Investigating the genetic origin of three *Fucus* morphotypes using microsatellite analysis

Frida Catharina Skovereng Knoop

Master of Marine Biology, June 2021

Supervisors: Inga Kjersti Sjøtun, Pedro Miguel de Azevedo Ribeiro, Geir Dahle

Department of Biological Sciences, University of Bergen

Acknowledgements

First, I would like to say thank you Kjersti, for shaping the thesis and for giving me the opportunity to participate in this project. Without exception, you have been so kind and supportive throughout the whole process. Although I only got to explore a small part of the vast world of algae, it surely has been an inspirational and interesting journey full of new learnings. Thank you for your guidance and patience in the field, the lab, and for always answering my questions. I could not ask for a better supervisor, and it has been a pleasure to work with you.

Pedro, thank you for being an excellent co-supervisor. During this thesis, I very much appreciated your positive attitude and patience. Thank you for taking your time to explain the processes behind the molecular work and for guiding me through the statistical part, which I found particularly challenging. During stressful times, your support kept me calm and made sure I did not lose focus. Also, your feedback was very much appreciated.

A special thank you to co-supervisor Geir Dahle at the Institute of Marine Science (IMR) for taking your time to help with the genetic analysis, the ABI Machine, and allele scoring, which was only possible at IMR. I also want to thank you for sharing your knowledge regarding microsatellite analysis, being helpful with the statistics, and providing good feedback. My thesis could not be completed without your help.

Louise, thank you for your guidance during the microsatellite course involving lab procedures such as DNA extraction, PCR, and gel electrophoresis. Despite the extra high demand for laboratory equipment due to the global pandemic crisis, you were able to provide all the necessary equipment so the lab work could be completed in time. Also want to say thank you Kenneth Mæland, for helping with practicalities in the lab. Thank you, Rafael Martín-Martín for contributing with the material from Spain.

Lastly, thank you all for the extra flexibility and countless zoom meetings. I am very grateful that I was able to complete my master thesis, despite the extra challenges that COVID-19 has brought upon us all.

Cover photographs: *Fucus chalonii* taken in Spain, by Rafael Martín-Martín (2016). *Fucus cottonii* taken in Eggholmane, Norway (2020). *Fucus spiralis* forma *nanus* taken in Gulo, Norway (2020).

Abstract

Species in the *Fucus* genus play an important ecological role for intertidal communities in the northern hemisphere. Studies in recent years have attempted to unwind the complexity of the *Fucus* genus. Confusing morphology, intricate phylogeographic history and frequent hybridization are factors that challenge a full understanding of the relationship between species. Therefore, targeting knowledge gaps to understand the fundamental processes behind evolution and the significance for intertidal communities globally is necessary. Also, current climate change imposes potential threats to the survival of intertidal organisms.

This study aims to investigate the genetic relationship between three miniaturized *Fucus* and the connection to closely related taxa. While *Fucus cottonii* may have different origins, *Fucus spiralis* forma *nanus* is believed to be closely related to *Fucus spiralis*. Moreover, little is known about the rare *Fucus chalonii*, only found in a few localities in Northern Spain. However, relationships between *F. cottonii*, *F. spiralis* f. *nanus*, and *F. chalonii* and their connection to *Fucus guiryi*, *F. spiralis* and *Fucus vesiculosus* have not been properly investigated. The findings may provide new data for morphotype fucoid and contribute to improving conservation efforts for vulnerable species.

Tissue samples of the *Fucus* species were collected from several sites in both Norway and Spain. The microsatellite analysis of samples from Norway revealed *F. cottonii* were cloned individuals with close connection to *F. vesiculosus*. *Fucus spiralis* f. *nanus* had the closest connection to the nearby sampled *F. spiralis*. The Spanish samples could not be fully resolved. However, two separate clusters for *F. chalonii* were suggested.

Table of contents

1. Introduction 7
1.1 Biology of the *Fucus* genus 8
1.1.1 Morphology 8
1.1.2 Life cycle 10
1.1.3 Habitat and distribution 12
1.1.4 Threats to the *Fucus* genus 13
1.2 Study species 14
1.2.1 Three closely related *Fucus* species 15
1.2.2 Three small varieties within the *Fucus* genus 15
1.3 Evolutionary history and speciation of the *Fucus* genus 16
1.4 Objectives 17
2. Material and Methods 19
2.1 Fieldwork 19
2.1.1 Sampling 21
2.1.2 Morphological descriptions 22
2.2 Laboratory work 23
2.2.1 DNA Extraction 25
2.2.2 PCR amplification of microsatellite markers 26
2.2.3 Microsatellite genotyping 28
2.3 Population genetics analysis 28
2.3.1 Quality control of the data 28
2.3.2 Genetic diversity 29
2.3.3 Genetic structure 29
3. Results 31
3.1 Morphological descriptions 31
3.1.1 Norwegian samples 31
3.1.2 Spanish samples 33
3.2 Quality control of molecular data 35
3.2.1 Three alleles in three loci 35
3.2.2 Suspected null alleles and potential loci under selection for all eight loci 35
3.2.3 Hardy-Weinberg equilibrium and linkage disequilibrium for five loci 35
3.3 Genetic diversity 36
3.4 Genetic structure 40

4. Discussion 44
4.1 Discussion of the thesis results 44
4.1.1 Does *F. cottonii* in Norway originate from *F. spiralis* or *F. vesiculosus*, or is it a hybrid of the two? 44
4.1.2 Is *F. spiralis* f. *nanus* genetically similar to *F. spiralis* in Norway? 47
4.1.3 Does *F. chalonii* originate from *F. vesiculosus?* 49
4.1.4 Remarks on three closely related *Fucus* species 50
4.2 Methodological issues 50
4.2.1 Fieldwork and sampling 50
4.2.2 Laboratory work and statistics 51
4.3 Future perspectives 52
Bibliography 54
Appendix I – Fieldwork 62
A. Fieldwork Norway 62
B. The Norwegian herbarium 63
Appendix II - Labwork 65
A. Detailed protocol 65
B. Preparing for DNA extraction 66
C. DNA extraction stage 67
D. Technical Data Sheet 68
E. PCR process 69
Appendix III – Data 70
A. Raw data 70
B. Linkage disequilibrium 80
C. Summary statistics 84
D. Allele frequency 89
E. Evanno table outputs 90

1. Introduction

Phaeophycea (brown algae) is a large class of macroalgae that dominate the temperate Arctic and Antarctic coasts (Lüning, 1990; Steinberg, 2019; Wernberg et al., 2019) and currently comprise 2059 described species (Guiry & Guiry, 2021). Two of the main orders, *Fucales* (rockweed, wracks) and *Laminariales* (kelp), are categorized among the largest autotrophs in the marine ecosystem, due to unique features concerning growth, internal transportation, cell communication and tissue differentiation (Bringloe et al., 2020). In comparison to other brown alga, members of the orders *Laminariales* and *Fucales* are perennial and long-lived (Lubchenco, 1980; Råberg & Kautsky, 2007; Zardi et al., 2011; Steinberg, 2019).

Fucales inhabit mainly intertidal communities in the northern hemisphere (Lüning, 1990; Serrão et al., 1999a; Laughinghouse et al., 2015) and are considered essential ecosystem components for the coastal fauna (Coyer et al., 2011). The genus *Fucus* includes ecologically important foundation species such as *Fucus radicans* L. Bergström & Kautsky 2005, *Fucus serratus* Linnaeus 1753 and *Fucus vesiculosus* Linnaeus 1753 (Dudgeon & Petraitis, 2005; Wahl et al., 2011; Duarte et al., 2015; Kautsky et al., 2019). Foundation species provide crucial habitat and nursery ground for other organisms (Steneck et al., 2002; Korpinen et al., 2010), increase the structure complexity (Wikström & Kautsky, 2007), alter local environmental factors such as light and sedimentation (Bringloe et al., 2020), in addition to increasing primary production (Kautsky et al., 1986; Steneck et al., 2002). Moreover, *Fucus* also has industrial value through food supplements and commercial compounds (Ferreira et al., 2019; Bringloe et al., 2020; Torres et al., 2020).

Several studies in recent years have attempted to unwind the complexity of the *Fucus* genus. Its evolutionary history has been interpreted in the light of various mating systems, reproductive strategies, and abilities for hybridization (Mathieson et al., 2006; Neiva et al., 2012; Sjøtun et al., 2017). Especially in the North Atlantic, the evolution and diversification within *Fucus* is identified as challenging (Coyer et al., 2011). Certain taxa are not recognized as separate species and may represent incipient species evolving into new lineages (Wallace et al., 2004; Cánovas et al., 2011; Neiva et al., 2012; Sjøtun et al., 2017). Therefore, targeting knowledge gaps to understand the fundamental processes behind evolution and the significance for intertidal communities globally is necessary. Furthermore, these studies contribute to amend conservation efforts and management.

To set the scene for my thesis, in the following sections I will provide a brief account of the biology, ecology and evolution of *Fucus.*

1.1 Biology of the *Fucus* genus

The family Fucaceae presents large morphological variation between taxa (Hardy et al., 1998). At present, the order *Fucales* has 559 described species, where 18 species belong to Fucacea, and nine to genus *Fucus* (Guiry & Guiry, 2021). The *Fucus* genus is monophyletic and two main lineages are identified (Serrão et al., 1999a). *Fucus serratus* and *Fucus distichus* Linnaeus 1767 belong to the first lineage (Coyer et al., 2006a), and the second lineage comprises *Fucus ceranoides* Linnaeus 1753, *Fucus chalonii* Feldmann 1941, *Fucus cottonii* M.C.Wynne & Magne 1991, *F. radicans*, *Fucus spiralis* Linnaeus 1753, *F. vesiculosus* and *Fucus virsoides* J. Agardh 1868 (Coyer et al., 2011). Confusing morphology, intricate phylogeographic history and frequent hybridization are factors that challenge the study and full understanding of the species belonging to the second lineage (Neiva et al., 2010; Coyer et al., 2011).

1.1.1 Morphology

The general morphology within the Fucacea family consists of parenchymatous thallus, with various forms of holdfast, stipe, branches and air vesicles (Bringloe et al., 2020). In the *Fucus* genus the terminal buds have dichotomous branching (Kucera & Saunders, 2008), and on the apical tips, reproductive organs (receptacles) are developed (Monteiro et al., 2012). In general, the nine species in the *Fucus* genus have olive-green leathery blades, a midrib, seasonal receptacles and adventitious branches that often form during regeneration (Guiry & Guiry, 2021). However, minor differences are observed in the thallus shape, branching patterns, presence of air vesicles, midrib and holdfast (Table 1).

Table 1. Main characteristics for the ten species in the *Fucus* genus. X = Presence of a character. (X) = Species which occasionally develop indistinct midribs.

Species	Holdfast	Thallus	Branching	Adv. branching	Midrib	Vesicles	Receptacles	Reproduction
F. ceranoides	X	Flat	Dichotomous	X	X		X	Dioecious
F. chalonii	X	Narrow, flat	Irregular	X	(X)		X	Dioecious
F. cottonii		Narrow, flat	Dichotomous, irregular	X	(X)			Vegetative
F. distichus	X	Flat	Dichotomous	X	X		X	Monoecious
F. guiryi	X	Flat, spiraled	Monopodial	X	X		X	Monoecious
F. radicans	X	Flat	Dichotomous		X		X	Dioecious
F. serratus	X	Flat, spiraled	Dichotomous	X	X		X	Dioecious
F. spiralis	X	Flat, spiraled	Dichotomous	X	X		X	Monoecious
F. vesiculosus	X	Flat	Dichotomous	X	X	X	X	Dioecious
F. virsoides	X	Flat, spiraled	Dichotomous	X	X		X	Monoecious

1.1.2 Life cycle

The mating system is an essential component for understanding the distribution of genetic diversity and gene flow between and within populations (Perrin et al., 2007). Within the *Fucus* genus there is a wide range of mating systems (Billard et al., 2005; Heesch et al., 2019).

Monoecious species (Table 1), such as *F. distichus* (Maier & Muller, 1986; Pearson & Brawley, 1996), *F. virsoides* (Serrão et al., 1999a) and *F. spiralis*, develop sperm and oocytes in the same conceptacle and are therefore characterized as hermaphroditic (Monteiro et al., 2012). This mode of reproduction can lead to high levels of inbreeding within a population (Zardi et al., 2011) due to occasional self-fertilization that occurs prior to gamete release (Müller & Gassmann, 1985). According to Serrão et al. (1996) gamete dispersal among *F. spiralis* is very restricted, which may contribute to high levels of genetic structuring.

The mating system of the dioecious *F. vesiculosus* (Figure 1) normally depends on two individuals, since sperm and oocytes mature separately in male and female individuals (Wynne & Bold, 1985; Heesch et al., 2019). Furthermore, species that possess air vesicles conferring the ability for buoyancy may have enhanced dispersal capacity (Tatarenkov et al., 2007). Although rarely observed, a few populations of *F. vesiculosus* in the Baltic Sea have been found to develop vegetatively (Tatarenkov et al., 2005). Studies report high genetic subdivision within a small geographic range for *F. vesiculosus* (Pereyra et al., 2013). Other dioecious *Fucus* species (Table 1) are *F. ceranoides* (horned wrack) (Brawley, 1992; Neiva et al., 2010), *F. chalonii* (Feldmann, 1941), *F. radicans* (Bergström et al., 2005) and *F. serratus* (d'Avack & Tyler-Walters, 2015).

Vegetative reproduction (Figure 2) is characterized for species with asexual mating systems (Neiva et al., 2012), e.g., *F. cottonii* (Wynne & Magne, 1991). New individuals, often genetically identical, emerge from adventitious branches (Cotton, 1912).

