp *Laminaria hyperborean* is mainly shaped by extreme (warm and cold) ocean temperatures during winter months. (Assis et al. 2016). Long term maximum ice thickness of summer months, long-term lowest salinity of winter months and long-term ocean temperature of the warmest summer month were also used (next to the most important variables, namely long term ocean temperature of the warmest winter month and long-term ocean temperature of the coldest winter month) to investigate the distribution of this kelp.

In conclusion these studies already provided the basic knowledge on abiotic factors that shape the broad-scale pattern of distribution of seaweeds and these environmental variables will also be influential for the species complex of *Fucus spiralis* and *Fucus guiryi*. According to the examples described we can generally assume that the extremes of temperatures are the main drivers of the distribution where the poleward limits are set by the tolerance of extreme minimum temperatures and low latitudinal limits are mainly influenced by maximum temperatures. Mean or ranges of temperatures might not be as influential as extremes. Furthermore, depending on the extent of the study area nutrients might also play an important role as well as salinity, humidity, precipitation and chlorophyll concentration. All this knowledge was integrated when establishing a species distribution model for the *Fucus spiralis / guiryi* complex and lead to the final selection of variables (described in Methods).

1.5 Side Study: Cryptic long-term Persistence of Fucus during unfavorable Conditions?

A study carried out in 2016 by Lourenco et al. highlighted the influence of recent warming leading to scattered fucoid populations in the southern marginal areas by impairing reproduction for example. A population in Santa Eulalía (South Portugal) vanished totally within not even a decade, for unknown reasons but possibly related to short-term climatic conditions, long-term (mean and maximum SST) or extreme climatic events such as marine heat waves. Surprisingly, some individuals flourished again at this location in 2020. These were sampled in order to identify the source of the recruits after apparent local extirpation.
Firstly the new recruits could be received via cryptic, long-term dormant stages persisting in the area despite stressful conditions, or secondly via immigration from the South West coast of Portugal, Gibraltar, Morocco or another regions. Genetic data was available from populations before they apparently got extinct and individuals were analysed which are now present in this location. Especially because the genetic signature of these populations was different to adjacent populations it was possible to investigate the two mentioned hypothesis of recruit sources. This side study is highly interesting because the hypothesis of long-term dormant stages for Fucus species especially the more heat tolerant Fucus guiryi, had not been investigated previously. *Fucus guiryi* might be able to withstand years of chronic exposure to

stressful thermal conditions as cryptic stages or shows the yet unexplored capacity of long-distance immigration in (re)colonization.

2. Objectives

I will explore how past climate shaping, range shifts and potentially species interactions, such as hybridization and competition, have been affecting the evolution, biogeography and phylogeography of two sister species potentially capable of hybridization – *Fucus spiralis* and *Fucus guiryi.* The phylogeography regarding lineage I of the genus *Fucus* is well understood, however the extinction and colonisation dynamics of lineage II remain largely unknown. I am also interested in predicting the consequences of climate change for species biogeography and gene-pools. This thesis aims to clarify these gaps in the current knowledge. When *Fucus guiryi* was firstly described as a separate species rather than a morphological variation of *Fucus spiralis* the individuals that were described were sampled from Viana (North Portugal). The species however encompasses all the southern range populations (where *Fucus guiryi* and *Fucus vesiculosus* occur in allopatry) despite apparent differences in ecology, morphology and genetics. For these reasons, I hypothesize *Fucus guiryi* actually consists of two entities, as suggested in previous studies (e.g. Coyer et al. 2011).

A genetic diversity and differentiation analysis will be applied, using microsatellites and mitochondrial DNA for assessing the influence of historical and ongoing processes in explaining species distributions, range limits and gene-pools. Examples include the hypothesis of potential recent colonisation by *Fucus guiryi* in marginal habitats and even in not yet recorded areas, such as the coastlines of North-West America. Moreover, the potential role of hybridization at local and biogeographical scales is of interest, where I hypothesize that hybridization is rare but occurs at least in some areas. Both, large spatial and temporal scales will be taken into account, since the last glacial maximum, had a dramatic influence on the distribution patterns of many marine species of today.

Furthermore, I hypothesize that distinct (unique) alleles will be present in more isolated populations and in persistent areas. With the genetic data that I will receive throughout the laboratory work it may enable me to identify the phylogeography within the *Fucus spiralis/guiryi* complex and track signatures of past and ongoing hybridization. I eventually intend to relate the genetic structure to refugia that had been previously identified and estimate the effects of the LGM on *Fucus spiralis* and *Fucus guiryi* relative to other species of *Fucus spp.*. Especially in the North West Iberian Peninsula I expect to find higher haplotype diversities and potentially even the presence of endemic haplotypes given that this area reflects refugium for other marine macroalgae during the Pleistocene era (Hoarau et al., 2007). I also hypothesize that this area may show evidence for hybridization between different entities.

In addition to the genetic methods, species distribution models (SDMs) will be used to investigate the potential and realized exploitation of the ecological niche of *Fucus spiralis* and *Fucus guiryi* respectively, as well as differences between these sister species related to niche conservation. This study also aims to reveal potential connectivity patterns of *Fucus guiryi* and tries to get a holistic approach through understanding its reproduction dynamics, life history, and ecological niche in relation to its sister species and sometimes sympatric *Fucus spiralis*. The main expected result of this study is to get a broader picture of the actual global distribution of each species and analysing the potential habitat for these species. The potential habitat gains or habitat loss for the future will be investigated under two contrasting climate change scenarios (RCP2.6 and RCP8.5). Following results from previous gained knowledge I hypothesize that populations dealing with elevated temperatures due to global warming will show a poleward shift. Especially those populations in the south will experience a decrease in suitable habitat and populations in the north will gain suitable habitat in the future. I will investigate to what extent the populations will shift in the future, which populations will be affected the most and are likely to disappear and which populations might have a greater probability to survive in the southern regions. The most important variables that are responsible for determining and limiting the distribution range of this species complex will be identified and the possible differences between the ecological niche of *Fucus spiralis* and *Fucus guiryi* will be analysed.

In conclusion, I aim in this thesis to provide insight into the evolutionary history of the recently diverged intertidal macroalgae *Fucus spiralis* and *Fucus guiryi,* with the tools provided by molecular methods and additionally integrate these findings on a macroecological scale, in order to predict the fate of these species and gene pools under different climate change scenarios.

3. Material and Methods

3.1 Laboratory Work

To start with this project a list was analysed, which included all samples that are available in the lab. The data was sorted, and populations were mapped. This process and visualization of the data was done to help identifying relevant populations for this study. *Fucus* species tend to have very low diversity within each site, as common in selfing hermaphroditic fucoid algae (Billard, et al., 2005; Engel et al., 2005; Perrin et al., 2007) but can be fixed for different alleles across nearby sites and even distinct genetic groups in different locations/habitats in the same site. For this reason, we choose to genotype individuals from many distinct locations but starting with a relatively low number (4 individuals) per site to assess if this is the case. Samples were collected since 2000 and were stored in plastic bags on silica gel. This desiccant ensures a long-term storage and proved to sufficiently maintain the integrity of the DNA of plant material (Chase & Hills, 1991). The main criteria in choosing populations was to capture the overall distributional range of the species.

3.1.1 DNA Extraction

The genomic DNA was extracted from the selected samples with the NucleoSpin® Plant II kit. 10mg of lamina tissue was used and is it was assured that the tissue was sufficiently dry. If that was not the case, the samples were lyophilized. The samples were homogenized by vigorous shaking using a steal bead of 3 mm diameter. Cells were lysed by adding a buffer in combination with RNAseA. After incubating the samples at 65°C for 30min the samples were cleared by centrifugation. The supernatant was further processed and was transferred to the Nucleospin ® Plant II Binding Plate and the DNA was bound to the silica membrane by centrifugation. Finally, the samples experienced three phases of washing to make sure that excessive non-DNA residues were removed. By adding a mixture of elution buffer, which was preheated for 2 minutes at 70°C the final DNA extract was eluted and stored in the freezer.

3.1.2 Application of Mitochondrial and Microsatellite Markers

For all polymerase chain reactions (PCRs) dilutions plates with a DNA concentration of 1:100 were prepared with the priorly gained DNA extract. The 500-600 bp region of a mitochondrial intergenic spacer (mtIGS) was sequenced using the developed forward and reverse primers (F 5’ CGTTTGGCGAGAACCTTACC; R 5’ TACCACTGAGTTATTGCTCCC) by Coyer et al., (2006). All PCRs were conducted with reagents provided by the GoTaq® G2 Flexi DNA

Polymerase kit. The PCR reactions (20µl total volume) for this analysis contained 6.95µl of H2O, 4µl 5x GoTaq ® Flexi Buffer, 1.6µl MgCl2 solution (25mM), 1.25 µl of dNTPs (8mM – 2mM each), 0.5µl of forward and reverse primer (10mM) each, 0.2 µl GoTaq® Flexi DNA Polymerase (5µl/µl) and 5µl DNA dilution (1:100). PCRs were performed with an ABI 2720 Thermal Cycler. The protocol used started with an initiation denaturation step of 94°C at 5min followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 50°C for 30 sec, elongation at 72°C for 1 minute and a final extension to make sure any remaining single stranded DNA is fully elongated. The sample temperature was brought down to 4°C and the samples were ready for further analysis. These amplification products were sequenced in an automated capillary sequencer (applied Biosystems) at the Centro de Ciencias do Mar (CCMAR Portugal).

For the analysis of microsatellites, loci were chosen in respect to recent studies that were found to be phylogenetically informative for *F.spiralis* and *F.guiryi*. Especially the makers L20, L78, F21, F34, Fsp1, F9, F36, F42 seemed to be highly informative on both, distinguishing between *F.spiralis and F.guiryi* and the population structure within this species complex. The combination of L78, F21 and F34 allows to determine members of each species where other microsatellite markers (e.g. L20, F9, F36) on the other hand show a higher allele diversity within the populations rather than on a species level. In order to gain genetic results in a cost and time efficient way, some microsatellite markers were amplified as a duplex (F21 + F34 and F9 + F36) whereas the remaining loci were amplified in simplex reactions. The PCR mix for the markers consisted out of 4.45 µl H2O or 3.7µl H2O for a simplex or a duplex mix respectively, 3µl of 5x GoTaq ® Flexi Buffer of buffer, 1.2 µl MgCl2 (25mM), 0.5µl dNTPs (8mM), 0.25µl forward primer (5uM) tagged with a fluorophore, 0.5 µl of reverse primer (10uM) for each marker, 0.1µl Taq and 5µl DNA dilution making a total volume of 15 µl. The protocol for all the markers differed slightly in the annealing temperature for each specific primer (Table 1). All markers followed the same protocol (5 min at 95°C, 30 sec at 95°C, 35 sec at Ta, 40sec at 72°C and 72°C for 20min for 30 cycles) except the PRC protocol for the marker Fsp1 (5 min at 95°C, 30 sec at 95°C, 15 sec at Ta, 30 sec at 72°C and 72°C for 10min for 35 cycles).

Table 1: Markers that were used for genotyping the individuals with the corresponding, primer sequence, repeat array reference and annealing temperature (Ta).

Locus	Primer Sequence (5’-3’)	Repeat array	Reference	Ta (°C)
Fsp1	F: TCAAAAGCCAGCAGGGGTG R:TCTTCTGGGAGCTGTAAAATAGTC	(AG)11	Perrin et al. (2007)	56
L20	F:ACTCCATGCTGCGAGACTTC R: CCTCGGTGATCAGCAATCAT	CTGG(CTG)8(TTG)3CTT(CTG)2	Engel et al. (2003)	54
L78	F: CGTGAGGGCAGGAATGTC R: GATTTCCGGCATCATCAATC	(TGC)11TGT(TGC)2	Engel et al. (2003)	55
F9	F: GGCGGAAGTCGATTTGAATA R: ACTTGGCTGACGTCCAGAAT	(GT)16	Coyer et al. (2009)	55
F21	F: CATGTAGCGTGAAGCGTTTG R: CACGCAAACAAAACGTCAAC	(TG)15	Coyer et al. (2009)	55
F34	F: TGCCGAAGTACCGCATCTAC R: CTCCACTGGCATGCTGTTTA	(TG)6	Coyer et al. (2009)	55
F36	F: TTTGCGGGATTGAAAGAGAG R: CCAGAATGGATGGGAAGAAA	(TG)8	Coyer et al. (2009)	55
F42	F: AGTGTGACTGCCCATTAGGG R: AGACGTAACCCAGTGCTGCT	(AGC)5	Coyer et al. (2009)	55
mtIGS	F: GTGCAAGAGCTGCGAAGTTT R: CCCAAATGTAGGCGTATTGG		Coyer et al. (2006)	58

The DNA extraction and PCR products were always checked via gel electrophoresis for sufficient quality. However, in some samples degradation was observed making it not possible to be amplified/extracted. Furthermore, if the PCR products were not visible enough in the gel and if the amplification did not seem to be successful enough the PCRs were repeated with an adjusted dilution (1:10 or 1:50) or a higher number of cycles. In some cases, the annealing temperatures was also altered (lowered) in order to gain a less stringent but a more successful result.

3.1.3 Genotyping

After PCRs were performed and showed sufficient amplification corresponding samples with different markers were merged for the genotyping processed. The concentration of each marker was depended on the intensity of the band, which was priory assessed with agarose gel electrophoresis. 1µl of the PCR-Marker-Mix was added to a mixture of 0.25 µl of a size standard (LIZ500) and 9.75 µl of formamide. The samples were processed in a thermocycler at 95°C for 5 minutes which assured that the samples were separated before running in an ABI PRISM 3130xm (Applied Biosystems, CCMAR,Portugal) automated capillary sequencer.

3.2 Genetic Data Analysis

mtIGS sequences were aligned, proofread and edited with the software GENEIOUS 4.8 (Biomatters; http://www.geneious.com). Through these alignments the nucleotide polymorphisms were detected. These alignments were used to produce haplotype networks with the software PopArt, which provides a number or algorithms to illustrate the phylogenies of the species of interest. A Median Joining network (Bandelt et al., 1999) was used in this study.

The produced multi-locus genotypes (MLG) for all individuals were obtained by screening the chromatograms. Alleles were manually scored with the software STRand (Veterinary Genetics Laboratory, University of California, Davis; http://www.vgl.ucdavis.edu/STRand) binned and checked for discontinuities using the R package MsatAllele (Alberto, 2009). The package standArich (Alberto, 2006) was applied to standardize the populations sample size and comparing allelic richness (Â) among populations.

Patterns of differentiation and diversity in space were investigated, and accordingly the levels of similarity between populations, to infer patterns of connectivity, hybridization and signatures of past range-shifts were detected with GENETIX (Version 4.05, Belkhir et al. 1996 -2004). Expected heterozygosity on a species levels within specified regions was as well calculated with GENETIX. The R package Adegenet (Jombart & Ahmed, 2011) was used to delimit species by clusters through the iterative K-approach, to perform principal components analysis (PCA) (Hotelling, 1933) and a discriminant analysis of principal components (DAPC) (Jombart et al., 2010). A PCA itself lacks essential features for investigating the genetic structure of biological populations because it does not provide a computational group assessment. A PCA simply aims to summarize the overall variability among individuals, which include the divergence between groups and the variation occurring within groups. A DAPC retains all assents of a discriminant analysis (DA), which maximizes between-group components while minimizes/neglecting within-group variation. A DAPC is using a sequential K-means approach by applying a multivariate method to infer clusters based on a Bayesian Information Criterion (BIC). The lowest BIC for a number of clusters is supposed to determine the number of clusters.

To see how and if this groupings will be supported by another analytical approach, the MLG data was analyzed with the software STRUCTURE (Pritchard et al., 2000). STRUCTURE is a model-based clustering method that uses a Bayesian approach (Markoc Chain Monte Carlo - MCMC) and detects the underlying genetic populations among a set of individuals genotyped at multiple markers. The parameter settings to run STRUCTURE were defined with a length of Burnin period of 500.000, the number of MCMC repetitions being 1.000.000, assuming an ancestry model of admixture and correlating allele frequencies among populations. Additionally, different Fst values for different subpopulations were assumed. The model assumed K=1 to K=10 with 10 independent runs for each K. The variability between the different number of K was assessed and taken into account when interpreting the data. To investigate hybridization on a local scale and the integrity of species across latitudinal and vertical gradients we produced additional plots with selected sub-sets of data.

The output of STRUCTURE was analysed via STRUCTURE HARVESTER (Earl & Holdt, 2012), which is an online website that enables the visualization of results provided by STRUCTURE and allows a fast analysis of a large number of replicates over many successive values of K. STRUCTURE is in comparison to a DAPC more computer-intensive, yet it shows many other functions to explore the data. Finally, the congruence between mtDNA- and microsatellite- based delimitation of species was compared.

Additionally, packages in R (R Core Team, 2019), such as pegas (Knaus & Winter, 2020), diveRsity (Keenan, 2017) and hierfstat (Jerome, Archer, Hardy, & Goudet, 2020) were used to analyse potential long-term persistence areas via calculating the nucleotide diversity, allele richness, and the mean number of alleles for each entity in each geographic region.

3.3 Species Distribution Modelling

All analyses were performed in R. In combination with a variety of packages such as dismo, ENVMeval, SDMtune and raster the basic functions and analysis or species distribution modelling were performed. Furthermore, the packages leaflet, leaflet.extras, rgbif and robis were used to obtain occurrence data.

3.3.1 Occurrence Records

Two different models were developed: one for *F.spiralis* and *F.guiryi* together and another for a new entity inferred from genetic data (see results). The occurrence records for the former mentioned were obtained from several citizen science platforms such as the Global Biodiversity Information Facility (GBIF), the Ocean Biogeographic Information System (OBIS) and iNaturalist. These platforms provide a great amount of data that contributes to species distribution modelling. The genetically verified data could have been used for the model of *F.spiralis* and *F.guiryi*, yet these citizen science platforms firstly represented the distribution range better than the available genetic data and secondly was present in greater numbers or records. Records of the new entity were restricted to genetical verified data set because no occurrence records under this name exist. Due to the fact that there might be a high degree in uncertainty regarding the correct identification that is only based on the morphological traits of the species *F.spiralis* and *F.guiryi*, all records that were appearing under the name “Fucus spiralis” and “Fucus guiryi” respectively were merged together – because the model was anyway developed for both species. These records also included observations of *Fucus spiralis var. platycarpus* for example which is now considered to be a taxonomic synonym to *Fucus guiryi*. As already mentioned, many records that occur in the southern extends might have been falsely identified as *Fucus spirals* where in fact the species is *Fucus guiryi*. Records were processed by the following cleaning steps. Firstly, the longitudinal and latitudinal coordinates of all records were extracted and the ones that did not provide exact coordinates were deleted. Duplicates and records that were outside the known distribution range were as well removed. *F.spiralis* and *F.guiryi* are intertidal macroalgae which implied the necessity that records that are supposed to be on land had to be relocated to the closest marine surface. This means that some records were considered to be on land and therefore would not get a marine environmental variable assigned which resultingly would cause errors in the later modelling process. The relocation distance was 9km, which corresponds to one raster cell and seems to be a reasonable distance to still gain non-biased results. Finally, all the remaining records were checked on the correct vertical distribution.

This step was done by extracting the depth of each record from a bathymetry raster layer. The fact that some occurrence records were found to be outside of the known distribution range (below tidal ranges) was related to a resolution problem within the 9x9 km^2 raster cells. Due to very steep coastal shelfs, for example on the Azores, the mean depth was far below the intertidal zone even though the occurrence record is still found at the coastline. Therefore, all records were still used for the model. One approach that is commonly used to avoid this problem is clipping the environmental layers to the known vertical distribution of the species. However, this is also not exactly ideal considering the variability of environmental factors throughout the global ocean and the different bathymetric characteristics of the coasts as it is here the case. Furthermore, within a resolution of 9x9 km^2 used in this model the bathymetry can also change quickly to more shallow areas and in the end not affect the final model.

The final step to gain the best model possible was to minimize spatial autocorrelation. Spatial autocorrelation is challenging for species distribution modelling and often not taken into account leading to a poor output of the predictive power. The phenomenon of spatial autocorrelation represents the disagreement of datasets and the real occurrence, which in so many cases simply represent the sampling afford of a species. The environmental values sampled at nearby locations to the occurrence records are therefore not independent because they correlate with each other and are overrepresenting the distributional range in this area. In order to get evenly distribution occurrence records a correlogram was produced, which assesses the correlation of each variable predictor within a range of geographic distances. The minimum non-significant autocorrelated distance (significance level of test = 0.05) is determined by testing the effect of correlation with geographic distance for each distance class. Eventually the average of the minimum non-significant distances found per environmental variable used in the model, prunes the occurrence records and leaves only one records within such calculated distance.

3.3.2 Environmental Data

Environmental data was extracted from Bio-Oracle (Assis et al., 2018; Tyberghein et al., 2012). The data is derived from remote sensing data and showed a resolution of 9x9 km^2 (equals one cell size or raster layers). Most of the Bio-Oracle layers were measured in the time from 2000 until 2014 and were received from several sources: NASA's Ocean Color Web, Copernicus Marine Environment Monitoring Service and Nasa Earth Observations (NEO).

The environmental variables were chosen according to three criteria: (1) the ecological relevance for intertidal Fucus, (2) published SDMs dealing with intertidal macroalgae or fucoids and (3) the availability of environmental layers for future climatic scenarios. Finally, the gathering of relevant biodiversity and environmental data and assessing its adequacy, the decision on which correlated variables to exclude and selecting the appropriate modelling algorithm provided the basis of the following model.

By running the model several times with a preselection of environmental variables extracted from the literature that might be influential to the model it revealed that the most important variables were SST and air temperature. Every other variable (nutrients and chlorophyll concentration, etc…) had a relatively low contribution in the model and was therefore were not included for the model. As it is desired to decrease the complexity in predictive models only SST (maximum and minimum) and air temperature were selected for developing the final model regarding the present distribution. For the model that was used to predict future range shifts, only SST was used, because firstly of its important indicated by prior studies and secondly data on the future climate scenarios on air temperature was not available.

The environmental layers were cropped to the study area and the coastlines in order to match the intertidal distributions of the Fucus species complex. Background information (n=10.000) was generated by randomly selecting points that would still be within the potential suitable habitat meaning along the coastlines. The extent in which these background points were created reached from the most southern to the most northern ranges within the northern hemisphere that were defined by the occurrence records gathered previously. With this background information, the environmental values were extracted according to the occurrence records and a data frame was created that comprises pseudo-absence data. Cross-validation was used to train a Maxent-model where the folds used to gain independent data sets for this fitting were 4 different blocks.

As a next step all possible combinations of regularization hyperparameter values were tested, where the one with the highest test score was chosen. The model was fitted to this chosen hyperparameter value (reg = 1) and the relative variable contribution was determined.

The variables were reduced where the relative variable contribution was determined by fitting the model with and without each variable. This determined the potential increase in model performance. Without an important variable a model should reduce performance. In the end a threshold of the predicted habitat suitability was determined that lead to maps, which identified suitable and non-suitable habitat. Further details on the modelling procedure can be found in Supplementary 1.

Environmental values of temperature and salinity were extracted from the occurrence records, that were gained to identify potential significant differences in the environment within each scenario. Temperature was the main focus because it showed to be the most influential factor regarding the distribution of macroalgae and secondly because temperatures showed to have the most drastic increase within the future. The mean and change for the present and each emission scenario were calculated as well as the minimum and maximum values illustrating in a broad sense under which temperatures the species complex is commonly occurring.

To see if there might be a significant difference in the environmental use between the genetically verified clusters the variables that were found in the literature to be influential on the distribution were tested for significant differences between entities. The variables that were screened were air temperature (min. and max.), SST (min. and max.), salinity at the surface (min. and max.), precipitation, chlorophyll concentration (max and min), cloud cover (min. and max.) and nutrients (phosphate, nitrate and calcite). Furthermore, the set of the aforementioned variables was collected for each location of the genetically verified data and the differences of the averaged values among groups was compared in a matrix. A multidimensional scaling plot was also performed to investigate potential niche clusters/differences among entities.

4. Results

4.1 Genetic Data

4.1.1 Sampling and Genetic Analyses

Overall 876 individuals of Fucus spp. were analysed from in 130 different locations across the entire range of the species complex, including the North-East Atlantic (n= 820) as well as the Atlantic (n= 28) and Pacific coast (n= 28) of North America.

4.1.2 Mitochondrial DNA Phylogeography

The mitochondrial intergenetic spacer (mtIGS) produced four haplotype groups (Figure 5), which are spread across the known distribution range and will be referred to as such in the following: *F. spiralis* (cycled in green, previously referred to as *F. spiralis High*), *F. macroguiryi* (in blue, previously referred to as *F. spiralis Low*) and *F. guiryi* (red, previously referred to as *F. spiralis-South*). Finally, samples from Azores also produced a separate family of haplotypes, that we referred to as “Azores”. The mtIGS sequences of the Azores were separated from the rest of the *F.gruiyi* haplogroup by at least 4 mutation (in light-orange).

The *F.spiralis* haplogroup was the most common/frequent one. It consisted out of 26 haplotypes arranged in a star-shape network. Most of the haplotypes surrounding the core haplotype were separated by one or sometimes two mutations. This haplotype group was mainly present in Iceland, Scandinavia, the UK and Ireland and as well along the coasts of France, and even on the South West coast of Portugal. It was the only haplogroup present along the East and West coasts of North America.

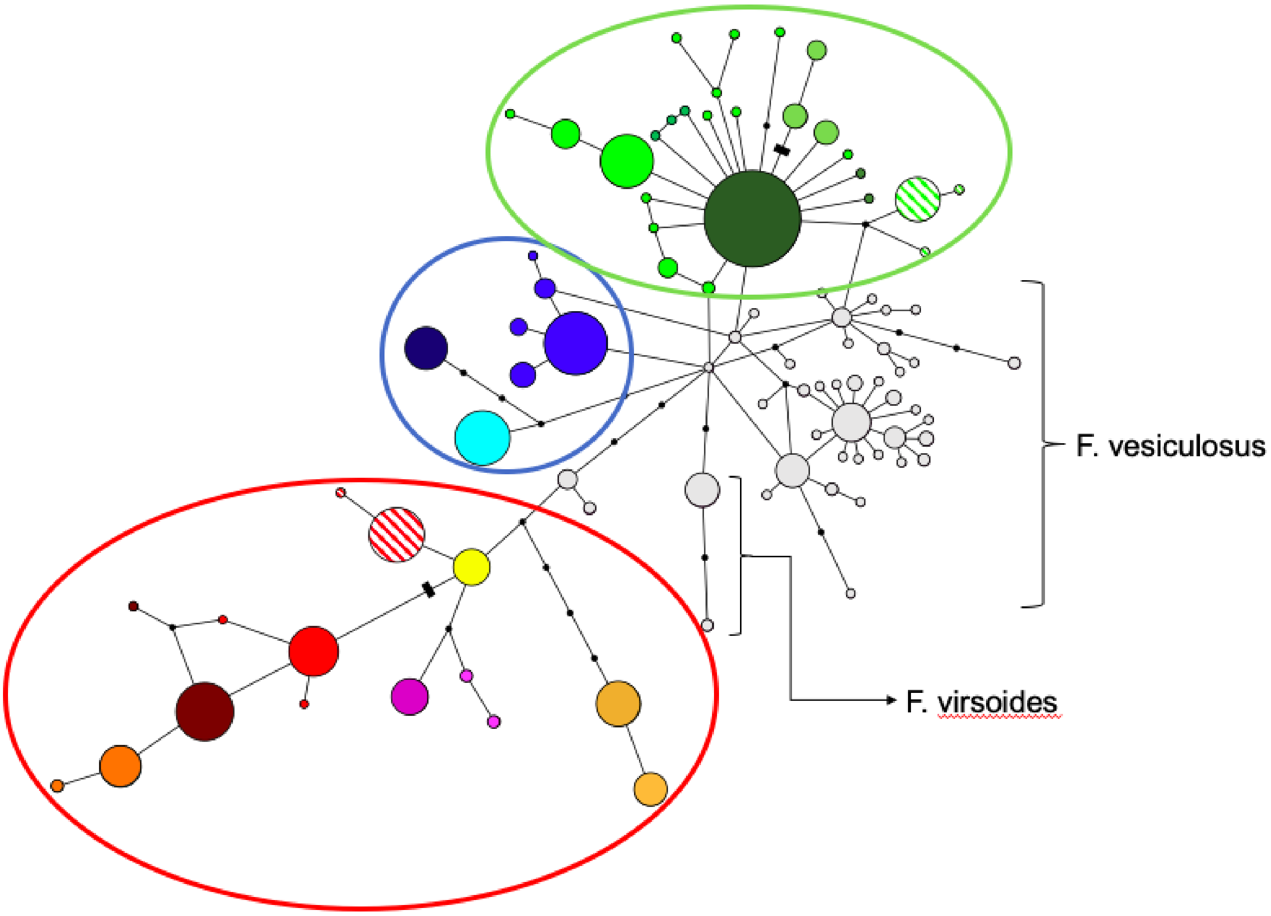


Figure 5: Median joining mtIGS haplotype network of Fucus illustrating F. spiralis (northern entity) in green, F.macroguiryi in blue and F. guiryi (southern entity) in red. The haplotype network was produced in PopArt. The size of each single haplotype corresponds to the number of individuals/sequences. Smaller grey circles represent F.vesiculosus and F.virsoides (from the Mediterranean) respectively, which were used as a reference in order to investigate the relative position of each entity to one another. Each black dot represents one mutation step of the 500 bp sequence and black bars represent indels.

The southern haplogroup (*Fucus guiryi(mt)*) seems to be very unique and quite separated from the other two entities by at least five mutations. By comparison, *F.spiralis(mt)* and *F.macroguiyi(mt)* are in some cases just two mutations apart. *F.guiryi(mt)* was distributed in North West Africa along the coasts of West Sahara and Morocco, in South West Portugal as well as in one isolated pocket in the North of Spain (Asturias). Within this southern haplogroup, two haplotypes found at the Azores (in light-orange) represent their own very distinct clade. Furthermore, the haplotypes that were found from the Gran Canaria island were as well very characteristic and even separated by an indel from almost the entire other southern haplotypes.

Finally, *F.macroguiryi* haplogroup comprised seven different haplotypes and occurred at two locations in central Morocco, in North West Iberia, in France and in the South of the UK. The network showed that this entity is clearly differentiated from *F. guiryi(mt)* (with whom it is currently synonymized) and actually implied a closer relationship with *F. spiralis(mt)* and *F. vesiculosus(mt)*. However, to get more into detail regarding the distribution of each haplotypes and the potential distributional ranges of the suggested entities were mapped geographically.

F.guiryi haplogroup (Figure 6A), described in prior studies as "Southern *F.spiralis* entity" or as "allopatric F.guiryi" was, as the name already suggests, found in the most southern distributional ranges of this species complex, having its known southern limit around Ad-Dakhla, West Sahara (DAK). This entity was also detected in populations of Tarfaya (TAR), Essaouira (ESS), Plage Val D'or (Val) and partly in Oualidia (Oua). On the Canary Islands only one single haplotype was detected being very particular as priory mentioned. Regarding the Azores (Figure 6B) only one of the two haplotypes were found in Faial, Pico and Terceria, whereas on São Miguel both haplotypes were present.

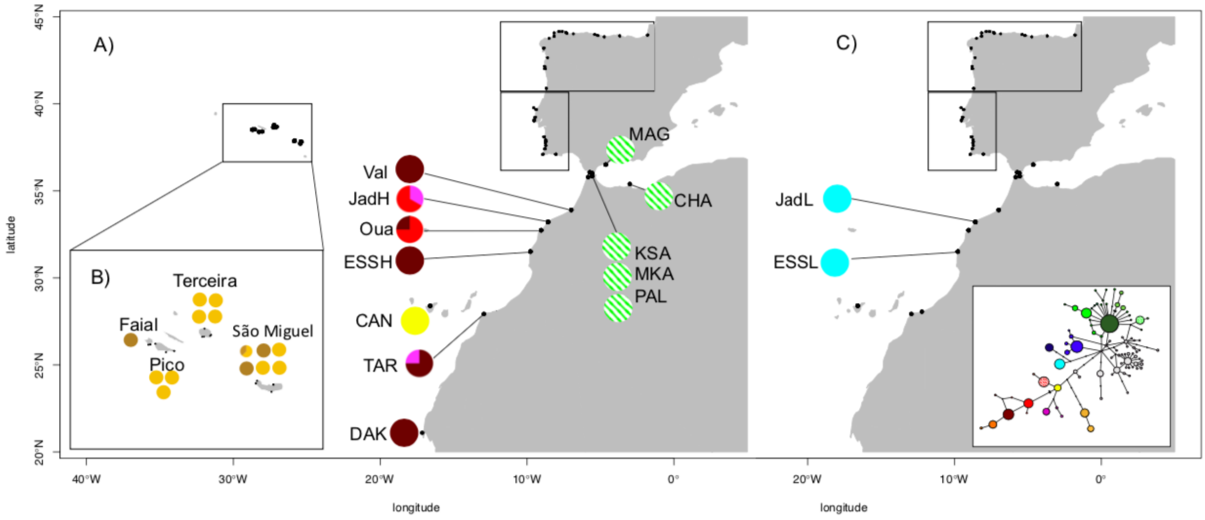


Figure 6: Phylogeography of Fucus sp. along Morocco, Gibraltar and Macaronesia. The left map shows F. spiralis and F.guiryi haplotypes (A) including the Azores (B), the right map indicates the presence and haplotypes of F.macroguiryi (C). Cut outs of the South West coast of Portugal and North West Iberia are illustrated more detailed in the following. An inset of the mtIGS network is shown in low left corner. Note that also in the following, samples that end with a capital H or L (e.g. JadH and JadL or ESSH and ESSL) indicate that the vertical sampling position along the shore was known (H= High and L=Low).