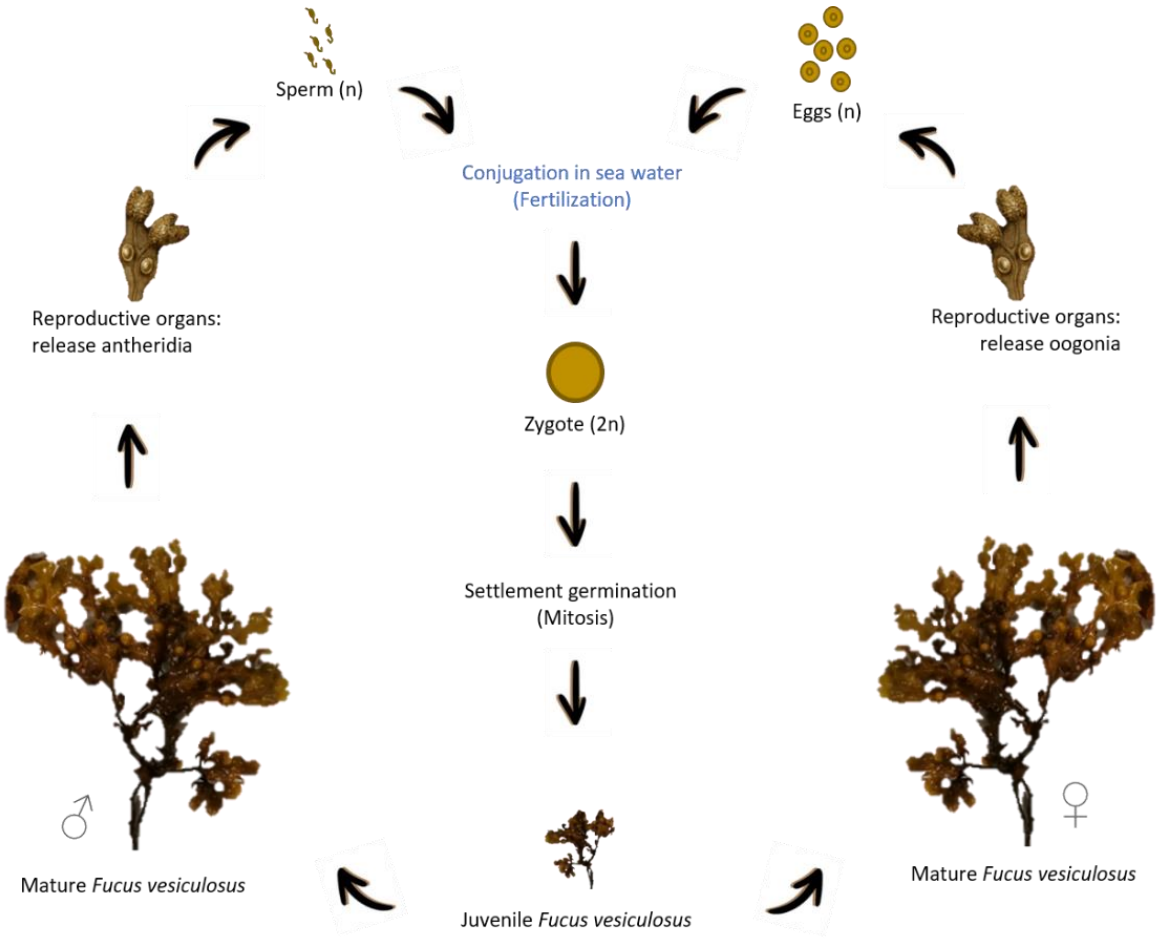


Figure 1. Life cycle in the dioecious *F. vesiculosus*. Mature male individuals develop receptacles that release antheridia with spermatozoids, and female individuals release oogonia with oocytes. Fertilization takes place when a sperm cell (n) and the oocytes (n) connect and develop into a diploid zygote (2n). The zygote will germinate into a new juvenile individual which can repeat the cycle.

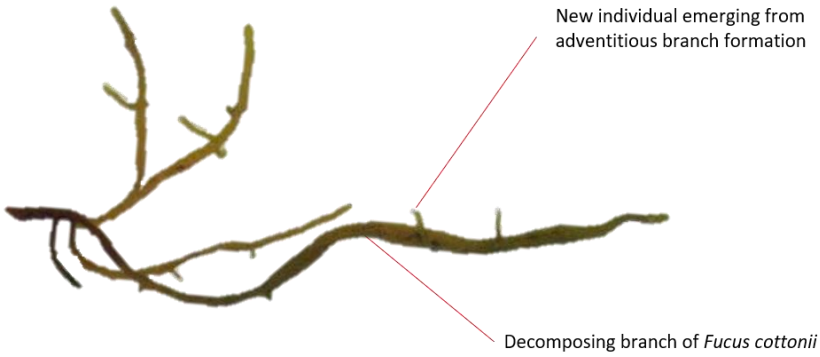


Figure 2. Asexual life cycle in *F. cottonii*. Modified photograph by Kjersti Sjøtun, 2014 (seaweeds.uib.no).

1.1.3 Habitat and distribution

Rocky shores are typical *Fucus* habitat where the species grow in a fucoid zonation (Lubchenco, 1980). In this vertical gradient, abiotic factors such as wave exposure, light, desiccation, temperature and salinity, in addition to competition and predation (biotic interactions), define the species distribution (Zardi et al., 2011). *Fucus cottonii* is only found in high tide salt marshes in sheltered bays (Wallace et al., 2004), *F. ceranoides* lives in the upper parts of estuaries (Neiva et al., 2010) and *F. radicans* inhabit brackish waters in the sublittoral zone (Bergström et al., 2005). *Fucus spiralis* normally grows in the upper littoral zone in areas sheltered from wave exposure (Perrin et al., 2007). *Fucus vesiculosus* inhabits the littoral zone and *F. serratus* the lower littoral zone (Lubchenco, 1980), but also in semi-exposed areas (Arrontes, 1993; Nicastro et al., 2013). Due to overlapping habitat, they compete for space in the intertidal zone (Zardi et al., 2011). *Fucus virsoides* grows in the mid-littoral zone, in sheltered and semi-exposed areas (Verlaque et al., 2019). While some species thrive in sheltered or semi-exposed sites, others like *F. chalonii* (Feldmann, 1941) and *F. distichus* (Laughinghouse et al., 2015) grow in very wave-exposed sites.

The geographical distribution of *Fucus* is extensive as they are considered dominant structural species in the North Atlantic and North Pacific coast (Lüning, 1990; Coyer et al., 2006a; Billard et al., 2010; Coyer et al., 2011). The species in lineage one (consisting of *F. serratus* and *F. distichus*) has a more northern distribution, *F. distichus* are located in the North Pacific and North Atlantic (Laughinghouse et al., 2015) and *F. serratus* is restricted to northeast and northwest Atlantic (Edelstein et al., 1974; Lüning, 1990). *Fucus vesiculosus* and *F. spiralis* are generally distributed from the Sub-Arctic to South of Portugal, on the east Atlantic (Wahl et al., 2011), and from Canada to USA on the western margin (Lüning, 1990; Coyer et al., 2006a). However, recent studies have seen *F. spiralis* and *F. vesiculosus* in Al-Hoceima, National Park of Morocco (Moussa et al., 2018). *Fucus vesiculosus* also forms the main sublittoral vegetation on bottom substrate in the Baltic Sea (Ruuskanen & Bäck, 2002) and is the only fucoid species in the gulf of Bothnia (Torn et al., 2006). *Fucus cottonii* is located in Europe (Guiry, 2012), the northwest Atlantic (Mathieson et al., 2001) and northeast Pacific (Ruiz et al., 2000). Other *Fucus* species have more limited distribution e.g., *F. ceranoides* is endemic to Europe (Neiva et al., 2010), *F. radicans* to the Baltic Sea (Pereyra et al., 2009; Rinne et al., 2018) and *F. virsoides* are exclusively found in

the Adriatic Sea (Verlaque et al., 2019). Furthermore, the *F. chalonii* is only located in a small area in North Spain.

1.1.4 Threats to the *Fucus* genus

In contrast to freshwater systems, the ocean biome is more stable in regards to environmental variabilities (Steele et al., 2019). However, small physiological changes can have a large effect on marine organisms. This is particularly true for intertidal organisms, which already live near their physiological tolerance threshold. According to the latest IPCC report (Bindoff et al., 2019) the ocean temperature has increased by 3.22 ZJ between 1969 – 1993 and 6.28 ZJ from 1993 – 2017, suggesting a two-fold increase in ocean heat uptake. The Institute of Marine Research, has recorded the temperature in the Norwegian coastal waters since 1940, and revealed that the surface layer and deep water temperature was above the normal in 2020 (Havforskningsinstituttet, 2021). Due to a more northern distribution for species in the first *Fucus* lineage, *F. serratus* and *F. distichus* are more exposed to temperature stress (Coyer et al., 2006a) than the species in the second lineage (Cánovas et al., 2011). Lüning (1984) performed a temperature-tolerance experiment on algal species collected on intervals during a 2-year time, in the North sea. After one week exposure time, *F. serratus* upper survival limit was 25°C and *F. vesiculosus* and *F. spiralis* limit was 28°C (Lüning, 1984). However, other studies demonstrate that temperature changes cause retreat or change of species distribution in the North Atlantic (Lima et al., 2007; Fernández, 2016). In the coast of North Spain, ocean warming is causing the Spanish distribution of *F. vesiculosus* to move westward (Fernández, 2016), and *F. chalonii* is already under potential pressure to become locally extinct. The conservation status for other species with limited distribution, such as *F. virsoides* is listed as vulnerable (V) (Verlaque et al., 2019). In addition, *F. cottonii* is categorized as near threatened (NT) in the Norway red list (Artsdatabanken, 2015). Furthermore, combining warming with other physiological factors impose even greater threats due to potential cumulative effects. Schonbeck & Norton (1978) found increasing tissue damage in *F. spiralis* when exposed to high air temperature, in addition to desiccation and neap tides. In the Baltic Sea there has been a major decline of *F. vesiculosus*, due to ocean acidification combined with elevated sea surface temperature (Graiff et al., 2017). Moreover, global warming can cause increased runoff which will decrease salinity levels in brackish water basins such as the Baltic Sea (Saraiva et al., 2019). As a result, foundation species (such as *F. vesiculosus*) are exposed to salinity stress that impacts growth

rate (Kinnby et al., 2020). Other threats to photosynthetic organisms are excess nutrients and eutrophication (Sahla et al., 2020). In the Mediterranean there are reports of fucoid algae loss due to destruction of habitat, eutrophication and overgrazing (Thibaut et al., 2015).

1.2 Study species

In my thesis, the focus will be on three morphotypes, *F. chalonii*, *F. cottonii*, *Fucus spiralis* forma *nanus* Kjellmann Batters 1902 and their genetic affinity to close relatives *Fucus guiryi* Zardi, Nicastro, E.S.Serrão & G.A. Pearson 2011, *F. spiralis* and *F. vesiculosus* (Figure 3). The study species in this thesis are associated with the second lineage in the *Fucus* genus.

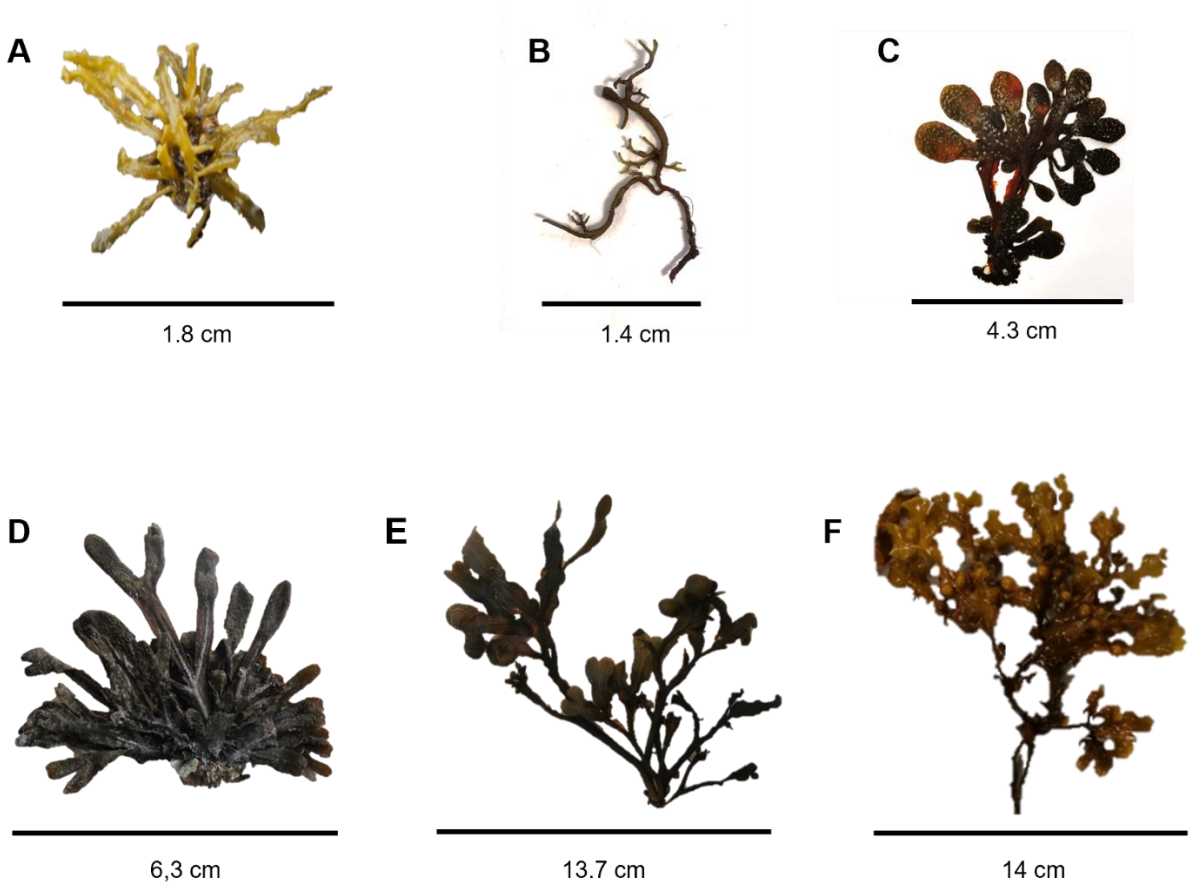


Figure 3. Morphological variation of the six study species. **A**. *Fucus chalonii* from Spain, photo taken by Raphael Martín-Martín (2020). **B.** *Fucus cottonii* from Indre Eggholmane, Norway (2020). **C.** *Fucus spiralis* f. *nanus* from Ytre Gulo, Norway (2020). **D.** *Fucus guiryi* from Bakio, Spain. Photo taken by Kjersti Sjøtun (2016). **E.** *Fucus spiralis* from Indre Eggholmane, Norway (2020). **F.** *Fucus vesiculosus* from Indre Eggholmane, Norway (2020). Images are not to scale.

1.2.1 Three closely related *Fucus* species

Fucus spiralis, F. vesiculosus and *F. guiryi*, the sister species of *F. spiralis,* are genetically closely related (Cánovas et al., 2011; Zardi et al., 2011). Their morphology is also similar, except *F. vesiculosus* has pneumatocysts (air bladders) for buoyancy (Bringloe et al., 2020), and *F. guiryi* has receptacles with sterile rim and monopodial branching (Guiry & Guiry, 2021). While *F. guiryi* and *F. spiralis* are hermaphroditic (Monteiro et al., 2012), *F. vesiculosus* is dioecious (Pereyra et al., 2013). Despite contrasting reproductive strategies and frequent hybridization (Engel et al., 2005), these sister species are able to coexist (Monteiro et al., 2012). In the intertidal zone, *F. guiryi* grows between *F. spiralis* and *F. vesiculosus* (Monteiro et al., 2012). Since *F. vesiculosus* grows on a lower level in the intertidal, it is generally less resilient for desiccation stress (Zardi et al., 2011). The distribution of the three species is largely sympatric. However, *F. guiryi* has the most southern distribution, from the British isles, along the shores of Iberia and Canary islands, to the Moroccan coasts (Zardi et al., 2011; de Pedro et al., 2019). According to Nicastro et al. (2013) current climate changes have impacted the abundance and distribution of the species in the south.

1.2.2 Three small varieties within the *Fucus* genus

The rare *F. chalonii* is exclusively found in a few areas in North Spain, growing attached to rock substrate areas in wave exposed sites (described by Feldmann, 1941). This dioecious miniaturized species has irregular to dichotomous branching and develop verrucose receptacles (Gómez-Garreta et al., 2001). Due to limited species distribution, conservation concerns highly apply to this poorly studied species.

Fucus cottonii was first discovered in Ireland (Cotton, 1912). Since then, the species has been given other names until it was revised by Wynne Magne (1991). *Fucus cottonii* is now considered to be a morphotype with different genetic origin and not a separate *Fucus* species (e.g., Sjøtun et al., 2017), but is still being referred to by its scientific name. Molecular studies suggest that *F. cottonii* may be a morphotype of *F. spiralis* or *F. vesiculosus,* (Coyer et al., 2006b; Neiva et al., 2012; Sjøtun et al., 2017), or a hybrid between them (Wallace et al., 2004). In contrast to other *Fucus* species, this moss-like fucoid is missing the holdfast, which may be due to the habitat with reduced water motion (Coyer et al., 2006b), as they grow unattached in high tide sites (Wallace et al., 2004). Another morphological deviation from other *Fucus* species is irregular branching

pattern (Mathieson et al., 2006) and the lack of receptacles, except for a few populations found in Ireland (Sjøtun et al., 2017). Other places in Europe where *F. cottonii* is located are Britain (Wynne & Magne, 1991), France (Loiseaux-de Goër & Noailles, 2008) and Norway (Wynne & Magne, 1991). Studies suggest convergent growth forms, due to other miniature species (e.g., *F. distichus*, *F. serratus*) associated with salt marsh habitats (Neiva et al., 2012). The uncertain taxonomic status of the *F. cottonii* calls for more information about this entity also out of conservation interest.

Fucus spiralis f. *nanus* is a smaller version of the hermaphroditic *F. spiralis* (Hardy et al., 1998; Scott et al., 2001; Mathieson et al., 2006). The species are significantly shorter, have fewer branches, and more units from one singular holdfast when compared with *F. spiralis* (Scott et al., 2001). In addition, the species develop smaller receptacles than those of *F. spiralis*, which may be seen as a competitive advantage (Norton, 1991). While this is the case, other studies suggest that small thallus size may be related to reproductive disadvantages (Vernet & Harper, 1980). Earlier studies have observed the species in wave exposed sites in Shetland, Orkney (Powell, 1963) the North East coast of UK, and the west and north coast of Norway (Rueness, 1977; Scott et al., 2000). In this study, *F. spiralis* f. *nanus* were exclusively found in the wave exposed locality in Bømlo, Norway. Considering its limited distribution outside the normal habitat (sheltered shores) in Norway, this variety was included in the study in order to investigate its origin.

1.3 Evolutionary history and speciation of the *Fucus* genus

The *Fucus* genus originated 5.5-2.3 million years ago (Mya), right after the geographical opening of the Bering Strait (Coyer et al., 2011). The opening of the Bering Strait created an arctic passage between the Pacific and Atlantic oceans, allowing species to radiate to new waters (Cánovas et al., 2011). The ancestors of *Fucus* originated from the North Pacific, and then dispersed and diverged to the North Atlantic around 3-1 Mya (Coyer et al., 2011).

The Fucacea family provides essential community structures in the Northern hemisphere (Cánovas et al., 2011) However, in the South the abundance is significantly smaller, a similarity observed in sister families (Cánovas et al., 2011). Cycles of global ice ages took place 1.8 Mya resulting in closing and opening the trans-oceanic corridor in the Bering Strait up to six times (Cánovas et al.,

2011). In the event of warmer periods the water exchange and flow were higher towards the Atlantic ocean, and therefore a contributing factor for higher species radiation in this direction (Cánovas et al., 2011). In the marine environment there are few barriers that potentially obstruct the gene flow (Cánovas et al., 2011). Thus, full understanding of the mechanism behind speciation is more challenging than in areas with natural barriers.

Several factors contribute to drive marine speciation. Adaptation to environment with various stress factors (desiccation, temperature, wave exposure, competition, predation), biogeographic history, divergent selection and reproductive strategies play important role for how species thrive and evolve (Cánovas et al., 2011). The majority of the Fucacea genera exhibit small species variation, and are therefore considered monospecific (Cánovas et al., 2011). However, the *Fucus* genus is highly diverse and species rich.

In Europe, the salt marsh version of *F. cottonii* has been found to originate either from *F. spiralis* or *F. vesiculosus* in Ireland (Coyer et al., 2006b; Neiva et al., 2012), or being a hybrid between *F. spiralis and F. vesiculosus* (Wallace et al., 2004; Coyer et al., 2006b). *Fucus spiralis* f. *nanus*, is known from very wave exposed rocky shores (Hardy et al., 1998). Since *F. spiralis* is associated with sheltered sites, the wave exposed example may be a genetically adapted form. While *F. cottonii* may have different origins, the *F. spiralis* f. *nanus* is believed to be closely related to *F. spiralis*. However, relationships between *F. cottonii*, *F. spiralis* f. *nanus*, and their connection to *F. spiralis* and *F. vesiculosus* have not been properly investigated in Norway. Moreover, little is known about *F. chalonii* that was only found in Northern Spain. Due to lack of studies, its origin is not yet fully understood. In addition to exploring the genetic origin of the morphotypes, there is also an important conservation aspect due to their limited distribution in Norway and Spain.

1.4 Objectives

Assessing the genetic variation from closely related taxa can provide new genetic data for morphologically separated fucoids and contribute improving conservation efforts for red-list species such as *F. cottonii* and *F. chalonii.*

Using traditional markers (nuclear ITS, mitochondrial DNA) to resolve the evolutionary relationship of species in lineage 2, has been unsuccessful (Zardi et al., 2011; Pereyra et al., 2013). For accurate assessment of genetic diversity in seaweeds, highly polymorphic markers (such as microsatellites) are suggested (Valero et al., 2001). Therefore, for my thesis, genotyping analysis was carried out using eight microsatellite markers developed in previous studies (Engel et al., 2003; Perrin et al., 2007). Morphological description was also accomplished to describe the morphology of each entity and to assess reproductive stage at sampling time.

In order to investigate the genetic relationship between the three morphotypes (*F. chalonii, F. cottonii, F. spiralis* f. *nanus*) and the connection to closely related taxa (*F. guiryi, F. spiralis, F. vesiculosus*), the following research questions were addressed:

1) Does *F. cottonii* in Norway originate from *F. spiralis* or *F. vesiculosus,* or is it a hybrid between the two?
2) Is *F. spiralis* f. *nanus* genetically similar to *F. spiralis* in Norway?
3) Does *F. chalonii* originate from *F. vesiculosus?*

2. Material and Methods

2.1 Fieldwork

Samples were collected from three locations in southwest Norway (Figure 4) and four locations along the northern coast of the Iberian Peninsula (Figure 5). The samples from Spain were collected by Rafael P. Martín-Martín from the University of Barcelona and Kjersti Sjøtun from the University of Bergen, then stored at the Department of Biological Sciences, University of Bergen. These samples were included as part of the project after agreement with the Spanish group. A total of 304 individuals were collected from 18 sites over a four-year period (details in Table 2).

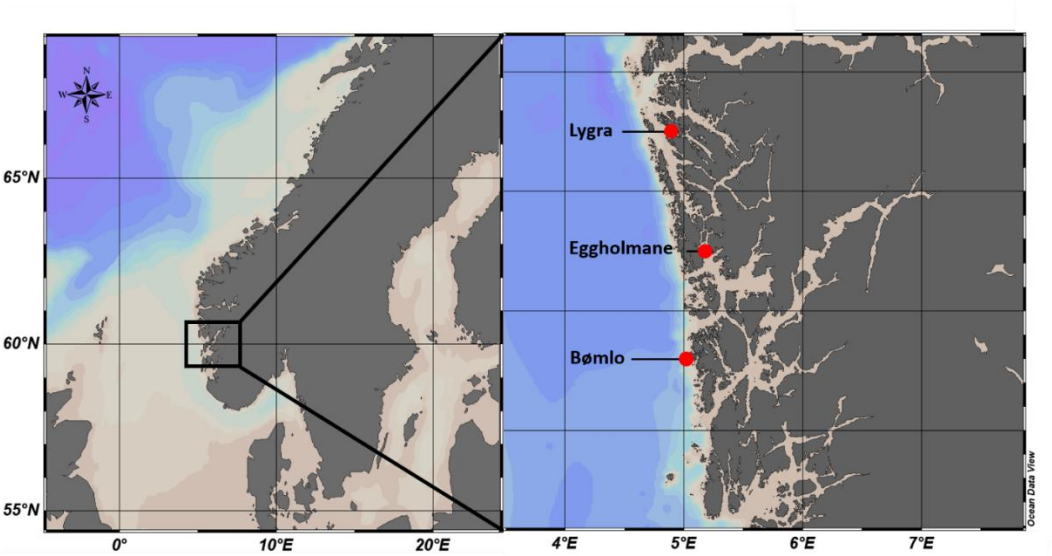


Figure 4. The three study locations in the Hordaland region, Norway (Source Ocean Data View, 2021).

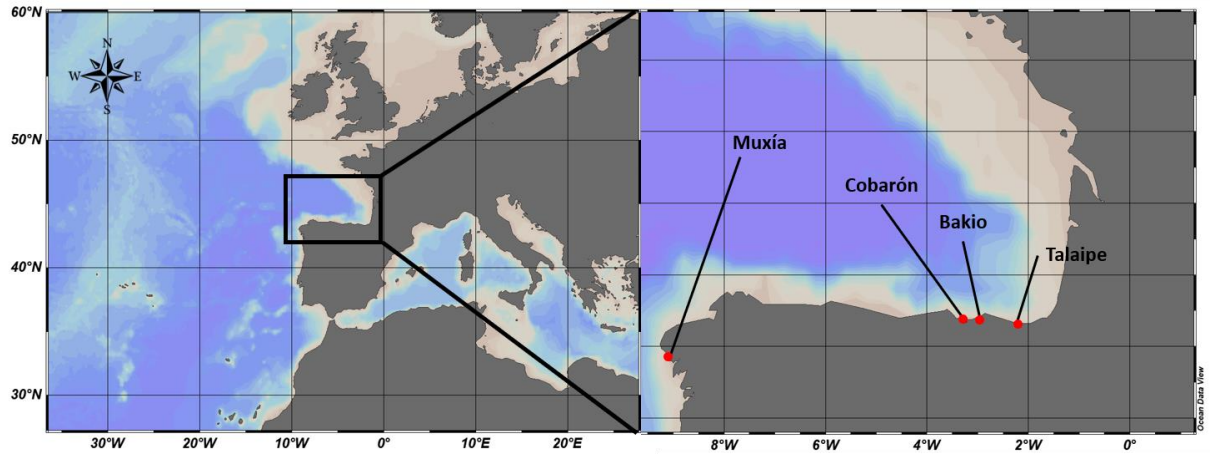


Figure 5. The four study locations in North Spain (Source Ocean Data View, 2021).

Table 2. Summary of the data collection from north to south and by taxa. N = number of individuals. Bold type indicates the three morphotypes.

Region	Location	Site	Sample ID	Taxon	Coordinates	N	Date	Collected by
Norway	Lygra	Lygra	N_LYGFs	*F. spiralis*	60°42'09.8"N, 5°05'24.8"E	20	06.10.2020	Sjøtun
Norway	Eggholmane	Indre Eggholmane	N_IEGFs	*F. spiralis*	60°15'36.2"N, 5°12'44.6"E	20	09.09.2020	Knoop, Sjøtun
Norway	Eggholmane	Ytre Eggholmane	N_YEGFs1	*F. spiralis*	60°15'36.7"N, 5°12'25.9"E	10	28.06.2019	Sjøtun
Norway	Eggholmane	Ytre Eggholmane	N_YEGFs2	*F. spiralis*	60°15'36.7"N, 5°12'25.9"E	20	09.09.2020	Knoop, Sjøtun
Norway	Bømlo	Indre Gulo	N_IGUFs	*F. spiralis*	59°44'01.3"N, 5°06'55.5"E	30	13.08.2020	Knoop, Sjøtun
Norway	Bømlo	Indre Toska	N_ITOFs	*F. spiralis*	59°42'42.5"N, 5°07'05.6"E	30	13.08.2020	Knoop, Sjøtun
Spain	North Spain	Cobarón	S_COBFs	*F. spiralis*	-	8	07.07.2016	Martín
Norway	Eggholmane	Ytre Eggholmane	N_YGUFsfn	***F. spiralis* f. *nanus***	59°43'59.2"N, 5°06'48.7"E	30	13.08.2020	Knoop, Sjøtun
Spain	North Spain	Bakio	S_BAKFg	*F. guiryi*	-	8	31.08.2016	Martín
Norway	Lygra	Lygra	N_LYGFv	*F. vesiculosus*	60°42'09.8"N, 5°05'24.8"E	18	06.10.2020	Sjøtun
Norway	Lygra	Lygra	N_IEGFv1	*F. vesiculosus*	60°15'36.2"N, 5°12'44.6"E	10	28.06.2019	Sjøtun
Norway	Lygra	Indre Eggholmane	N_IEGFv2	*F. vesiculosus*	60°15'36.2"N, 5°12'44.6"E	10	09.09.2020	Knoop, Sjøtun
Spain	North Spain	Muxía	S_MUXFv	*F. vesiculosus*	-	18	25.11.2019	Martín, Sjøtun
Norway	Lygra	Lygra	N_LYGFc	***F. cottonii***	60°42'09.8"N, 5°05'24.8"E	20	06.10.2020	Sjøtun
Norway	Eggholmane	Indre Eggholmane	N_IEGFc	***F. cottonii***	60°15'36.2"N, 5°12'44.6"E	30	28.06.2019	Sjøtun
Spain	North Spain	Cobarón	S_COBFch	***F. chalonii***	-	7	07.07.2016	Martín
Spain	North Spain	Talaipe	S_TALFch	***F. chalonii***	-	7	30.08.2016	Martín
Norway	Eggholmane	Indre Eggholmane	N_IEGFsp	*Fucus sp.*	60°15'36.2"N, 5°12'44.6"E	8	09.09.2020	Knoop, Sjøtun

2.1.1 Sampling

Specimens of *F. cottonii, F. spiralis* f. *nanus, F. spiralis, F. vesiculosus* were sampled in Norway between 2019 and 2020 (Figure 6; Appendix I, A, Figure I). The locations were situated approximately 60 km apart, and a total of 13 samples were collected (Table 2). The red-listed *F. cottonii* was growing on muddy substrate on the sheltered side in Lygra and Eggholmane. High abundance of loose-laying *Ascophyllum nodosum* Linnaeus was also observed on these sites. The two common species *F. spiralis* and *F. vesiculosus,* were attached to rocky substrate on the sheltered sites in all three locations. *Fucus spiralis* f. *nanus,* was exclusively found in the wave exposed site in Gulo (Bømlo), growing alongside *F. distichus*. One sample from Indre Eggholmane could not be morphologically distinguished from *F. spiralis* and *F. vesiculosus* and was therefore named *Fucus* sp. (N_IEGFsp).