Individuals belonging to the *F. macroguiryi(mt)* (Figure 6C) were restricted to Essaouira and in El Jadida in central Morocco.

Interestingly, one haplotype of the northern haplogroup (*F.spiralis(mt)* in green striped) was found at the strait of Gibraltar at three different populations (PAL, MKA, KSA) and even the nearby populations inside the Mediterranean - one in Spain in Malaga (MAG) and another one in Morocco at Charrana Beach (CHA). None of the adjacent populations share this distinct one.

F. guiryi haplotypes were also found along the South West coast of Portugal (Figure 7). The same haplotypes partly found in Oualidia (Oua) and El Jadida (JADH) (red) were also present in Vila Nova de Milfontes (VNMF), Azenhas do Mar (AZE) and some individuals of Cabo de Sines (SINH), which were located in the upper intertidal zone. In Peniche (PenH and PenL) *F.spiralis* and *F.guiryi* haplotypes were present at low and high intertidal positions, respectively. Even the same locations containing both, *F.spiralis* and *F.guiryi* mtDNA could be detected (e.g. LIZ, RIh, ZAM and SAG).

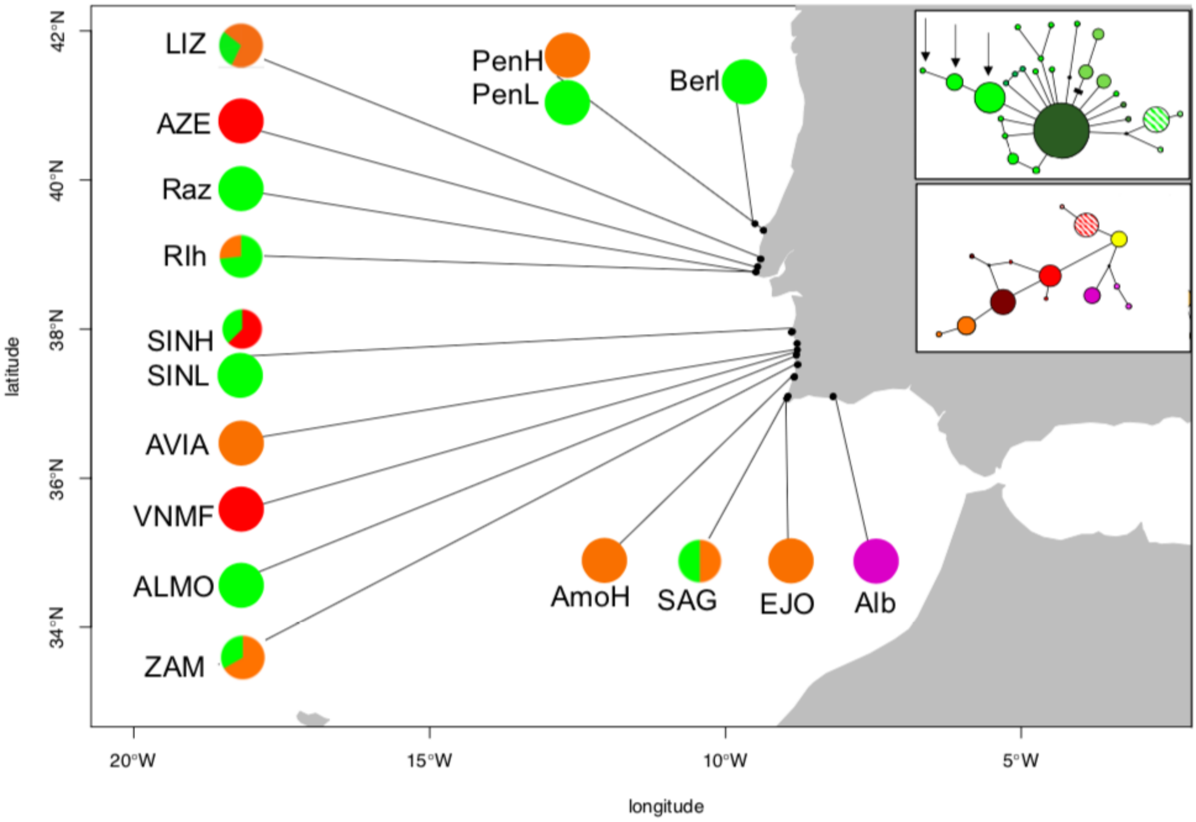


Figure 7: Phylogeography of Fucus sp. along South West coast of Portugal with its northern (F.spiralis(mt)) and southern (F.guiryi(mt)) entities. Corresponding haplotype networks in the top right corner for F.spiralis(mt) (upper) and F.guiryi(mt) (lower) network. Arrows in the F.spiralis network indicate the haplotypes displayed in the map.

Mitochondrial data of the lower situated population in Amoreira (AmoL) could not be obtained. In any case, the data clearly establishes the limits of the *F.spiralis* mtDNA in Sagres (SAG). Moreover, the South of Portugal (including the strait of Gibraltar) seems to be the new southern boundary of the *F.spiralis* haplogroup, which was priory assumed to be further north in Viana do Castelo (ViaH and ViaL respectively). No *F.macroguiyi* haplotypes were found in this area. The very unique haplotype that was fixed in Albufeira populations (in purple) before its putative extinction was identical to the haplotype found in the samples collected from this year of 2020.

Unexpectedly, an outstanding pocket of *F guiryi* haplotypes was also found in Asturias, a region in the north of Spain (Figure 8A). These four unique populations (Cas, PeiH, Pcast, Rib) all possessed the same unique haplotype (red striped) and were not present anywhere else in the surrounding geographic areas – a similar case as reported above in Gibraltar for mtDNA of *F.spiralis*. These populations define the most northern limit of the *F.guiryi* haplogroup in a region otherwise dominated by *F.spiralis(mt)* and *F.macroguiryi(mt)*.

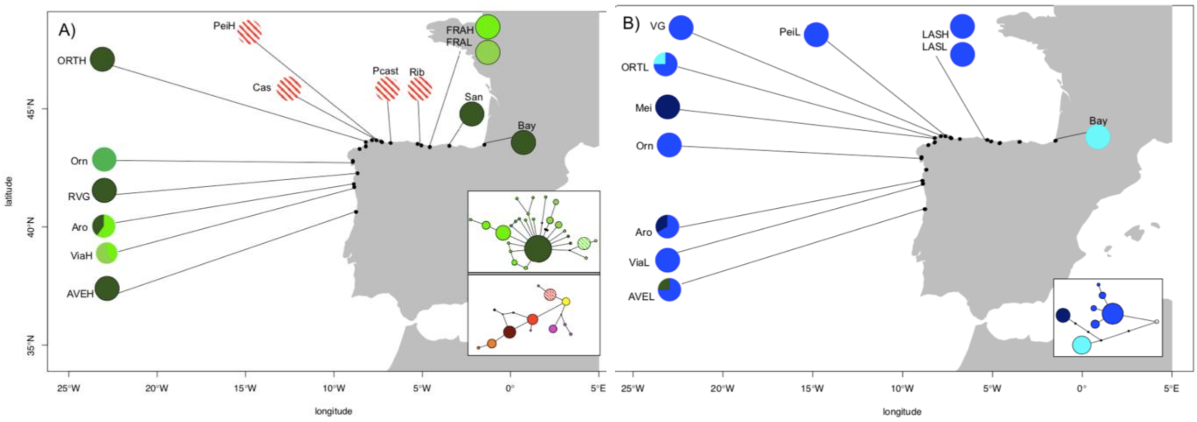


Figure 8: Phylogeography of Fucus sp. along North West Iberia of F.spiralis and F.guiryi (left) and F.macroguiryi (right). Haplotype networks are shown as insets in the respective maps.

In Peinzás (PeiH) the population, which belongs to the southern entity occurs in sympatry with other *Fucus* populations on the lower shore corresponding to *F.macroguiryi(mt)* (PeiL). Unlike in the previously described areas, in NW Iberia *F.macrogiuryi(mt)* is highly abundant in an almost continuous pattern (Figure 8 B). Praia do Rio Meirás in Galicia (Mei) had as well a very unique haplotype (dark blue), which was found in some individuals in Ilha de Arousa (Aro). The haplotypes found in Bayonne (Bay) and in Porto de Ortigueira (ORTL) where shared with Morocco (Essauria and El Jadida). Again there was a clear vertical zonation between *F.spiralis(mt)* (along the upper intertidal) and *F.macroguiryi(mt)* (lower intertidal) in Aveiro (AVEH vs. AVEL), in Viana do Castelo (ViaH vs ViaL) and Porto de Ortigueira (ORTH vs ORTL). Individuals corresponding to *F.spiralis(mt)* in Viana do Castelo even showed to have a special position within the haplotype network, being separated as well by an indel from the core *F.spiralis* haplotype. In Porto de Ortigueira (Orn), Ilha de Arousa (Aro) and Bayonne (Bay) the sampling procedure (details about the vertical level, where the samples were collected from) was not known. In La Franca (FRAH and FRAL) and Lastres (LASH and LASL) only *F.spiralis(mt)* and *F.macroguiryi(mt)* respectively were found.

The more we proceed towards colder more northern areas the more dominant the main *F.spiralis* haplogroup became (Figure 9A in dark green).

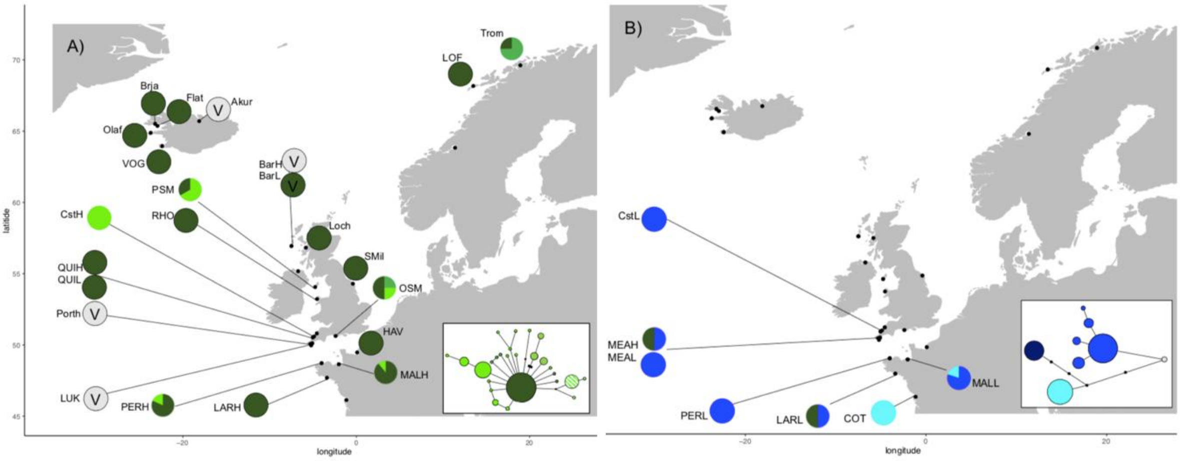


Figure 9: Phylogeography of Fucus sp. along North Europe illustrating the F.spiralis entity (left) and F.macroguiryi (right). Populations where F. vesiculosus was abundant are marked with a V in a grey circle. Dots with neither labels of F.spiralis nor F.macroguiryi indicate that no data was available for these locations.

Both *F.spiralis(mt)* and *F.macroguiryi(mt)* were present along the French coast. However, the most northern boundary for *F.macroguiyi(mt)* seems to be the at the English Channel on the rocky shores of Cornwall (Figure 9B). In this exact same geographic area *F.vesiculosus(mt)* was sampled, as well e.g. Lizard (LUK) and Porthleven (Porth). Other *F.vesiculosus* (as assessed by mtDNA) were found in and Akureyri, Iceland (Akur) and Barra, Orasaigh (BarH and BarL). The populations of the lower version of the latter mentioned even had individuals assigned to both, *F.spiralis(mt)* and *F.vesiculosus(mt)* leaving suspicion of hybridization between these individuals.

Looking at the vertical zonation and comparison of high and low situated populations in Constantine Bay, UK (CstH and CstL), Perharidi (PERH and PERL) and Saint Malo (MALH and MALL), both in the Bretagne in France, there was a clear separation between these species (in respect of their mitochondrial signature), whereas in Meanporth, Cornwall (MEAH and MEAL) and Larmor, Bretagne (LARH and LARL) individuals were mixed (*F.spiralis(mt)* and *F.macroguiryi(mt)*). In Port Quin, (QUIH and QUIL) both populations in the upper and lower interdental correspond to the typical *F.spiralis(mt)* group. Moreover, in La Cotinière (COT) and partly in Saint Malo again the characteristic southern haplotype version (in turquoise) of *F.macroguiryi* was present. To sum up, *F.guiryi(mt)* and *F.macroguiryi(mt)* occur together in Morocco as well as in Asturias (however, just isolated), whereas *F.spiralis(mt)* and *F.macroguiryi(mt)* occur in sympatry from Aveiro (most southern limit) to the English Channel on the rocky shores of Cornwall (most northern limit).

The core *F.spiralis* haplotype dominated the shores of North America (Figure 10). Unanticipated, the very unique haplotype found at Gibraltar, which was considered to be exclusive of this very marginal area, appeared also on the Atlantic coast of North America in Logy Bay, Canada (CANA) and at the Pemaquid Peninsula in Maine (Pem2). The "Gibraltar-Sequences" here in CANA and Pem2 fully correspond to the main Gibraltar core.

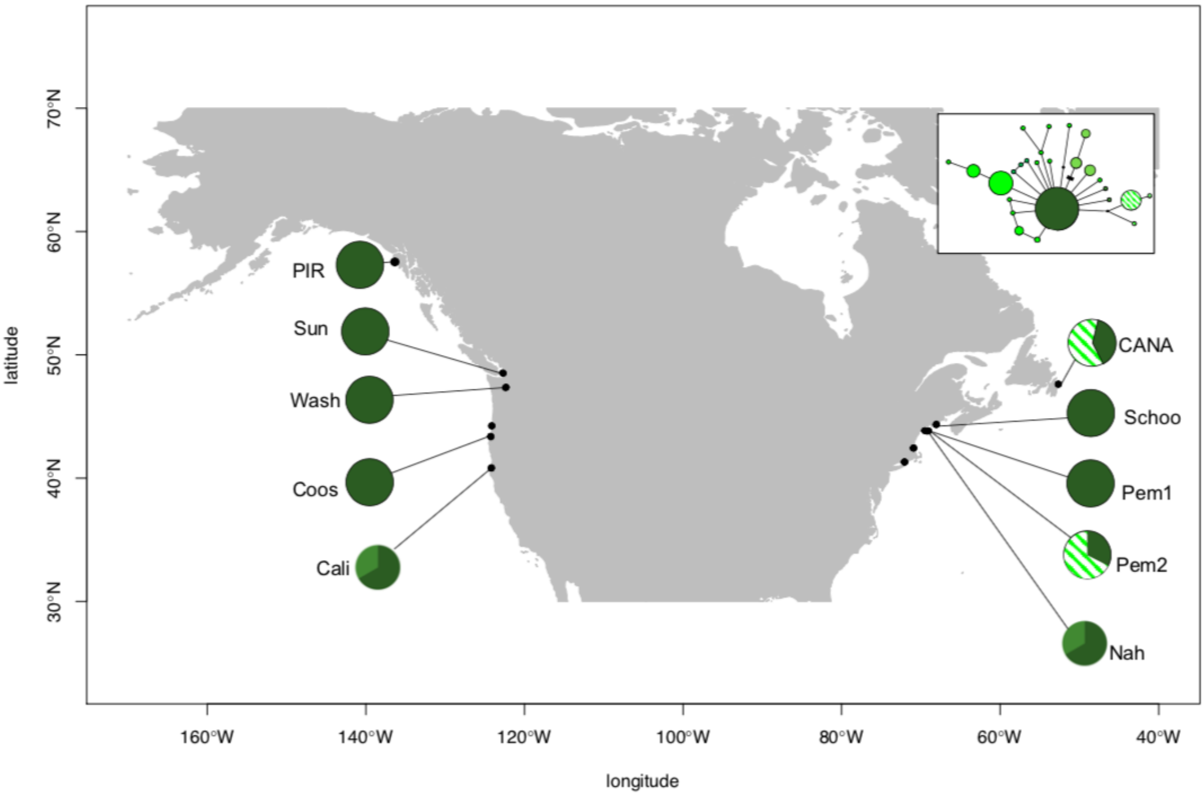


Figure 10: Phylogeography of Fucus sp. along North American distributional range of the Fucus complex. Note the presence of only one of the three entities, which is the F. spiralis(mt) entity. Dots without labels indicate that no data was available for these locations.

4.1.3 Microsatellite Data

The microsatellites at the loci L78, F21, F34, Fsp1, L20, F36 and F9 revealed valuable insight into the genetic structure of this Fucus complex. Polymorphism was variable and ranged between 4 alleles in locus F21, and 19 alleles in locus L20.

Allele frequency plots (Figure 11 and Figure 12) show that in a broad sense the marker L78 in combination with F21 and F34 can be used to distinguish *F.spiralis(msat)* (L78:136, F21:207, F34:194), *F.guiryi(msat)* (L78:154, F21:201, F34:194), and *F.macroguiryi(msat)* (L78:154, F21:201, F34:192) as well as the Azores (L78; 154, F21:207, F34:192). The overall allele frequencies were calculated (Table 2) to provide an overview about each marker and the level of polymorphism.

Even though the populations from Gibraltar belong to the northern entity (regarding their mtDNA) and population from Asturias show the southern mtDNA signature, they do not reflect this pattern of typical north and south discrimination rather than the allele signature of the corresponding geographic area making them the exception of this rule. F21 does discriminate between the Asturias populations and the ones surrounding them in NW Iberia, whereas the populations of Gibraltar still fall in the pattern of the southern populations (*F.guiryi(msat)*). The boundaries in NW Iberia and SW Portugal are blurry, indicated by both alleles present in some populations for the marker F21.

Fsp1 draws a sharp line to recognize individuals that belong to the geographic north and south, where individuals northwards from SW Portugal (including populations from North America) show a characteristic size of 140 and populations from SW Portugal towards the equator have their typical size of Fsp1 at 142. There are exceptions in Asturias (even with a unique allele at 144), Ribeira de Ilhas (RIh), Canary Island and Oualidia (Oua). The geographical separation of the north and the south is similarly reflected within the *F.macroguiryi* entity, where population in the more northern areas have a typical size of 140 and southern ones at 142, which illustrates a geographical component that these marker can contribute to the overall populations structure analysis.

The size 164 for the marker L20, which had the highest polymorphism in its sizes, was consistent for the *F.spiralis(msat)* entity as well as for populations from the Azores. Moreover, a size of 134 was characteristic for individuals belonging to *F.macroguiryi(msat).* The southern populations in the North (Asturias at 148) and the northern populations in the South (Gibraltar at 151) had their own very unique configuration of L20. In NW Iberia, SW Portugal and in the south (Morocco and West Sahara) the greatest variabilities were found for that marker. Notable Albufeira represents a very unique allele size as well at this locus (181).

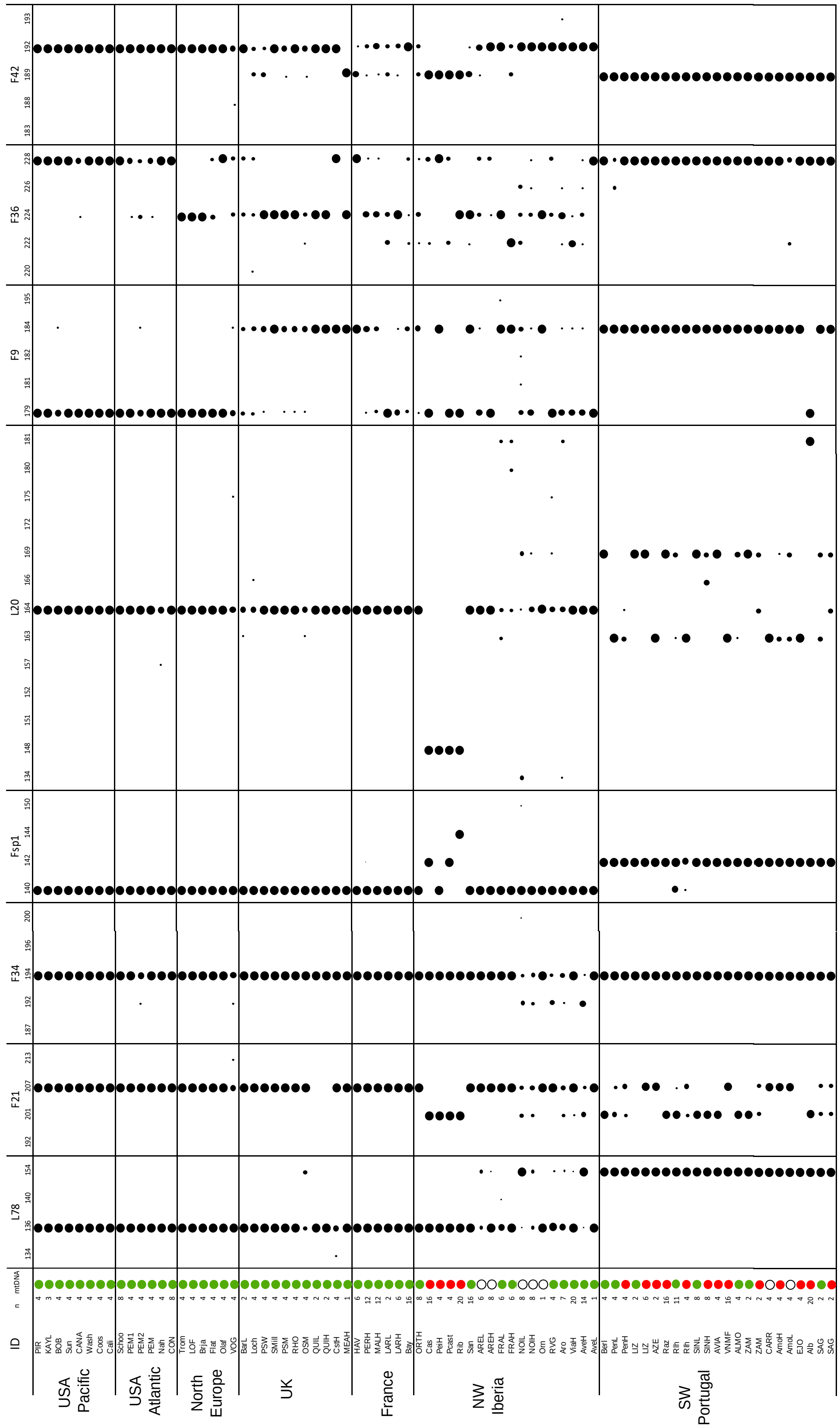


Figure 11: Allele frequency plots per population for all analysed loci. Sample size was standardized, and samples are sorted from North to South starting with the samples from the USA followed by northern Europe to South West Portugal. The number of samples for each analysed population is indicated as well as the mtDNA haplogroup (green = F.spiralis, red = F.guiryi, empty circles mean that no data on the mtDNA was available).

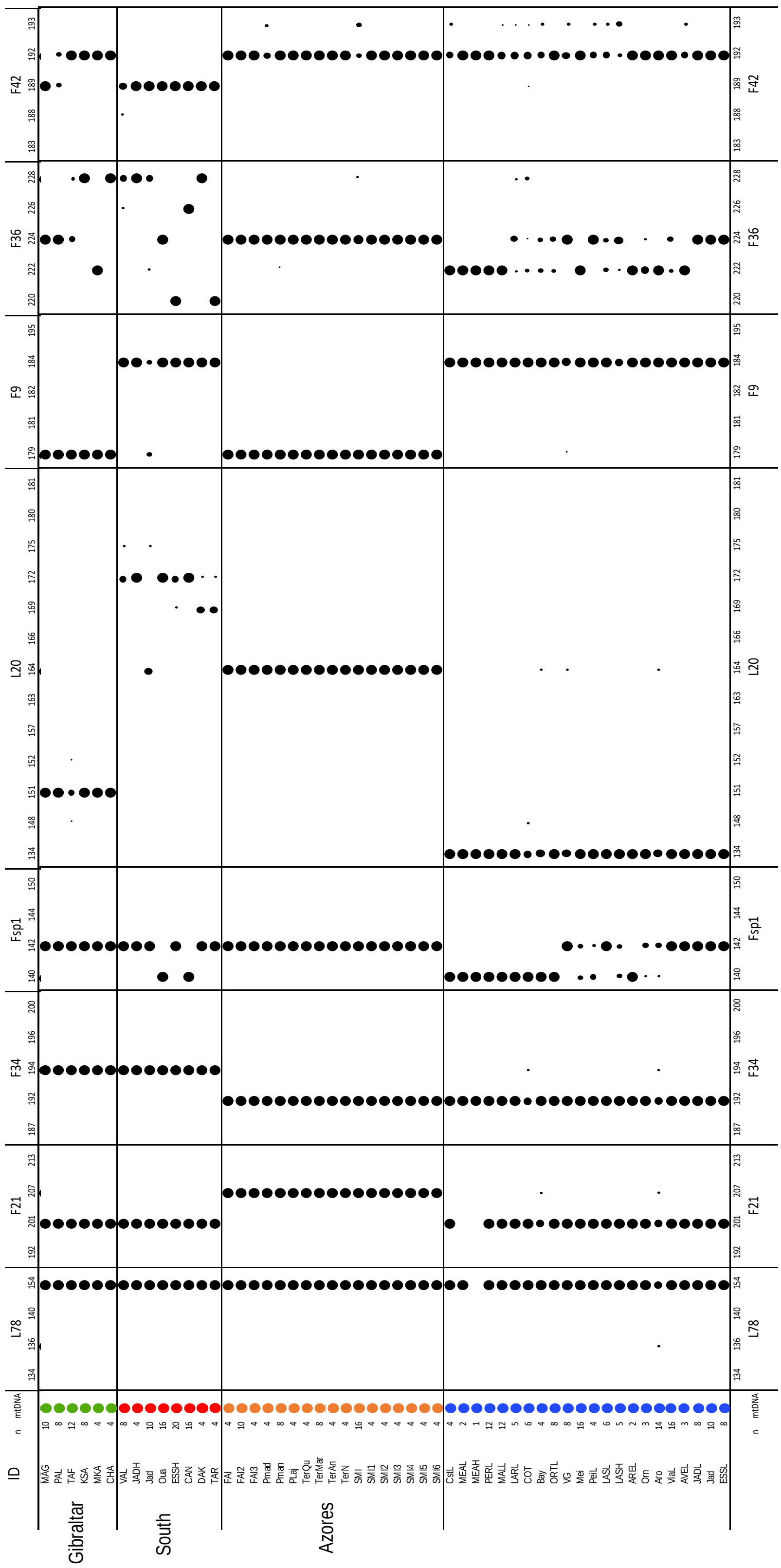


Figure 12: Allele Frequency plots for southern populations in Gibraltar, South (Morocco and West Sahara) and Azores. All F.macroguiryi samples are listed last and sorted as well from North to South. The mitochondrial DNA is as well indicated with additionally highlighting the unique mtDNA from the populations of the Azores (orange) and F.macroguiryi (blue). FAI, FAI2 and FAI3 are the ones from Faial, Pmad, Pman, PLaj correspond to Pico, TerQu, TerMar and TerAn are located on Terceira and SMI (SMI1 – SMI5) are the ones from São Miguel from the Azores. The same plot for individuals that presumably belong to F. vesiculosus can be found in the supplementary data.

Table 2: General information about the microsatellites that were used in this study. Most frequent alleles are defined as alleles with a presence of > 30% and were in all cases informative about whether the geographic details or regarding the entity. Note that these values also include individuals belonging to F.vesiculosus.

Locus	Nr. of different Alleles	Range of sizes	Most frequent Alleles	
			Size	Frequency (%)
L78	14	134 - 187	136	34.5
			154	62.89
F21	4	192 - 213	207	48.23
			201	51.44
F34	8	187 - 200	192	33.16
			194	66.02
Fsp1	14	134 - 165	142	47.26
			140	48.13
L20	19	134 - 181	164	42.39
F9	6	171 - 195	179	42.98
			184	55.96
F36	7	220 - 230	228	35.17
			224	41.97
F42	9	183 - 194	189	36.04
			192	58.94

F9 with its two most dominant alleles at 179 and 184 provided again interesting details because does not only separate individuals on a species level but also within the geographic northern and southern distributional ranges within *F.spiralis(msat)*.

F36 had a size range of 220 to 230 and its most common alleles at 228 and 224. This marker revealed regional divergence either within *F.spiralis(msat)* or *F. macroguiryi(msat)*, suggesting geographical differentiation within clusters, just like the marker Fsp1 and F9 (but only regarding *F.spiralis(msat)*).

Unique alleles were found on the Canary Island (CAN), Ribadesella (Rib), Tarifa (TAR), on the Azores and Ribeira de Ilhas (Rih) for example (for more detail see Supplementary 2). Yet, the majority of the unique alleles could be assigned to the group of *F.vesiculosus* haplogroup (mainly samples from the UK e.g. Porth, LUK, BarH,…), which were highly polymorphic, since this is an dioecious species (Supplementary 3).

4.1.4 Microsatellite based Population Structure (PCA, DAPC and STRUCTURE)

The PCA revealed patterns of structure that were largely congruent with the mitochondrial data. Firstly, there is a broad separation between the *F.spiralis* (green), *F.macroguiryi* (blue) and the southern (*F.guiryi*) populations (red) regarding the microsatellite clusters (Figure 13). The cluster of the Azores (orange) represent their own very unique genotypic signature. The outlier populations from Gibraltar and Asturias (indicated by empty circles) that exhibited a haplogroup atypical for their geography can be distinguished as well from the other clusters. Most individuals form the SW coast of Portugal which contained the mtDNA of *F.spiralis* were not distinguishable from the co-occurring individuals possessing *F. guiryi* haplotypes, as all grouped into the southern cluster (SNMix). In other words, these individuals actually could be defined as *F. guiryi* with *F- spiralis* mtDNA. When taking a closer look to the cluster of the Azores, one outlier, (in green) can be found which corresponds to individuals from Aveiro (AVEH).

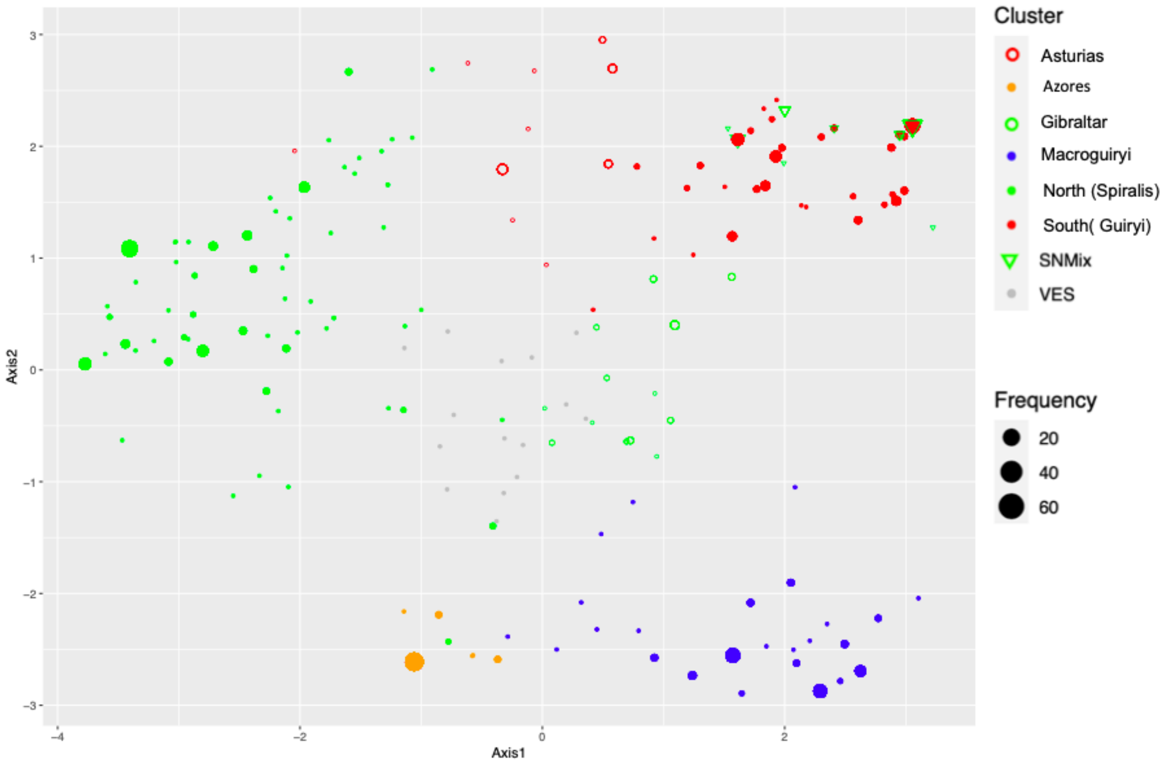


Figure 13: Principal component analysis (PCA) showing the multi locus genotypes. The colours chosen for this graph correspond to the mtDNA described in the above. The size of the dots represents the frequency of the corresponding genotypes. SNMix are individuals from the South West coast of Portugal, that have the mitochondrial mtDNA of the F.spiralis entity, but still fall within the F.guiryi cluster according to microsatellites; VES corresponds to individuals with F.vesiculosus mtDNA.