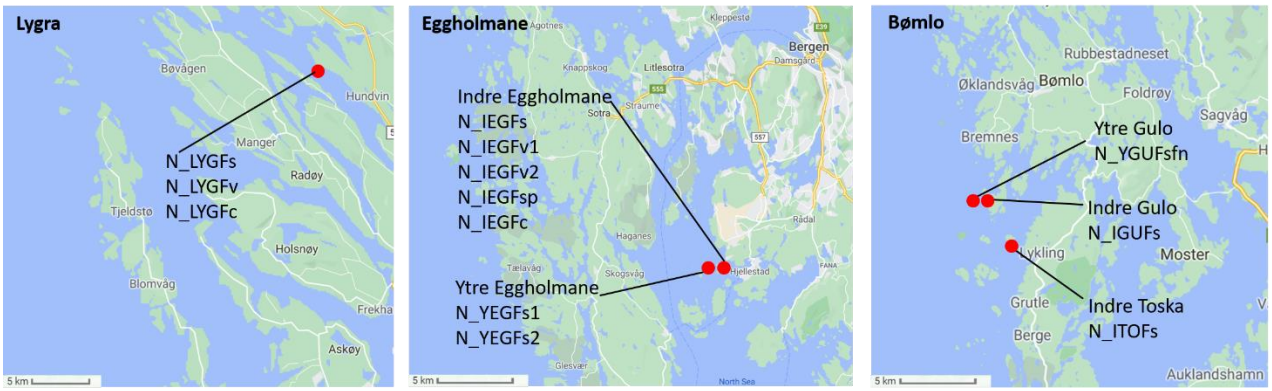


Figure 6. Overview of the three locations (Lygra, Eggholmane, Bømlo) in Norway. Sample IDs are given in table 2 (Source Google maps, 2021).

In Norway, the target species were haphazardly collected by hand along a 10-30 m transect parallel to the shoreline during low tide. A minimum of 0.5 m intervals was used to avoid sampling species from the same clone. Since *F. cottonii* grows unattached and intertwined, each individual per sample was carefully picked out. The remaining species grew attached and were picked from the holdfast, then placed into plastic bags and stored in cooling bags. Between 8-30 individuals were collected per station, and a few samples from Eggholmane were sampled two successive years (Table 2). Directly after sampling, a clean piece of ca 0.5 cm^2 tissue was cut off the tip of each individual, preferably without receptacles for purer DNA extraction. The tissue was placed into 5.0 ml screw-capped tubes filled with silica gel orange (Sigma-Aldrich), then stored dry at 4°C.

In addition, 2-9 individuals from 10 samples were mounted on herbarium sheets for morphological descriptions (Appendix I, B, Figure II).

Specimens of *F. guiryi*, *F. vesiculosus, F. spiralis* and *F. chalonii* were sampled in Spain between 2016 and 2019. The samples of *F. guiryi* (originally sampled as *F. spiralis* var. *limetaneus*), *F. spiralis* and *F. chalonii* were collected during the summer of 2016 along the Basque coast, and the samples of *F. vesiculosus* were collected in 2019 at Muxía, Galicia. Three of the sites, Talaipe, Bakio, Cobarón, are located 30-50 km apart on the Eastern side of the Bay (Figure 7). *Fucus vesiculosus* were not observed at any of these localities. Muxía is situated approximately 500 km further to the west. *Fucus chalonii* was found attached to rocky substrate in two sites (Cobarón, Talaipe), and *F. spiralis* was growing in the intertidal zone alongside with *F. chalonii*, in Cobarón (Rafael Martín-Martín pers. comm). *Fucus vesiculosus* was exclusively found in Muxía, whereas *F. guiryi* was only found in Bakio.

Figure 7. Overview of the sites and the samples from Spain. Sample IDs are given in table 2 (Source Google maps, 2021).

2.1.2 Morphological descriptions

Descriptions of morphological characters of the samples, was carried out in the Systematics lab at the Department of Biological Sciences, University of Bergen. A total of 50 individuals of the Norwegian samples were mounted on herbarium sheets and analyzed, in order to describe the morphology of each entity (Appendix I, B, Figure II). In addition, five specimens from the Spanish herbarium were included in the morphological descriptions (Figure 12D-H). Five individuals from each site in Bømlo (IGUFs, ITOFs, YGUFsfn) were measured by hand for morphometric recordings (Table 8). The conditions of the receptacles were also documented, as this may inform about the reproductive stage of the individuals. Total length (cm), leaf width from five branches

(chosen haphazardly), tips with receptacles, tips without receptacles, in addition to presence of adventitious branches, holdfast and midrib was recorded (Figure 8).

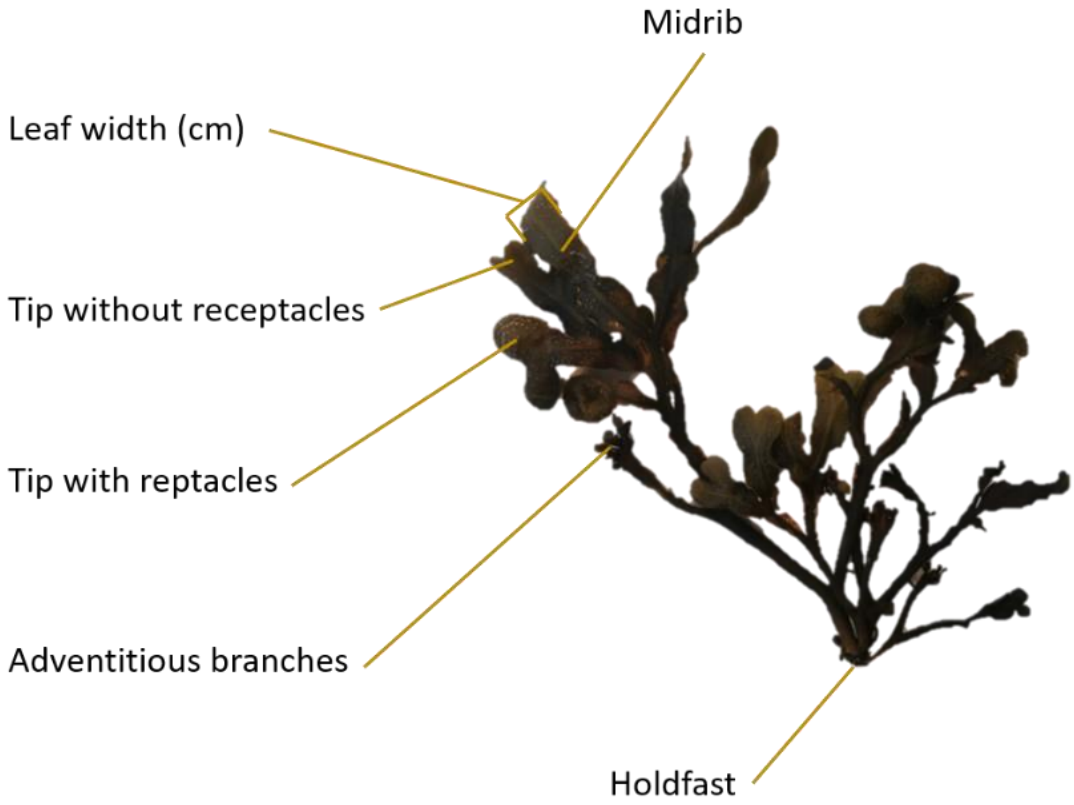


Figure 8. Characteristics of the thallus in *F. spiralis*. The total length was measured from holdfast to the highest point of the thallus.

2.2 Laboratory work

The laboratory work was carried out in the DNA lab at the Department of Biological Sciences, University of Bergen. Genomic DNA extraction was performed using the NucleoMag® Plant (Macherey Nagel) on all the 304 individuals. For microsatellite analysis, eight polymorphic microsatellite markers were used (Table 3). Subsequently, the PCR-amplified microsatellites were run on an ABI 3730 DNA Analyzer (Applied Biosystems) at the Institute of Marine Research (IMR) in Bergen and fragments identified using the Genemapper 6.0 software (Applied Biosystems).

Table 3. Characteristics of the eight microsatellite loci used in this study. Ta = annealing temperature. GenBank accession number DQ314269-DQ314273 for the three loci from Perrin et al. (2007) and AY158011-AY158019 for the five loci from Engel et al. (2003).

Locus	Sequence (5´- 3´)	Repeat array	Ta (°C)	Size range (bp)	Source
L20	F-ACTCCATGCTGCGAGACTTC	$CTGG(CTG)_8(TTG)_3CTT(CTG)_2$	55°	120-159	Engel et al., 2003
	R-CCTCGGTGATCAGCAATCAT				
L38	F-TGCTAGCTGCTCTTGTGTGC	$(GCT)_{11}GCC(GVT)_7$	55°	169-199	Engel et al., 2003
	R-TAACCTGTCGGTCGCAACG				
L58	F-AAACGAAAATGGCACAGTGA	$(GA)_{19}$	55°	103-115	Engel et al., 2003
	R-CCTTGCATGTAGGAGGGAAC				
L78	F-CGTGAGGGCAGGAATGTC	$(TGC)_{11}TGT(TGC)_{32}$	55°	121-158	Engel et al., 2003
	R-GATTTCCGGCATCATCAATC	$TGGCGGTGCTGT(TGC)_3$			
L94	F-TTAGGAATGGGCGGGATG	$(GCA)_3GACGAT(GCA)_5$	55°	136-166	Engel et al., 2003
	R-GATTTCGTGAGGCTGGTTCA	$ACA(GCA)_5[GCT(VCA)_6]_{12}$			
Fsp1	F: TCAAAAGCCAGCAGGGGTG	$(AG)_{11}$	55°	140-158	Perrin et al., 2007
	R-TCTTCTGGGAGCTGTAAAATAGTC				
Fsp2	F: GCATCTGGTGTCATTCCTTGTTC	$(TC)_6CT(TC)_3G(CT)_5$	55°	153-194	Perrin et al., 2007
	R-TTGTTTGAGTGCCACCTTGC				
Fsp4	F: ATGACCGGGCCGGATTGC R-GTGCTTCCCCTCCTTGTTCTGTTG	$(AG)_6AA(AG)_{22}$	55°	128-168	Perrin et al., 2007

2.2.1 DNA Extraction

For DNA extraction, the NucleoMag® Plant (Macherey Nagel) user manual was followed with small modifications from Fort et al. (2018). The detailed protocol and modifications can be seen in Appendix II, A (Figure III). The DNA extraction is divided into tissue lysis, DNA isolation and DNA quantification. Using magnetic beads for DNA extraction has been identified as an efficient and affordable method for large sampling sets (Fort et al., 2018). Fort et al. (2018) compared several methods for DNA extraction (NucleoMag®, PowerPlant, DNEasy, CTAB) on algae and concluded that the NucleoMag® Plant method produced the highest yield of purified DNA. Marine plants contain a considerable amount of polyphenols and polysaccharides (Fort et al., 2018). Therefore, the four-step washing procedure in the NucleoMag® Plant is extensive due to removing a large proportion of supernatants.

To prepare for DNA extraction (Appendix II, B, Figure IV) the seaweed tissue was homogenized by mechanical disruption using mixer mil TissueLyser II (Qiagen), in order to release the DNA material in the nucleus. According to the protocol, the Lysis Buffer MC1 is added in step 1. However, using a dry sample in the mixing mill provides better disruption of the cells and prevents contamination. The program for grinding the samples was followed according to Næss (2019), two rounds of 20 seconds at 20 Hz. The samples were stored dry in room temperature (21°C) ready for DNA extraction.

The DNA extraction stage (Appendix II, C, Figure V) starts with tissue lysis, when a mixture of proteinase K, RNase A and buffer MC1 is added to the homogenized tissue material and incubated for 2 hours at 56°C. In the following process, the NucleoMag® C-Beads (Macherey Nagel) and binding buffer was added to each sample in a Square-well Block to attach the DNA to the NucleoMag® C-Beads (Macherey Nagel). Thereafter, the Square-well block is placed on a NucleoMag® SEP (Macherey Nagel) that attracts the beads containing the DNA, while contaminants are removed and discarded by pipetting. In the last step, the DNA is eluted (re-suspended) in 100 µl of Buffer MC6 into an Axygen 96-well plate elution tube. The extracted DNA was stored at 4°C.

To prepare working DNA solutions for PCR, 10 µl of the stock DNA extracts were diluted 1:2 by adding 10 µl ddH_2O. In this process the 96-well plate is placed on the NucleoMag® SEP (Macherey Nagel), to avoid contamination of NucleoMag® C-Beads (Macherey Nagel). DNA concentration was measured with the Invitrogen QUBIT® fluorometer (ds DNA HS assay kit), to determine if the DNA concentration was adequate. Working DNA solutions were stored at 4°C.

2.2.2 PCR amplification of microsatellite markers

For amplification, each of the eight forward microsatellite primers including a 18 bp-long M13-tail, were ordered from Sigma-Aldrich (Appendix II, D, Figure VI). In the PCR-mix one universal M13 primer labeled with a specific fluorescent dye (FAM, VIC, PET, NED) was included (Table 4). The eight primers were assembled into two groups (2x4) post PCR to speed the sequencing step. Properties of the dye are summarized in Table 5.

Table 4. Overview of loci, base pair size, dye and allocated group.

Primer	Size (bp)	Dye	Group
L58	103-115	FAM	1
L38	169-199	FAM	1
L20	120-159	VIC	1
Fsp4	128-168	PET	1
L78	121-158	FAM	2
L94	136-166	VIC	2
Fsp1	140-158	PET	2
Fsp2	153-194	NED	2

Table 5. Summarize the M13 dye properties.

Dye	Color	Absorption (nm)	Emission (nm)	Intensity
FAM	Blue	494	520	100
VIC	Green	538	554	100
NED	Yellow	546	575	40
PET	Red	558	595	25

A stock solution of 100 µ mol per primer was prepared according to specifications from Sigma-Aldrich (Appendix II, D, Figure VI). The stock solution of 100 µ mol was diluted ten-fold (1:10) by transferring 10 µl stock and 90 µl ddH_2O into 1.5 ml Eppendorf tubes. The stock solutions were stored in the freezer (-18°C). A PCR cocktail (Table 6) was prepared for each primer that was amplified independently. Master mixes comprised of 2.4 µl ddH_2O, 0.1 µl forward primer, 0.2 µl reverse primer, 6.1 µl AmpliTaq 360 mix (Applied Biosystems) and 0.2 µl M13. A total of 9 µl PCR cocktail were added into each well of the 96-cassette and 1 µl DNA extraction (1:2 diluted) was added to the 96-cassette with the PCR cocktail.

Table 6. Reaction master mix for one locus. For a 96-cassette the mix was multiplied by100.

PCR cocktail	
Reagents	Volume (µl)
ddH_2O	2.4
Fwd primer	0.1
Rev primer	0.2
AmpliTaq 360 mix	6.1
M13	0.2
Total (1 sample)	9
Total (100 samples)	900

All PCR reactions were run using the C1000 Thermal Cycler (Bio-Rad). Several trials with different temperatures were tested before a midrange annealing temperature of 55°C was demonstrating positive results. A two-step PCR was applied. In the first PCR-cycles the M13-forward primer was incorporated into the PCR products. In subsequent cycles (touchdown step) it is these products that are the targets for the labelled M13 primers. The same PCR program was selected for all eight primers (Table 7).

Table 7. PCR program used for all eight primers. The annealing temperature (step 3) was set to 55°C and 30 cycles.

PCR Program		
Step	Degrees (°C)	Time
1	95	5 min
2	95	30s
3	55	45s
4	72	45s
5	<-- step 2 x 30	
6	95	30s
7	53	45s
8	72	45s
9	<-- step 6 x 7	
10	72	30min
	4	∞

Amplified PCR products were added together for microsatellite genotyping (Table 4). In the first group, 2 µl of L58, L38, L20 and Fsp4 amplicons were mixed and in the second group, 2 µl of L78, L94, Fsp1 and Fsp2 were mixed. A total mix of 8 µl per group was stored at 4°C and protected from ambient light prior to microsatellite genotyping. Photo documentation of the PCR process is provided as supplementary material (Appendix II, E, Figure VII).

2.2.3 Microsatellite genotyping

Genetic variation for all 304 individuals was evaluated at eight microsatellite loci (Table 3). The genetic analysis of the PCR products was carried out in the laboratory at the Institute of Marine Science (IMR), Bergen. The ABI 3730 DNA analyzer (Applied Biosystems) is a sequencer using capillary electrophoresis to separate and identify fluorescent labelled DNA fragments. The post PCR products were diluted 1:10 with ddH_2O and then 2 µl were transferred to a customized ABI 96-plate. A mixture of Genescan™ 500 Liz standard (Applied Biosystems) and formamide was prepared, and 8 µl of this mix was added to each of the samples. The Genescan™ 500 Liz standard is composed of 16 DNA fragments ranging from 35-500 bp making it possible to identify each fragment. The fragments are allocated to pre-determined bins (size range of each allele) with the GeneMapper 6.0 software (Applied Biosystems). PCR processes were repeated for samples with uncertainties, background noise, or missing peaks.

2.3 Population genetics analysis

In three loci, three alleles were observed for certain individuals. Since statistical programs are developed for managing two alleles (diploid population) or one allele (haploid population), individuals with three alleles cannot be analyzed correctly. Considering not knowing what caused the three alleles, the three loci (L38, L78, Fsp2) were removed from most of the analyses. The raw genotype data are provided as supplementary material (Appendix III, A, Table A).

2.3.1 Quality control of the data

Genotyping errors (null alleles, large allele dropout and scoring failure) that may occur during the PCR process, were identified for all eight loci using software program MICRO-CHECKER version 2.2.3 (Van Oosterhout et al., 2004). LOSITAN 1.0.0 (Antao et al., 2008), a workbench to

detect molecular adaptation based on a F_{ST} -outlier method, was used in order to recognize potential loci under selection. The following parameters were applied for all eight loci. CPU Cores: 2, x1000 simulations, confidence interval 0.95, attempted F_{ST} 0.528.

When inbreeding is suspected, the proportion of homozygotes in the population will increase when performing a test for deficit of heterozygotes in Hardy-Weinberg Equilibrium (HWE) assumptions (Wigginton et al., 2005). A global HW test (H1 = Heterozygote deficiency), was computed in the web version of GENEPOP 4.7.5 (Rousset, 2008) in order to explore the level of inbreeding. This was done by measuring the inbreeding coefficients (F_{IS}) and associated P-values for five loci. A linkage disequilibrium test for each pair of loci in each sample was also calculated in GENEPOP 4.7.5 (Rousset, 2008) in order to search for correlation between alleles at the five loci (Flint-Garcia et al., 2003), using the Fisher's method. Default settings were applied (Marcov chain parameters: dememorization = 1000, batches = 100, iterations per batch = 1000). Sequential Bonferroni correction was used in order to correct for type 1 errors, which may occur during multiple statistical tests (Armstrong, 2014).

2.3.2 Genetic diversity

F-STAT version 2.9.4 (Goudet, 2003) was used to estimate and test population genetics parameters such as number of alleles (N_a), allelic richness (Ar), and Fixation index (F_{ST}) and P-values. The allelic richness, which is considered one of the most commonly reported measures of genetic variation, is referred to as the mean number of alleles per locus (Leberg, 2002). F_{ST} and P-values were generated after 15300 permutations and adjusted after Bonferroni correction.

GENEPOP 4.7.5 (Rousset, 2008) was used to investigate allele frequency, observed heterozygosity (H_O), expected heterozygosity (H_E), and the inbreeding coefficient (F_{IS}). A positive F_{IS} value implies heterozygote deficit and negative heterozygote excess within the populations (Wallace et al., 2004). The analyses were calculated per sample and per locus.

2.3.3 Genetic structure

GenAlEx 6.5 (Peakall & Smouse, 2006) was used for principal coordinate analysis (PCoA) via covariance matrix with data standardization, for sampled from Norway and Spain separately.

PCoA was calculated using Nei's genetic distance and represents similarities and dissimilarities between the populations based on allele distribution.

The genetic structure was further analysed in STRUCTURE version 2.3.4 (Pritchard et al., 2000), which allows to explore properties of samples by utilizing multiple locus genotyped information. Analysis was carried out using a burn-in of 500000, 1000000 reps of Markov Chain Monte Carlo (MCMC) and 10 iterations. For the samples from Norway and Spain, assumed number of clusters (K) = 2 to 5. Previous studies show that *F. guiryi* can be defined by using loci L20 and L78 (Zardi et al., 2011). Therefore, additional STRUCTURE analysis was carried out for the Spanish material with loci L20 and L78, in order to separate *F. guiryi* from *F. spiralis* and *F. vesiculosus.* The web version of Structure Harvester (Earl, 2012) was used for identifying the most likely number of clusters (K) using the method by Evanno et al. (2005), in accordance with the STRUCTURE analyses.

The analysis of molecular variance (AMOVA) was carried out in Arlequin version 3.5.2.2 (Excoffier et al., 2005) in order to investigate the genetic and demographic connections between and among individuals. The populations were grouped after taxa and run with 10000 permutations.

3. Results

3.1 Morphological descriptions

Morphological descriptions were carried out on 50 individuals from Norway. Photography of the complete Norwegian herbarium are provided as supplementary material (Appendix I, B, Figure II). The material from Spain included five individuals that were mounted on herbarium sheets (Figure 12E-H).

3.1.1 Norwegian samples

Fucus cottonii from Indre Eggholmane and Lygra was growing unattached and entangled within each other, and the total length of thallus varied between 1 cm to 2 cm approximately (Figure 9AB). Large abundance of irregular and adventitious branching was seen on most of the individuals.

Figure 9. Morphological traits and scale bar of *F. cottonii* sampled in Norway. **A.** Three individuals sampled from Lygra, October 2020. **B.** Six individuals sampled from Indre Eggholmane, September 2020. Photo by Kjersti Sjøtun, May 2021.

Fucus spiralis from Eggholmane and Lygra was growing attached and had a total thallus length that varied between 10 cm to 25 cm approximately (Figure 10A-F). The specimens had a holdfast, midrib, dichotomous branching and several tips with receptacles. The receptacles were generally in poor condition. One population from Indre Eggholmane, could not be distinguished as *F. spiralis* or *F. vesiculosus* and was named *Fucus* sp. (Figure 10G).

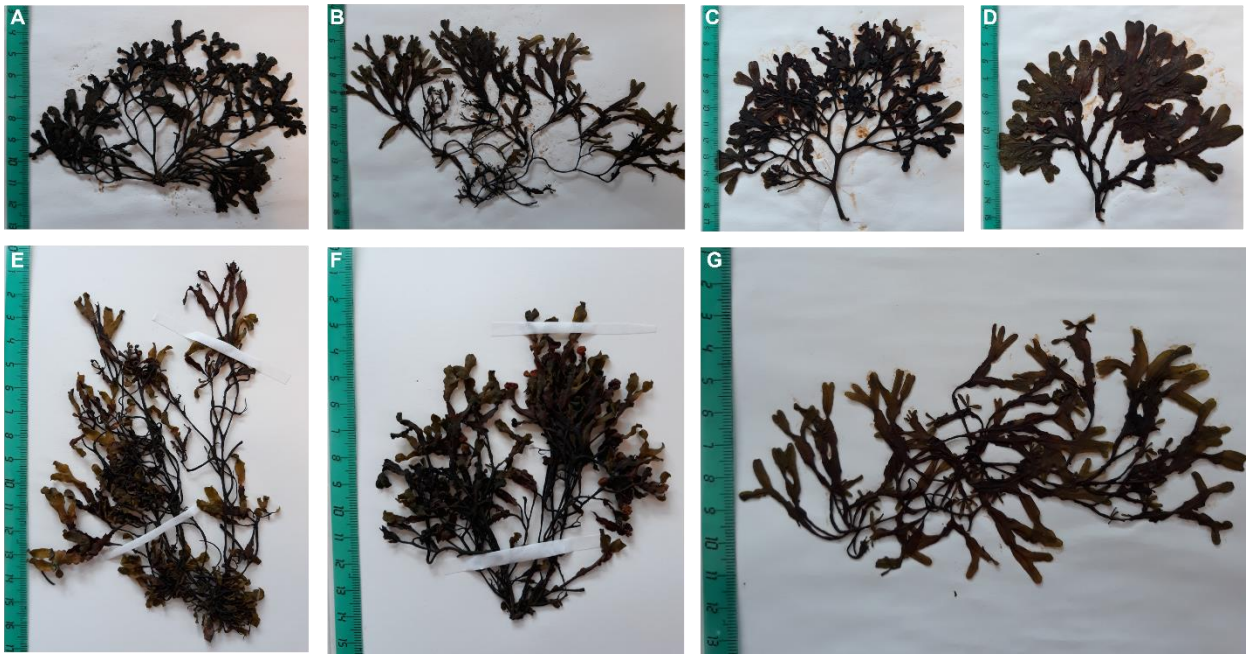


Figure 10. Morphological traits and scale bar of *F. spiralis* sampled in Eggholmane. **A-B.** *Fucus spiralis* (IEGFs12, IEGFs15) sampled from Indre Eggholmane, September 2020. **C-D.** *Fucus spiralis* (YEGFs14, YEGFs15) sampled from Ytre Eggholmane, September 2020. **E-F.** *Fucus spiralis* (LYGFs1, LYGFs6) from Lygra sampled 2019. **G.** *Fucus* sp. (IEGFsp2) sampled from Indre Eggholmane September 2020. Photo by Kjersti Sjøtun, May 2021.

Due to variation in sampling time, it was decided to only carry out morphometric recordings on the samples from Bømlo (Appendix I, B, Figure IID-E, G). The mean number of total length (TL) for the 15 individuals ranged from 4.02 cm to 19.14 cm (Table 8). *Fucus spiralis* f. *nanus* was smallest in size (4.02 cm) and had a strong holdfast (Figure 8E-G). *Fucus spiralis* sampled in Indre Gulo was longest (19.14 cm) (Figure 8AB). The mean number of total leaf width (TLW) was smallest for *F. spiralis* f. *nanus* (0.284 cm) and largest for *F. spiralis* from Indre Gulo (0.682 cm).

Tips with receptacles presence (RP) ranged from 10 to 25 and tips with receptacles absence (RA) ranged from 4 to 35 (Table 8). *Fucus spiralis* f. *nanus* had fewer receptacles presence (RP = 10)

and absent (RA = 4). *Fucus spiralis* from Indre Gulo (Figure 11AB) was found with most receptacles' presence (RP = 25) and *F. spiralis* from Indre Toska (Figure 11CD) had most receptacles' absent (RA = 35). The majority of the *F. spiralis* individuals had receptacles in poor condition. However, *F. spiralis* f. *nanus* had receptacles in good conditions, and high abundance of adventitious branching (*).

Table 8. Morphometric recordings of specimens from Bømlo, Norway. N = number of individuals, TL = mean number of total length, TLW = mean number of total leaf width x 5, RP = tips with receptacles presence, RA = tips with receptacles absent, and standard deviations (S. D). Asterisk indicates the presence of adventitious branching.

Sample	N	TL (cm)	S.D	TLW (cm)	S.D	RP	S.D	RA	S.D
IGUFs	5	19.14	4.477	0.508	0.224	25	20.216	26	12.502
ITOFs	5	13.92	0.622	0.682	0.234	22	10.232	35	32.706
YGUFsfn	5	4.02	0.701	0.284	0.225	10	4.393	4*	2.775

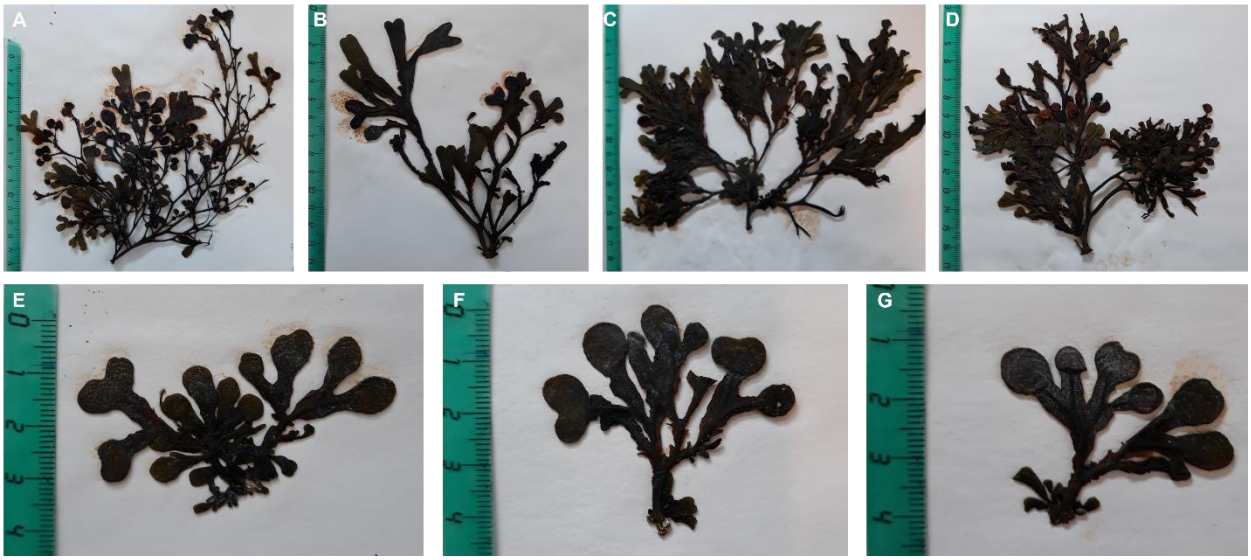


Figure 11. Morphological traits and scale bar of *Fucus* specimens sampled in Bølmo, Norway August 2020. **AB.** *Fucus spiralis* (IGUFs26, IGUFs30) sampled from Indre Gulo. **CD.** *Fucus spiralis* (ITOFs17, ITOFs24) sampled from Indre Toska. ***EFG.*** *Fucus spiralis* forma *nanus* (YGUFsfn2, YGUFsfn3, YGUFsfn8) sampled from a wave exposed site in Ytre Gulo. Photo by Kjersti Sjøtun, May 2021.

3.1.2 Spanish samples

Fucus chalonii was growing attached to rock substrate with a strong holdfast (Figure 12A-E). The total length of thallus varied between 1 cm to 2 cm approximately. The specimens had small thallus with anchoring point from holdfast, a midrib, and dichotomous and irregular branching. One individual from Cobarón was observed with a fertile verrucose receptacle that was relatively

larger than the other tips (Figure 12C). *Fucus guiryi* had a monopodial branching pattern and more elongated receptacles (Figure F-G). The sterile rim around the thallus could not be seen on the dried samples. A small example of *F. spiralis* had a thallus size of approximately 5 cm, a holdfast, midrib, dichotomous branching, and presence of receptacles (Figure 12H).