F. macroguiryi(msat) is very isolated and genotypically distinct from F.spiralis(msat) and southern (F.guiryi(msat)) clusters, which stands in conflict with the haplotype network. The distinct position of F.macroguiryi(msat) in relation to F.spiralis(msat) and F.guiryi(msat) is even more evident when performing a discriminant analysis of principal components (DAPC) (Figure 14). Finding the true number of clusters within this data set was challenging, given that the lowest associated BIC was not as clear as it should be when following the directions provided by the R-package for this analysis (curve did not show a typical “elbow” shape). Prior knowledge from the PCA however was considered and the number of clusters was determined as K=5 (F.guiryi, F.spiralis, F.macroguriyi, F.vesiculosus and individuals from

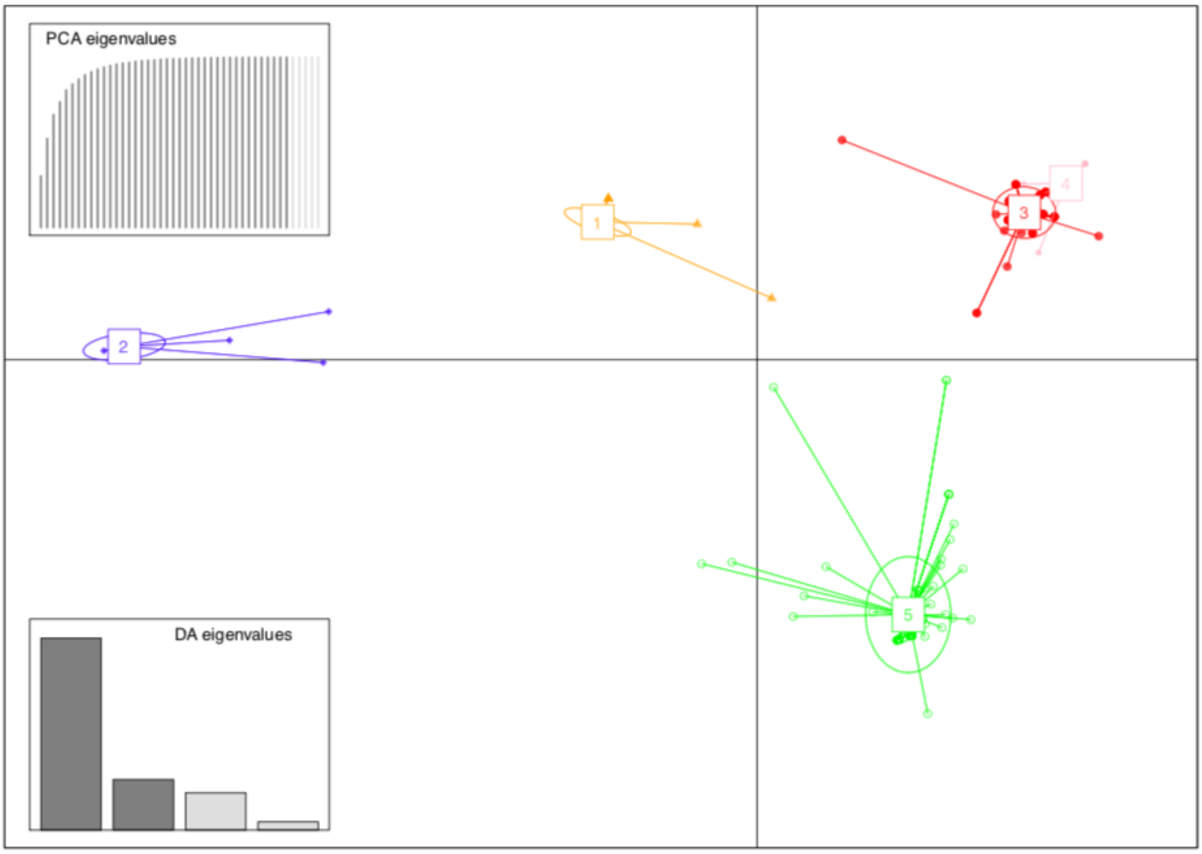


Figure 14: DAPC plot when considering five genetic clusters: , 1) Azores in orange , 2) F.macroguiryi in blue, 3) Southern population and populations from the SW coast of Portugal in red, 4) Albufeira, El Jadida and Gibraltar populations as well as Asturias in pink and 5) F.spiralis(msat) and F.vesiculosus(msat) in green. Each dot represents an individual.

the Azores). The more scattered distribution of the points within the *F.spiralis(msat)* cluster indicate that this group has a higher inter-population diversity. The DAPC clustered the following groups: Azores (1), *F.macroguiryi(msat)* (2), southern populations from Morocco and West Sahara together with populations form the SW of Portugal (3), Albufeira, El Jadida, Gibraltar and Asturias (4) and *F.spiralis(msat)* and *F.vesiculosus(msat)* (5).

The output of STRUCTURE (Figure 15), suggested that the most likely number of K would be 3, which was based on the distribution for the posterior probability of the data for a given K and was represented and the highest ΔK value (7.314724). Still this ΔK value was not outstanding huge in comparison to the other suggested number of clusters (K), which ranged between 0.34 to 4. 39. Therefore the identification of the numbers of clusters was challenging, given these vague ΔK values. Therefore, it was needed to take a closer look on the assignment of populations to the clusters. When testing the data for K=2 the Null - hypothesis would be that the individuals that were found to be *F.vesiculosus* (according to their mtDNA) would be

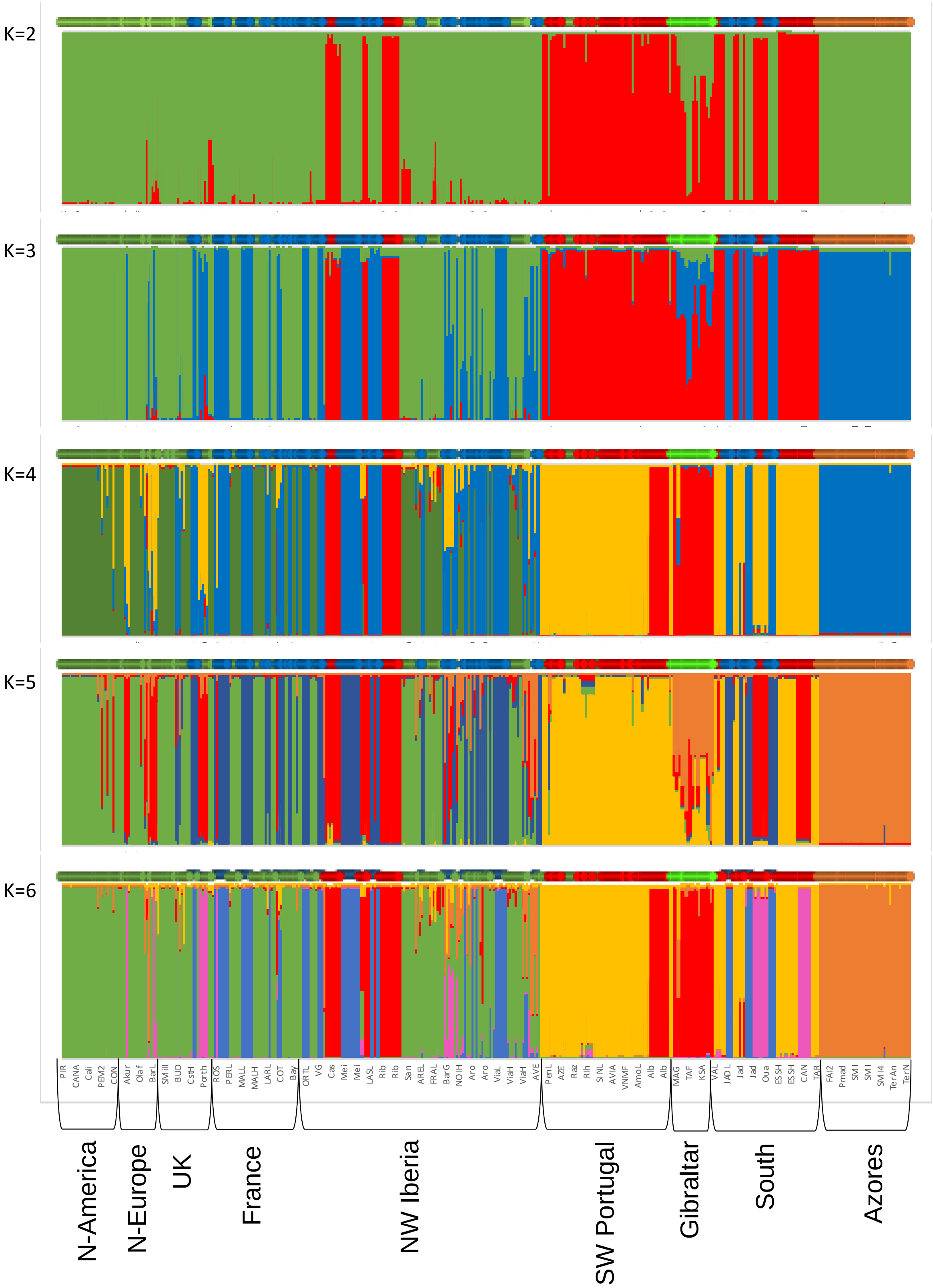


Figure 15: Clustering from Structure for K= 2, 3, 4, 5 and 6. The computed proportion of the genome for each number of clusters is illustrated as bar plots where each vertical bar represents one individual. The top line represents the mtDNA sequence corresponding to the sample (F.spiralis in green, F.guiryi in red, F.macroguiryi in blue, individuals from Gibraltar in bright green and Azores in orange).

separated from the entire data set. However, this was not confirmed - only Fucus individuals from the South (red) including the isolated population in Asturias versus Fucus from the North (green) together with the populations from the Azores and *F.macroguiryi(msat)* were differentiated. Gibraltar shows admixture for K=2. The Null-hypothesis is rejected in favour of the alternative hypothesis, that individuals with *F.vesiculosus* mtDNA apparently would correspond to the same cluster as *F.spiralis*. K=3, which is assumed to be the best fit of K, separates quite well the three entities - *F.spiralis* (green), *F.guiryi* (red) and *F.macroguiryi* (blue) as also reflected by the corresponding mtDNA haplogroups. The main exceptions are perhaps the populations from the Azores that are considered to be within the same cluster as *F.macroguiryi*, which in a biological and geographical context is improbable. By increasing the number of K to 4 to test that this number of clusters would separate the Azores from the *F.macroguiryi(msat),* SW Portugal and individuals form Morocco and the West-Sahara were grouped with potential "*F.vesiculosus*" from North Europe and the UK (yellow). The scenario of K=4 considers Gibraltar and Asturias to be part of the same cluster (red), whereby *F.macroguiryi* and the Azores still belong to the same group (blue). Finally, the number of K to be 5 generally does differentiate between F.spiralis (green), F.macroguiryi (blue), the Azores (including most of Gibraltar in orange), SW Portugal (yellow) and populations from the South (ESS and TAR), together with Asturias, Oua and CAN (red). These clusters were similar to the result from the PCA, yet not fully consistent between the analytical approaches.

The expected separation of *F.vesiculosus* was not established even for K=6, instead, "F.vesicuolus" in the north (in N-Europe and the UK in pink) grouped with a sub-group of *F. guiryi* (e.g. Oua and CAN). Yet the conclusion can be made, when assuming K=3 that individuals from the SW coast of Portugal all belong to the southern entity, even though individuals in some populations incorporated mtDNA of the *F.spiralis* entity. These findings are identical with the results in the PCA.

The integrity of species, as illustrated in the bar plots with the proportion of the genome to each cluster of the "best fit" of K=3, revealed no admixture between *F.macroguiryi* and *F.spiralis* or *F.guiryi* despite close proximity. What is evident is that *F.macroguiryi* is consistently maintaining its integrity when co-occurring with *F.guiryi* (in PeiH vs. PeiL, JADH vs. JADL and ESSH vs ESSL) and sharp lines in the genetic structure are visible (Figure 16). The isolated population of *F.guiryi(msat)* in Asturias shows a minimal proportion of the msat-cluster of *F.spiralis* (green). *F.macroguiryi(msat)* shows a well-defined barrier to *F.guiryi(msat)* and no gene flow was inferred from the analyses.

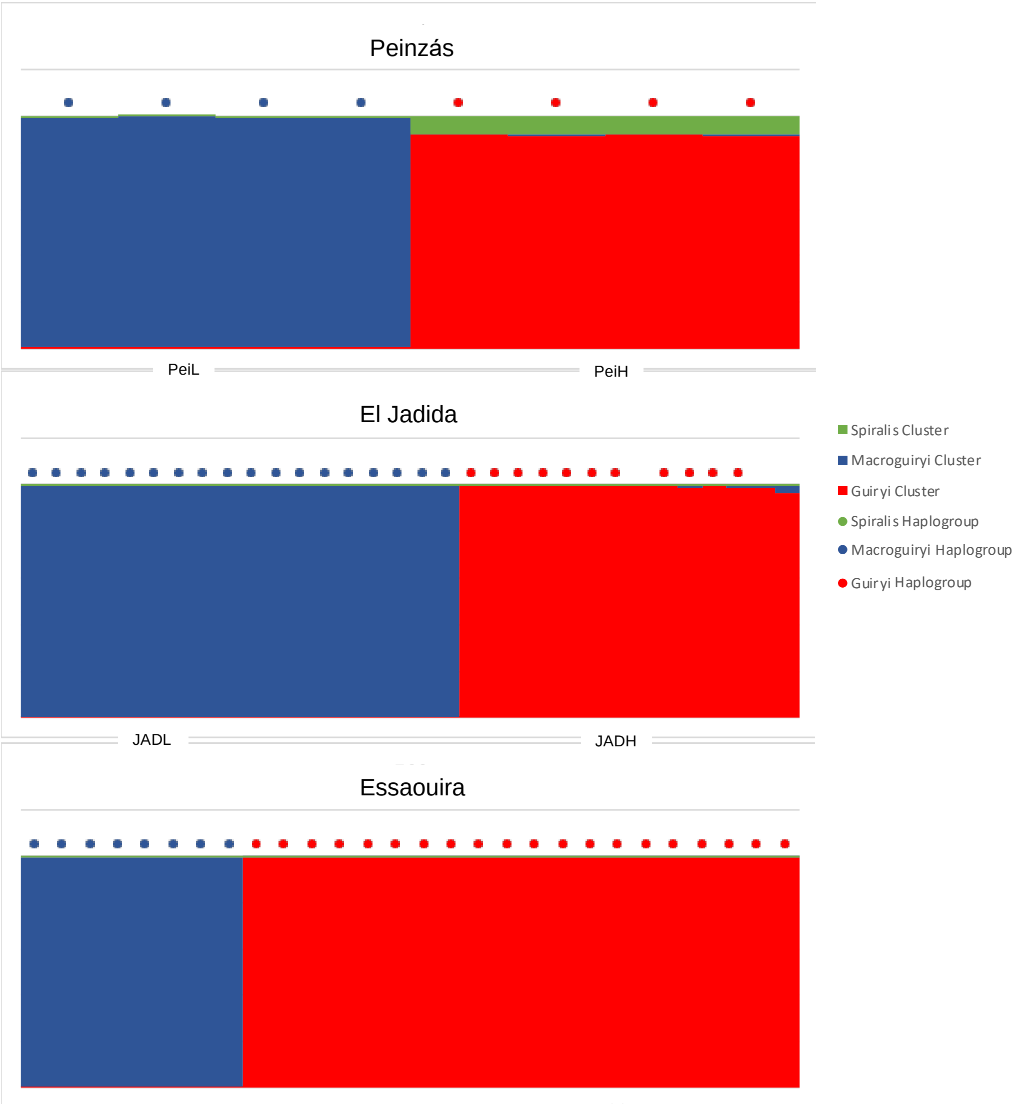


Figure 16: Genetic Structure of sympatric F.guiryi (red) and F.macroguiryi (blue) in NW Iberia (Peinzás), and Morocco (El Jadida and Essauria). The dotes on the top of each bar represent the mtDNA (note that for two samples in El Jadida the mtDNA was not available). The membership for each individual to a cluster is indicated by portioning into different colours.

A simmilar situation was observed in populations where *F.spiralis(msat)* and *F.macroguiryi(msat)* were present (Figure 17). No admixture was detected in samples from the UK namely Meanporth (MEA) and Constantine Bay (Cst) as well as in France in Perhardi (PER), Saint Malo (MAL) and Bayonne (Bay). In NW Iberia, the population from Porto de Ortigueira (ORT) was the only one that showed such an sharp and well defined species boundary between *F.spiralis(msat)* and *F.macroguiryi(msat)*.

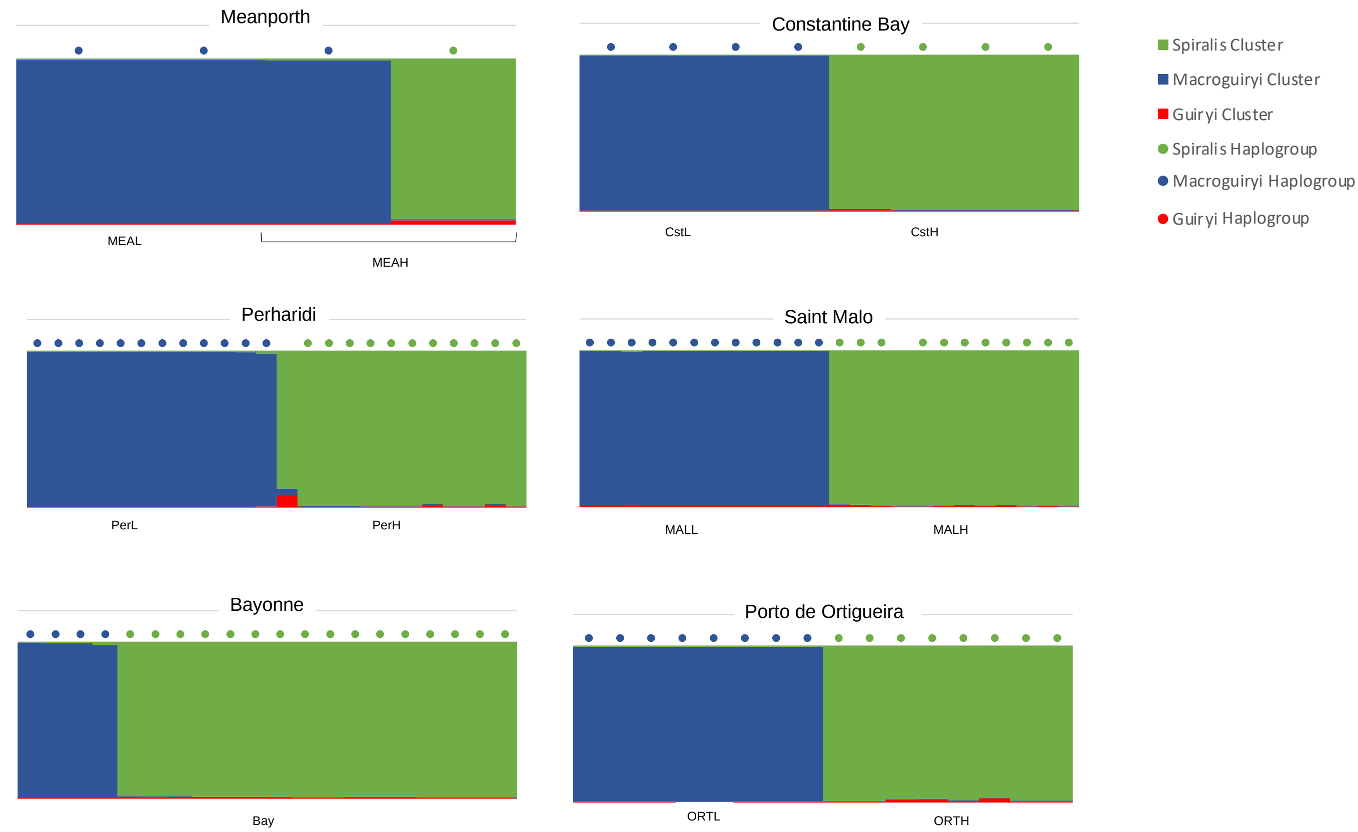


Figure 17: Genetic Structure of sympatric F.macroguiryi (blue) and F.spiralis (green) in the UK (Meanporth and Constantine Bay) together with samples from France (Perhardi, Saint Malo and Bayonne). The samples from Porto de Ortigueira are from NW Iberia. The membership for each individual to a cluster is indicated by portioning into different colours with the corresponding mtDNA data (dots). Information of the vertical position of Bayonne samples was not available.

The delimitation between species was not as clear in other locations (Figure 18). In Ilha de Arousa (Aro), one individual which contained the mitochondrial DNA of *F. spiralis* showed an admixture of all three entities. The relative high contribution of the "southern cluster" (in red) is clearly related to its very distinct allele at L20 with a size of 181. This allele size was as well found in samples form Albufeira and might lead to this result. In Viana do Castelo (Via), being the most southern limit of a sympatric distribution of *F.vesiculosus*, *F.macroguiryi* and *F.spiralis*, gives reason to assume that the admixed individuals containing the mtDNA of *F.spiralis* might probably be hybrids between two of the three entities. Again, the failure of STRUCTURE for K=3 to discriminate between *F.macroguiryi*, individuals from the Azores and *F.vesiculosus*, should be acknowledged. In Viana do Castelo the admixed individuals showing a genomic proportion of "*F.macroguiryi*" might not actually correspond to this entity and rather belong to *F.vesiculosus*.

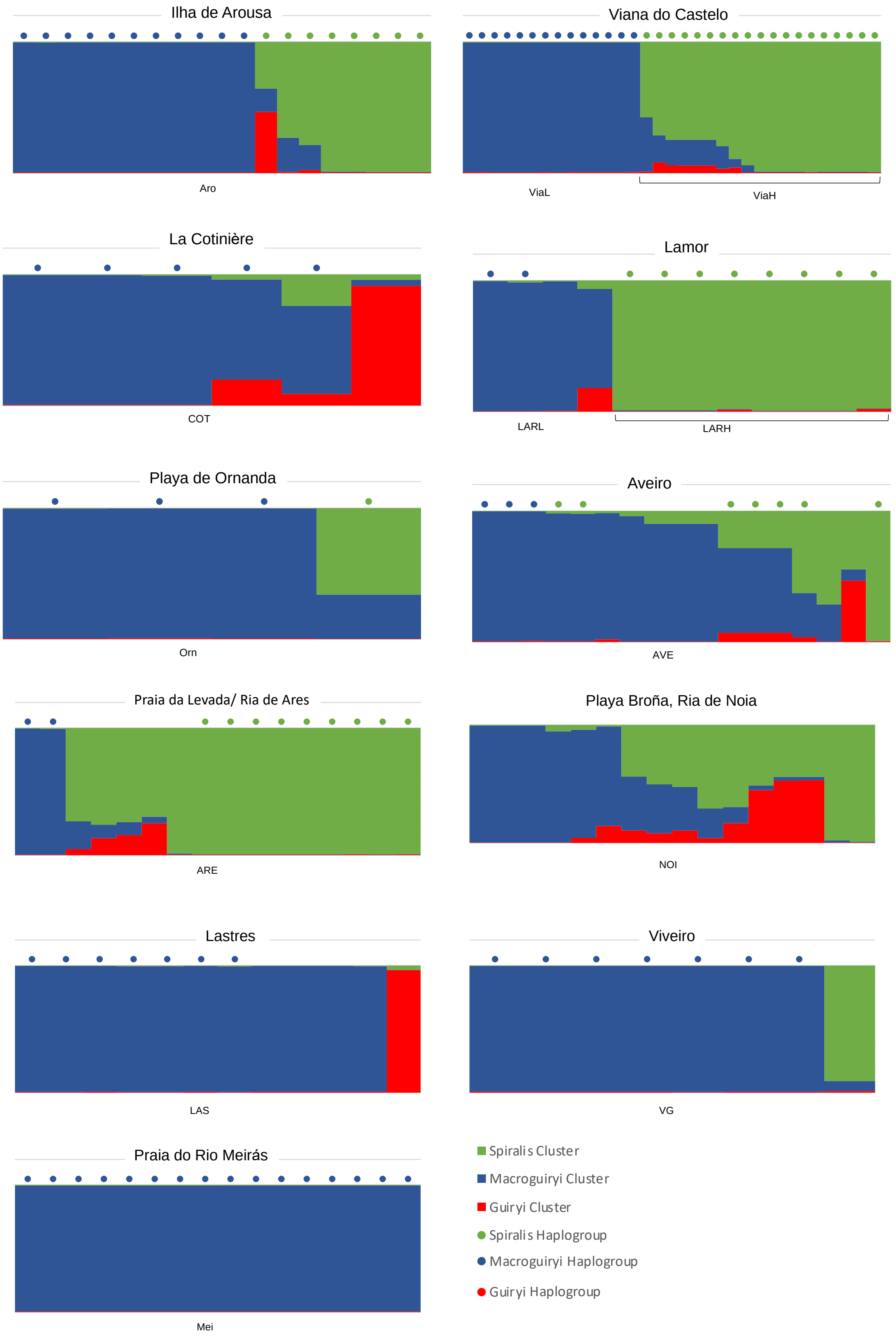


Figure 18: Genetic Structure of sympatric F.macroguiryi (blue) and F.spiralis (green). The membership for each individual to a cluster is indicated by portioning into different colours with the corresponding mtDNA data (dots). All samples except of the ones from France (La Cotiniere and Lamor) are from NW Iberia.

In La Cotinière (COT) one putative *F. guiryi* individual was also identified, which can be related to its allele sizes of F42 at 189, characteristic for individulas form SW Portugal, and L20 at 148 (also found in the isolated *F.guiryi* populations in Asturias), which are typical for the cluster of *F.guirryi(msat)*. In Lar (Larmor) and Playa de Ornanda (Orn), Aveiro (AVE), Praia da Levada/ Ria de Ares (ARE) and Playa Broña (NOI) the same can apply for the proportion of *F.guiryi(msat)* in their genome as well as for the "admixture of *F.spiralis(msat)* with *F.macroguiryi(msat*)" wich de- facto is a admixture with *F.vesicuolsus.*

F.macroguiryi individuals form Lastres (North Spain), as well as at Praia do Rio Meirás (Mei) did not knowingly cooccured with either *F.spiralis* or *F.guiryi* which is again confirmed in their genomic cluster attibution according to STRUCTURE for the samples form Praia do Rio Meirás. In Lastres one of the individuals was asigned to the *F.guiryi* entity (regarding the MLG), which is likeley to be the case since it is known that a pocket of Fucus harboring the *F.guiryi* haplogroup is well established in Asturias. However, the mtDNA for this specific individuals could not be recoverred.

Populations from the SW coast of Portugal harbouring a mix of *F.spiralis* and *F.guiryi* haplotypes did not show, as could be expected, any signs of admixture (Figure 19). Irrespective of the mtDNA of the populations, also even from the same location (e.g. Sines, and Peniche populations showed a relatively genetic homogeneity, with individuals from this area assigned to the cluster of *F.guiryi(msat)*. These data sets of the most northern limit for *F.guiryi(msat)* to Peniche.

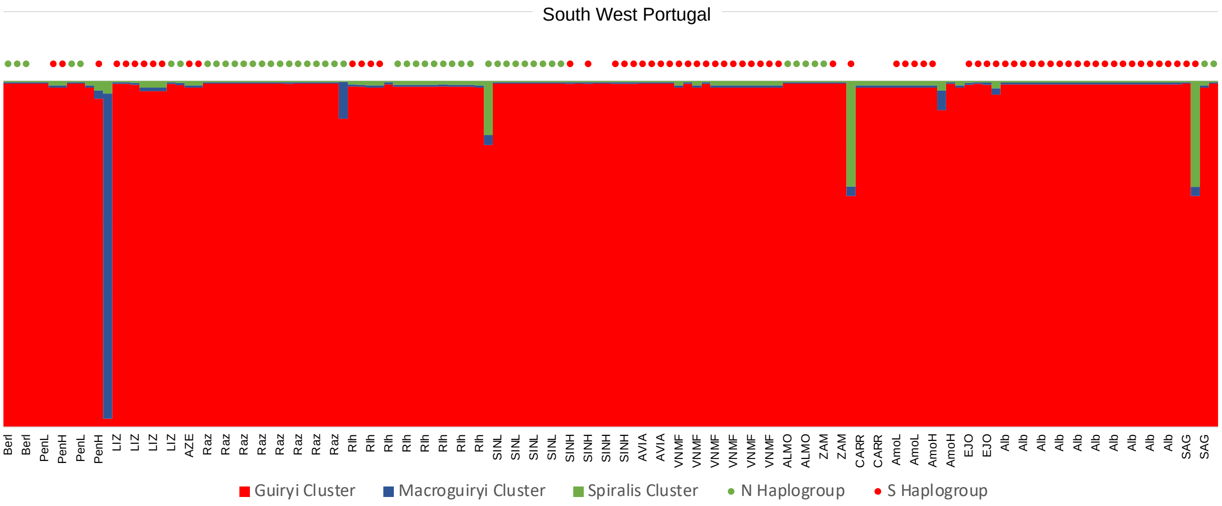


Figure 19: Genetic structure of individuals form SW Portugal with individuals possessing mt-F.spiralis DNA however being very homogenous in their genetic structure and assigned to the F.guiryi (msat-) cluster.

4.1.5 Diversity Analysis

For each species, different indices of diversities were calculated for each region (Azores, South, NW Iberia, North Europe and North America) with the occurring entities in these areas (Table 3). The extend of each region was chosen according to genetic (haplotypic and genotypic) data and "natural" biogeographic regions. South includes all individuals from Peniche towards the equator, NW Iberia had its most northern limit in Bayonne and North Europe included France, the UK, Scandinavia and Iceland.

The populations from the Azores showed relatively low levels in their diversities, due to their almost fixed allele sizes and just two different haplotypes, of which these population consist of.

The lowest nucleotide diversity was found for the *F.guiryi(mt)* populations in NW Iberia, in Asturias (0.20680 $*10^3$). At the same time this haplogroup had the highest nucleotide diversity in its southern ranges (6.03756 $* 10^3$). The number of different haplotypes within one region (in%) corresponding for each haplotype group were further calculated. These numbers indicate similar patterns like the nucleotide diversity and can be used as an estimator of diversity, even though the calculations do not consider evenness. A great number of haplotypes were found for *F.guiryi(mt)* in the South (22.92%). The number of alleles per locus displayed a similar pattern as the other diversity measures for *F.guiryi* in the South (3.125) showing the highest values.

For calculating the nucleotide diversities of *F.spiralis(mt)* in the south, all individuals that also possessed *F.spiralis* mtDNA were summed, including the individuals from SW Portugal that according to microsatellites are *F.guiryi*. If one would consider "mt-F.spiralis" individuals together with all *F.guiryi* haplotypes the diversity in the South would be even higher (10.64 * 10^3; not shown in Table) and much lower for *F.spiralis(mt)* when only considering the individuals form Gibraltar as the "true *F.spiralis* in the South". Allele richness (Â), expected, observed heterozygosity (H_{exp} and H_{obs}) and the number of alleles per locus varied between the msat-clusters as identified with STRUCTURE and were considered irrespective of the mtDNA haplogoups. For instance, individuals along the SW coast of Portugal that had mitochondrial *F.spiralis* DNA were still considered to belong to *F.guiryi(msat)* in the south. *F. guiryi* in the South had the highest allele richness (2.519)

Haplotypes corresponding to *F.spiralis* had moderate nucleotide diversity levels in the northern ranges (2.2768 * 10^3), and were relatively high in the southern ranges (4.35393* 10^3). Most haplotypes of *F.spiralis(mt)* were present in the North (18.75%). Regarding the genotype data *F.spiralis* in the south consisted only out of the populations of Gibraltar with the highest allele richness of 2.462 in North Europe followed by NW Iberia (2.388), the South (2.244) and North America (1.9).

The haplotypes of *F.macroguiryi(mt)* had their highest nucleotide diversity in NW Iberia (5.33304 *10^3). Notably the diversity for *F.macroguiryi(mt)* is zero in the South (Mococco) because only one single haplotype was found which was identical in both locations (JadL and ESSL). The highest number of haplotypes for *F.macroguiryi(mt*) was found as well in NW Iberia (12.5%) together with the allele richness (1.95) and the number of alleles per locus (2.125) being the highest within this region as well for this entity.

Overserved heterozygosity was in all groups lower than the expected heterozygosity (except of *F.macroguiryi* in the south where $H_{exp} = H_{obs}$) which can be attributed to forces such as inbreeding.

Table 3: Regional genetic diversity within species: Calculations for the mtDNA diversities (nucleotide diversities and relative frequency of haplotypes for each region and each entity in %) are shown and calculations for the genotype data (allele richness, expected and observed heterozygosity and the number of alleles per locus) were done independently irrespective of the probable introgressions. Allele richness was standardized to the number of individuals of the smaller group. N° of Ind = Number of individuals.

			Mitochondrial Data		Genotype Data				
Species	Region	N° of Ind	Nucleotide Diversity (10^{-3})	N° Haplo-types (%)	Allele Richness (Â)		H_{exp}	H_{obs}	N° of alleles per locus
Azores	Azores	94	0.91378	4.17	1.381	±0.174	0.0202	0.0041	1.875
F. guiryi	NW Iberia (Asturias)	44	0.20680	2.08	1.812	±0.188	0.1818	0.0086	1.875
	South	215	6.03756	22.92	2.519	±0.282	0.2562	0.003	3.125
F. macro-guiryi	North Europe	41	1.63073	6.25	1.894	±0.261	0.0882	0	1.75
	NW Iberia	90	5.33304	12.50	1.95	±0.234	0.2206	0.0115	2.125
	South (Morocco)	26	0	2.08	1.163	±0.059	0.0048	0.0048	1.125
F. spiralis	North Europe	103	2.2768	18.75	2.462	±0.475	0.2441	0.0439	4.25
	NW Iberia	86	1.13296	12.50	2.388	±0.222	0.2619	0.0595	3.25
	South*	42	4.35393	10.42	2.244	±0.261	0.1407	0	1.625
	N-America	56	0.71120	8.33	1.9	±0.36	0.0811	0.0451	2.5

**F.spiralis* in the South considered all individuals with mt-F.spiralis DNA for calculating the nucleotide diversity and the N° of haplotypes, whereas for calculating allele richness, observed and expected heterozygosity only the populations from Gibraltar were considered.

Moreover, the differences in the allele frequencies were analysed within the corresponding entities (Figure 20) in order determine which marker could be used as a diagnostic one for each entity. The frequencies of each alleles were extracted from the STRUCTURE output for *F.spiralis* and *F.guiryi* for K=3 and for *F.macroguiryi* and the Azores for K=5. Notably, the Azores show very little to almost no variation in their allele sizes and display very well-defined sizes indicated by frequencies close to almost 1 (i.e. near fixation). The same applies for *F.macroguiryi* with the exception of the marker Fsp1 (yellow) and F36 (pink) which displays the aforementioned geographical separation of northern versus southern populations. *F.guiryi* is the entity which showed the highest polymorphism in the marker L20 (light-blue), whereas contrastingly *F.spiralis* only had one significant size at this marker (at 164).

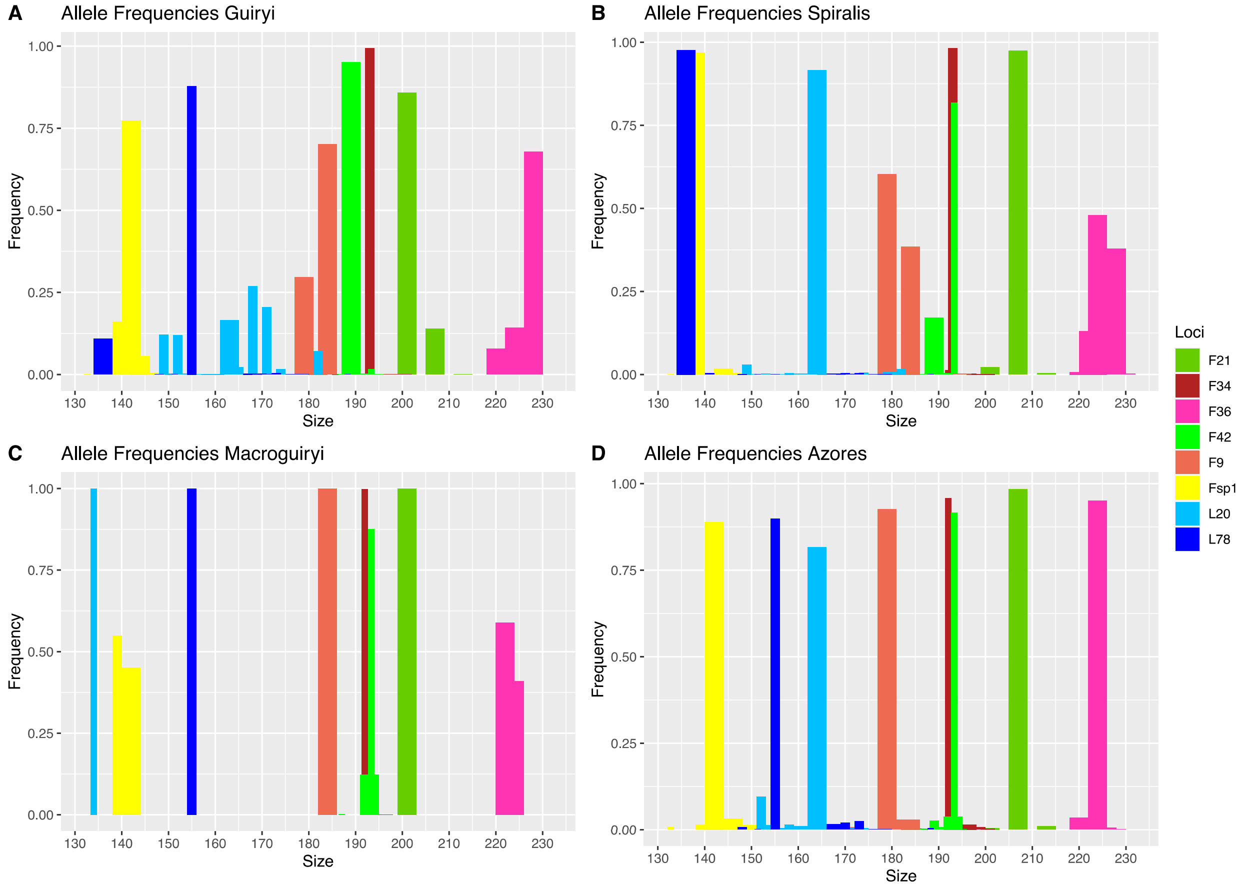


Figure 20: Allele Frequencies for each entity (F.spiralis, F.guiryi, F.macroguiryi) and the Azores based on the genomic assignation to each cluster. The averaged allele frequencies were extracted for F.spiralis and F.guiryi from the structure output for K=3 and for the Azores and for Macroguiryi for K=5.

To sum up, the marker L20 (at 134) is the most outstanding diagnostic marker for *F.macroguiryi*, whereas for the Azores, *F.spiralis* and *F.guiryi* only a combination of several markers allow their identification.

In conclusion, genetic analyses revealed the existence of three main genetic entities within this species complex together with one separated group within the southern entity, namely the Azores. This subdivision was largely concordant between the mitochondrial data and the MLG data notwithstanding the confounding effects of *F.vesiculosus,* and some intermediate/introgressed populations. Yet, the genealogic relationships *F.macroguiryi* to *F.spiralis* and *F.guiryi* inferred with nuclear DNA stand in clear disagreement with independent genomic data.

4.2 Species Distribution Modelling

Overall 12.267 records were extracted form citizen science platforms. The number of records extracted from these platforms therefore mostly consist of records of *Fucus spiralis* (Table 4).

Table 4: Number of occurrence records gathered from GBIF,OBIS and iNaturalist for each species (F.spiralis and F.guiryi)

	Fucus spiralis	Fucus guiryi	Total (by source)
iNaturalist	273	13	286
GBIF	9815	94	9909
OBIS	2072	0	2072
Total (by species)	12160	107	12267

After all the cleaning steps (described in methods) 50 occurrence records (of *F.spiralis* and *F.guiryi*) were left for the modelling process (Figure 21A). Distributions were modelled for *F.spiralis* and *F.guiryi* versus *F.macroguiryi* separately, as *F.macroguiryi* occurs in distinct habitat further lower on the shore. Secondly, the genotypic data showed as well that *F.macroguiryi* is much more distinct from *F.spiralis* and *F.guiryi* (see graph from DAPC).

The occurrence records that were gained from the citizen science platforms were used for the model of *F.spiralis* and *F.guiryi* (n=50) due to the fact that these records represented the overall distribution range better than the genetically analysed records. Additionally, the records form the citizen science platforms were present in a higher number after treating the data to reduce spatial autocorrelation as priorly mentioned. The model that was developed for *F.macroguiryi* just used the genetically verified occurrence records (n=18). Records for *F.macroguiryi* (Figure 21B) were not "cleaned" with reducing spatial autocorrelation because the number of records was already extremely low. Yet this will be taken into account, when interpreting the outcome.

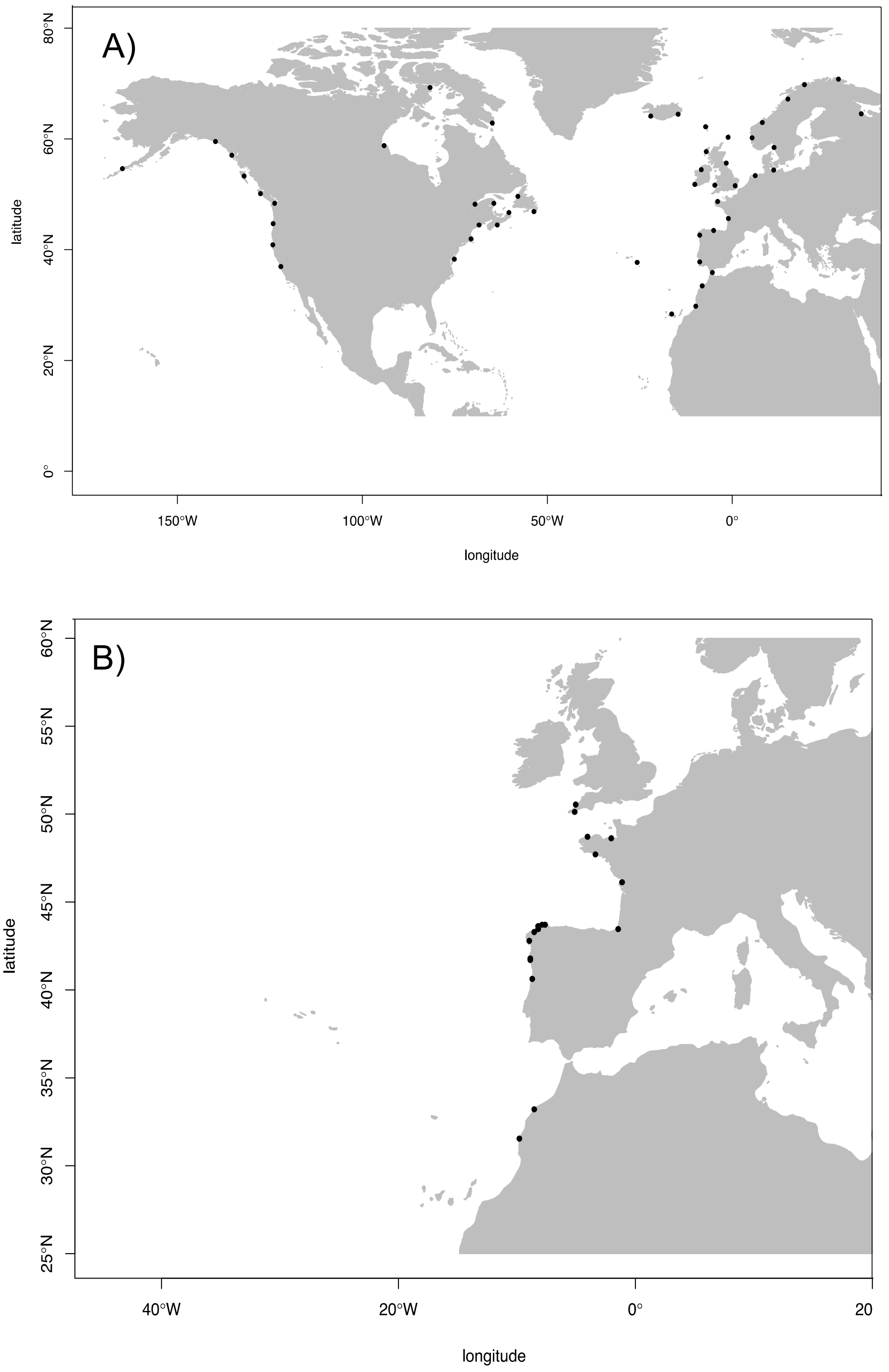


Figure 21: Final occurrence records of the F.spiralis complex consisting of F.spiralis and F.guiryi (n=50; upper map (A)). Records used for the model of F.macroguiryi (n=18; lower image (B)).

4.2.1 General Environment

A general analysis of environment for the present, RCP2.6 and RCP8.5 was done in the beginning of the analysis.

Table 5: Extracted values of occurrence records(citizen science platforms) from the environmental conditions (sea surface temperature) of the present and future scenarios (RCP2.6 and RCP8.5) Mean, maximum and minimum values are displayed as well as the change of the future scenarios in comparison to the present. Lower and upper limit are defined by excluding the outliers (values that fall more than 1.5 times the interquartile range above the third quartile or below the first quartile following basic statistical analysis).

	maxSST			minSST		
	Present	maxSSTRCP26	maxSSTRCP85	Present	minSSTRCP26	minSSTRCP85
Mean	16.35 ± 4.74	17.30 ± 4.86	20.25 ± 4.75	5.17 ±5.40	5.72 ±5.34	7.73 ± 5.33
Change in Mean		+0.95	+3.90		+0.55	+2.56
Max	25.35	26.48	29.05	18.15	18.65	20.51
Min	3.35	3.25	7.10	-1.90	-1.89	-1.86
Lower limit	13.61	14.31	17.40	0.84	1.80	4.34
Upper Limit	19.52	20.60	23.89	8.52	9.10	11.19

Both extremes (minimum and maximum) of SST were measured between the years of 2000 to 2014 and represent the mean of all of those years. Therefore, the maximum and minimum values can exceed in some periods these values described due to seasonal fluctuations. In the worst-case-scenario there had been the prediction of increasing temperatures and decreasing salinity, where existing Fucus populations will have to deal in average with an increase of 3.9°C in their present locations in the future climate scenario RCP8.5, which is a dramatical change in a short period of time - within the next 80 years (Figure 22).

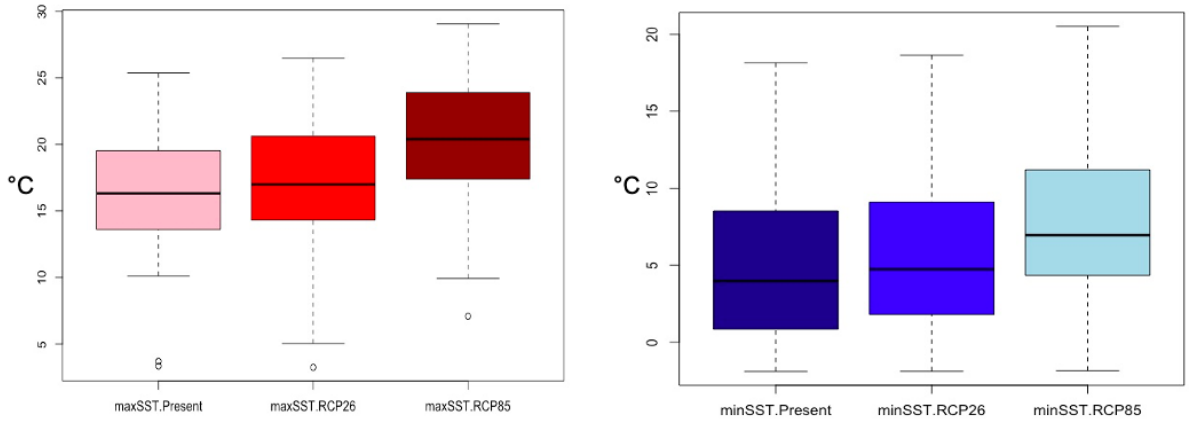


Figure 22: Extracted values from occurrence records (n=50) that were used for the model of F.spiralis and F.guiryi. Maximum (left) and minimum (right) SST for the present, RCP2.6 and RCP8.5.

The RCP2.6 scenario shows an increase of maximum SST of 0.95°C. Logically, there were not as severe changes in the environment with the present and low-emission scenario (RCP2.6), however an increase of almost 1 °C might be threatening to populations in the southern marginal ranges. Decreasing overall salinity, as a result of melting sea ice that capture freshwater, was noted but did not show significance.

The Kruskal-Wallace Test and Tukey HSD test revealed that the most striking differences in the present environmental use were between the populations from the Azores and populations from the northern (*F.spiralis*) cluster. This is not surprising given the clearly distinct climatic environments of these areas. The Azores have a special status in their data set, and therefore it is just a logical consequence that this island group represents very unique climatic conditions, being totally exposed in the ocean and isolated from the mainland.

Neither from the comparison matrix of environmental variables between entities nor from the multi-dimensional scaling plot (described in methods) patterns of niche differentiation could be detected, indicating that all individuals show highly similar ecological niches. Yet, it can be concluded, that the entity *F.spiralis* having a greater distributional range, in general seems to have a boarder niche than the populations of *F.macroguiryi* or *F.guiryi*.

When investigating the suggested variables (air temperature and SST) for the model, these variables were unsurprisingly found to be correlated with each other in the suggested study area. Even though SST and air temperature were highly correlated exceeding a threshold of 0.85, they were still used for the model used (Figure 23). This was done to capture as well terrestrial influences on the distribution if this intertidal species rather than just focusing on marine variables. Air temperature and SST seem to be limiting in regional scales. For example, along the coast of France, to be more specific, in the Bay of Biscay SST are higher than in the rest of the coasts (Spain and France). The Bay of Biscay acts like a pocket gathering warm water masses limiting the establishment of Fucus species. The aerial temperatures are suitable for the species however the SST are too warm. On the other hand, along the coasts of Portugal it is the other way around. The areal temperatures are the limiting aspect whereas the SST are favourable for Fucus species showing upwelling dynamics bringing cold water masses up the coast.

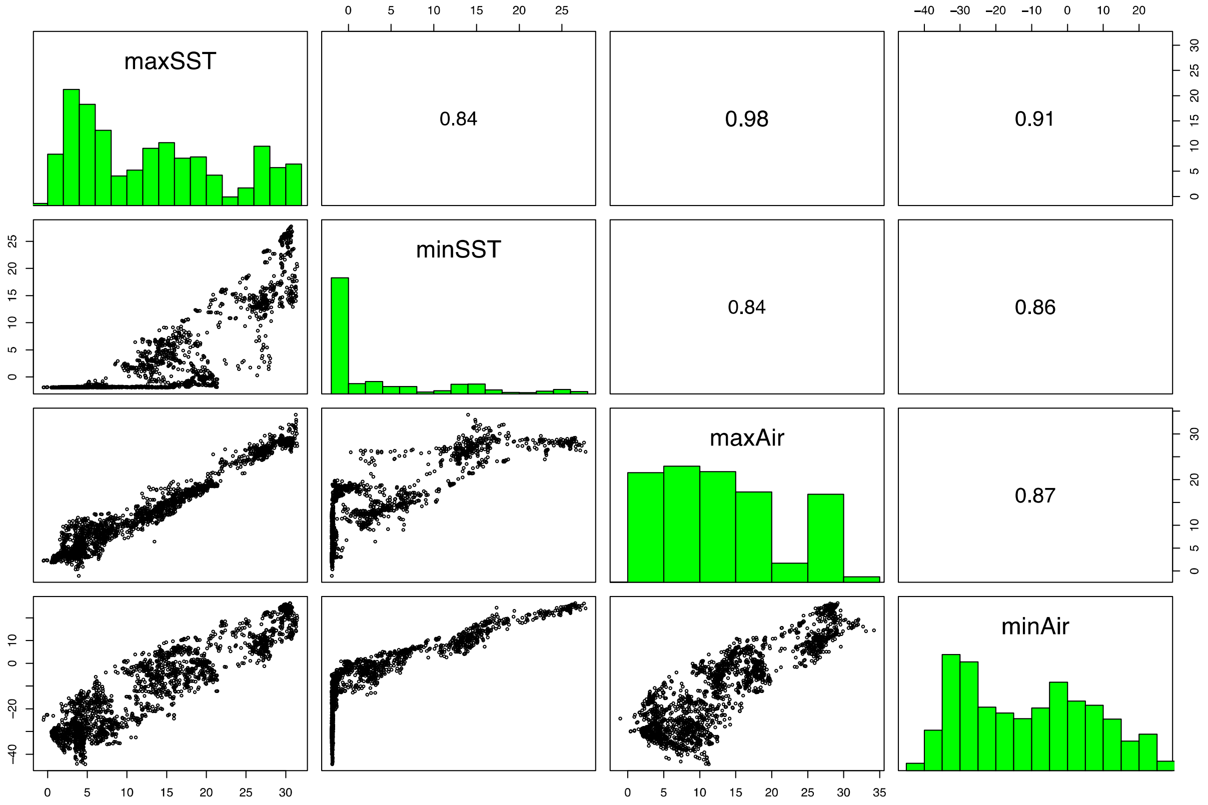


Figure 23: Collinearity analysis among the environmental variables used for the model. Variables were cropped to the coastline to represent intertidal habitat and potential correlation were tested within the study area.

4.2.2 Present Distribution and Future Predictions

The model for the present distribution of *F.spiralis* and *F.guiryi* together showed that maximum SST contributed the most (67.95%) and maximum air temperature contributed the least to the model (less than 5%). For *F.macroguiryi* minimum air temperature played a minor role in the model and contrastingly to the model of *F.spiralis* and *F.guiryi*, minimum SST was the variable that was the most explanatory (66.85%) for the distribution (Table 6).

Even though the number of records used for the model of *F.spiralis* and *F.guiryi* were higher in comparison to the model of *F.macroguiryi*, this model had a lower AUC (0.703) in comparison to the model for *F.macroguiryi* (0.909). To evaluate the models and their reliability and accuracy, elements of the true skill statistics are considered. The sensitivity (the proportion of presences that are correctly predicted) were in both models high (0.918 for *F.spiralis* and 0.933 for *F.macroguiryi*). However, the specificity, for the model of *F.spiralis* and *F.guiryi*, which is the proportion of absences correctly predicted was very poor (0.68).

Table 6: Variable contribution to the models that were developed for the present

	Spiralis + Guiryi (n=50)		Macroguiryi (n=18)	
Variable	Permutation Importance (%)	SD	Permutation Importance (%)	SD
MaxSST	67.95	±16.82	28.03	±24.4
MinAir	23.68	±12.76		
MinSST	8.38	±8.01	66.85	±21.12
MaxAir			5.13	±8.09
AUC	0.703		0.909	
Sensitivity	0.918		0.933	
Specificity	0.685		0.967	
Threshold for reclassification	0.36		0.28	

Therefore is can be suggested that the model for *F.spiralis* and *F.guiryi* will potentially show an overestimation of the likelihood of occurrences given its poor prediction of absences. Predictions for the occurrence of the species were made, where in the next step a threshold was identified to maximizing the sum of sensitivity and specificity. The threshold for each model to gain reclassification maps for the present were 0.36 (for *F.spiralis* + *F.guiryi*) and 0.28 (for *F.macroguiryi*). This application of the threshold generated the desired reclassification map that distinguishes from suitable to non-suitable habitat.

The models that were developed for future projection only used maximum and minimum SST. For the species complex of *F.spiralis* and *F.guiryi* maximum SST was the most important variable (76.9 % ± 11.22), whereas for the model for *F.macroguiryi* minimum SST (72.075 ±21.07) was highly influential (Table 7), which was consistently found within the models that also incorporated air temperatures.

Table 7: Model information which were used for future predictions

	Spiralis + Guiryi (n=50)		Macroguiryi (n=18)	
Variable	Permutation Importance (%)	SD	Permutation Importance (%)	SD
MaxSST	76.9	±11.215	27.925	±21.068
MinSST	23.1	±11.215	72.075	± 21.068
AUC	0.730		0.862	
Sensitivity	0.898		0.867	
Specificity	0.705		0.99	
Threshold for reclassification	0.44		0.61	

As for the models for future scenarios the AUC was lower (0.73) for the model of *F.spiralis* and *F.guiryi* in comparison to the model for *F.macroguiryi* (0.86) indicating a generally lower predictive power. The values for the true skill statistics (1 – specificity + sensitivity), when only considering SST were still relatively high (above 0.6) and therefore it can be generally considered as good models.

According to the produced model for the present distribution, *F.spiralis* together with *F.guiryi* show and extremely wide potential distribution range (Figure 24) in the Pacific from Alaska to California along the whole coastline of north America as well as on the Atlantic coast, around the southern Canadian coasts and USA in from Maine to New Jersey. Iceland appears to be a suitable habitat as well together with vast areas in Scandinavia. Notably the Baltic Sea and parts in the Mediterranean are as well considered as a suitable habitat, which is clearly a flaw in this model. The Baltic has a gradient that ranges from marine to nearly freshwater conditions, where *F.spiralis* is known of being incapable to survive. This model also predicts suitable habitat throughout Iberia and Morocco until the costs of West-Sahara. To see if the involvement of the variable salinity might show a different output the model was performed again by including salinity. In this case the Baltic Sea was still considered as a suitable habitat (but in such great magnitude).

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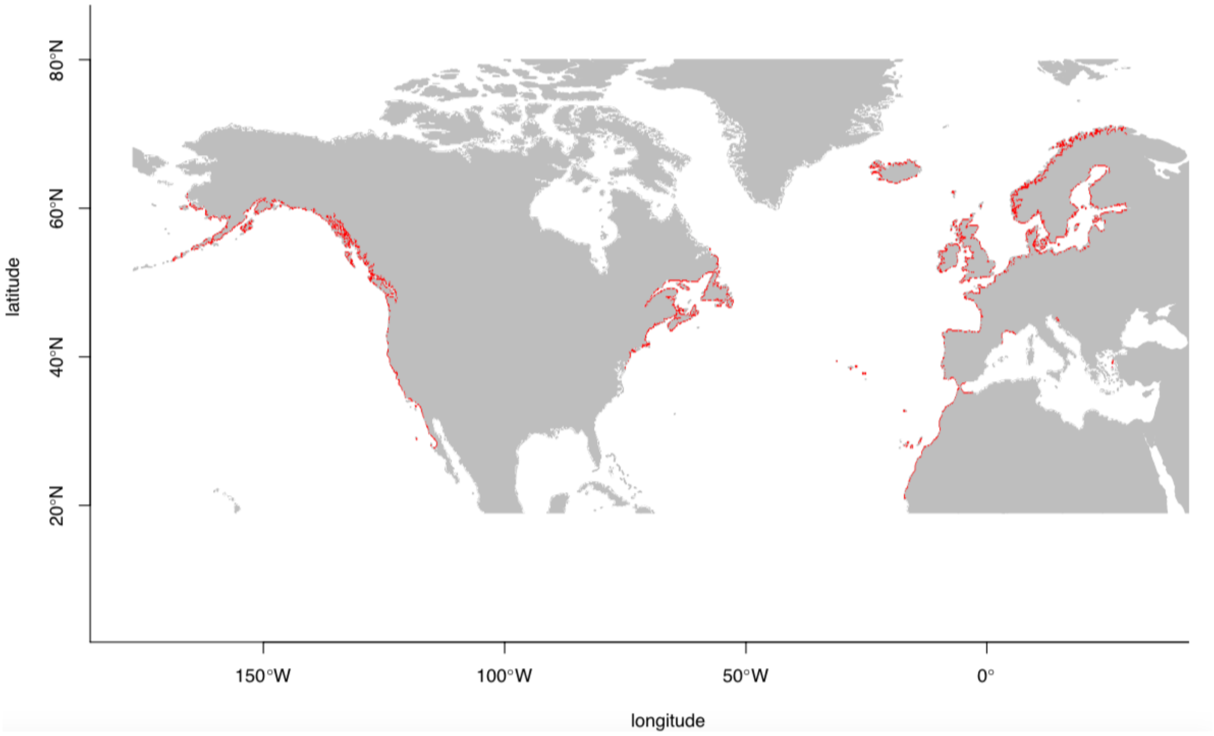

Figure 24: Present distribution model for the species complex of F.spiralis and F.guiryi, where red areas indicate suitable habitat.

The prediction for the future scenario RCP2.6 (not shown) displayed only little to no differences in the habitat suitability, which is not surprising since the overall global climate will not severely change in this scenario. Yet the results from the worst-case scenario are even more striking (Figure 25). The species complex of *F.spiralis* and *F.guiryi* will experience a great poleward expansion period within the next 80 years and might be able to colonize vast areas in the North. The coastal areas in North Canada will become suitable in the year 2100 for this species complex reaching areas even in Greenland. Scandinavia, will remain the same in its suitability for Fucus to exist. The southern margin, such as the Canary Islands and the Azores will very likely become unsuitable challenging them in their existence. The same applies for populations in Gibraltar, which reflects in a decrease of likelihood of existing of 40%. The coast of North Spain was as well identified as a vulnerable and threatened region hosting Fucus populations in the future.

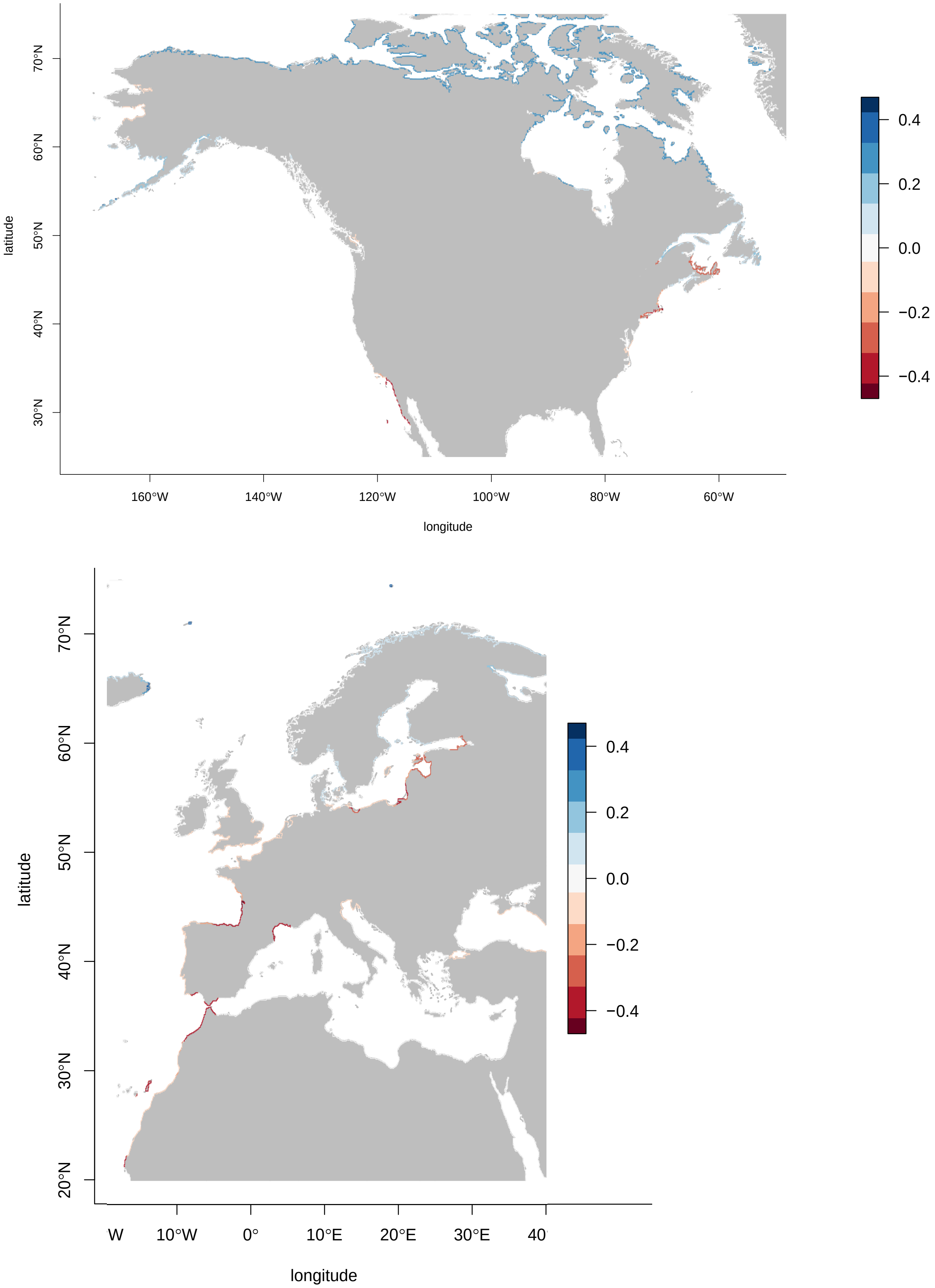

Figure 25: Difference in habitat suitability between the present and the future scenario RCP8.5. Red areas indicate a decrease in suitability and blue areas an increase in suitability.