Figure 12. Morphological traits and scale bar of *Fucus* species sampled in Spain. **A.** *Fucus chalonii* attached to rock in Talaipe, documented during fieldwork 30.08.2016. **B.** *Fucus chalonii* from Cobarón, documented during fieldwork 07.07.2016. **C.** Fertile *F. chalonii* from Cobarón. **D-E.** *Fucus chalonii* from Cobarón, mounted on herbarium sheet. **F-G.** Two individuals of *F. guiryi* sampled in Cobarón, 07.07.2016. **H.** *Fucus spiralis* from Cobarón sampled 07.07.2016. Photo by Kjersti Sjøtun.

3.2 Quality control of molecular data

3.2.1 Three alleles in three loci

The initial dataset consisted of 304 individuals genotyped at eight microsatellite loci (Table 3). The results of the genotyping demonstrated that 232 individuals were diploid (2n) for all loci (Appendix III, A, Table A). However, 72 individuals were observed with three alleles in three loci (L38, Fsp2, L78). Three alleles were exclusively found in two taxa (*F. cottonii, F. vesiculosus*). Three alleles in two loci (L38, Fsp2) were found in 29 of 30 *F. cottonii* individuals sampled in Indre Eggholmane. In addition, 9 of 18 individuals from *F. vesiculosus* (N_LYGFv) sampled in Lygra, Norway had three alleles in two loci (Fsp2, L78) and 4 of 18 individuals from *F. vesiculosus* (S_MUXFv) sampled in Muxía, Spain was observed with three alleles of one locus (L78). The complexity of finding three alleles when genotyping presents certain challenges regarding the data analysis, and lack of information on how the alleles are inherited. Therefore, the three loci (L38, L78 and Fsp2) were omitted from most of the analysis.

3.2.2 Suspected null alleles and potential loci under selection for all eight loci

All eight loci were quality checked in MICRO-CHECKER for the presence of null alleles and in LOSITAN for potential influence of selection. Suspected null alleles were detected in two loci (Fsp2, Fsp4). However, comparison of analysis (STRUCTURE, AMOVA and F-STAT) demonstrated minor differences when Fsp4 was removed. One genetic group of *F. spiralis* disappeared in the Norwegian samples, and *F. guiryi* disappeared from the Spanish samples. Since the absence of these genetic groups was not relevant for the study questions, it was decided to carry out the rest of the analysis including Fsp4. LOSITAN analysis showed balancing selection for L38 ($P = 0.0144$) and for Fsp2 ($P = 0.0070$). The remaining six loci were candidates for neutral selection ($P > 0.05$). No candidates were potentially under positive selection. After removing the problematic loci (L30, L78, Fsp2), all subsequent analyses were performed on the remaining five loci.

3.2.3 Hardy-Weinberg equilibrium and linkage disequilibrium for five loci

The global test of HWE (Table 9) showed 7 of 18 samples with heterozygote deficit to HWE expectations after Bonferroni correction ($P \leq 0.0028$). Global values of genetic diversity showed significant deviation from HWE for 15 of 90 exact testes (Appendix III, C, Table C). The global test of linkage disequilibrium (Table 10) showed significant linkage disequilibrium for five locus

pairs after Bonferroni correction ($P \leq 0.005$). The linkage disequilibrium test per sample at 160 pairs of loci (Appendix III, B, Table B) showed 11 pairs of *F. spiralis* with significant values ($P \leq 0.0003$), four from N_IEGFs, and seven from N_LYGFs. Furthermore, 74 pairs were not significant ($P > 0.0003$), and for 35 pairs, linkage disequilibrium could not be calculated.

Table 9. Global HWE Exact test for the 18 samples showing P-value and Standard Error (S.E.). Asterisk meaning significant deviation from Hardy–Weinberg expectations after Bonferroni correction; $\alpha = 0.05$; $P \leq 0.0028$.

Samples	P-value	S. E
N_LYGFs	0.1155	0.0068
N_IEGFs	0.0000*	0.0000
N_YEGFs1	0.0025*	0.0006
N_YEGFs2	0.0000*	0.0000
N_IGUFs	0.0052	0.0010
N_ITOFs	0.0000*	0.0000
S_COBFs	0.0000*	0.0000
N_YGUFsfn	0.0167	0.0008
S_BAKFg	0.0200	0.0007
N_LYGFv	0.0067	0.0035
N_IEGFv1	0.0654	0.0066
N_IEGFv2	0.0002*	0.0001
S_MUXFv	0.0643	0.0035
N_LYGFc	1.0000	0.0000
N_IEGFc	1.0000	0.0000
S_COBFch	0.3467	0.0086
S_TALFch	0.0000*	0.0000
N_IEGFsp	0.9989	0.0002

Table 10. Linkage disequilibrium test for each locus pair using the Fisher's method. Asterisk indicates significant P-values after Bonferroni correction; $\alpha = 0.05$; $P \leq 0.005$.

Locus pair	Chi2	df	P-value
Fsp4 & L20	62.7395	18	0.0067
Fsp4 & L58	44.5452	18	0.0005*
L20 & L58	48.7274	20	0.0003*
Fsp4 & Fsp1	76.9583	18	0.0004*
L20 & Fsp1	72.0984	20	0.0028*
L58 & Fsp1	56.3384	20	0.0174
Fsp4 & L94	62.0322	16	0.0216
L20 & L94	44.1568	14	0.0377
L58 & L94	45.3020	14	0.0245
Fsp1 & L94	59.2700	14	0.0014*

3.3 Genetic diversity

The number of alleles (*Na*) ranged from 6 to 34, with mean number 14.89 (Table 11). The mean number was uniformly low for most taxa (*F. chalonii* = 13, *F. cottonii* = 9.5, *F. guiryi* = 6, *F. spiralis* f. *nanus* = 6, *F. spiralis* = 12,4), except for *F. vesiculosus* (28). The allelic richness (*Ar*) ranged from 1.0730 (N_YGUFsfn) to 4.9189 (N_IEGFv2). Also, the allelic richness was generally

low for most taxa except *F. vesiculosus*. Estimates of observed heterozygosity (H_O) ranged from 0.000 to 0.9933 and expected heterozygosity (H_E) from 0.0133 to 0.7878 (Table 11). The general trend showed 15 of 18 samples with lower observed (H_O) than expected heterozygosity (H_E). Four samples (N_YEGFs1, N_YGUFsfn, S_BAKFg, S_TALFch) had extremly low numbers (H_O = 0.000). In contrast, high numbers of H_O were found for *F. cottonii* (0.9933, 0.8000). *Fucus spiralis* f. *nanus* (N_YGUFsfn) had lowest H_E (0.0133). Furthermore, the inbreeding coefficient (F_{IS}) (Table 11), were extremely high for N_YEGFs, N_YGUFsfn, S_BAKFg, S_TALFch (F_{IS} = 1.0000), and very low for *F. cottonii* (-1.000, -0.9866). Estimates of the genetic diversity per sample and per loci are provided as supplementary material (Appendix III, C, Table C). Summary statistics per loci for all samples (Appendix III, C, Table D) showed significant P-values for Fsp4 after Bonferroni correction ($P \leq 0.01$).

Table 11. Genetic diversity estimates per samples genotyped using five microsatellite loci: Number of individuals (*N*), Number of alleles (*Na*), allelic richness (*Ar*), observed heterozygosity (H_O), expected heterozygosity (H_E), inbreeding coefficient (F_{IS}).

Region	Location	Sample ID	*N*	*Na*	*Ar*	H_O	H_E	F_{IS}
Norway	Lygra	N_LYGFs	20	22	2.1150	0.2200	0.2929	0.2489
Norway	Eggholmane	N_IEGFs	20	25	3.0820	0.1300	0.4221	0.6920
Norway	Eggholmane	N_YEGFs1	10	7	1.3410	0.0000	0.0756	1.0000
Norway	Eggholmane	N_YEGFs2	20	7	1.3920	0.0100	0.1284	0.9221
Norway	Gulo	N_IGUFs	30	8	1.2170	0.0200	0.0443	0.5481
Norway	Gulo	N_ITOFs	30	6	1.2000	0.0133	0.1014	0.8685
Spain	Biscaya	S_COBFs	8	12	2.1700	0.0500	0.2500	0.8000
Norway	Gulo	N_YGUFsfn	30	6	1.0730	0.0000	0.0133	1.0000
Spain	Biscaya	S_BAKFg	8	6	1.2000	0.0000	0.0855	1.0000
Norway	Lygra	N_LYGFv	18	34	4.6210	0.6889	0.7180	0.0405
Norway	Lygra	N_IEGFv1	10	28	4.9150	0.6800	0.7867	0.1356
Norway	Lygra	N_IEGFv2	10	30	4.9180	0.5800	0.7878	0.2638
Spain	Biscaya	S_MUXFv	18	20	3.0560	0.4444	0.4712	0.0569
Norway	Lygra	N_LYGFc	20	9	1.8000	0.8000	0.4000	-1.0000
Norway	Eggholmane	N_IEGFc	30	10	2.0000	0.9933	0.5000	-0.9866
Spain	Biscaya	S_COBFch	7	17	3.2550	0.5143	0.5262	0.0226
Spain	Biscaya	S_TALFch	7	9	1.7930	0.0000	0.2788	1.0000
Norway	Eggholmane	N_IEGFsp	8	12	2.2800	0.5500	0.3839	-0.4326

The pairwise F_{ST} comparisons gave insight for inter and intraspecific relations, proving significant genetic differentiation after Bonferroni correction ($P \leq 0.000327$) for 130 of the 150 pairs (Table 12). All pairs of *F. cottonii* showed high and significant genetic differentiation from each other and the other species.

For *F. spiralis* f. *nanus* significant genetic differentiation was observed for all pairs, except IGUFs, which was geographically the closest site where *F. spiralis* was sampled (Table 12). Comparing the 15 pairs of *F. spiralis*, suggest no significant genetic differentiation for seven pairs. No significant differentiation was found for four pairs of *F. vesiculosus,* including *Fucus* sp..

F_{ST} values for *F. chalonii*, revealed that seven pairs were not significantly different, including the pairs between them (Table 12). In addition, both *F. chalonii* samples had little genetic differentiation from *F. spiralis* sampled in Cobarón (COBFs), and *F. chalonii* from Talaipe showed little genetic differentiation from *F. guiryi* from Bakio (BAKFg). On the other hand, high and significant genetic differentiation from *F. vesiculosus* sampled in Muxía (MUXFv) was found.

Table 12. Genetic differentiation between all 18 samples. The pairwise F_{ST} (below) and P-value (above) after 15.300 permutations. Indicative adjusted nominal level (5%) for multiple comparisons was: 0.000327 after standard Bonferroni corrections.

	LYGFs	IEGFs	YEGFs1	YEGFs2	IGUFs	ITOFs	COBFs	YGUFsfn	BAKFg	LYGFv	IEGFv1	IEGFv2	MUXFv	LYGFc	IEGFc	COBFch	TALFch	IEGFsp
LYGFs		NS	NS	*	**	**	**	**	**	**	**	**	**	**	**	**	**	**
IEGFs	0.2984		NS	NS	*	**	**	**	*	**	**	**	**	**	**	**	NS	**
YEGFs1	0.0641	0.1505		NS	**	**	*	**	*	**	**	*	**	**	**	*	*	*
YEGFs2	0.1401	0.1112	0.0399		NS	NS	**	*	**	**	**	**	**	**	**	**	**	**
IGUFs	0.2048	0.6934	0.2284	0.4336		**	**	NS	**	**	**	**	**	**	**	**	**	**
ITOFs	0.1302	0.5021	0.1167	0.1745	0.4178		**	**	**	**	**	**	**	**	**	**	**	**
COBFs	0.7498	0.7744	0.5050	0.5978	0.8611	0.7892		**	*	**	*	*	**	**	**	NS	NS	*
YGUFsfn	0.3397	0.8359	0.2882	0.5110	0.0293	0.5139	0.9008		**	**	**	**	**	**	**	**	**	**
BAKFg	0.7181	0.8150	0.4226	0.5290	0.8697	0.7575	0.7573	0.9335		**	**	**	**	**	**	NS	NS	*
LYGFv	0.6793	0.6578	0.4895	0.5743	0.7633	0.7233	0.5715	0.7856	0.6360		NS	NS	**	**	**	**	**	**
IEGFv1	0.6018	0.5577	0.3833	0.4763	0.7128	0.6663	0.4460	0.7405	0.5232	0.2012		NS	**	**	**	**	*	NS
IEGFv2	0.5776	0.5315	0.3552	0.4480	0.6950	0.6441	0.4190	0.7243	0.5004	0.1738	0.0183		**	**	**	*	*	**
MUXFv	0.5520	0.5146	0.3650	0.4433	0.6486	0.6078	0.4151	0.6709	0.4917	0.1022	0.0332	0.0241		**	**	**	**	**
LYGFc	0.5743	0.5555	0.4227	0.4880	0.6540	0.6193	0.5350	0.6742	0.5802	0.3721	0.3048	0.2627	0.2399		**	**	**	**
IEGFc	0.6652	0.6496	0.4924	0.5589	0.7518	0.7097	0.5633	0.7751	0.6274	0.4287	0.2546	0.2724	0.2760	0.4843		**	**	**
COBFch	0.7455	0.7289	0.5242	0.6032	0.8452	0.7890	0.5802	0.8738	0.7046	0.3426	0.1809	0.1480	0.1677	0.4262	0.3995		NS	NS
TALFch	0.7090	0.7446	0.4330	0.5370	0.8424	0.7564	0.0204	0.8917	0.7253	0.5556	0.4147	0.3922	0.3907	0.5126	0.5510	0,5725		NS
IEGFsp	0.7881	0.7820	0.5526	0.6621	0.8723	0.8262	0.6786	0.8989	0.7633	0.2412	0.1995	0.2045	0.1673	0.4380	0.5441	0,4397	0,6571	

The allele frequency distribution, given by 18 samples, varied across the five loci (Figure 13). Graphs with allele frequency distribution for *F. cottonii*, *F. spiralis f. nanus* and for the Spanish samples are provided as supplementary material (Appendix III, D, Figure VIII).