The habitat that will be newly available will however just enable *F.spiralis* to expand northwards. Conversely, *F.guiryi* will be the most affected with predicted contractions and potential extinctions along its southern range (e.g. Gibraltar, Morocco, West Sahara etc.). However, upwelling along the coast of North west Africa could lower heat induced stress levels on populations in the south and enables them to persist.

For *F.macroguiryi* a poleward range shift as it was observed for *F.spiralis* and *F.guiryi* was noted as well within the different climate change scenarios. At its present distribution (Figure 26) its centre of occurrence is in North West Iberia, partly in Brittany and in the South West of the UK. The areas form Morocco were not considered as suitable habitat (probably due to the low number of samples in this area).

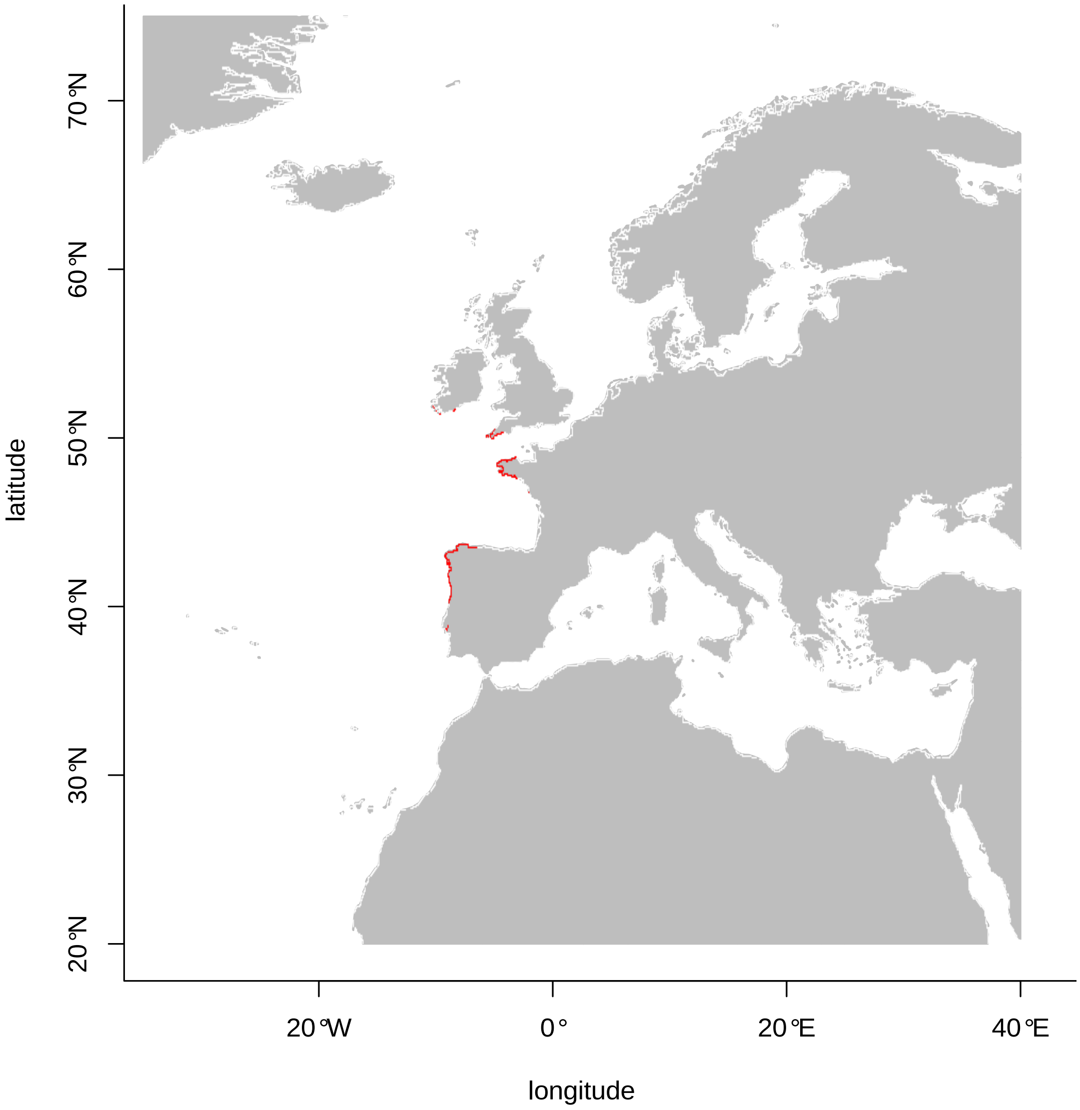

Figure 26: Predicted distribution for the present of F.macroguiryi. Red areas indicate suitable habitat.

Interestingly the prediction for the RCP scenario 2.6 (not shown) would suggest that there might be already a shift into more cooler regions e.g. South Ireland, which is even more emphasised when looking at the prediction for RCP8.5, where *F.macroguiryi* totally vanishes form its native occurrence, namely North West Iberia (Figure 27), which is becoming largely unsuitable for the year 2100 due to increasing SST.

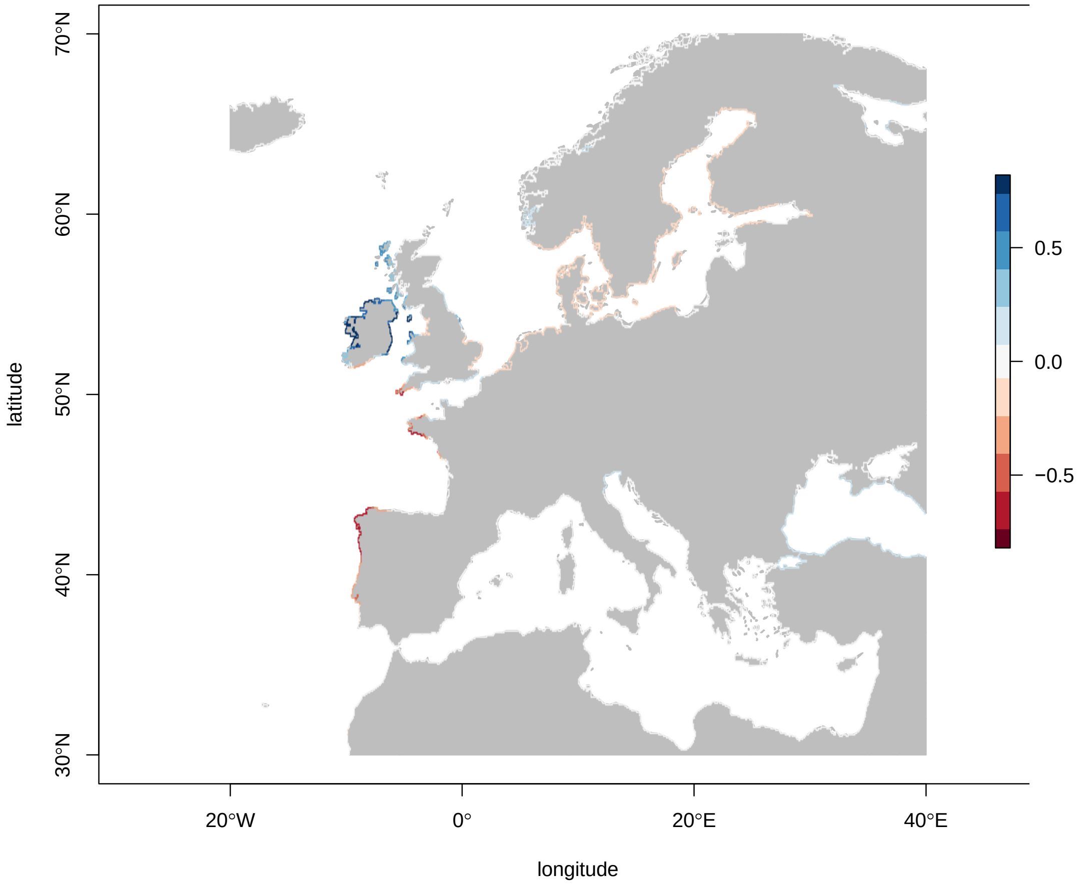

Figure 27: Difference within the present prediction for the occurrence of Fucus macroguiryi and the climate scenario RCP.8.5. Red areas indicate a decrease in suitability and blue areas an increase in suitability.

Gains and losses of suitable habitat were quantified by calculating the square kilometres that were considered as suitable habitat of the produced raster layers (Figure 28). The areas were calculated for the combined model (*F.piralis* + *F.guiryi*) in respect to the northern and southern limits that were revealed by the genetic verified data. *F. spiralis* is experiencing especially in North America and enormous increase in suitable habitat according to these calculations. In the north of Europe, however, the calculated square kilometres of suitable coastal areas will remain the same for the “business-as-usual” future climate scenario, contrastingly to the RCP2.6 scenario where a slight increase in suitable habitat was noted.

For the species complex of *F.spiralis* and *F.guiryi*, only *F.guiryi* will suffer from a severe decrease of suitable habitat and will even totally disappear from the Azores.

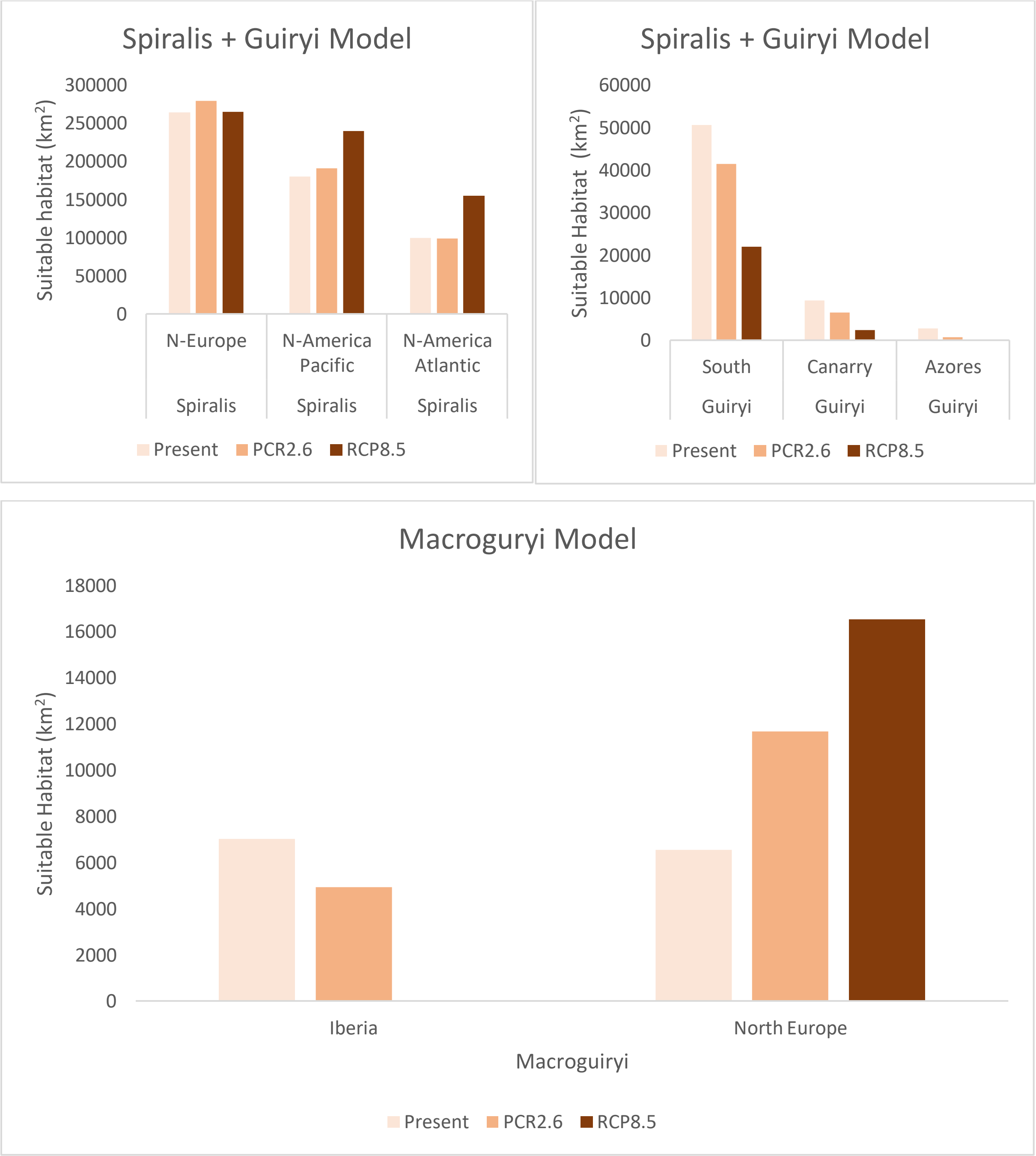

Figure 28: Calculated habitat gain and loss for the present and each climate change scenario separately for each model.

The true numbers of the actual habitat gain and loss remain unknown because the model does not consider suitable substrate e.g. rocky shores. Therefore, this number is very likely an overestimation, yet it gives us an idea about the different outcomes for each entity under contrasting climate change scenarios.

5. Discussion

5.1 Genetic Data

5.1.1 Mitochondrial DNA

The biogeography, phylography and niche modelling of *F.spiralis*, *F.macroguiryi* and *F.guiryi* provides many indication of possible evolutionary dynamics. These include poleward range shifts induced by global warming, and areas of high genetic diversity, which are likely a result of long-term persistence. As expected, the results provided in this study, showed that the investigated Fucus species, which lack of a planktonic phase in their life history, show genetically well-defined populations structures in comparison to highly mobile species, that are often characterized by the absence of genetic structure indicating high levels of connectivity.

The East Atlantic would represent the starting point of the compressed distributional range of the investigated Fucus species. To apply this for the data in this thesis, this would represent the populations which were in NW Iberia at the end of the LGM. It is assumed that populations of *F.guiryi* are abundant in the south and *F.spiralis* might have had its centred occurrence along the NW Coast up to Brittany. This is supported by a hindcast model from another study of *F.vesiculosus* having its main distribution as well NW Iberia at the end of the LGM (Assis et al., 2014). The divergence of *F.spiralis* hermaphrodite lineage and *F.vesiculosus* is estimated to have occurred approximately at the end of the Pilocene (Cánovas et al., 2011), well before the range expansion of both taxa through the English Channel to the North Sea after the LGM according to Engel et al. (2005) and that genetical intermediates as the product of interspecific gene flow occurred after the divergence of the two taxa. *F.macroguiryi* could have been distributed across the entire east Atlantic because according to the genetic and biogeographic data populations from Morocco could be seen as relict populations from past, colder climates, persisting in buffered upwelling areas.

Presumable *F.spiralis* was able to colonize new available areas after deglaciation in northern Europe. Spatial sorting might have contributed to this successful colonization. It can be assumed that leading edge populations after deglaciation consisted mainly out of *F.spiralis* individuals which represented the leading edge dispersal alleles together with *F.vesiculosus*. Spatial sorting reduces the likelihood of genetic exchange between weak and strong dispersers (e.g. *F.spiralis* and *F.guiryi*) and thus promotes one genotype, that further concentrated in higher-dispersal alleles at the expansion front. It is expected that “spatial sporting favours the evolution of increased dispersal ability at the leading edge and resultingly increasing expansion speed through a positive feedback according to Miller et al. (2020). *F.spiralis* and *F.vesiculosus* could have been equally efficient in dispersal, which is reflected by their occurrences in northern Europe. Spatial sorting does not involve the aspects of fitness or dispersal as it is the case when considering natural selection and is a process unique to expanding populations. When strong dispersers (*F.spiralis* and *F.vesiculosus*) escape density dependent competition which was shown to drive genetic structural patterns for *F.cernaoides* in Galicia (Neiva et al., 2012b), then “spatial selection” will favour the evolution of increased dispersal ability with greater reproductive output. As density dependent limitations for a population will decrease, it will eventually lead to high reproduction rates (r-selection). *F.guiryi* might have the same expansion capacity just like *F.spiralis* or *F.vesiculosus* and potentially could have as well dispersed into northern ranges and adapting to colder temperatures. However, because of higher heat tolerance levels and potentially the random circumstances of *F.guiryi* not being within the leading edge populations, *F.guiryi* never managed to expand beyond southern ranges, where this entity could adapt even better to a warmer climate in combination with a decrease of competition with the other entities, while these continued to expand northwards.

Bottlenecks, which probably occurred as a logical consequence of founder events, where a few individuals were colonizing the North of Europe, it was likely that their offspring were continuing to colonize the next generations front edge. Eventually these mechanisms lead to lower levels of diversity among entities, but high diversities within the entity of *F.spiralis*. Additionally, alleles that were originally present at the leading edge and those that arose from mutations could “surf” to higher frequency on the front. Even though brown algae show a quite simple body structure, they are the most complex macroalgae, which can rapidly adjust their morphology to environmental conditions by modifying their molecular mechanisms accordingly (Charrier et al., 2012). Given that *F.spiralis* is still radiating and shows high adaptation capacities to very heterogenous habitats it can be assumed that the expansion wave of colonization towards the North happened evolutionary speaking at a fast speed during the post-glacial periods within the last 16K years.

The nuclear genomic data showed in the study of Cánovas (2011), implies that *F.spiralis* and *F.guiryi* are closely related, having diverged in the past 800K years. The nuclear genomic data furthermore suggests that *F.vesiculosus* represents a very distinct lineage clearly separated from the hermaphrodite species complex that includes *F.spiralis*, *F.virsoides*, *F.macroguiryi* and *F.guiryi* and stands in conflict with our mtDNA sequence data. Indeed, the IGS network suggests that *F. spiralis* and *F.vesiculosus* together with F.*macroguiryi* are closely related, while the southern entity representing *F.guiryi* seems to be well separated. Therefore, the first conclusion that can be drawn is that the mitochondrial genome does not reflect the nuclear genome (meaning the actual species tree and relationships among Fucus). Phylogenetically these results do not coincide with each other and have to be looked at separately. The haplotype network based on the mitochondrial DNA is clearly driven by more complex processes such as hybridization, random processes or selection. The fixed genetic differences presented here indicated that *F.macroguiryi* is clearly a distinct species with a specific ecology and morphology in respect to *F.spiralis* and *F. guiryi*. The fact that *F.macroguiryi* is more heat susceptible, proven by differences in selective pressures by using emersion times as proxies (Billard et al., 2010), might leave room for the hypothesis that the ancestor of *F.macroguiryi* might have had split from the *F.vesicuolosus* lineage at the end of the Pilocene/beginning of the Pleistocene before the divergence of *F. spiralis* and *F.guiryi*. However, an updated multi-gene phylogenetic approach including *F.macroguiryi* is necessary to investigate this hypothesis.

Zardi et al., (2011) suggested that “allopatric F.guiryi” in the south is considered to be a pure entity, whereas “sympatric F.guiryi”, which was proven here is actually *F.macroguiryi,* would have resulted from hybridization/introgression with *F.spiralis* and *F.vesiculosus*. According to this study, sympatric *F.guiryi* (=*F.macroguiryi*) morphotypes are polyphyletic, which is suggestive of extensive hybridization and introgression with both other taxa (*F.spiralis* and *F.vesiculosus*). However, if *F.macroguiryi* would be a result of hybridization between *F.spiralis* and *F.vesiculosus*, genotypes that had a proportion of both parental species would be found. Yet, this study shows that *F.macroguiryi* is a well delimited species with its own evolutionary history, indicated by not only its genetic pattern but also ecologically and morphologically. Furthermore, it does not seem to admix in most places even when in sympatry.

Moreover, the authors suggest that the current data of the distribution of F.guiryi supports a model of secondary contact in the northern range (in South West Portugal), where speciation in sympatry is strongly driven by steep environmental gradients. Recurrent environmental processes together with weak ongoing geneflow between entities contributing to the differentiation of F.macroguiryi and its relatives F.spiralis and F.guiryi are still unknown. The characteristic ecological position of F.macroguiryi lower on the shore relative to the other entities, supports the argument of being a separate delimited species.

Since the genus Fucus successfully colonized and diversified in the East Atlantic, we hypothesize that all species regularly underwent severe periods of contractions and expansions for hundreds and thousands of years. Within the last 10.000 years the climate had been relatively stable and with deglaciation processes in the northern hemisphere, it allowed Fucus species to colonize new areas towards the north pole as described above. Furthermore, we assume that by the end of the LGM NW Iberia might have been an area of long-term persistence because all three entities are currently present in this area. Another indication leading to this assumption is that the haplotype of isolated *F.guiryi* populations in Asturias was not present in the southern areas. If these populations would happen to have recently colonized Asturias, the same haplotype should have been found the region where these individuals would have sourced from. However, the haplotypes found in Asturias for *F.guiryi* were highly unique. Unknown oceanographic barriers in Asturias and along the North Iberian coast in combination with generally warmer water masses that are trapped in this distinct area could have led to the long term persistence and restriction of *F.guiryi* populations in this area. A comparison of terrestrial vegetation (pollen data) and oceanographic data from deep ocean cores revealed that approximately 23 – 19K years ago the climate in Iberia was quite stable and appears to have been rather warmer and moister. Even though vast ice sheets were dominating the northern hemisphere in the time of the LGM, the global climate might not “necessarily see the most severe climatic conditions everywhere” (Roucoux et al., 2005), making it a suitable area for these intertidal algae to persist. In marine environments barriers to geneflow or dispersal, are often less obvious than in terrestrial environments. Vicariance events are mainly mentioned as the main drivers for genetic intraspecies divergence over time. However, a study on *F. ceranoides* in Galicia showed that there are density dependent barriers between populations (Neiva et al., 2012b), which could have as well contributed to the Asturias populations persisting. Despite the difficulty of determining which of the aforementioned factors and in which magnitude in the end was responsible for this isolated population of *F.guiryi* in Asturias, it can be said that historical patterns of isolation and colonization can explain the population structure better than more recent events, especially for a species with such poor dispersal ability. Not only for the entity of *F.guiryi* endemic populations were found (Asturias) but also haplotypes corresponding to *F.macroguiryi* were unique in Praia do Rio Meirás in Galicia (Mei) and in Ilha de Arousa (Aro) and found nowhere else.

The distinct haplotype corresponding to *F.macroguiryi* in NW Iberia (Bay) and in France (COT and partly in MALL) also indicate that there might be an impact of past warmer climate periods, which shaped the genetical structure in this area. The same haplotypes found in Mococco (JadL and ESSL) are likely to be are relict populations that were able to persist because of upwelling dynamics in this area (Lourenço et al., 2016), while in the SW of Portugal the environmental conditions are not suitable enough for this entity in present times.

All of the three entities showed different distributional centres. The high diversity of *F.macroguiryi* in NW Iberia and of *F.guiryi* in the south suggests that the entities have the longest persisted in these areas. High levels of endemism and diversity of the Iberian region agree with the expectation of long term persistence of seaweeds in glacial refugia and was as well confirmed in other studies (Hoarau et al., 2007). A typical star shaped haplotype network, as it is shown for the *F.spiralis* entity, indicates a long evolutionary history. With time mutations in the mitochondrial DNA inter genetic spacer accumulate leading as well to higher nucleotide diversity levels within this entity. However, the high diversity of *F.spiralis* in the north (when excluding introgressed populations in SW Portugal) might indicate a recent colonization an still on-going radiation in combination with a lack of competition leading to such high diversity levels rather than long term persistence.

Individuals form Gibraltar, are likely a result of long-term persistence in combination with historical events. The populations are exceptionally interesting because this haplotype was not found in nearby located populations. With the environmental conditions being slightly cooler at the strait of Gibraltar on a broad timescale and relatively stable over time, this could be a reason for the presence of these populations. Mediterranean outflow water (MOW) is known to exit the Mediterranean basin in greater depth contributing to the North Atlantic deep-water formation, whereas water from the Atlantic, which is colder, is entering the Mediterranean on the surface layers. The exchange of these water masses, driven by the difference of salinity, and as a result a difference in the density of the water, can create internal waves along the strait of Gibraltar. Internal waves, which enter from the Atlantic towards the direction of the Mediterranean might create a sort of “mini-upwelling” bringing very cold-water masses from the deep to the surface. It should be noted that the “Gibraltar-Sequences” fount in North America in CANA and Pem2 fully correspond to the main Gibraltar core (- two very closely related haplotypes, separated by just one mutation were found just in Gibraltar itself). Therefore, it could be assumed that this haplotype originated in Gibraltar and was likely anthropogenically brought to these locations in North America via shipping, inducing a genetic exchange via secondary contact with populations from Europe. Other dispersal mechanisms such as drifting can be ignored because the distances between Gibraltar and the East coast of North America are simply too large. If this haplotype would have evolved in North America, there would be a higher diversity of these haplotypes, which is not the case.

By looking at the mtDNA found in North America and the absence of other entities of the investigated Fucus complex, it could imply that other *F.spiralis* species might have a stronger capability to outcompete *F.guiryi* and *F.macroguiryi*. However, if *F.spiralis* would have been present in North America for a long time, the genetic diversities would be higher, especially if there is no competition induced by *F.guiryi* or *F.macroguiryi*. The likeliest scenario to explain the little diversity in North America its due to recent colonization. As mentioned before, the genus *Fucus* likely originated from the Pacific and gradually colonized the easter Atlantic, where the leading-edge populations radiated, and the rear edge populations went extinct. This makes the East Atlantic in general to the hot spot of diversity for Fucus species, which supports the idea that the *F.spiralis* individuals analyzed here just recently colonized North America.

The adaptation of *F.guiryi* haplotypes to greater heat influence giving its presence in the southern margins of this species complex, is well presented in this data. The same haplotypes from Morocco were also present in South West Portugal, which were located in the upper intertidal zone in comparison to mitochondrial DNA corresponding to the *F.spiralis* haplogroup. Mitochondrial data of the lower situated population in Amoreira (AmoL) could not be obtained, but we hypothesize that the same partitioning that is present in Peniche and Sines since the most southern distribution of *mt-F.spiralis* is in Sagres (SAG). In general, the southern as well as the northern haplotypes are equally dominant in South West Portugal, which therefore is considered to be a contact zone of these two entities. A reason why no *F.macroguiyi* sequence could be detected could be because the competition with *F.spiralis* and *F.guiryi* is anyway extremely high in this area and does not allow *F.macroguiyi* to establish itself. The influence on temperatures surely cannot be ruled out. *F.macroguiryi*, generally occurs in areas with upwelling dynamics and cooler temperatures, additionally to a lower vertical position relatively to *F.spiralis* and *F.guiryi* on the shore. A clear zonation pattern was illustrated by the data, yet in some location e.g. in Aveiro, individuals form the lower population owned partly "core F.spiralis" mitochondrial sequences, which is probably simply a biased sampling effect. Potentially the topographical characteristics of these areas blurred the zones of "high" and "low". In other locations in NW Iberia (e.g. La Franca and Lastres) only *F.spiralis* and *F.macroguiryi* respectively were found, indicating no such coexistence of both species according to a vertical gradient or, which is more plausible can as well be explained by the mentioned sampling design bias and the possibility of presence of *F.spiralis* haplotypes or *F.macroguiryi* haplotypes can surely not be excluded in these areas. The same applies to Meanporth, Cornwall (MEAH and MEAL) and Larmor (LARH and LARL) where mixed proportions of *F.spiralis* and *F.macroguiryi haplotypes* according to the vertical zonation were found – probably again related to the sampling design. In a broad sense, this data showed that with some exceptions *F.macorguiryi* occurs on the lower shore in comparison to *F.spiralis,* a well-defined characteristic of this species.

The reason, why the haplotypes found at the Azores were so different than the other southern sequences from the mainland can probably be led back to a long-term isolation to the original mitochondrial pool. In combination with selection and time passing it resulted in a group of very distinct haplotypes, which probably also were more successful in this geographic area regarding certain gene expressions as heat responses etc. Only in São Miguel both haplotypes of the "Azores-haplotype-clade" were found. São Miguel being the largest island of this island group and resultingly being able to provide the most resources (e.g. potentially suitable habitat like suitable substrate etc.), its populations could serve as a genetic pool / source populations for the other islands (Pico, Terceira and Faial), which as a result could be considered as sink populations. Combining these findings with MacArthur and Wilsons island theory and metapopulation models might be too far-fetched with such little data and could be investigated in another study in the future. Additionally, more samples and more genetic data could be gained to make better and clear hypothesis regarding the degree of differentiation within these two Azorean haplotype groups. The distinct genetic signature of samples from the Azores could as well be related to the findings of Hoek et al., (1990), who postulated that many temperate seaweeds are believed to be restricted to this area (together with the Canary Island and NW Africa) during the LGM.

5.1.2 Cryptic long-term Persistence in Santa Eulália

The analysis of the mtIGS marker for the population in Albufeira (Santa Eulalia) confirmed the hypothesis of a long-term persistence capacity allowing Fucus individuals some resilience to short-term perturbations that kill macroscopic individuals. The first hypothesis of long-distance migration was rejected. Dispersal of the investigated *Fucus* species is very limited due to their inherent selfing reproductive mode and lack of floating organs. Only *F.vesiculosus* is known to be able to drift far distances because of the presence of floating organs for this species. Anthropogenic inducement to non-native locations i.e. via shipping was overserved in the past (Brawley et al., 2009), yet there has not been outstanding evidence that would prove that *F.spiralis*, *F.guiryi* or *F.macroguiryi* are capable of rafting great distances in order to colonize new areas. In situ survival of cryptic stages had been reported with growing evidence for marine macroalgae, but research on this topic is still scares. Many organisms, both marine and terrestrial, rely on banks of microscopic stages to survive unfavourable periods. Especially microscopic life forms, which in the literature are in referred to as “banks of microscopic forms” (Chapman, 1986) for marine macroalgae tend to be more tolerant to unfavourable light, nutrient and especially temperature conditions. There had been evidence that for example microscopic gametophytes of *Macrocystis* are able to survive weeks and even months (Deysher & Dean, 1984) in the field. Several mechanisms were as well studied in the past and showed that biological control from neighbouring conspecific may also be important. Cues from individuals in close proximity might induce the production of microscopic stages when unfavourable condition are about to happen. According to Carney & Edwards (2006) microscopic stages of marine algae are very likely to remain physiologically active and highly sensitive to changes in the environment. Especially because knowledge the field of long-term persisting microscopic stages in marine macroalgae is scares, it also might be possible that some large brown seaweeds vary in their forms of these microscopic life stages and research should focus on topics as such. In comparison to terrestrial seed banks, which are usually located within the sediment, the microscopic forms of marine macroalgae are present on rocky substrates in close proximity to the adults facilitating their investigation with advanced technology such as fluorescence microscopy to identify the different stages. Edwards (2000), for example showed that microscopic life stages of *Desmarestia ligulate* can persist overwinter during periods when the macroscopic thali are absent. Surely, recognizing the entire life-cycle of marine organisms is critical to understand their ecology and determine their susceptibility to climate change, as it is the case for the populations located in the South of Portugal.

Although the mtIGS confirmed the hypothesis of long-term persistence instead of immigration from near-by populations, there is severe reason for criticism. Firstly, this investigation lacks proper scientific monitoring design. No continuous data (e.g. density counts or presence/absence data) was collected for these populations. The apparent extinction is simply based on empirical knowledge from other researchers. Secondly, the study of Zardi et al. (2011) used samples that were from that location to morphologically characterize traits of “allopatric F.guiryi”. In this paper it was mentioned that “at this sheltered location individuals reach a larger size that allows greater confidence in morphological characterization, relative to the typically stunted and wave- damaged individuals encountered on the more exposed southwest coast of Portugal”. The authors also mention that only undamaged individuals were collect to avoid morphological biased identification caused by wave brakeage”. This is clearly not congruent regarding the state of the population now. When the samples were obtained this year, the individuals looked like as if they experienced great levels of grazing pressure. Many individuals showed clearly marks of predation and were in general very small (- not even outstandingly existent from far away; Figure 29).

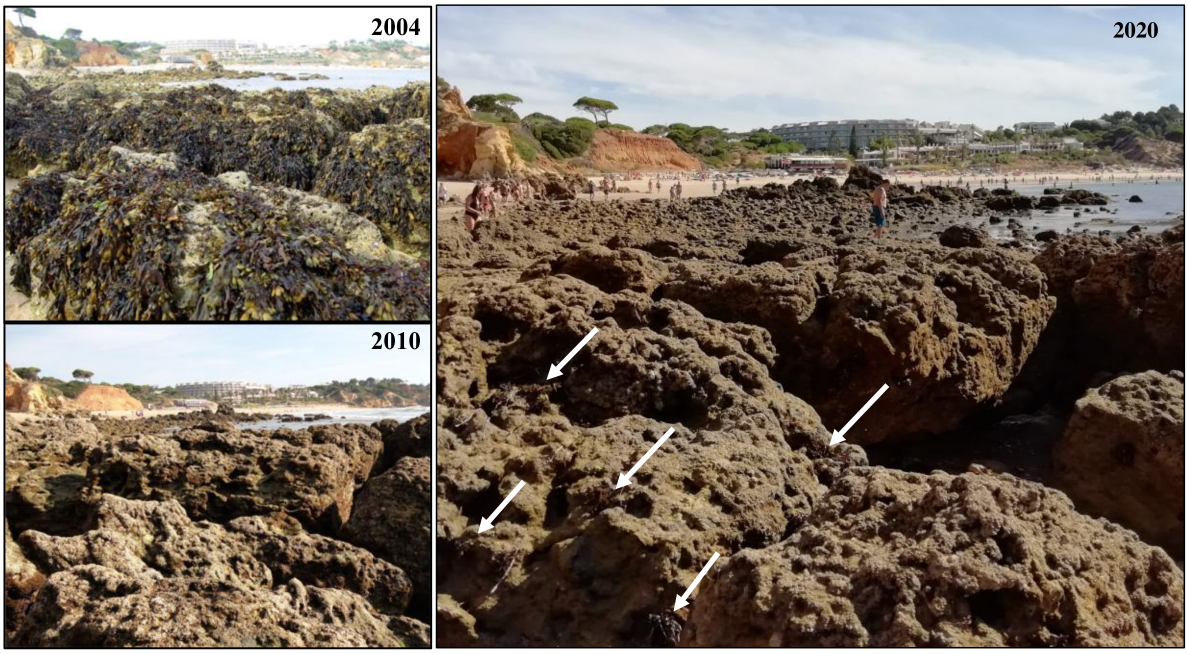

Figure 29: F.guiryi cover in 2004, 2010 an 2020 in Santa Eulália, Albufeira (South of Portugal). Pictures from 2004 and 2010 extracted from the study of Lourenço et al., (2016). Fucus populations present in 2020 had a very small size and are indicated by white arrows.

According to Lourenço et al., (2016) individuals vanished in 2010, whereas the samples list for this thesis indicated that individuals were sampled from 2011 and 2013 from this exact location. The time frame is quite inconsistent and the reasons for the severe decrease in population size is unknown. Even if not temperature but grazing might have driven the population size in Santa Eulália to a minimum, where no functioning populations that neither build up biomass, nor reproduce nor showed any indication of their presence, it can still be assumed that Fucus species are able to restart their populations from a potential cryptic/dormant microscopic stage. Clearly a follow-up study with a better monitoring and sampling design is necessary in order to clear all doubts that are mentioned. Additionally, the confirmation of the marker L20 with a size of 181 will also contribute in providing more robust results.

5.1.3 Microsatellite based Population Structure (PCA, DAPC and STRUCTURE)

The markers that were used to determine the belonging of each entity were sufficient enough even though some exceptional patterns were observed. The populations in Osmington Mills (OSM) in the UK, for example had both alleles in L78 corresponding to the assignment of *F.macroguiryi* and *F.spiralis* indicating potential hybridization within this area. Likewise, in North West Iberia (e.g. population in AREL, NIOH, RVG or Aro) the typical assignation to one or another entity, when using this locus (L78) could not be clearly made.

The majority of the unique alleles could be assigned to the group of *F.vesiculosus* haplogroup (mainly samples from the UK e.g. Porth, LUK, BarH,…), which were highly polymorphic, since this is an dioecious species. In general, the great heterozygote deficiencies in the hermaphroditic species complex can be explained by high rates of selfing. Selfing also increases premating isolation, and thereby creates a barrier to complete mixing of *F.vesiculosus* and *F.spiralis*. Being on the upper shore (in comparison to *F.vesiculosus*), *F.spiralis* has to deal with severe desiccation stress that may actually favour self-fertilization. This reproductive system maintains favourable gene combinations by reducing recombination. Brown algae are known to harbour a high diversity in biological mechanisms that governs their development, which could further show great morphological plasticity. This makes them the group with the highest morphological diversity overall of the three groups of macroalgae (green, red and brown.).

Samples that showed to have a mtDNA corresponding to *F.vesiuclosus* should as well be discussed in respect to the genomic data found. First of all, many studies illustrated that *F.spiralis* and *F.vesiculosus* are clearly defined and distinct species (Billard, et al., 2005; Billard et al., 2010; G. Zardi et al., 2011) and at the same time hybridization is possible (Moalic et al., 2011). A number of studies independently come to the agreement that reproductive isolation between *F.vesiculosus* and *F.spiralis* are not complete (Billard, et al., 2005; Engel et al., 2005; Wallace et al., 2004). The unweighted pair group method using an arithmetic average (UPGMA) used for the reconstruction of the phylogeny of *F.ceraniodes, F.spiralis* and *F.vesiculosus* also showed, that *F.vesiculosus* was scattered within this phylogenetic tree and implied that this taxon is not as genetically cohesive as the other taxa of (Billard et al., 2005). The presence of intermediates between *F.spiralis* and *F.vesicuolsus* is considerately related to geneflow occurring after the divergence of these taxa. Interspecific geneflow is most likely the product of rare, unfertilized *F.spiralis* eggs crossed with *F.vesiculosus* sperm. In general, dioecious *F.vesiculosus* showed to have a much higher contribution of male gametes, given that the distances between eggs and sperm are generally larger, because the originate from different individuals (male and female), than the other hermaphroditic species This provides sufficient likelihood of fertilization.

The "vesiculosus" – individuals that were dealt with in this analysis presented, might already show a high admixture with *F.spiralis*, (showing maybe not only genetically but also morphological), and could explain as well how these samples got into the data set, since they were sampled under the name of “F.spiralis”. However, this hypothesis of morphological similarity of *F.vesiculosus* to *F.spiralis* remains unclear for these exact samples because the original morphological traits were not documented. Introgressive hybridization of mtDNA, which might have been possible during range expansion periods (e.g. in the south during the LGM) could be another reason to explain this observation. It is also known that mtDNA experiences a much higher genetic exchange frequency between individuals, than genomic parts of the DNA. Moreover, “*F.vesiculosus*” individuals do not represent a cohesive group in the data set, which also could be a reason for the non-existent separation between *F.spiralis* and *F.vesiculosus* regarding the results of STRUCTURE for K=2. If we take the evolutionary history into account, it could also be hypothesized that the hermaphroditic lineage (*F. vesiculosus*) did not have one common ancestor of which this hermaphroditic lineage all arose. Instead, each hermaphroditic entity arose from a common ancestor with *F.vesiculosus,* of which *F.guiryi* is the most recently evolved one. Thirdly, selection in favour of the most heat resistant mtDNA, might as well explain the disagreement of mtDNA found and cluster assignation of the genomic data. Clearly the complexity that is enforced with probable hybridization blurs species distinctions and complicates the identification of the best structure for this species complex. On the one hand, the PCA stood in great agreement with the mtDNA data, separating *F.spiralis*, *F.guiryi*, *F.macroguiryi* and the Azores with sub-clusters of Gibraltar and Asturias and on the other hand the DAPC seemed to represented an intermediated grouping of the STRUCTURE results for K=5 and K=6. It is hard to determine the true number of K as well as the differentiation within these clusters that would help to identify subpopulations. In a study, where simulated data was tested to identify flaws in the interpretation protocol for STRUCTURE (Lawson et al., 2018), it was proved that generally speaking a DAPC was found to perform better than STRUCTURE at characterizing population sub-division and allowed to unravel complex populations structures. One has to keep in mind that “the true value of K may not always possible to asses, but it should be aimed to capture the smallest value of K that illustrated the major structure in the data”, according to Pritchard et al., (2000). Model based approaches rely on assumptions such as the type of population subdivision, which are often difficult to verify and can restrict their applicability. Overall the results of STRUCTURE have to be looked at with caution considering firstly, the low value of ΔK, secondly the lack of differentiation between *F.spiralis* and *F.vesiculosus* and thirdly the inability to separate species on the expected level of *F.vesiculosus, F.spiralis, F.guiryi, F.macroguiryi* and the populations from the Azores. Yet these clusters provide broadly illustrate the populations structure and leave room further discussion. Regarding the output of STRUCTURE on a more detailed level, populations from the South West coast of Portugal harbouring a mix of *F.spiralis* and *F.guiryi* haplotypes did not show, as could be expected, any signs of admixture. Irrespective of the mtDNA of the populations, also even from the same location (e.g. Sines, and Peniche) populations showed a relative genetic homogeneity, with individuals from this area assigned to the cluster of *F. guiryi*. Therefore, it can be concluded that individuals containing mtDNA of *F. spiralis* are a result of introgression of mtDNA. Mitochondrial DNA of *F. spiralis* might also be a remnant of the female contribution when reproducing with each other.

At this point it should be referred to the origin of the microsatellite markers that were used. All of these markers were originally developed for *F.vesiculosus* and therefore the observed lack of biodiversity for the investigated species can be considered as an artifact. This lack of biodiversity within the loci is very likely not the case and cannot be applied to the whole genome. A comparison with other studies using the same makers entails difficulties as well. Although the same markers were used, allele sizes differ because of the usage of different sequencing machines or different size standards (e.g. LIZ 500 vs Li-Cor 4200 used in Billard et al., 2010). These factors hinder the comparison of many studies and complicate the calibration of the results gained with these microsatellite markers. However, there are implication that these markers are diagnostic for *F.spiralis* (L78:122) and *F.ceranoides* (L78:131) - allele sizes from Billard et al., (2005)) but can only be diagnostic for *F.vesiculosus* by the absence of these fixed alleles.

The number of markers and the differences in the weight that they contribute to clustering the data is as well another point that lead to odd results. Regarding the principal component analysis one individuals (AveH) was close to the very distinct unit of the Azores. One could come to the conclusion that this indicates that individuals from Aveiro could be the ancestor populations but another explanation for this result should be acknowledged instead. A reason for that could be the application of the eight markers chosen for this study. The weight of each marker contributes to the formation of the components of which the analysis is based on. Therefore, the number of markers and their weighted values determine the assignation to a cluster. If only one marker falls out of the common pattern that would be expected due to its geographic locations and the surrounding populations, these individuals are falsely attributed to a certain cluster. A higher number of microsatellites would in theory enhance the discriminatory power of each cluster and additionally provide greater insight on diversities among entities, populations and regions.

5.1.4 An Approach to define the term "Species" for this Species Complex

Overall this study illustrates three entities (or four entity when counting the Azores separately) of a species complex regarding the genetic traits and their biogeography. However, one has to keep in mind that this study does not consider the aspect of morphology of these entities, which often plays as well a crucial role in determining a species and describing it. Morphological descriptions were carried out in the past (Zardi et al., 2011). However, hybrids might exist showing not only genetical variants of both entities (e.g. *F.macroguiryi* and *F.spiralis*) but as well morphological traits of these species units making it even more difficult to determine and define the term species for this species complex. Empirical observations showed for example that different morphotypes exist, but at the same time it is possible to assign the individual to a clearly defined genetic entity (e.g. Fucus vesiculosus var. volubilis in the Ria Formosa, which lacks of its typical air bladders and is morphologically very unique – but genetically shows clearly the signature of the *F.vesiculosus* entity). An interplay of environmental forces such as wave exposure and desiccation levels additionally contribute to different morphological characteristics leading to an extremely phenotypical diverse species complex. The morphology of a species, being the most eye- catching and easy to assess for human beings in comparison to genetic patterns, might be the first hint of a development of a new species and extremely recent or fast radiation. Already in 1993 three different varieties of *F. spiralis* were found through a study of morphological variation patterns (Pérez-Ruzafa et al., 1993). If these three different varieties described in the past might also be congruent with the three genetic units displayed in this thesis remains unsolved yet is very likely to be the case. In fact a morphological characterization of *F.spiralis*, *F.guiryi* and *F.vesiculosus* was performed (Zardi et al., 2011) but this assessment just considered individuals from two sites only (Viana do Castello and Roscoff). In the study of Zardi et al (2011) the results showed that even if STRUCTURE clearly assigned individuals of *F.guiryi* and *F.vesiculosus* to their corresponding cluster genetically (admixture analysis based on two microsatellites - L20 and L78- , which were found to be diagnostic), several individuals morphologically identified as *F.spiralis* and *F.vesiculosus* displayed genetic characteristics of the other entity. The most inconsistencies between morphology and genetic signature involved *F.guiryi* (=*F.macroguiryi*) and

F.vesiculosus. Vegetative morphological continuity between *F.spiralis* and *F.vesiculosus* is generally well documented (Burrows & Lodge, 1951; Pérez-Ruzafa et al., 1993).

Several studies had been undertaken in the past to investigate the integrity and genetic structure of cooccurring Fucus species in sympatry. Especially, the differences in the mating systems of *F.vesiculosus* in comparison to the three entities (*F.spiralis, F.guiryi* and *F.macroguiryi*) stands in the focus of ongoing science and implies several different expectations. Many studies were performed on the reproduction success of *F.spiralis* and *F.guiryi* versus *F.vesicuolsus*, however in previous studies certain factors need to be clarified. Firstly, many fertilization experiments were performed with individuals from Viana do Castelo, which represents the most southern limit of these species occurring in sympatry and is additionally protected from severe wave action making it a very attractive location to perform such experiments. Knowing in the data that is reported in this thesis, it is obvious that the species that were investigated under the name “F.guiryi” were actually *F.macroguiryi*. Secondly, genetically confirmed data should be included when performing such experiments on egg release in order to surely identify mechanisms that might contribute to enforcing species boundaries together with the avoidance of including hybrids masking the effects. Broadly speaking, the main difference seen in commonly performed egg release experiments is that *F.spiralis* shows an significantly larger egg dispersal radius in comparison to *F.vesiculosus* (Ladah et al., 2008). *F.vesiculosus* is more in synchrony with the environment and may have developed a specific adaptation for timing the egg release, where most eggs are released later in the day at a lower tide than *F.spiralis*, which releases fewer eggs throughout the day at all tides. This proved, that interspecific asynchrony between *F.vesiculosus* and *F.spiralis* is acting as prezygotic barriers and enforcing species boundaries. Interestingly an earlier study from the same author on the fertilization success of *F.vesiculosus* and *F.spiralis* (Ladah et al., 2003) used species from Viana do Castelo and transplanted these individuals to a location in the South of Portugal (in Sines and Vila Nova de Milfones). It is very unlikely, that the *F.spiralis* mtDNA found in these location could result from such transplantation experiments, however this can hypothetically be taken into account. However, if this would be the case, it would be possible to see the genomic signature (together with the mitochondrial genome) of a pure *F.spiralis* at this location, which was not the case. Anyway, the anthropogenic inducement of non-native species still might play regionally a role and could be investigated in a study dealing at a smaller scale. By 2012, when *F.guiryi* was already identified as a own species, Monteiro et al., (2012) illustrated interspecific patterns of egg release for *F.guiryi*, *F.spiralis* and *F.vesiculosus* that were coincident at seasonal to semilunar timescales. Again, individuals were derived from Viana do Castelo and the results do not apply for *F.guiryi* but only for *F.macroguiryi*. Apparently, the largest events of gamete release on an hourly scale during the tidal cycle were shared mainly by *F. spiralis* and *F.macroguiryi* (in the paper referred to as *F.guiryi*). Lastly, a study was undertaken on a fine circadian timescale (Monteiro et al., 2016), comparing the temporal windows of egg release of sympatric *F.vesiculosus* and *F.serratus* versus *F.spiralis* and "*F.guiryi*" (in fact *F.macroguiryi* because individuals were again from Viana do Castelo). Major egg release events were observed during high tides, when the individuals were immersed, and the water movement is reduced during four neap tide periods over two months in June and July 2015. Minor differences were shown between *F.spiralis* (peak at 3:00) and *F.macroguiryi* (with a peak at 1:00 and 3:00). The greatest differences were illustrated between the different reproductive modes of the aforementioned species, which is supported by their divergence time that could be less than 1MYA (Cánovas et al., 2011) implying an ancestral characteristic. Earlier-diverging dioecious members of the genus were all spawning during daytime at high tide (Monteiro et al., 2012). Early morning spawners in the hermaphrodites (*F.macroguiryi* and *F.spiralis*) could indicate a recent change to a modified or alternate signal – response pathway. The water motion sensing system within species having different reproduction modes is clearly different. As the differences within the hermaphrodites are not as significant in comparison to the diecious species there is the need for further investigation on the drivers that still maintain their species boundaries.

The main focus of these studies were on differences in the reproductive systems however future research should as well explicitly focus on cooccurring hermaphroditic *F.macroguiryi* and "the true" *F.guiryi*. Research in the past found that reproductive strategies are the most likely to be involved in maintaining the different species and therefore other factors might contribute to the clear delimitation of *F.macroguiryi* to *F.spiralis* and *F.guiryi*.

F.macroguiryi follows the same reproduction system as *F.spiralis* and *F.guiryi* , which lead to the question of how they are still retaining their species integrity. The answerer might be the (partial) habitat segregation. As described *F.macroguiryi* is ecologically much more restricted to the lower shore and therefore individuals might not be in close proximity to hybridize among each other. Geneflow in the past might have even facilitated divergence induced by adaptation and did not have a homogenizing effect, which would have counteracted the diversifying effects of local selection. Individuals that are locally adapted like *F.macroguriyi* to colder temperatures may be maladapted to contrasting environments, such as the higher intertidal zone and experiences reduced fitness, leading to diminished rates of gene flow. Diversifying selection might play a role in maintaining genetic groupings despite evidence of gene flow between them. In future research emphasis should lay on the potential temporal or spatial differences in the spawning between the hermaphroditic species. It would be interesting to see if there is as well such an partitioning in egg release on a very fine temporal or spatial scale for *F.spiralis*, *F.macroguiryi* and *F.guiryi*, which could explain how they keep their integrity as species apart from the hypothesis of strong environmental forces. Previous literature lacks in the clear identification of the species, which usually based on the morphology previously described in Zardi et al., (2011) and therefore all results presented just apply for the entity of *F.macroguiryi*. What can certainly be extracted from the literature is, that there might not be severe differences in the spawning mechanisms between hermaphrodites and therefore it gives reason to assume that environmental forces, selection and selfing itself reinforced species boundaries. Clearly the determination of the question of species integrity within the hermaphroditic clades remains unsolved. Moreover the data presented in this work also calls to investigate not only *F.macorguiryi* and *F.spiralis* in sympatry, but also *F.guiryi* and *F.macroguiryi* form Asturias and Morocco because all these results presented in the literature just apply for *F.macroguiryi* and *F.spiralis* in sympatry. Given that the populations in Morocco face severe reductions and threats through global warming in the future and might even get extinct before being able to perform such experiments and bring more light in evolutionary mechanisms that results in evolutionary novelties within the genus Fucus highlights another aspect of urgency for future research.

In some locations (for example in Viana do Castelo, Praia da Lavada/ria de Ares, Aveiro, etc), the species integrity was not as strong/clear, which was indicated by genomic proportions of *F.macroguiryi(msat)* mixed with individuals of *F.spiralis(mt and msat)*. Firstly, one reason for that could be the general lack in resolution by the results from STRUCTURE (discussed above), however other studies found as well that depending on the location the lines between species are expressed in different magnitudes (Billard et al., 2010). According to Billard et al. (2010), in Portugal a clear gradient in the distribution of the 3 clusters , namely *F.vesiculosus*, *F.spiralis* and *F.macroguiryi* (called *F.spiralis Low* in this study), were found wherease in France *F.macroguiryi* (*F.spiralis Low*) was mostly found admixed with *F.vesiculosus*. There was no overlap in the distribution of the two types of *F.spiralis* (*F.spiralis* and *F.spiralis Low)* in the study of Billard which gives reason to suggest that *F.vesiculosus* plays a major role in hybridization dynamics. Genetic admixture of *F.vesiculosus* and *F.macoguiryi* might be as well be the case in the data here, where the clear identification of *F.vesiculosus* was not possible because introgression or hybridization prior in generations would mask delimiting between *F.spiralis* and *F.vesiculosus*.

The classical definition of a species is due to this mix in genomic and mitochondrial patterns hardly possible. Still, it lays within us humans to have the urge of ordering nature according to a systematic way. It seems to be impossible to determine rules of classifying this species complex that are dominated by exceptions in their genomic patterns and driven by introgression of organelle genomes and a high level of hybridization. The term species can therefore be meant on a mitochondrial level, a genomic level, in ecological means and as well in a morphological way and should be used in respect to the research questions and the scale of the study.

5.2 Species Distribution Modelling

The models that were provided in this thesis show that the potential current distributional range of the sister species *F.spiralis* and *F.guriyi* is very wide ranging from the West Sahara in the South to Scandinavia in northern Europe and comprises vast areas along the North American coastlines. Contrastingly, it was illustrated that the current suitable habitat for *F.macroguiryi* is much more restricted to colder habitats highlighting the ecological segregation of *F.macroguiryi* from the above mentioned. In both models a poleward shift was observed. *F. spiralis* will have an increase in suitable habitat in comparison to *F. guiryi*, which will most likely experience severe habitat loss in the southern margins. The results of the observed shift of the species confirms the outcome of other studies (e.g. Nicastro et al., 2013a). Species distribution models in combination with genetic data are extremely powerful tools to implement protection measures where necessary especially if highly diverse areas are affected by global warming. The most commonly observed pattern in this field of study are that firstly in refugia areas, which are commonly in more southern region (when speaking of the northern hemisphere) harbour higher levels of diversity because of long term persistence and secondly that especially these areas are the most susceptible towards climate change. A decrease of highly diverse genetic pools of populations goes hand in hand with an increase of vulnerability therefore creating a feedback by making them less able to adapt to future ecological disturbances. Fucus species are not only ecosystem engineers by increasing spatial complexity, they also facilitate the presence of other species (Seed & O’Connor, 1981). The ecosystems in which the investigated organisms appear are such heterogenous habitats forming microclimates and show great differences in their characteristics on a very small scale and therefore the consequences of extinction or disappearance will doubtlessly affect organisms on upper trophic levels. Species distribution models not only for a single species but for species assemblages could be applied in the future to investigate the magnitude of impact on higher trophic levels.

The models that are presented should be interpreted with caution for many reasons. Firstly, these models do not take the substrate suitability into account, and therefore the number of the calculated habitat loss and gain for the future are surely an overestimation. This model simply assumes the presence of rocky substrates. For example in the Bay of Biscay there is potentially a continuous area that is suitable for Fucus however this long stripe of sand is considered to be a barrier for Iberian kelps and wracks, which are physically separated to the ones in Brittany by these large stretches of soft and sandy substrate creating distances between populations that can very hardly be bridged through their low levels of dispersal. However, these large stretches of sand are still considered to be suitable. The model simply assumes that if a rocky substrate will be present at this area the species will flourish. The gathering of global data on the substrate composition in the intertidal zone will be hopefully available with newly developed technological advancements such as satellite images or new computer image analysis technology. Interdisciplinary collaborations between data scientists as well as geologists and remote sensing experts will be necessary together with funding for projects on such a global scale. This will allow a more precise refinement of suitable habitat apart from the impact that environmental variables will have on the biogeography of marine intertidal species.

The reason for the weakness of also considering the Baltic sea as suitable habitat in the model could be referred back to the geographic extent of the study area on which the variables that are considered to be the most important ones depend. For example in a study of Jonsson et al., (2018), who investigated climate driven range shift of *F.vesiculosus* in the Baltic Sea (for future scenarios until 2099) surely incorporated salinity because it is such a crucial variable on a regional scale. Another study (Assis et al., 2014) which dealt with the same species however, on a much broader scale, both geographically (from Northern Norway to Western Morocco and the West Atlantic from Canada to North Carolina) and regarding the time frame (hindcast models form the LGM and future scenario for the year 2100) never choose ocean salinity for their best model.

Another disagreement with the predicted probability of occurrence of *F.spiralis* and *F. guiryi* is the area falsely identified in the Gulf of Lion in the Mediterranean and isolated patches in Slovenia and Greece. Yet the same applies for these areas as is it the case for the Baltic Sea. Surely, the model would need further refinement, however for the purpose of checking if populations abundant in the most southern margins will experience range shift or extinctions under difference climate scenarios, this seemed to be sufficient enough.

The models presented here are just an attempt to capture the current distribution range of the investigated species and predict the possible shifts for the future. Knowing that especially the East Atlantic harbours the most diversity for this species complex some unique genetic pools will face severe reduction and even might go extinct. Especially the fate of *F.macroguiryi* illustrated by the poleward shift in 2100 raises certain concerns. Such an extreme geographical shift and also the decrease of likelihood (50%) of this species to be found in NW Iberia in the next 80 years is astonishing and leaves the question open if this shift will actually happen. This range shift might be at such a speed that populations might not be able to keep up with it and will potentially face major reduction in abundances. It might not be possible for *F.macroguiryi* to disperse as fast into the north and colonize new areas. The genetic pool of the haplotypes found only in the south (JadL, ESSL) as well as in Bayonne for example, might be lost completely. As a result, this entity could suffer a decrease in genetic diversity due to the forthcoming bottleneck leaving only individuals from the south of the UK to colonize South Ireland. On a global scale it does not seem profitable trying to protect certain genetically unique population against global warming. Well selected areas with special environmental conditions, such as upwelling and shaded areas could buffer the increase in temperatures allowing population to continue to exist. There might also be the idea of transplanting genetically unique populations to areas, which are predicted to be suitable. However, the anthropogenic transplant of a genetical pool into a non-native area can have unforeseeable consequences on an ecological scale. When looking more detailed on the aspect of temperatures there are two different kinds of temperature boundaries: in seaweeds, lethal boundaries and growth or reproduction boundaries (Breeman 1988; Hoek & Breeman 1989).

The optimum growth temperature ranges of *F.vesiculosus* for example were found between 10 – 24°C (Graiff et al., 2015). The upper survival temperature, however, reaches up to 27°C. Together with the suspecting of cryptic survival in microscopic stages it is not possible to tell if the populations in the south will actually get extinct and the likeliness of the prediction in the models has to be assessed. These populations could return and re-establish themselves after unfavourable temperatures had passed, but under such increasing temperatures and political steps that are not efficient enough for a drastic change to stop or at least slow down climate change until alternative solutions can be found it might be that these unfavourable periods will last too long. On the other hand, it is not explored how long these potential dormant microscopic stages are.

Unfortunately, it was not possible to explore of the environmental niche in respect to the genetically defined entities apart from obvious statements, that could be done such as that *F.spiralis* appearing in the north is highly different than the populations found on the Azores. The reason why no differences within entities could be found can be explained by the very recent radiation within their evolutionary history. It seems like as if environmental forces at the time scale investigated do not contribute as much to the separation of these entities rather than dynamics on a genetic level (e.g. hybridization). The genetic signature that was found for this species complex is also a result of a much broader timescale. The climatic and oceanographic changes on planet earth especially in the North Atlantic regions during the last 65KY were dramatic (Schönfeld, et al., 2003; Taylor et al., 1993) and extremely fluctuating especially when looking at the time scales, that we are dealing with when analysing genetic data. Periods of expansions and contractions as well as the stochastic effect of populations being the leading-edge ones after deglaciation lead to the genetic patterns and random fixation of alleles and traits we see today. The application of paleoclimatic data might have been more suitable to investigate niche differences, contraction and expansions. This was done in prior studies using high-resolution data from HYCOM (Hybrid Coordinate Ocean Model) on *Laminaria ochroleuca* (Assis et al., 2018). To detect biogeographic patterns long-term data series directed to address macroecological questions on a broad scale should gain importance not only to understand patterns that contributed in the past to the ones we see today, but also to draw a better and more accurate picture for the future. Models on a smaller ecological scale would potentially make more sense to investigate niche partitioning within the entities that occur in sympatry.

Another reason why these models should not be overinterpreted is that they show variations in the variable importance and predicted habitat suitability based on the input data. Maxent developers (Phillips et al., 2004) noted that Maxent is vulnerable to biases in input data sets. Still, Pearson et al., (2007) showed in their study that Maxent achieved better predictive success rates, particularly at small sample sized, which is the case for the model of *F.macroguiryi*, with only 18 occurrence records. Even though the threshold according to which the reclassification maps were creating discriminating between suitable and not suitable habitat was quite low (0.41 for the model of *F.guiriy+F.spiralis* and 0.61 for *F.macroguiryi*) it still seems acceptable for the algorithm used. Townsend Peterson et al., (2007) showed that Maxent models show and overall lower threshold in comparison to other algorithms, however these models also have higher levels of predictions.

According to which scale and extends the models are performed the outcome will vary (Collingham et al., 2000). Especially, when considering phenology and the fact that species may display different responses climate change and to certain environmental variables deepening on their life stages. The variables that will be influential furthermore are determined by the seasons for example. For instance, sea temperature, air temperature, wave action, wind speed and turbulence during spring influence the spawning dynamics of *Patella depressa*, whereas currents and storm events are determining the dispersal during summer. In autumn sea and air temperature together with desiccation are responsible for the settlement, metamorphosis and development of juvenile individuals and food availability, sea temperature and desiccation are the drivers that influence the emergence from a cryptic habitat in winter (Helmuth et al., 2006). The scale of the research question is the key to determine the most influential variable. Many studies rely on environmental proxies for physiological stress (e.g., air or water temperature) and fail to consider the environment at the scale of the organism separately.

Another deficit of the model is that only temperature was considered firstly due to simplicity reasons and secondly because in the literature temperature was found to be the most influential especially for organisms that inhabit the intertidal zone. The influence that other variables - apart from sea surface temperature – have on organisms in the intertidal zone can certainly not be neglected. Within several prior model runs including all variables that were discussed in the literature review nutrients or chlorophyll concentration were never within the most predictive variables. However, precipitation is suspected to play a role in determining the distribution of Fucus species. Sea surface temperature and precipitation together seem quite plausible to be responsible for the persistence of Fucus especially because these two variables represent of both, marine and terrestrial factors. The importance of precipitations can be interpreted in a way that it is a proxy for humidity. When intertidal algae are experiencing desiccation stress and are exposed to the air, they tend to reduce their metabolism in order to survive. When these organisms are still in a humid environment, they stay wet and the metabolism is not reduced.

Salinity, of course acts as well as an important environmental variable excluding the Baltic Sea with nearly freshwater conditions and the Mediterranean with higher concentrations in salinity from the suitable range of the Atlantic and Pacific open oceans. Still, because of the overall geographic extent of this study salinity was not recognized as an important variable. In another species distribution modelling study for *F.distichus* in the Arctic (Jueterbock et al., 2016) calcite, nitrate and chlorophyll were the most explanatory variables. Calcite and chlorophyll might be indirectly correlated to other variables that were not included in this model, however higher calcite concentration might be favourable for crustose and coralline algae or calcified herbivorous species and therefore influence the grazing pressure on *Fucus disticus*. Chlorophyll concentration, which is positively correlated to water visibility might be negatively correlated with light availability. So even if *F.distichus* is ecologically similar, growing as well on rocky shores, however has its main core populations in such cold waters with different ecosystem drivers, the outcome is totally different to the results for *F.spiralis* and *F.guiryi* respectively. The aspect of collinearity by the inclusion of air temperature together with sea surface temperature should also be discussed. Usually, collinearity of variables should be avoided in modelling procedures, however sometimes models include both, air and water temperatures, which are variables that are highly correlated (Assis et al., 2014; Neiva et al., 2015), when investigating intertidal species, which are influenced by both terrestrial and marine forces.

When building a species distribution model one of the main assumption is an equilibrium with the environment (Elith, Kearney, & Phillips, 2010), however this assumption of often violated due to recent colonization of newly available habitats where the ecosystem has probably not reached the full equilibrium yet - especially for representatives of the genus Fucus, that are still radiating. In order to gain more robust results in species distribution models the average output of different algorithms can be used to minimize flaws of each single model. These so called “ensembled models” reduce the degree of uncertainty with general statistics (mean, median, average) and synthesize the results. To sum up, the output of the predicted distribution of species distribution model is highly dependent on the input data, the extend of the study and the variables that were chosen. Variations within these three factors together can show very different results.

Range shifts and loss of unique genetic diversity at the rear edge where predicted for the future climate scenario – especially for RCP8.5. The observed poleward shift in all species highlights and supports the hypothesis of niche conservatism. A review by Townsend Peterson (2011) mentions that niche conservatism breaks down over time and therefore the evidence for niche conservatism is mixed. The shorter the time scale, the more exists a tendency of conservatism in comparison to long-term events. However, because the genus Fucus has undergone evolutionary speaking relatively recent and extensive radiation process and recolonization processes are still ongoing since the LGM the results from this study leave us without doubt that niche conservatism exists for this species complex. Some criticism regarding the selection of the chosen RCP could as well be addressed. There were almost no differences in the distribution range visible when comparing the present to the “optimistic” climate scenario (RCP2.6). Yet, how realistic is this scenario? Some people might argue that the usage of the future climate scenario RCP2.6 might be too optimistic because human society may already exceed the point of which a reduction in greenhouse gases could have taken place. Looking at the current state of climate change and consequently destabilisation of the world’s climate and severe biodiversity loss induced by overexploitation, which creates a feedback loop and making ecosystems even more vulnerable, it seems as if we have already passed this reversible phase of climate change/ the threshold of limiting our CO2 emissions within this scenario. Therefore, the scenario with the next lower emissions namely the RCP4.5 (Clarke et al., 2007; Wise et al., 2009) might be more accurate. Wang, et al., (2017) conducted a study assessing numerous different climate projections for the future. Their results revealed that the increase of global sea surface temperature will be lower than 2.6°C (compared to pre-industrial level) the atmospheric CO2 concentration will not exceed 610 ppm which leads to the conclusion that the “worst-case-scenario” with an estimate 936 ppm is highly overestimated.