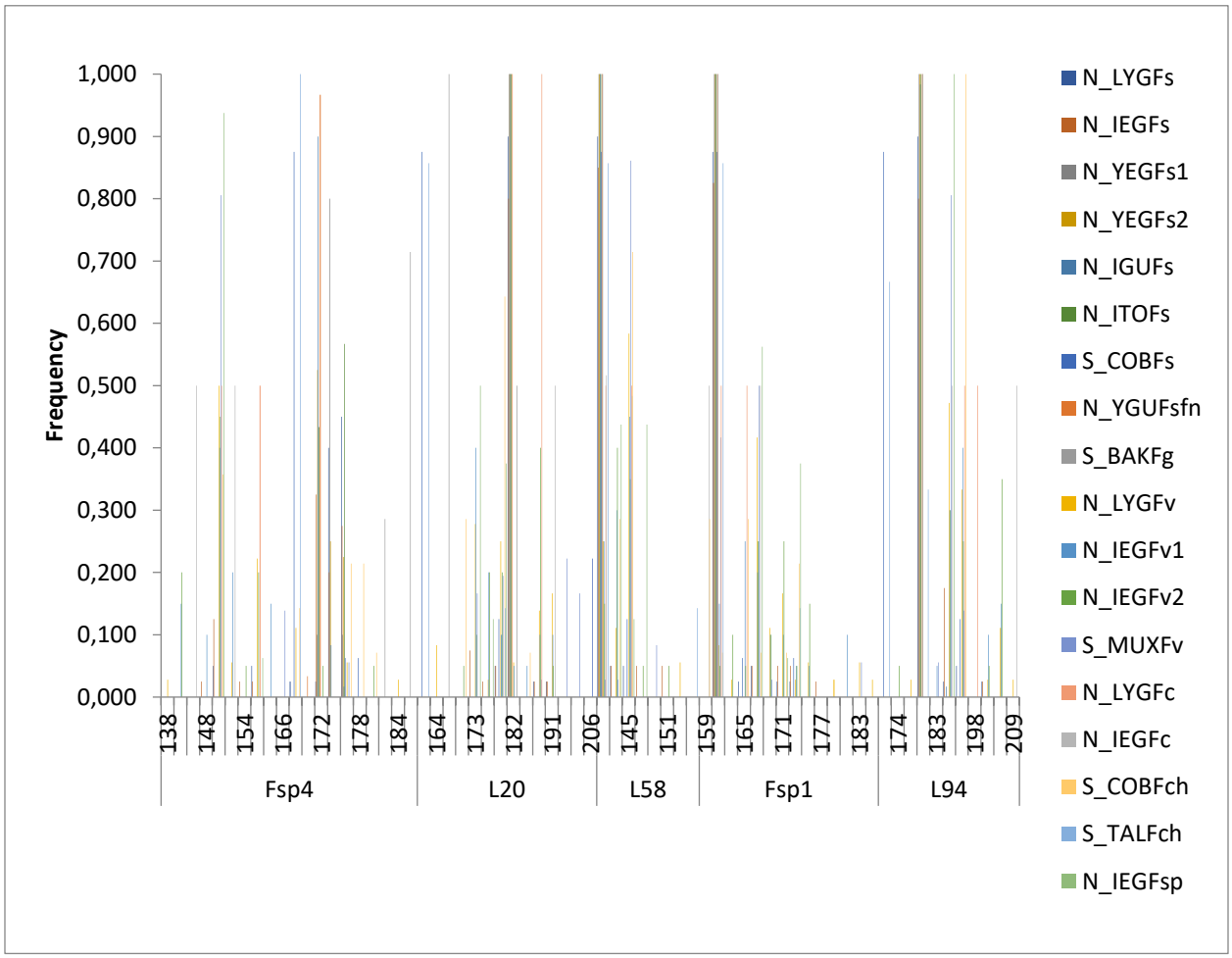


Figure 13. The allele frequency for all 18 samples using five loci. GenAlEx calculated 12% missing data in S_BAKFg at the locus Fsp4. For S_TALFc, there was 14% missing data at the locus Fsp4 and L94.

3.4 Genetic structure

The principal coordinate analysis (PCoA) for the Norwegian samples (Figure 14) showed four groups. One group included five samples of *F. spiralis*, the second group included *F. cottonii*, *F. vesiculosus* and *Fucus* sp., then *Fucus spiralis* f. *nanus* was presented as a separate group, again with closest affinity to *F. spiralis* (N_IGUFs). *Fucus spiralis* (N_YEGFs1) appeared as a separate

group. This site was sampled from the same locality as N_YEGFs2, but in the previous year (2019) and contained only ten individuals.

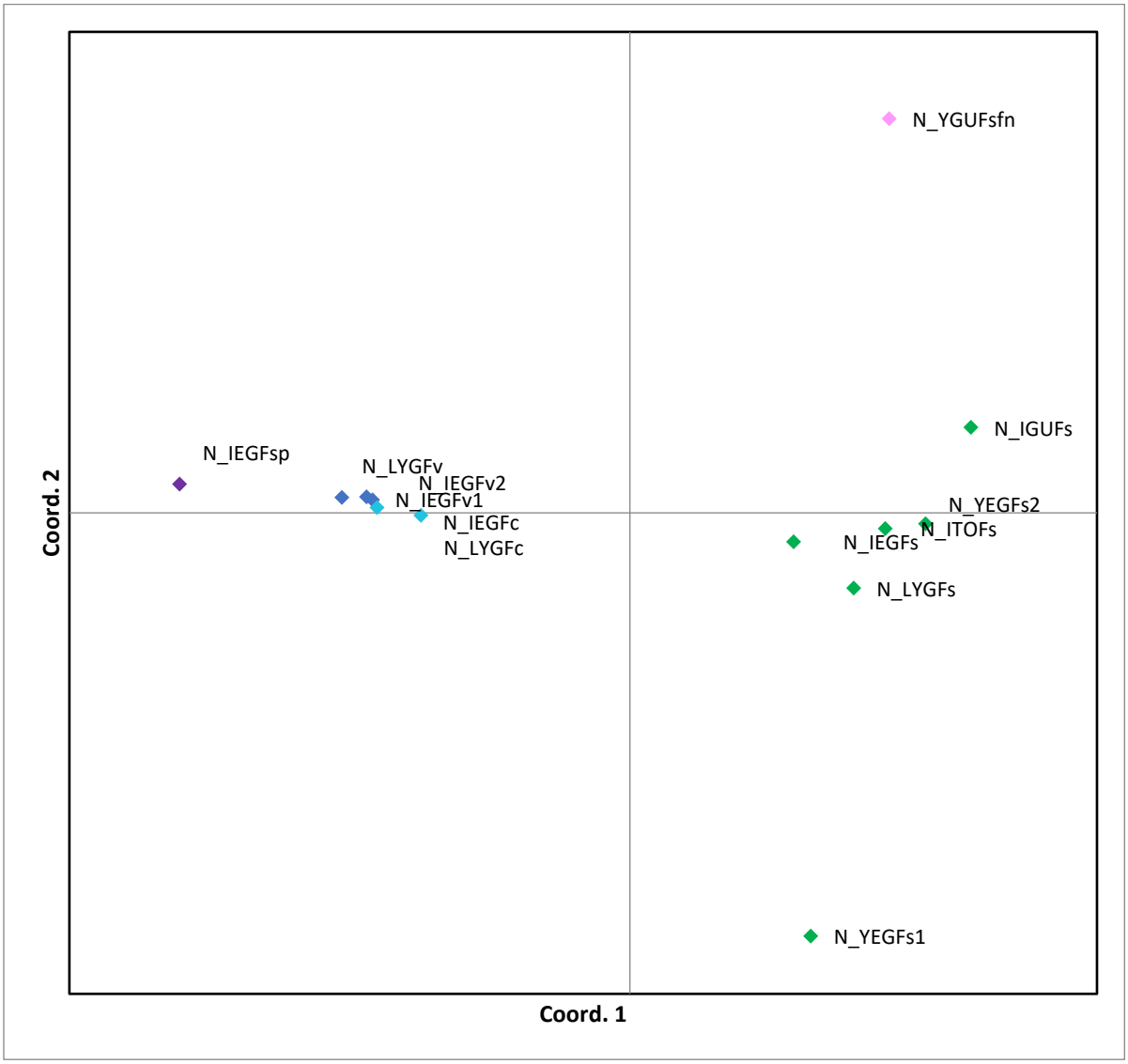


Figure 14. PCoA plot for the 13 samples from Norway. The five species are assigned a unique color code. *Fucus spiralis* (green), *F. vesiculosus* (blue), *F. spiralis f. nanus* (pink), *F. cottonii* (light blue) and *Fucus* sp. (purple).

The PCoA for the Spanish samples showed three separate groups (Figure 15). *Fucus spiralis* sampled in Cobarón (S_COBFs) appeared in the same group as *F. chalonii* sampled in Talaipe (S_TALFch). *Fucus chalonii* sampled in Cobarón (S_COBFch) was grouping with *F. vesiculosus* sampled in Muxía (S_MUXFv). On the other hand, *F. guiryi* appeared to be in a separate group.

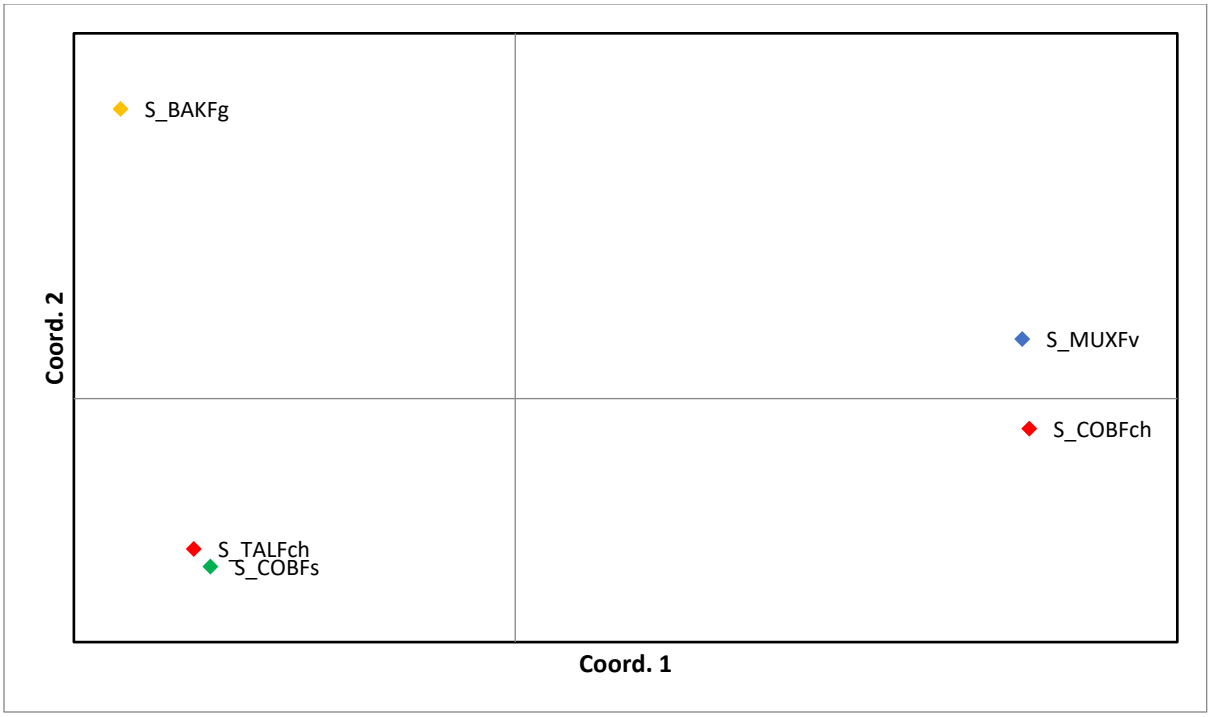


Figure 15. PCoA plot for the five samples from Spain. The four species are assigned a unique color code. *Fucus spiralis* (green), *F. vesiculosus* (blue), *F. guiryi* (orange) and *F. chalonii* (red).

Two STRUCTURE analyses were calculated for Norway and Spain separately. The assumed number of clusters (K) was suggested as four for the Norwegian samples (Figure 16A). Two genetic clusters appeared in *F. vesiculosus*, and in *F. spiralis.* A mixture between genetic groups of the two taxa was seen. *Fucus spiralis* f. *nanus*, appeared to belong to *F. spiralis*. Strong genetic structure for *F. cottonii*, and the two samples appeared as an isolated group with no variation within the individuals. However, some genetic connection to *F. vesiculosus* is suggested by the analysis. *Fucus* sp. from Ytre Eggholmane appeared to be in the same cluster as *F. vesiculosus*.

In the Spanish samples, the assumed number of clusters (K) are suggested as four (Figure 16B). Within the individuals the STRUCTURE analysis suggested minimal variation, but clear species differentiation was observed. *Fucus chalonii* appeared in two different clusters. *Fucus chalonii* from Talaipe was in the same cluster as *F. spiralis* from Cobarón. However, there seems to be a mix of one individual that is suggested to belong to *F. chalonii* from Cobarón. *Fucus guiryi* was suggested as a separate cluster, with minor similarities to two individuals in sample 17. Lastly, *F. vesiculosus* appeared as a genetically isolated group with small variations within the individuals.

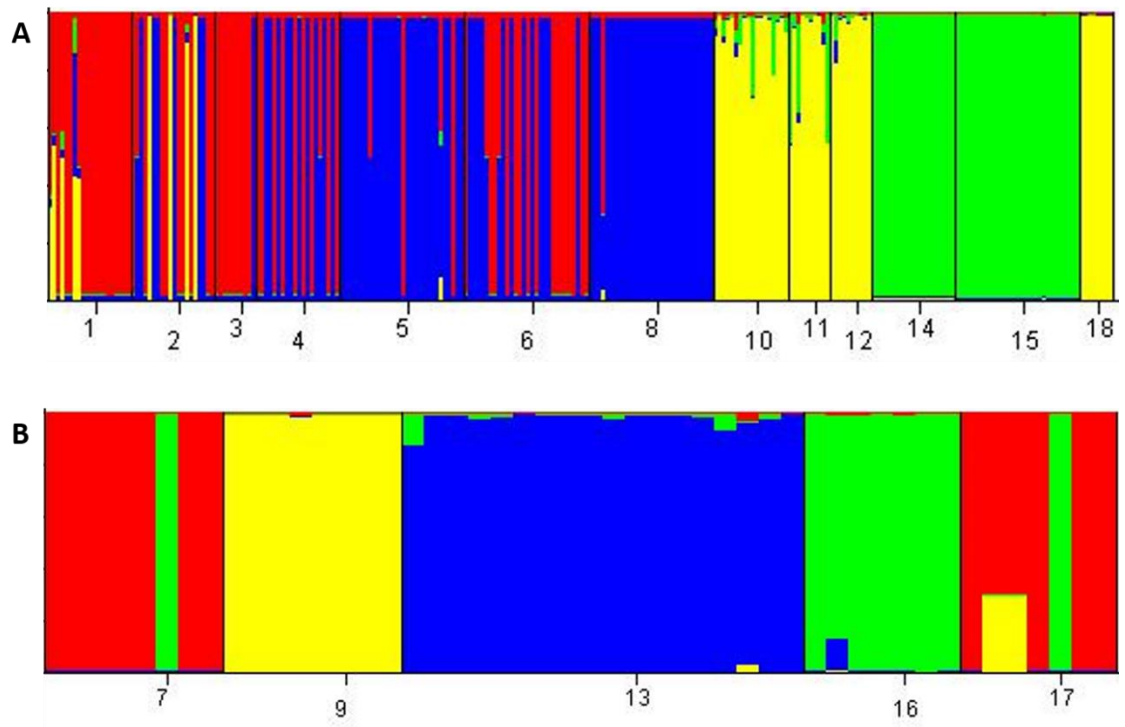


Figure 16. STRUCTURE analysis with vertical bars representing different individuals, and the colors are the proportion of genotypes assigned to each genetic group. The number on the x-axis represents samples. 1-7 = *F. spiralis*, 8 = *F. spiralis f. nanus*, 9 = *F. guiryi*, 10-13 = *F. vesiculosus*, 14-15 = *F. cottonii*, 16-17 = *F. chalonii*, 18 = *Fucus* sp.. **A.** The Norwegian samples (K=4). **B.** The Spanish samples (K=4).