6. Conclusion

Overall, the genealogic relationships of *F. macroguiryi* to *F.spiralis* and *F.guiryi* inferred with nuclear DNA stand in clear disagreement with independent genomic data. Sharp species delimiting features could be identified as well as introgression and hybridization within species (e.g with *F.vesiculosus*). Knowledge on hybridization dynamics and the role and magnitude of interaction and genetic exchange of *F.vesicuolus* with *F.spiralis* (and *F.macroguiryi*) remains partly in the dark, especially for the locations which did not show such sharp species delimitation. A cohesive sample size on the genomic DNA of *F.vesiculosus* might be the key to bring more light into solving these inconsistencies and should increase the resolution of the results gained via STRUCTURE. Furthermore, the integrity of *F.macroguiryi* as a own species could be proved by firstly though its vertical distinct location on the shore in respect to *F.spiralis* and *F.guiryi*, while knowing the background of the analysis approach and its weaknesses. The second indication of *F.macroguiryi* being a own species that likely developed independently from *F.spiralis* and *F.guiryi* is its ecological characteristics being less heat resistant. Despite some questions that still remain open, it was possible to illustrate the genetic structure of this species complex and the markers that were used, showed to be sufficient enough to provide insight on both, an entity level and on a geographic level.

Species distribution modelling is very powerful if done properly by considering all the above mentioned (e.g. interpreting models in the ecological context, adapted to the scale of research, computational refinements such as layer clipping, ensemble models, etc,). The general purpose of investigating poleward shifts, which as overserved for all entities, and the prediction of habitat reduction/gains in southern versus northern areas was performed. This revealed two contrasting outcomes for *F.spiralis* and *F.guiryi*, where the first will experience great increases in suitable habitat in respect to temperatures and the latter will face extreme habitat loss and potential extinction.

This thesis aims to give impulses for future research on topics such as the microscopic long-term persisting stages or hybridization and fertilization experiments among entities including *F.vesiculosus*. The knowledge gained from this thesis supports studies that had been done in the past with identifying e.g the Iberian Peninsula as an extremely crucial area of long-term persistence for intertidal macroalga. Additionally the results presented might call out for updating current known algae data bases and acknowledge *F.macroguiryi* as its own entity.

References

Alberto, F. (2006). standArich _ v1 . 00 : an R package to estimate population allelic richness using standardized sample size. 1–8.

Alberto, F. (2009). MsatAllele-1.0: An R package to visualize the binning of microsatellite alleles. *Journal of Heredity*, *100*(3), 394–397. https://doi.org/10.1093/jhered/esn110

Anderson, C. I. H., & Scott, G. W. (1998). The Occurrence of Distinct Morphotypes within a Population of Fucus Spiralis . *Journal of the Marine Biological Association of the United Kingdom*, *78*(3), 1003–1006. https://doi.org/10.1017/s0025315400044933

Assis, J., Serrao, E. A., Claro, B., Perrin, C., & Pearson, G. A. (2014). Climate-driven range shifts explain the distribution of extant gene pools and predict future loss of unique lineages in a marine brown alga. *Molecular Ecology*, *23*(11), 2797–2810. https://doi.org/10.1111/mec.12772

Assis, Jorge, Araújo, M. B., & Serrão, E. A. (2018). Projected climate changes threaten ancient refugia of kelp forests in the North Atlantic. *Global Change Biology*, *24*(1), e55–e66. https://doi.org/10.1111/gcb.13818

Assis, Jorge, Lucas, A. V., Bárbara, I., & Serrão, E. Á. (2016). Future climate change is predicted to shift long-term persistence zones in the cold-temperate kelp Laminaria hyperborea. *Marine Environmental Research*, *113*, 174–182. https://doi.org/10.1016/j.marenvres.2015.11.005

Assis, Jorge, Serrão, E. Á., Coelho, N. C., Tempera, F., Valero, M., & Alberto, F. (2018). Past climate changes and strong oceanographic barriers structured low-latitude genetic relics for the golden kelp Laminaria ochroleuca. *Journal of Biogeography*, *45*(10), 2326–2336. https://doi.org/10.1111/jbi.13425

Assis, Jorge, Tyberghein, L., Bosch, S., Verbruggen, H., Serrão, E. A., & De Clerck, O. (2018). Bio-ORACLE v2.0: Extending marine data layers for bioclimatic modelling. *Global Ecology and Biogeography*, *27*(3), 277–284. https://doi.org/10.1111/geb.12693

Bandelt, H., Forster, P., & Röhl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Mol Biol Evol*, *16*(1), 37–48. https://doi.org/10.1146/annurev.es.18.110187.002421

Barbet-Massin, M., Jiguet, F., Albert, C. H., & Thuiller, W. (2012). Selecting pseudo-absences for species distribution models: How, where and how many? *Methods in Ecology and Evolution*, *3*(2), 327–338. https://doi.org/10.1111/j.2041-210X.2011.00172.x

Bartsch, I., Wiencke, C., & Laepple, T. (2012). Global Seaweed Biogeography Under a Changing Climate: The Prospected Effects of Temperature. *Seaweed Biology*, 383–406. https://doi.org/10.1007/978-3-642-28451-9

Bewley, J. D. (1995). Physiological aspects of desiccation tolerance. *International Journal of Plant Sciences*, *156*(4), 393–403. https://doi.org/10.1086/297261

Billard, E., Daguin, C., Pearson, G., Serrão, E., Engel, C., & Valero, M. (2005). Genetic Isolation between three closely related Taxa: Fucus Vesiculosus, F. spiralis, and F.veranoides (Phaophyceae). *Journal of Phycology*, *41*(4), 900–905. https://doi.org/10.1111/j.1529-8817.2005.00113.x

Billard, E., Serrão, E. A., Pearson, G. A., Engel, C. R., Destombe, C., & Valero, M. (2005). Analysis of sexual phenotype and prezygotic fertility in natural populations of Fucus spiralis, F. vesiculosus (Fucaceae, Phaeophyceae) and their putative hybrids. *European Journal of Phycology*, *40*(4), 397–407. https://doi.org/10.1080/09670260500334354

Billard, E., Serrão, E., Pearson, G., Destombe, C., & Valero, M. (2010). Fucus vesiculosus and spiralis species complex: A nested model of local adaptation at the shore level. *Marine Ecology Progress Series*, *405*(April 2019), 163–174. https://doi.org/10.3354/meps08517

Boltovskoy, D., & Correa, N. (2016). Biogeography of Radiolaria Polycystina (Protista) in the World Ocean. *Progress in Oceanography*, *149*, 82–105. https://doi.org/10.1016/j.pocean.2016.09.006

Brawley, S. H., Coyer, J. A., Blakeslee, A. M. H., Hoarau, G., Johnson, L. E., Byers, J. E., … Olsen, J. L. (2009). Historical invasions of the intertidal zone of Atlantic North America associated with distinctive patterns of trade and emigration. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(20), 8239–8244. https://doi.org/10.1073/pnas.0812300106

Breeman, A. M. (1988). Relative importance of temperature and other factors in determining geographic boundaries of seaweeds: Experimental and phenological evidence. *Helgoländer Meeresuntersuchungen*, *42*(2), 199–241. https://doi.org/10.1007/BF02366043

Breeman, A. M. (1990). Expected Effects of Changing Seawater Temperatures on the Geographic Distribution of Seaweed Species. *Expected Effects of Climatic Change on Marine Coastal Ecosystems*, 69–76. https://doi.org/10.1007/978-94-009-2003-3_9

Burrows, E. M., & Lodge, S. (1951). Autecology and the species problem in fucus. *Journal of the Marine Biological Association of the United Kingdom*, *30*(1), 161–176. https://doi.org/10.1017/S0025315400012650

Cánovas, F. G., Mota, C. F., Serrão, E. A., & Pearson, G. A. (2011). Driving south: A multi-gene phylogeny of the brown algal family Fucaceae reveals relationships and recent drivers of a marine radiation. *BMC Evolutionary Biology*, *11*(1). https://doi.org/10.1186/1471-2148-11-371

Carney, L. T., & Edwards, M. S. (2006). Cryptic Processes in the Sea: A Review of Delayed Development in theMicroscopic Life Stages of Marine Macroalgae. *Algae*, *21*(2), 161–168. https://doi.org/10.4490/algae.2006.21.2.161

Charrier, B., Le Bail, A., & De Reviers, B. (2012). Plant Proteus: Brown algal morphological plasticity and underlying developmental mechanisms. *Trends in Plant Science*, *17*(8), 468–477. https://doi.org/10.1016/j.tplants.2012.03.003

Chase, M. W., & Hills, H. H. (1991). Silica Gel : An Ideal Material for Field Preservation of Leaf Samples for DNA Studies. *Taxon*, *40*(2), 215–220.

Clark, P. U., Dyke, A. S., Shakun, J. D., Carlson, A. E., Clark, J., Wohlfarth, B., … McCabe, A. M. (2009). The Last Glacial Maximum. *Science*, *325*(5941), 710–714. https://doi.org/10.1126/science.1172873

Clarke, L. E., Jacoby, H., Pitcher, H., Reilly, J., & Richels, R. (2007). Scenarios of Greenhouse Gas Emissions and Atmospheric. *US Department of Engergy*, (July), 154.

Coleman, M. A., & Brawley, S. H. (2005). Erratum: Are life history characteristics good predictors of genetic diversity and structure? A case study of the intertidal alga Fucus spiralis (Heterokontophyta; Phaeophyceae) (Journal of Phycology 41 (753-762)). *Journal of Phycology*, *41*(6), 1287. https://doi.org/10.1111/j.1529-8817.2005.00158.x

Collingham, Y. C., Wadsworth, R. A., Huntley, B., & Hulme, P. E. (2000). Predicting the spatial distribution of non-indigenous riparian weeds: Issues of spatial scale and extent. *Journal of Applied Ecology*, *37*(SUPPL. 1), 13–27. https://doi.org/10.1046/j.1365-2664.2000.00556.x

Connell, J. H. (1972). Community Interactions on Marine Rocky Intertidal Shores. *Annual Review of Ecology and Systematics*, *3*(1972), 169–192.

Coyer, J. A., Hoarau, G., Beszteri, B., Pearson, G., & Olsen, J. L. (2009). Expressed sequence tag-derived polymorphic SSR markers for Fucus serratus and amplification in other species of Fucus. *Molecular Ecology Resources*, *9*(1), 168–170. https://doi.org/10.1111/j.1755-0998.2008.02406.x

Coyer, J. A., Hoarau, G., Costa, J. F., Hogerdijk, B., Serrão, E. A., Billard, E., … Olsen, J. L. (2011). Evolution and diversification within the intertidal brown macroalgae Fucus spiralis/F.

vesiculosus species complex in the North Atlantic. *Molecular Phylogenetics and Evolution*, *58*(2), 283–296. https://doi.org/10.1016/j.ympev.2010.11.015

Coyer, J. A., Hoarau, G., Stam, W. T., & Olsen, J. L. (2007). Hybridization and introgression in a mixed population of the intertidal seaweeds Fucus evanescens and F. serratus. *Journal of Evolutionary Biology*, *20*(6), 2322–2333. https://doi.org/10.1111/j.1420-9101.2007.01411.x

Coyer, James A., Hoarau, G., Oudot-Le Secq, M. P., Stam, W. T., & Olsen, J. L. (2006). A mtDNA-based phylogeny of the brown algal genus Fucus (Heterokontophyta; Phaeophyta). *Molecular Phylogenetics and Evolution*, *39*(1), 209–222. https://doi.org/10.1016/j.ympev.2006.01.019

Creed, J. C., Norton, T. A., & Kain, J. M. (1997). Intraspecific competition in Fucus serratus germlings: The interaction of light, nutrients and density. *Journal of Experimental Marine Biology and Ecology*, *212*(2), 211–223. https://doi.org/10.1016/S0022-0981(96)02748-7

Dayton, P. K. (1985). Ecology of kelp communities. *Annual Review of Ecology and Systematics. Vol. 16*, (63), 215–245.

Deysher, L. E., & Dean, T. A. (1984). Critical irradiance levels and the interactive effects of quantum irradiance and does on gametogenesis in the giant kelp. *Journal of Phycology, 20*(4), 520–524.

Dongen, A. Van. (1956). The Preference of Littorina obtusata for Fucaceae. *Archives Néerlandaises de Zoologie*, 373–386.

Dring, M., & Brown, F. (1982). Photosynthesis of Intertidal Brown Algae During and After Periods of Emersion: A Renewed Search for Physiological Causes of Zonation. *Marine Ecology Progress Series*, *8*(1961), 301–308. https://doi.org/10.3354/meps008301

Earl, D. A., & Holdt, B. M. Von. (2012). STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, *4*(2), 359–361. https://doi.org/10.1007/s12686-011-9548-7

Edmonds, C. A., Lillie, A. S., & Cavalli-Sforza, L. L. (2004). Mutations arising in the wave front of an expanding population. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(4), 975–979. https://doi.org/10.1073/pnas.0308064100

Edwards, M. S. (2000). The role of alternate life-history stages of a marine macroalga: A seed bank analogue? *Ecology*, *81*(9), 2404–2415. https://doi.org/10.1890/0012-9658(2000)081[2404:TROALH]2.0.CO;2

Elith, J., & Graham, C. H. (2009). Do they? How do they? WHY do they differ? on finding reasons for differing performances of species distribution models. *Ecography*, *32*(1), 66–77. https://doi.org/10.1111/j.1600-0587.2008.05505.x

Elith, J., Kearney, M., & Phillips, S. (2010). The art of modelling range-shifting species. *Methods in Ecology and Evolution*, *1*(4), 330–342. https://doi.org/10.1111/j.2041-210x.2010.00036.x

Elith, J., & Leathwick, J. R. (2009). Species Distribution Models: Ecological Explanation and Prediction Across Space and Time. *Annual Review of Ecology, Evolution, and Systematics*, *40*(1), 677–697. https://doi.org/10.1146/annurev.ecolsys.110308.120159

Engel, C. R., Daguin, C., & Serrão, E. A. (2005). Genetic entities and mating system in hermaphroditic Fucus spiralis and its close dioecious relative F. vesiculosus (Fucaceae, Phaeophyceae). *Molecular Ecology*, *14*(7), 2033–2046. https://doi.org/10.1111/j.1365-294X.2005.02558.x

Engel, Carolyn R., Brawley, S. H., Edwards, K. J., & Serrão, E. (2003). Isolation and cross-species amplification of microsatellite loci from the fucoid seaweeds fucus vesiculosus, f. serratus and Ascophyllum nodosum (heterokontophyta, fucaceae). *Molecular Ecology Notes*, *3*(2), 180–182. https://doi.org/10.1046/j.1471-8286.2003.00390.x

Estep, M. Y. F., & Dabrowski, H. (1980). Tracing Food Webs with Stable Hydrogen Isotopes. *Science*, *209*(4464), 1538–1541. https://doi.org/10.1126/science.6159680

Fielding, A. H., & Bell, J. F. (1997). A review of methods for the assessment of prediction errors in conservation presence/absence models. *Environmental Conservation*, *24*(1), 38–49. https://doi.org/10.1017/S0376892997000088

Flores-Molina, M. R., Thomas, D., Lovazzano, C., Núñez, A., Zapata, J., Kumar, M., … Contreras-Porcia, L. (2014). Desiccation stress in intertidal seaweeds: Effects on morphology, antioxidant responses and photosynthetic performance. *Aquatic Botany*, *113*, 90–99. https://doi.org/10.1016/j.aquabot.2013.11.004

Frenzel, B. (1992). Atlas of Paleoclimates and Paleoenvironments of the Northern Hemisphere: Late Pleistocene-Holocene. *Arctic and Alpine Research*, *25*(2), 161. https://doi.org/10.2307/1551555

Gallien, L., Douzet, R., Pratte, S., Zimmermann, N. E., & Thuiller, W. (2012). Invasive species distribution models - how violating the equilibrium assumption can create new insights. *Global Ecology and Biogeography*, *21*(11), 1126–1136. https://doi.org/10.1111/j.1466-8238.2012.00768.x

Gauch, H. G. . J., & Chase, G. B. . (1974). Fitting the Gaussian Curve to Ecological Data. *Ecological Society of America*, *55*(6), 1377–1381.

Graiff, A., Liesner, D., Karsten, U., & Bartsch, I. (2015). Temperature tolerance of western Baltic Sea Fucus vesiculosus - growth, photosynthesis and survival. *Journal of Experimental Marine Biology and Ecology*, *471*, 8–16. https://doi.org/10.1016/j.jembe.2015.05.009

Hagerman, L. (1966). The macro- and microfauna associated with Fucus serratus L., with some ecological remarks. *Ophelia*, *3*(1), 1–43. https://doi.org/10.1080/00785326.1966.10409631

Hawkins, S., & Hartnoll, R. (1985). Factors determining the upper limits of intertidal canopy-forming algae. *Marine Ecology Progress Series*, *20*(1953), 265–271. https://doi.org/10.3354/meps020265

Helmuth, B., Mieszkowska, N., Moore, P., & Hawkins, S. J. (2006). Living on the Edge of Two Changing Worlds: Forecasting the Responses of Rocky Intertidal Ecosystems to Climate Change. *Annual Review of Ecology, Evolution, and Systematics*, *37*(1), 373–404. https://doi.org/10.1146/annurev.ecolsys.37.091305.110149

Hernandez, P. A., Graham, C. H., Master, L. L., & Albert, D. L. (2006). The effect of sample size and species characteristics on performance of different species distribution modeling methods. *Ecography*, *29*(5), 773–785. https://doi.org/10.1111/j.0906-7590.2006.04700.x

Hewitt, G. (1996). Some genetic consequences of ice ages, and thier role in divergence. *Biological Journal of the Linnean Society*, *58*(September 1994), 247–276.

Hewitt, G. (2004). Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *359*(1442), 183–195. https://doi.org/10.1098/rstb.2003.1388

Hoarau, G., Coyer, J. A., Veldsink, J. H., Stam, W. T., & Olsen, J. L. (2007). Glacial refugia and recolonization pathways in the brown seaweed Fucus serratus. *Molecular Ecology*, *16*(17), 3606–3616. https://doi.org/10.1111/j.1365-294X.2007.03408.x

Hoek, C. V. Den, Breeman, A. M., & Stam, W. T. (1990). The Geographic Distribution of Seaweed Species in Relation to Temperature: Present and Past. *Expected Effects of Climatic Change on Marine Coastal Ecosystems*, 55–67. https://doi.org/10.1007/978-94-009-2003-3_8

Holt, R. D. (2009). Bringing the Hutchinsonian niche into the 21st century: Ecological and evolutionary perspectives. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(SUPPL. 2), 19659–19665. https://doi.org/10.1073/pnas.0905137106

Holt, R. D., & Gaines, M. S. (1992). Analysis of adaptation in heterogeneous landscapes: Implications for the evolution of fundamental niches. *Evolutionary Ecology*, *6*(5), 433–447. https://doi.org/10.1007/BF02270702

Hotelling, H. (1933). Analysis of a complex of statistical variables into Principal Components. Jour. Educ. Psych., 24, 417-441, 498-520. *The Journal of Educational Psychology*, *24*, 417–441.

Hunt, L. J. H., & Denny, M. W. (2008). Desiccation protection and disruption: A trade-off for an intertidal marine alga. *Journal of Phycology*, *44*(5), 1164–1170. https://doi.org/10.1111/j.1529-8817.2008.00578.x

Hurd, C. L., Galvin, R. S., Norton, T. A., & Dring, M. J. (1993). Production of Hyaline Hairs By Intertidal Species of Fucus (Fucales) and Their Role in Phosphate Uptake. *Journal of Phycology*, Vol. 29, pp. 160–165. https://doi.org/10.1111/j.0022-3646.1993.00160.x

Hutchinson, G. E. (1957). Concluding Remarks. *Cold Spring Harbor Symposia on Quantitative Biology*, *22*(0), 415–427. https://doi.org/10.1101/sqb.1957.022.01.039

Jerome, A., Archer, E., Hardy, O., & Goudet, M. J. (2020). *Package ' hierfstat .'*

Jombart, T., & Ahmed, I. (2011). adegenet 1.3-1: New tools for the analysis of genome-wide SNP data. *Bioinformatics*, *27*(21), 3070–3071. https://doi.org/10.1093/bioinformatics/btr521

Jombart, T., Devillard, S., & Balloux, F. (2010). Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. *BMC Genetics*, *11*(1), 94. https://doi.org/10.1186/1471-2156-11-94

Jonsson, P. R., Kotta, J., Andersson, H. C., Herkül, K., Virtanen, E., Sandman, A. N., & Johannesson, K. (2018). High climate velocity and population fragmentation may constrain climate-driven range shift of the key habitat former Fucus vesiculosus. *Diversity and Distributions*, *24*(7), 892–905. https://doi.org/10.1111/ddi.12733

Jordan, A. J., & Vadas, R. L. (1972). Influence of environmental parameters on intraspecific variation in Fucus vesiculosus. *Marine Biology*, *14*(3), 248–252. https://doi.org/10.1007/BF00348287

Jueterbock, A., Smolina, I., Coyer, J. A., & Hoarau, G. (2016). The fate of the Arctic seaweed Fucus distichus under climate change: An ecological niche modeling approach. *Ecology and Evolution*, *6*(6), 1712–1724. https://doi.org/10.1002/ece3.2001

Jueterbock, A., Tyberghein, L., Verbruggen, H., Coyer, J. A., Olsen, J. L., & Hoarau, G. (2013). Climate change impact on seaweed meadow distribution in the North Atlantic rocky intertidal. *Ecology and Evolution*, *3*(5), 1356–1373. https://doi.org/10.1002/ece3.541

Karsten, U. (2012). Seaweed Acclimation to Salinity and Desiccation Stress. In *Seaweed Biology* (Vol. 219, pp. 87–107). https://doi.org/10.1007/978-3-642-28451-9

Keenan, K. (2017). Package "diveRsity" v1.9.90.

Klopfstein, S., Currat, M., & Excoffier, L. (2006). The fate of mutations surfing on the wave of a range expansion. *Molecular Biology and Evolution*, *23*(3), 482–490. https://doi.org/10.1093/molbev/msj057

Knaus, B., & Winter, D. (2020). *Package ' pegas .'*

Krug, L. A., Platt, T., & Barbosa, A. B. (2018). Delineation of ocean surface provinces over a complex marine domain (off SW Iberia): An objective abiotic-based approach. *Regional Studies in Marine Science*, *18*, 80–96. https://doi.org/10.1016/j.rsma.2018.01.003

Ladah, L. B., Feddersen, F., Pearson, G. A., & Serrão, E. A. (2008). Egg release and settlement patterns of dioecious and hermaphroditic fucoid algae during the tidal cycle. *Marine Biology*, *155*(6), 583–591. https://doi.org/10.1007/s00227-008-1054-4

Ladah, L., Bermudez, R., Pearson, G., & Serrão, E. (2003). Fertilization success and recruitment of dioecious and hermaphroditic fucoid seaweeds with contrasting distributions near their southern limit. *Marine Ecology Progress Series*, *262*, 173–183.

Lambeck, K., Esat, T. M., & Potter, E. K. (2002). Links between climate and sea levels for the past three million years. *Nature*, *419*(6903), 199–206. https://doi.org/10.1038/nature01089

Lawson, D. J., van Dorp, L., & Falush, D. (2018). A tutorial on how not to over-interpret STRUCTURE and ADMIXTURE bar plots. *Nature Communications*, *9*(1), 1–11. https://doi.org/10.1038/s41467-018-05257-7

Leclerc, M. C., Barriel, V., Lecointre, G., & De Reviers, B. (1998). Low divergence in rDNA ITS sequences among five species of Fucus (Phaeophyceae) suggests a very recent radiation. *Journal of Molecular Evolution*, *46*(1), 115–120. https://doi.org/10.1007/PL00006278

López-Cristoffanini, C., Zapata, J., Gaillard, F., Potin, P., Correa, J. A., & Contreras-Porcia, L. (2015). Identification of proteins involved in desiccation tolerance in the red seaweed Pyropia orbicularis (Rhodophyta, Bangiales). *Proteomics*, *15*(23–24), 3954–3968. https://doi.org/10.1002/pmic.201400625

Lourenço, C. R., Zardi, G. I., McQuaid, C. D., Serrão, E. A., Pearson, G. A., Jacinto, R., & Nicastro, K. R. (2016). Upwelling areas as climate change refugia for the distribution and genetic diversity of a marine macroalga. *Journal of Biogeography*, *43*(8), 1595–1607. https://doi.org/10.1111/jbi.12744

Lüning, K. (1984). Temperature tolerance and biogeography of seaweeds: The marine algal flora of Helgoland (North Sea) as an example. *Helgoländer Meeresuntersuchungen*, *29*(3), 305–317.

Maggs, C. A., Castilho, R., Foltz, D., Henzler, C., Jolly, M. T., Kelly, J., … Wares, J. (2008). Evaluating signatures of glacial refugia for north atlantic benthic marine taxa. *Ecology*, *89*(11 SUPPL.), 108–122. https://doi.org/10.1890/08-0257.1

Martínez, B., Arenas, F., Rubal, M., Burgués, S., Esteban, R., García-Plazaola, I., … Viejo, R. M. (2012). Physical factors driving intertidal macroalgae distribution: Physiological stress of a dominant fucoid at its southern limit. *Oecologia*, *170*(2), 341–353. https://doi.org/10.1007/s00442-012-2324-x

Mathieson, A. C., & Niemeck, R. A. (1978). Physiological Studies of Intertidal Fucoid Algae. *Botanica Marina*, *21*(4), 221–228. https://doi.org/10.1515/botm.1978.21.4.221

Melero-Jiménez, I. J., Enrique Salvo, A., Báez, J. C., Bañares-España, E., Reul, A., & Flores-Moya, A. (2017). North Atlantic Oscillation drives the annual occurrence of an isolated, peripheral population of the brown seaweed Fucus guiryi in the Western Mediterranean Sea. *PeerJ*, *2017*(11). https://doi.org/10.7717/peerj.4048

Miller, T. E. X., Angert, A. L., Brown, C. D., Lee-Yaw, J. A., Lewis, M., Lutscher, F., … Williams, J. L. (2020). Eco-evolutionary dynamics of range expansion. *Ecology*, *0*(0), 1–14. https://doi.org/10.1002/ecy.3139

Mineur, F., Arenas, F., Assis, J., Davies, A. J., Engelen, A. H., Fernandes, F., … De Clerck, O. (2015). European seaweeds under pressure: Consequences for communities and ecosystem functioning. *Journal of Sea Research*, *98*, 91–108. https://doi.org/10.1016/j.seares.2014.11.004

Moalic, Y., Arnaud-Haond, S., Perrin, C., Pearson, G. A., & Serrao, E. A. (2011). Travelling in time with networks: Revealing present day hybridization versus ancestral polymorphism between two species of brown algae, Fucus vesiculosus and F. spiralis. *BMC Evolutionary Biology*, *11*(1). https://doi.org/10.1186/1471-2148-11-33

Monteiro, C. A., Paulino, C., Jacinto, R., Serraõ, E. A., & Pearson, G. A. (2016). Temporal windows of reproductive opportunity reinforce species barriers in a marine broadcast spawning assemblage. *Scientific Reports*, *6*(December 2015), 35–38. https://doi.org/10.1038/srep29198

Monteiro, C. A., Serrão, E. A., & Pearson, G. A. (2012). Prezygotic barriers to hybridization in marine broadcast spawners: Reproductive timing and mating system variation. *PLoS ONE*, *7*(4). https://doi.org/10.1371/journal.pone.0035978

Monteiro, C., Zardi, G. I., McQuaid, C. D., Serrão, E. A., Pearson, G. A., & Nicastro, K. R. (2019). Canopy microclimate modification in central and marginal populations of a marine macroalga. *Marine Biodiversity*, *49*(1), 415–424. https://doi.org/10.1007/s12526-017-0824-y

Moss, R. H., Edmonds, J. A., Hibbard, K. A., Manning, M. R., Rose, S. K., Van Vuuren, D. P., … Wilbanks, T. J. (2010). The next generation of scenarios for climate change research and assessment. *Nature*, *463*(7282), 747–756. https://doi.org/10.1038/nature08823

Mota, C. F., Engelen, A. H., Serrao, E. A., Coelho, M. A. G., Marbà, N., Krause-Jensen, D., & Pearson, G. A. (2018). Differentiation in fitness-related traits in response to elevated temperatures between leading and trailing edge populations of marine macrophytes. *PLoS ONE*, *13*(9), 1–17. https://doi.org/10.1371/journal.pone.0203666

Muhlin, J. F., & Brawley, S. H. (2009). Recent versus relic: Discerning the genetic signature of fucus vesiculosus (Heterokontophyta; Phaeophyceae) in the northwestern atlantic1. *Journal of Phycology*, *45*(4), 828–837. https://doi.org/10.1111/j.1529-8817.2009.00715.x

Müller, R., Laepple, T., Bartsch, I., & Wiencke, C. (2009). Impact of oceanic warming on the distribution of seaweeds in polar and cold-temperate waters. *Botanica Marina*, *52*(6), 617–638. https://doi.org/10.1515/BOT.2009.080

Neiva, J., Assis, J., Coelho, N. C., Fernandes, F., Pearson, G. A., & Serrão, E. A. (2015). Genes left behind: Climate change threatens cryptic genetic diversity in the canopy-forming seaweed Bifurcaria bifurcata. *PLoS ONE*, *10*(7), 1–21. https://doi.org/10.1371/journal.pone.0131530

Neiva, J., Assis, J., Fernandes, F., Pearson, G. A., & Serrão, E. A. (2014). Species distribution models and mitochondrial DNA phylogeography suggest an extensive biogeographical shift in the high-intertidal seaweed Pelvetia canaliculata. *Journal of Biogeography*, *41*(6), 1137–1148. https://doi.org/10.1111/jbi.12278

Neiva, J., Pearson, G. A., Valero, M., & Serrão, E. A. (2010). Surfing the wave on a borrowed board: Range expansion and spread of introgressed organellar genomes in the seaweed Fucus ceranoides L. *Molecular Ecology*, *19*(21), 4812–4822. https://doi.org/10.1111/j.1365-294X.2010.04853.x

Neiva, J., Pearson, G. A., Valero, M., & Serrão, E. A. (2012a). Drifting fronds and drifting alleles: Range dynamics, local dispersal and habitat isolation shape the population structure of the estuarine seaweed Fucus ceranoides. *Journal of Biogeography*, *39*(6), 1167–1178. https://doi.org/10.1111/j.1365-2699.2011.02670.x

Neiva, J., Pearson, G. A., Valero, M., & Serrão, E. A. (2012b). Fine-scale genetic breaks driven by historical range dynamics and ongoing density-barrier effects in the estuarine seaweed Fucus ceranoides L. *BMC Evolutionary Biology*, *12*(1). https://doi.org/10.1186/1471-2148-12-78

Neiva, J., Serrão, E., Assis, J., Pearson, G. A., Coyer, J. A., Olsen, J. L., … Valero, M. (2016). Climate Oscillations, Range Shifts and Phylogeographic Patterns of North Atlantic Fucaceae. *Seaweed Phylogeography: Adaptation and Evolution of Seaweeds under Environmental Change, In Seaweed*, 279–308. https://doi.org/10.1007/978-94-017-7534-2

Nicastro, K. R., Zardi, G. I., Teixeira, S., Neiva, J., Serrão, E. A., & Pearson, G. A. (2013a). Shift happens: Trailing edge contraction associated with recent warming trends threatens a distinct genetic lineage in the marine macroalga Fucus vesiculosus. *BMC Biology*, *11*. https://doi.org/10.1186/1741-7007-11-6

Nicastro, K. R., Zardi, G. I., Teixeira, S., Neiva, J., Serrão, E. A., & Pearson, G. A. (2013b). Shift happens: Trailing edge contraction associated with recent warming trends threatens a distinct genetic lineage in the marine macroalga Fucus vesiculosus. *BMC Biology*, *11*(1), 6. https://doi.org/10.1186/1741-7007-11-6

Parmesan, C., Ryrholm, N., Stefanescus, C., Hill, J. K., Thomas, C. D., Descimon, H., … Warren, M. (1999). Poleward shifts in geographical ranges of butterfly species. *Nature*, *399*(June), 579–583. Retrieved from www.nature.com

Parmesan, Camille, Root, T. L., & Willig, M. R. (2000). Impacts of extreme weather and climate on terrestrial biota. *Bulletin of the American Meteorological Society*, *81*(3), 443–450. https://doi.org/10.1175/1520-0477(2000)081<0443:IOEWAC>2.3.CO;2

Pearson, G. A., & Brawley, S. H. (1996). Reproductive ecology of Fucus distichus (Phaeophyceae): An intertidal alga with successful external fertilization. *Marine Ecology Progress Series*, *143*(1–3), 211–223. https://doi.org/10.3354/meps143211

Pearson, G. A., Lago-Leston, A., & Mota, C. (2009). Frayed at the edges: Selective pressure and adaptive response to abiotic stressors are mismatched in low diversity edge populations. *Journal of Ecology*, *97*(3), 450–462. https://doi.org/10.1111/j.1365-2745.2009.01481.x

Pearson, R. G., Raxworthy, C. J., Nakamura, M., & Townsend Peterson, A. (2007). Predicting species distributions from small numbers of occurrence records: A test case using cryptic geckos in Madagascar. *Journal of Biogeography*, *34*(1), 102–117. https://doi.org/10.1111/j.1365-2699.2006.01594.x

Pecl, G. T., Araújo, M. B., Bell, J. D., Blanchard, J., Bonebrake, T. C., Chen, I. C., … Williams, S. E. (2017). Biodiversity redistribution under climate change: Impacts on ecosystems and human well-being. *Science*, *355*(6332). https://doi.org/10.1126/science.aai9214

Pérez-Ruzafa, I., Gallardo, T., & Gómez-Cancio, R. (1993). Numerical taxonomy of some taxa of the genus Fucus in the Iberian Peninsula. *Hydrobiologia*, *260–261*(1), 81–90. https://doi.org/10.1007/BF00049006

Perrin, C., Daguin, C., Van De Vliet, M., Engel, C. R., Pearson, G. A., & Serrão, E. A. (2007). Implications of mating system for genetic diversity of sister algal species: Fucus spiralis and Fucus vesiculosus (Heterokontophyta, Phaeophyceae). *European Journal of Phycology*, *42*(3), 219–230. https://doi.org/10.1080/09670260701336554

Peterson, A. T., Soberón, J., & Sánchez-Cordero, V. (1999). Conservatism of Ecological Niches in Evolutionary Time. *Science*, *285*(August), 1265–1267.

Peterson, A. Townsend. (2011). Ecological niche conservatism: A time-structured review of evidence. *Journal of Biogeography*, *38*(5), 817–827. https://doi.org/10.1111/j.1365-2699.2010.02456.x

Phillips, S. B., Aneja, V. P., Kang, D., & Arya, S. P. (2006). Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, *190*(3–4), 231–259. https://doi.org/10.1016/j.ecolmodel.2005.03.026

Phillips, S. J., & Dudik, M. (2008). Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. *Ecography*, *31*(2), 161–175. https://doi.org/10.1111/j.2007.0906-7590.05203.x

Phillips, S. J., Dudík, M., Elith, J., Graham, C. H., Lehmann, A., Leathwick, J., & Ferrier, S. (2009). Sample selection bias and presence-only distribution models: Implications for background and pseudo-absence data. *Ecological Applications*, *19*(1), 181–197. https://doi.org/10.1890/07-2153.1

Phillips, S. J., Dudík, M., & Schapire, R. E. (2004). A maximum entropy approach to species distribution modeling. *Proceedings, Twenty-First International Conference on Machine Learning, ICML 2004*, 655–662. https://doi.org/10.1145/1015330.1015412

Pritchard, J. K., Stephens, M., & Donnelley, P. (2000). Inference of Population Structure Using Multilocus Genotype Data. *Genetics*, *155*(2), 945–959. https://doi.org/10.1007/s10681-008-9788-0

R Core Team. (2019). R: A language and environment for statistical computing. *R Foundation for Statistical Computing, Vienna, Austria. URL Https://Www.R-Project.Org/*. https://doi.org/10.1108/eb003648

Raybaud, V., Beaugrand, G., Goberville, E., Delebecq, G., Destombe, C., Valero, M., … Gevaert, F. (2013). Decline in Kelp in West Europe and Climate. *PLoS ONE*, *8*(6), 1–10. https://doi.org/10.1371/journal.pone.0066044

Riahi, K., Grübler, A., & Nakicenovic, N. (2007). Scenarios of long-term socio-economic and environmental development under climate stabilization. *Technological Forecasting and Social Change*, *74*(7), 887–935. https://doi.org/10.1016/j.techfore.2006.05.026

Riera, R., Sangil, C., & Sansón, M. (2015). Long-term herbarium data reveal the decline of a temperate-water algae at its southern range. *Estuarine, Coastal and Shelf Science*, *165*, 159–165. https://doi.org/10.1016/j.ecss.2015.05.008

Roucoux, K. H., De Abreu, L., Shackleton, N. J., & Tzedakis, P. C. (2005). The response of NW Iberian vegetation to North Atlantic climate oscillations during the last 65 kyr. *Quaternary Science Reviews*, *24*(14–15), 1637–1653. https://doi.org/10.1016/j.quascirev.2004.08.022

Rushton, S. P., Ormerod, S. J., & Kerby, G. (2004). New paradigms for modelling species distributions? *Journal of Applied Ecology*, *41*(2), 193–200. https://doi.org/10.1111/j.0021-8901.2004.00903.x

Saada, G., Nicastro, K. R., Jacinto, R., McQuaid, C. D., Serrão, E. A., Pearson, G. A., & Zardi, G. I. (2016). Taking the heat: distinct vulnerability to thermal stress of central and threatened peripheral lineages of a marine macroalga. *Diversity and Distributions*, *22*(10), 1060–1068. https://doi.org/10.1111/ddi.12474

Sanchez de Pedro, R., Fernandez Nieves, A., Jesus Garcia-Sanchez, M., Flores-Moya, A., & Bañares-Espanña, E. (2019). When the microclimate does matter: differences in the demographic , morphometric and reproductive variables of Fucus guiryi from two nearby populations. 1–4.

Schagerl, M., & Möstl, M. (2011). Drought stress, rain and recovery of the intertidal seaweed Fucus spiralis. *Marine Biology*, *158*(11), 2471–2479. https://doi.org/10.1007/s00227-011-1748-x

Schonbeck, M. W., & Norton, T. A. (1980). Factors controlling the lower limits of fucoid algae on the shore. *Journal of Experimental Marine Biology and Ecology*, *43*, 131–150. https://doi.org/10.1016/0022-0981(80)90021-0

Schönfeld, J., Zahn, R., & De Abreu, L. (2003). Surface and deep water response to rapid climate changes at the Western Iberian margin. *Global and Planetary Change*, *36*(4), 237–264. https://doi.org/10.1016/S0921-8181(02)00197-2

Seed, R., & O’Connor, R. J. (1981). Community Organization in Marine Algal Epifaunas. *Annual Review of Ecology and Systematics*, *12*(1), 49–74. https://doi.org/10.1146/annurev.es.12.110181.000405

Serrão, E. A., Alice, L. A., & Brawley, S. H. (1999). Evolution of the Fucaceae (Phaeophyceae) inferred from nrDNA-ITS. *Journal of Phycology*, *35*(2), 382–394. https://doi.org/10.1046/j.1529-8817.1999.3520382.x

Serrão, E. A., Brawley, S. H., Hedman, J., Kautsky, L., & Samuelsson, G. (1999). Reproductive success of Fucus vesiculosus (Phaeophyceae) in the Baltic Sea. *Journal of Phycology*, *35*(2), 254–269. https://doi.org/10.1046/j.1529-8817.1999.3520254.x

Shine, R., Brown, G. P., & Phillips, B. L. (2011). An evolutionary process that assembles phenotypes through space rather than through time. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(14), 5708–5711. https://doi.org/10.1073/pnas.1018989108

Southward, A. J., Hawkins, S. J., & Burrows, M. T. (1995). Seventy years’ observations of changes in distribution and abundance of zooplankton and intertidal organisms in the western English Channel in relation to rising sea temperature. *Journal of Thermal Biology*, *20*(1–2), 127–155. https://doi.org/10.1016/0306-4565(94)00043-I

Spalding, M. D., Fox, H. E., Allen, G. R., Davidson, N., Ferdaña, Z. A., Finlayson, M., … Robertson, J. (2007). Marine Ecoregions of the World: A Bioregionalization of Coastal and Shelf Areas. *BioScience*, *57*(7), 573–583. https://doi.org/10.1641/b570707

Stockwell, D. R. B., & Peterson, A. T. (2002). Effects of sample size on accuracy of species distribution models. *Ecological Modelling*, *148*(1), 1–13. https://doi.org/10.1016/S0304-3800(01)00388-X

Stokland, J. N., Halvorsen, R., & Støa, B. (2011). Species distribution modelling-Effect of design and sample size of pseudo-absence observations. *Ecological Modelling*, *222*(11), 1800–1809. https://doi.org/10.1016/j.ecolmodel.2011.02.025

Takao, S., Kumagai, N. H., Yamano, H., Fujii, M., & Yamanaka, Y. (2015). Projecting the impacts of rising seawater temperatures on the distribution of seaweeds around Japan under multiple climate change scenarios. *Ecology and Evolution*, *5*(1), 213–223. https://doi.org/10.1002/ece3.1358

Taylor, K. C., Lamorey, G. W., Doyle, G. A., Alley, R. B., Grootes, P. M., Mayewski, P. A., … Barlow, L. K. (1993). The "flickering switch" of late Pleistocene climate change. *Nature*, *361*(6411), 432–436. https://doi.org/10.1038/361432a0

Tomanek, L. (2010). Variation in the heat shock response and its implication for predicting the effect of global climate change on species' biogeographical distribution ranges and metabolic costs. *Journal of Experimental Biology*, *213*(6), 971–979. https://doi.org/10.1242/jeb.038034

Townsend Peterson, A., Papeş, M., & Eaton, M. (2007). Transferability and model evaluation in ecological niche modeling: a comparison of GARP and Maxent. *Ecography*, *30*(4), 550–560. https://doi.org/10.1111/j.2007.0906-7590.05102.x

Tyberghein, L., Verbruggen, H., Pauly, K., Troupin, C., Mineur, F., & De Clerck, O. (2012). Bio-ORACLE: a global environmental dataset for marine species distribution modelling. *Global Ecology and Biogeography*, *21*, 272–281.

Václavík, T., & Meentemeyer, R. K. (2009). Invasive species distribution modeling (iSDM): Are absence data and dispersal constraints needed to predict actual distributions? *Ecological Modelling*, *220*(23), 3248–3258. https://doi.org/10.1016/j.ecolmodel.2009.08.013

Vuuren, D. P. van, Edmonds, J., Kainuma, M., Riahi, K., Thomson, A., Hibbard, K., … Rose, S. K. (2011). The representative concentration pathways: An overview. *Climatic Change*, *109*(1), 5–31. https://doi.org/10.1007/s10584-011-0148-z

Vuuren, D. P. van, Elzen, M. G. J. Den, Lucas, P. L., Eickhout, B., Strengers, B. J., Van Ruijven, B., … Van Houdt, R. (2007). Stabilizing greenhouse gas concentrations at low levels: An assessment of reduction strategies and costs. *Climatic Change*, *81*(2), 119–159. https://doi.org/10.1007/s10584-006-9172-9

Wallace, A. L., Klein, A. S., & Mathieson, A. C. (2004). Determining the affinities of salt marsh fucoids using microsatellite markers: Evidence of hybridization and introgression between two species of Fucus (Phaeophyta) in a Maine estuary. *Journal of Phycology*, *40*(6), 1013–1027. https://doi.org/10.1111/j.1529-8817.2004.04085.x

Wang, J., Feng, L., Tang, X., Bentley, Y., & Höök, M. (2017). The implications of fossil fuel supply constraints on climate change projections: A supply-side analysis. *Futures*, *86*, 58–72. https://doi.org/10.1016/j.futures.2016.04.007

Wethey, D. S., & Woodin, S. A. (2008). Ecological hindcasting of biogeographic responses to climate change in the European intertidal zone. *Hydrobiologia*, *606*(1), 139–151. https://doi.org/10.1007/s10750-008-9338-8

Whittaker, R. H., Levin, S. A., & Root, R. B. (1973). Niche, Habitat, and Ecotope. *The American Naturalist*, *107*(955), 321–338. https://doi.org/10.1086/282837

Wiens, J. J., Ackerly, D. D., Allen, A. P., Anacker, B. L., Buckley, L. B., Cornell, H. V., … Stephens, P. R. (2010). Niche conservatism as an emerging principle in ecology and conservation biology. *Ecology Letters*, *13*(10), 1310–1324. https://doi.org/10.1111/j.1461-0248.2010.01515.x

Wise, M. a., Calvin, K. V., Thomson, a. M., Clarke, L. E., Bond-Lamberty, B., Sands, R. D., … Edmonds, J. a. (2009). *The Implications of Limiting CO2 Concentrations for Agriculture, Land Use, Land Use Change Emissions and Bioenergy*. (February).

Young, M. L. (1975). The transfer of 65Zn and 59Fe along a fucus serratus (L.)→littorina obtusata (L.) food chain. *Journal of the Marine Biological Association of the United Kingdom*, *55*(3), 583–610. https://doi.org/10.1017/S0025315400017276

Zardi, G. I., Nicastro, K. R., Serrao, E. A., Jacinto, R., Monteiro, C. A., & Pearson, G. A. (2015). Closer to the rear edge: Ecology and genetic diversity down the core-edge gradient of a marine macroalga. *Ecosphere*, *6*(2). https://doi.org/10.1890/ES14-00460.1

Zardi, G., Nicastro, K., Canovas, F., Costa, J. F., Serrão, E., & Pearson, G. (2011). Adaptive Traits are maintained on Steep Selective Gradients despite Gene Flow and Hybridization in the Intertidal Zone. *PLoS ONE*, *6*(6). https://doi.org/10.1371/journal.pone.0019402

Appendix

Supplementary 1: Background and Theory for Species Distribution Models

The basic spatial data needed to perform a SDM is the location of the occurrence of the species of interest and environmental data, which is provided as raster files (images that represent a proportion of the earth and use a regular grid). Occurrence data is available on global biodiversity information centres, citizen science initiatives and reference collections such as museums and herbaria, zoological institutions, botanic gardens universities etc. Global biodiversity centres that show the biggest data collection are for example the Ocean Biogeographic Information System (OBIS) with around 60 million records and the Global biodiversity Information Facility (GBIF) with approximately 1.2 billion records. Commonly found taxonomic inaccuracy of records from these platforms emphasize the necessity of expert judgement and assessment of the overall distribution of the species that is investigated prior modelling. Peer-revered literatures is the most powerful and reliable source to do such due to the knowledge of qualified scientists.

Within recent decades there had been a dramatic increase and major improvements in open data from modelling approaches. The availability of reliable environmental up-to-date data from Bio-ORACLE (Assis et al., 2018b) largely contributes to an improvement of species modelling possibilities and exploring species distributions on earth. This set of geographical information system-raster involves geophysical, biotic and environmental data for both surface and benthic marine realms as well as recently updated data on future climatic scenarios. The environmental layers used in models are ideally with global coverage, biological significance and chosen according to the degree to which they impact physiological processes (directly, indirectly or as resources).

Several approaches are used to estimate the relationship between biodiversity observations and the environment. First of all, mechanistic modelling finds its application by incorporating detailed data on the physiological response of a species to its environmental conditions. A mechanistic distribution model is built by reclassifying environmental gradients with tolerance limits inferred from empirical physiological experiments These models aim to characterize the fundamental niche considering also resources, population dynamics, different life stages, life histories and energy requirements. This approach is especially useful for species that are not yet in an equilibrium with the environment and therefore the distribution of invasive species is investigated by this method. However, the research and data on the practice responses are often not available. Correlative modelling on the other hand is based on the assumption that the distribution of a species is an indicator of its ecological niche. By using the observed distribution of a species these models display the realized niche, when assuming that the species is in an equilibrium with the environment. An evaluation of "ecological realism" is in this case necessary to check if the values of the environment are as well consistent with prior ecological knowledge of limiting factor. Apart from which approach one decides to use, when models in the end can explain the relationship between distribution records and environmental variables for the present (baseline), predictions can be made for unknown samples. The output the SDM, which indicate the likelihood of a species to occur in a certain area can be used to understand which environmental variables are driving the distribution of a structuring species and transferring this data into the future or the past can reveal regions of persistence, extinctions and expansions in both, the past and the future. Even the genetic diversity that consists of differentiated genetic pools with maximal diversity in long-term-persistence areas and low levels of diversity in newly colonized areas can be explained by SDMs on the basis of bottleneck/founder effects. Predictions into geographical space on the other hand shows the potential niche that this species could occupy without considering inter-, and intraspecific competitions. Predictions for new sites within the training data are called interpolations, whereas predictions beyond the range of the data used to fit the model data are extrapolations. Transferring the model to new environments (extrapolating) leads in many cases to a high degree of uncertainty. To minimize this uncertainty extrapolation should be avoided in favour of interpolation. When prediction to the past or future though, extrapolation cannot be avoided, and the interpretation of the final model should be treated with a great deal of caution and more importantly with a sound ecological knowledge. To gain the most robust results, the complexity should be minimized by reducing the number of predictor variables, avoiding complex functions – simply said: using parsimonious models.

Transferability in time (future and past) and space of SDM can be achieved by reclassifying the projections which are continuous surfaces that show the probability of occurrence (ranging from 0 to 1) from the present (baseline) in a binary map by applying thresholds that are gained by extracting environmental data from a geographic location where the species is present. These thresholds can be transferred for future scenarios and sharply discriminate suitable from non-suitable habitat. All layers used in the baseline model have to be included in the transferability process.

Building a model is an iterative process following several steps. After gaining results of the first run for the predictions in time and space it is often needed to rethink the concept, re-prepare the data and adjusting the model until the final results are satisfying in respect to the research question and meeting the main objectives.

Sample size/the number of occurrence records has been found to be positively related to the performance of SMDs (Hernandez et al., 2006), however the absolute number of observations is less important than having observations that are equally distributed across the known range limits. Therefore the sampling design has a major contribution on predictive performance (Stokland et al., 2011). In general there had been found to exist an average success rate of 90% at predicting occurrence of a species with only 10 sample points and with 50 data points the success of predictions was near maximum (Stockwell & Peterson, 2002) Depending on the algorithm that is used for modelling presence- only or presence-absence data can be used. In MaxEnt (maximum entropy) presence-only data commonly finds its application while other software like BRT (boosted regression trees) and MARS (multivariate adaptive regression splines) use presence and absence data. Presence-only models can be considered to be more powerful because absence can be driven by dispersal and biotic interactions – and not only because of environmental data. The absence of certain species is often unknown leading to basic presence-only data sets. The broad use of models with presence -only data is therefore justifies by the lack of systematic surveys and the demand for making predictions, which have found to be more precise with presence-only data than with presence-absence data. “Pseudo-absences” can be used instead of real absence data. These “pseudo-absences” are generated from the study area where occurrences do not exist. Any regression (e.g. generalized linear model) can be implemented within these models. Maxent only uses presence data and the algorithm compares the locations of where a species has been found to all the environments that are available in the study region. It defines these available environments by sampling a large number of points throughout the study area, which are referred to as background points. Because background points can include locations where the species is known to occur, background points are not the same as pseudo-absences points. Background points define the available environment.

The number of absence records should cover beyond the range of environmental values where the species is present to allow describing the relationship between distributions and environment. The number of generated pseudo-absences or background locations to complement presence-only species data can reach a high number sometimes several magnitudes larger than the number of presences (Phillips et al., 2009) and was recommended to be around 10.000 (Barbet-Massin et al., 2012; Phillips & Dudik, 2008). Furthermore, the number of pseudo absences showed in previous studies to be highly influential on the predicted probability, which can be translated to different distribution areas (Stokland et al., 2011).

The selection of predictor variables, namely those environmental factors contributing the most to the species' distribution is another crucial process in SDM. Large data sets with a great number of environmental variables often gain criticism, because variables are randomly chosen and should be priory assessed instead. The approach of conducting a preselection by choosing those variables that are known to directly correspond to physiological rules requires extensive literatures research and should be guided by the research objectives and the hypothesis that are raised regarding the species-environment relationship. The number of environmental predictors depends on the scale of the question addressed, the complexity of the species ecology and the availability of the data. When incorporating too many variables collinearity issues can occur within the modelling process making the characterization of the niche in ecological terms more difficult. Resultingly, predictors that are correlated are discarded from the model that exceed a threshold of 0.85. Still the impact of the removed variables should be considered when dealing with the final results of the model. Prior to modelling a correlation analysis should be conducted.

The training data, which is used to fit (calibrate) a model is the observation of the response and predictor variables. Model fitting represents the heart of any SDM applications. Underfitting occurs when the model is too simple, which makes it inflexible in learning from the data set. Overfitting can be the case when noise of data interferes too much with the overall trend. By fitting the model predictions and the identification of the most important variables is possible.

Maxent is one of many machine learning models that construct a function, which estimated the effect of different environmental variables on the distribution of a species. If not properly parameterized these models tend to be susceptible to overfitting. Maxent is the most widely used algorithm and is based on the idea that the best explanation to unknown phenomena will maximize the entropy of the probability distribution, subject to the appropriate conditions (Phillips et al., 2006; Phillips et al., 2004). This algorithm uses known occurrences and pseudo absence data resampled from the known study area where the species is assumed to not occur. Even with small sample sizes Maxent showed to produce reliable results regarding the predictive performance (Hernandez et al., 2006). Generally speaking machine learning algorithms, like Maxent showed better performance than more simple statistical methods like generalized linear models (Elith & Graham, 2009).

Ultimately, transferability is directly related to under/overfitting. Improving transferability of ecological niche modelling includes a process called hyperparameter optimization. The best parameter is chosen that is used to control the machine-learning process. The same machine learning algorithm can require different parameter values to generalize different patterns. The approach relies on testing multiple parameter values that minimize a loss function on given independent data. Cross-validation is often used to estimate generalization performance.
Adjusting Maxent hyperparameters can reduce overfitting of the model in two ways: firstly, taking confidence intervals into account and therefore relaxing the constraints. This prevents the model from being fitted to closely around the input data. The higher the regularization (ß-multiplier) the smoother the response curve. The effects of changing the regularization multiplier affects the degree of generality in the resulting models (S. B. Phillips et al., 2006).
Secondly, the model can exclude feature types that to not add significant improvement tot the model, simply said: penalizing complexity. Excessive complexity risks overfitting and can falsely attribute patterns to random noise. Greater transferability is expected in parsimonious models with few predictors. As complexity grows, so do potential predictor combinations and the likelihood of mismatch between baseline and target condition, which can result in incorrect interpolation and extrapolation. One approach to prevent such mismatches is to remove all the variables that have a permutation importance lower than 5%. This removes the lowest ranked variable, trains a new model and computes a new rank. The process is repeated until all the remaining variables have an importance greater than 5%.

As the last step in the modelling process the evaluation of the predictive performance is needed by combining sensitivity (represents the proportion of presences that are correctly predicted) and specificity, which stands for the proportion of absences correctly predicted. Both of these elements combined together represent the “true skill statistics” (= 1 – sensitivity+ specificity). This in the end describes how well the model predicts presences and absences. The area under the receiver-operated characteristic curve (AUC) (Fielding & Bell, 1997) is another widely used measure to evaluate models and provides an overall picture of the predictive nature of models. AUC values can range between 0 to 1 where a value closer to one indicate a better fit between the observed and modelled data. In other words, the AUC is a measure of prediction errors to define false positives or false negative respectively. Using the AUC to evaluate a species distribution model is beneficial due to its well-known standard application in SDM. Additionally, it relies only on presence records and is therefore suitable for models based on random pseudo-absences or background information, like it is the case in this study. Yet, one should keep in mind that the AUC ignores the goodness of fit and the geographic extent to which models are generated influences false positive rates. The large the extent, the higher the AUC.

Another aspect in evaluating a model is determining the relative variable importance, where the model is with and without each variable, in order to determine the potential increase in model performance. Without an importance variable, the model should reduce performance. High accuracy scores are not linked to good transferability and depends how accuracy itself is measures. Testing accuracy with independent data is the approach to evaluate the model and its transferability. By doing that it generally leads to lower accuracy but at the same time to more reliable accuracy indices.

When independent data is missing portioning the data in k-fold cross-validation interactions can be performed. The data is split k times, which yields k estimates of accuracy that can be averaged. For example, a 10 fold cross validation leads to 9/10 of the observations that are used to train the model and the remaining 1/10 are used to estimate the performance. This is repeated 10 times and the estimated performance measures are averaged. For this model the independent data for cross validations sets were produced by sectioning the data into blocks.

The final maps can in the end display where the species is the most likely to occur and can distinguish between suitable and non-suitable habitat. Changes in distinct areas for the future can be highlighted and populations that are the most vulnerable to global warming can be identifies and conservational measures can be applied where necessary.

Supplementary 2 List of locations and corresponding abbreviations

Abbreviation	Country	Location
Akur	Iceland	Akureyri,
Alb	Portugal	Albufeira
ALMO	Portugal	Almograve, Odemira,Alentejo
AmoL	Portugal	Amoreira
AmoH	Portugal	Amoreira
AREH	Spain	Praia da Levada/ Ria de Ares, Galicia
AREL	Spain	Praia da Levada/ Ria de Ares, Galicia
Aro	Portugal	Ilha de Arousa
Asen	Norway	Asen, Tromdheinsfjord, Nord-trondelag
AveH	Portugal	Aveiro, Porto Pesca
AveL	Portugal	Aveiro, Porto Pesca
AVIA	Portugal	Praia dos Aivados,Alentejo
AZE	Portugal	Azenhas do Mar, Sintra
BarG	Spain	Barrañán,Galicia
BarH	Escocia	Barra, Orasaigh
BarL	Escocia	Barra, Orasaigh
Bay	France	Bayonne, outside Adour estuary
Berl	Portugal	Berlenga - Carreiro do Mosteiro
BOB	USA	Pacific
Brja	Iceland	Brjánslækur,
BUD	England (U.K.)	Bude, Cornwall
Cali	USA (Pacific)	Eureka, California
CAN	Canary Islands	Canary Islands
CANA	Canada	Logy Bay, N.L.
CARR	Portugal	Praia da Carriagem, Rogil,Alentejo
Cas	Spain	Playa de Castello
CHA	Morocco	Charrana Beach
CON	USA (Atlantic)	Avery Point, Conneticut
Coos	USA (Pacific)	Coos Bay, Oregon
COT	France	La Cotinière
CstL	UK (Cornwall)	Constantine Bay

CstH	UK (Cornwall)	Constantine Bay
DAK	W Sahara & canary	Dakhla
EJO	Portugal	Praia do Castelejo, Sagres
ESSH	Morocco	Essaouira
ESSL	Central Morocco	Essaouira
FAI	Portugal	Azores, Feteira, Faial
FAI2	Portugal	Azores , Ilha do Faial, Feteira
FAI3	Portugal	Azores Ilha do Faial, Castelo Branco
Flat	Iceland	Flatey,
FRAH	Spain	La Franca,Asturias
FRAL	Spain	La Franca,Asturias
HAV	France	Le Havre,Haute-Normandie
JADH	Central Morocco	Sidi Bouzid, El Jadida,
JADL	Central Morocco	Sidi Bouzid, near El Jadida
KAYL	USA	Alaska
KSA	Gibraltar & Medit.	Ksar Es Seghir, Tanger,
LARH	France	Larmor,Bretagne
LARL	France	Larmor,Bretagne
LASH	Spain	Lastres, Asturias
LASL	Spain	Lastres, Asturias
LIZ	Portugal	Foz do Lizandro,Estremadura
Loch	Escocia	Lochailort, Glenuig
LOF	Norway	Lofoten
LUK	England (U.K.)	Lizard
MAG	Gibraltar & Medit.	Calaburra, Malaga
MALH	France	Saint Malo,Bretagne
MALL	France	Saint Malo,Bretagne
MEAH	UK	Meanporth,Cornwall
MEAL	UK	Meanporth,Cornwall
Mei	Spain	Praia do Rio Meirás, Valdovino, Coruña, Galicia
MKA	Gibraltar & Medit.	Merkala
Nah	USA (Atlantic)	Nahant, Maine
NOIH	Spain	Playa Broña, Ria de Noia, Galicia

NOIL	Spain	Playa Broña, Ria de Noia, Galicia
Olaf	Iceland	Ólafsvík
Orn	Spain	Playa de Ornanda/Gaviotas, Ria de Noia y Muros
ORTH	Spain	Porto de Ortigueira,Galicia
ORTL	Spain	Playa Fornos, Ria de Ortigueira, Galicia
OSM	England (U.K.)	Osmington Mills
Oua	Central Morocco	Oualidia
PAL	Gibraltar & Medit.	Paloma Baja, Tarifa
Pcast	Spain	Praia Castello, W Asturias
PeiH	Spain	Peinzás Fazouro, Foz
PeiL	Spain	Peinzás, Fazouro, Foz
PEM	USA	Maine
PEM1	USA (Atlantic)	Pemaquid Peninsula, Maine
PEM2	USA (Atlantic)	Pemaquid Peninsula, Maine
PenH	Portugal	Peniche, Consolsao
PenL	Portugal	Peniche, Consolsao
PERH	France	Perharidi,Bretagne
PERL	France	Perharidi,Bretagne
PIR	USA	Alaska
PLaj	Portugal	Azores, Ilha do Pico, Lajes
Pmad	Portugal	Azores, Ilha do Pico, Madalena
Pman	Portugal	Azores, Ilha do Pico, Manhenha
Porth	England (U.K.)	Porthleven
PSM	England (U.K.)	Port St. Mary, Isle of Man
PSW	England (U.K.)	Portstewart, Northern Ireland
QUIH	UK	Port Quin,
QUIL	UK	Port Quin,
Raz	Portugal	SWCoast
RHO	UK (Wales)	Rhosneigr
Rib	Spain	Praia da Atalaia, Ribadesella, Asturias
RIh	Portugal	Ribeira de Ilhas
ROS	France	Roscoff,Bretagne
RVG	Spain	Redondela, Vigo,Galicia

SAG	Portugal	Sagres, Ponta Ruiva
San	Spain	Marismas de Santo
Schoo	USA (Atlantic)	Schoodic Peninsula, Maine
SINH	Portugal	Cabo de Sines, Sines,Alentejo
SINL	Portugal	Cabo de Sines, Sines,Alentejo
SMI	Portugal	Azores, Caloura, São Miguel
SMI1	Portugal	Azores, São Miguel Ferraria
SMI2	Portugal	Azores, São Miguel, Calhau da Areia
SMI3	Portugal	Vila Franca do Campo, São Miguel,Azores
SMI4	Portugal	Azores, São Miguel, Caloura
SMI5	Portugal	Azores, São Miguel, Ferraria
SMI6	Portugal	Azores, São Miguel, Mosteiros
SMill	England (U.K.)	Scalby Mills
Sun	USA (Pacific)	Sunset Beach, Fidalgo Island, Washington
TAF	Spain	Tarifa,Andaluzia
TAR	W Sahara & canary	Tarfaya
TerAn	Portugal	Azores, Ilha Terceira, Angra do Heroismo
TerMar	Portugal	Azores, Terceira, Porto Martins,Baía da Vila
TerMar1	Portugal	Azores, Ilha Terceira, Porto Martins
TerN	Portugal	Azores, Terceira, Negrito
TerQu	Portugal	Azores, Ilha Terceira, Quatro Ribeiras
Trom	Norway	Tromso
VAL	Central Morocco	Plage Val D'or, Temara,
VG	Spain	Viveiro, Galicia
ViaH	Portugal	Viana do Castelo
ViaL	Portugal	Viana do Castelo
VNMF	Portugal	Villa Nova de Milfontes
VOG	Iceland	Vogar
Wash	USA (Pacific)	Des Miones, Washington
ZAM	Portugal	Zambujeira do Mar,Alentejo

Supplementary 3 Table of unique alleles

Locus	Size	Population	Geographic Zone
F36	230	CON	N-America
L78	187	Akur	North
Fsp1	153	Akur	North
L78	134	CstL	UK
L78	150	Porth	UK
L78	170	Porth	UK
L78	175	LUK	UK
L78	178	ROS	UK
Fsp1	134	Porth	UK
Fsp1	141	LUK	UK
Fsp1	147	LUK	UK
Fsp1	157	BarH	UK
Fsp1	165	BarH	UK
L20	158	BarH	UK
F36	225	BarH	UK
F42	186	BarH	UK
F42	187	BUD	UK
F42	194	BarL	UK
L78	140	FRAL	NWIberia
F34	187	Rib	NWIberia
F34	188	BarG	NWIberia
F34	200	NOIL	NWIberia
Fsp1	150	NOIL	NWIberia
L20	180	FRAH	NWIberia
F9	195	FRAL	NWIberia
L20	150	TAF	GIB
L20	152	TAF	GIB
F42	183	RIh	South-West Coast
F21	192	CAN	South
F9	171	TerMar	Azores

Supplemenraty 4 Standadized allele frequency plots for individuals that correspond to F.vesicuolsus according to their mitochondrial DNA