The AMOVA analysis (Table 13) supports the STRUCTURE results, showing strong evidence for geographic differences within each species (P = 0.0000), and between groups of samples within species (P = 0.0000). Moreover, within populations, individuals are suggested as relatively similar (P = 0.7595).

Table 13: AMOVA results for all 18 samples at five loci. Fixation indices: FIS = Among individuals, within populations, FSC = Among populations, within groups (species), FCT = Among groups (species), FIT = within individuals. Asterisk indicates significant P-values.

Locus	**FIS**	**P-value**	**FSC**	**P-value**	**FCT**	**P-value**	**FIT**	**P-value**
Fsp4	0.29559	0.00000	0.35103	0.00000	0.22331	0.00069	0.64494	0.00000
L20	-0.09855	0.96676	0.42523	0.00000	0.36052	0.00196	0.59622	0.00000
L58	-0.15339	0.98970	0.15167	0.00000	0.46117	0.00020	0.47278	0.00000
Fsp1	-0.18301	0.99980	0.23988	0.00000	0.36149	0.00208	0.42583	0.00000
L94	-0.20907	0.99960	0.42494	0.00000	0.44055	0.00030	0.61103	0.00000
Total		0.75953		0.00000*		0.00000*		0.00000*

4. Discussion

Although several studies have attempted to unwind the complexity of the *Fucus* genus, the relationships between *F. chalonii*, *F. cottonii*, *F. spiralis* f. *nanus* and closely related taxa (*F. guiryi*, *F. spiralis* and *F. vesiculosus*) have not been properly investigated. The purpose of this thesis is to examine genetic affinity and origin of miniaturized *Fucus* species by morphological descriptions and microsatellites analysis to answer the following research questions: 1) Does *F. cottonii* in Norway originate from *F. spiralis* or *F. vesiculosus*, or is it a hybrid of the two? 2) Is *F. spiralis* f. *nanus* genetically similar to *F. spiralis* in Norway? 3) Does *F. chalonii* originate from *F. vesiculosus?* For the Norwegian samples, the main findings revealed *F. cottonii* were cloned individuals with close connection to *F. vesiculosus.* The findings concerning *F. spiralis* f. *nanus* suggested the closest connection to the nearby sampled *F. spiralis*. The Spanish samples could not be fully resolved. However, two separate clusters for *F. chalonii* were inferred.

4.1 Discussion of the thesis results

4.1.1 Does *F. cottonii* in Norway originate from *F. spiralis* or *F. vesiculosus,* or is it a hybrid of the two?

Fucus cottonii is still being referred to by its scientific name, despite being considered as a morphotype with different genetic origin and not a separate species. Studies from Ireland found evidence that *F. cottonii* derived from *F. spiralis*, *F. vesiculosus* or was a hybrid between them (Coyer et al., 2006b; Neiva et al., 2012; Sjøtun et al., 2017). Findings from Iceland (Coyer et al., 2006b) and Maine, USA (Wallace et al., 2004), also reported that *F. cottonii* originated from hybridization between *F. spiralis* and *F. vesiculosus*. In Oregon, North East Pacific, *F. cottonii* most likely originated from *Fucus gardneri* P.C Silva 1953, which is synonym for *F. distichus* (Kucera & Saunders, 2008; Neiva et al., 2012). The results in this thesis support the connection to *F. vesiculosus*.

Fucus cottonii from Indre Eggholmane and Lygra, Norway is suggested to be embedded in the *F. vesiculosus* cluster (Figure 14). The STRUCTURE analysis (Figure 16A) strongly supports the genetic affinity to *F. vesiculosus*. According to Coyer et al. (2006b), contributions of *F. vesiculosus* genes to the hybrid genome are crucial for local adaptation to salt marsh conditions. Furthermore,

the presence of unique alleles at loci L58, L78 and L94 was used as an argument opposing the hypothesis that *F. cottonii* in Ireland derived exclusively from local *F. spiralis* (Neiva et al., 2012). Studies found different allele frequency between *F. cottonii* from Oregon and Ireland (Neiva et al., 2012). A comparison with the present results (Figure 17) did not reveal any similarities with the unique alleles found by Neiva et. al (2012).

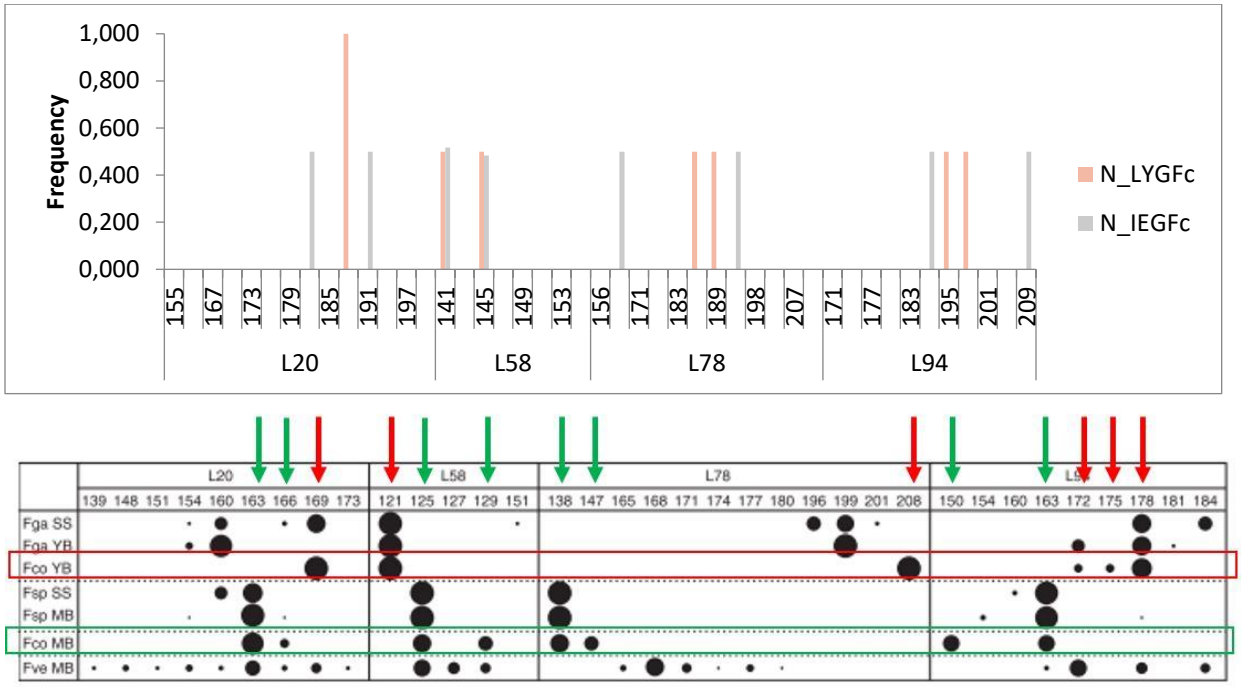


Figure 17: Comparison of the allele frequency in four loci (L20, L58, L78, L94). **A.** *Fucus cottonii* sampled in Norway. **B.** *Fucus cottonii* from Yaquina Bay, Oregon (red) and Mulroy Bay, Ireland (green), modified from (Neiva et al., 2012).

Microsatellite genotyping results (Appendix III, A, Table A) revealed that *F. cottonii* are cloned individuals. According to Sjøtun et al. (2017), *F. cottonii* sampled in Ireland was not reported as clones, but some were observed with receptacles. The morphological descriptions (Figure 9; Appendix I, B, Figure II) show adventitious branching among specimens from Lygra and Indre Eggholmane, an indication of vegetative reproduction (Figure 2). Also, extreme negative inbreeding coefficients were found (Table 11). A negative F_{IS} suggests that there was an excess of heterozygotes in relation to the expected value. Excess heterozygotes were found in seven out of the eight loci genotyped for LYGFc and in all loci for IEGFc (Appendix III, A, Table A).

Significant heterozygote excess has also been found in muscoides-like *Fucus* from Maine, USA (Wallace et al., 2004). A study from Antarctica, looking at asexual reproduction and heterozygote selection in demosponge *Stylocordyla chupachus*, suggested that heterozygote selection would help cloned species maintain some genetic diversity (Carella et al., 2019).

The STRUCTURE analysis (Figure 16A) suggested that within each population the individuals are similar. A closer look at the genotyping results (Appendix III, A, Table A), revealed different genotypes between the specimens from Indre Eggholmane and Lygra, but not within locations. The pairwise F_{ST} (Table 12) points out significant genetic differences when comparing the two locations. Strong evidence for geographic differences within the species was also supported by the AMOVA analysis (Table 13*).* Although rarely seen, there has been observation of vegetative reproduction among *F. vesiculosus* (Tatarenkov et al., 2005). It could be argued that *F. cottonii* settlements in Eggholmane and Lygra originated from tidal drifts of fragments from *F. vesiculosus.* The two *F. cottonii* populations may have emerged by source-sink relationship (Peck et al., 1998), and one individual was able to outcompete the others and establish a population. Or it could be because of a single colonization event by clonal propagation (Serrão et al., 1999b). A study of vegetative reproduction in the introduced red algae *Heterosiphonia japonica',* reported successful establishment of vegetative propagules in areas that are favorable (Husa & Sjøtun, 2006).

Three allele genotypes were found in two loci for *F. cottonii* from Indre Eggholmane (Appendix III, A, Table A). Normally diploid species (2n) inherit one allele from each parent. It is not sure how the three alleles were inherited in this study. However, it could be discussed if presence of three alleles was caused by independent mutation that duplicates a region of the genome. Another theory suggests triploid species (3n), with three chromosomes instead of two. Since very high values of observed heterozygosity were seen in both locations (Table 11), it may be the case that three alleles were exclusively expressed in specimens from Indre Egghomane, whereas the third allele was "masked" in those from Lygra. A higher observed heterozygosity than expected heterozygosity (Table 11) could indicate a mix of two previously isolated populations. Polyploidy is an important source of increased genetic diversity and adaptability (Wendel, 2000) and is a frequent feature in plants and more rare in animals (Dufresne et al., 2014). Since triploid animals

are genetically sterile, this has been used in research for controlling the reproduction in salmonids (Benfey et al., 1989). However, triploid *F. cottonii* would still be able to reproduce asexually. Moreover, three alleles were also found in half of the *F. vesiculosus* samples from Lygra, and in four out of 18 *F. vesiculosus* individuals from Muxía (Appendix III, A, Table A). Missing information regarding how the three alleles were inherited challenge to provide answers and fully understand the processes around triploid species. Therefore, further studies are advised.

In summary, the results suggest *F. cottonii* are cloned individuals, genetically similar to *F. vesiculosus*. Since this study only included two *F. cottonii* populations sampled from two locations, a connection to *F. spiralis* in Norway cannot be excluded. Further studies including more samples from several locations are needed to better understand the relationship between *F. cottonii* and closely related taxa in Norway.

4.1.2 Is *F. spiralis* f. *nanus* genetically similar to *F. spiralis* in Norway?

A study from Yorkshire used pyrolysis mass spectrometry to confirm the status of forma *nanus* as a small form of *F. spiralis* (Hardy et al., 1998). Also, in the North West Atlantic (Mathieson et al., 2006) and the northeast coast of the UK (Scott et al., 2000), *F. spiralis* f. *nanus* is suggested as a miniaturized version of *F. spiralis*. The results in this thesis support the connection to *F. spiralis.*

The STRUCTURE analysis (Figure 16A) revealed that *F. spiralis* f. *nanus* was included in the *F. spiralis* cluster, and that the genetic structure was comparable to the closest sampled *F. spiralis* from Indre Gulo. Moreover, pairwise F_{ST} comparisons (Table 12) and the PCoA (Figure 14) also suggest the closest connection to *F. spiralis* from Indre Gulo. It might be the case that *F. spiralis* f. *nanus* originated from a few migrants from the nearby population of *F. spiralis.* A study of implications of plant size in monotypic and polytypic populations of *F. spiralis,* suggested possibilities of inter-forma gene flow between *F. spiralis* and *F. spiralis f. nanus* in the UK (Scott et al., 2000).

The population of *F. spiralis* f. *nanus* appeared to be highly inbred given the extreme high inbreeding coefficient (Table 11). According to Zardi et al. (2011) high levels of inbreeding are seen among hermaphroditic species like *F. spiralis.* Moreover, extreme low observed

heterozygosity was seen (Table 11) and the microsatellite genotyping (Appendix III, A, Table A) revealed that all individuals were cloned and homozygote. A study from Wallace et al. (2004) suggests that heterozygote deficits could occur naturally among inbred populations. Furthermore, a private allele was observed in one individual (N_YEGFsfn04).

The fieldwork in Bømlo was carried out in August 2021 (Table 2). The morphological recordings (Table 8; Figure 11E-G) revealed more tips with receptacles than without, and that they were in good condition. *Fucus spiralis* sampled in Bømlo (IGUFs, ITOFs) had less receptacle presence and they were generally in poor condition (Table 8; Figure 11A-D). Based on these findings, it could be argued that the reproductive stage of *F. spiralis* f. *nanus* appears to be later than nearby *F. spiralis*. According to Monteiro et al. (2012) asynchronous gamete release constructs major prezygotic barriers. Other studies point out that egg size is impacting survival and that larger eggs were better resourced (Vernet & Harper, 1980). This was tested for *F. spiralis* f. *nanus* by Anderson & Scott (1998), which found evidence that the small size had reproductive cost in terms of absolute egg size, but not in production of number per unit size. Moreover, the small size is a prominent morphological feature (Table 8; Figure 11E-G; Appendix 1, B, Figure 2G). Small thallus and strong holdfast could be seen as an adaptation trait for exposed sites where wave action is stronger and the desiccation periods longer. Hardy et al. (1998) found *F. spiralis* f. *nanus* 5 m above high water mark and suggested that the miniaturized size was caused by increased exposure. Furthermore, a study of transplants of different *Fucus* taxa in Maine, confirmed that *F. spiralis* could transform into dwarf embedded thalli within the high intertidal (Mathieson et al., 2006).

In 1977, *F. spiralis* f. *nanus* was observed in very wave exposed site along the west- and north coast of Norway as well as in Skagerrak (Rueness). In this thesis, *F. spiralis* f. *nanus* was exclusively found in one wave exposed site in Gulo (Figure 6). It might be the case that the abundance of *F. spiralis* f. *nanus* in Norway has been reduced over time. According to Serrão et al. (1996) gamete dispersal among *F. spiralis* is very restricted, which may contribute to high levels of genetic structuring. Also, the lack of air vesicles may limit its distribution. Due to limited data, the results in this study could not confirm if the abundance of *F. spiralis* f. *nanus* in Norway has been reduced.

To sum up, the result suggests a close connection between *F. spiralis* f. *nanus* and nearby sampled populations of *F. spiralis.* However, considering the small number of samples, more studies are advised.

4.1.3 Does *F. chalonii* originate from *F. vesiculosus?*

Little is known about the rare *F. chalonii,* which has been observed in a few areas in North Spain (Feldmann, 1941). There has been little research about *F. chalonii,* thus its origin is not fully understood. Due to limited material, only a small number of individuals from two sites was included in this thesis (Table 2). Despite few studies and limited material, clear morphological features such as dichotomous branching and biparental reproduction may indicate that *F. chalonii* are connected to *F. vesiculosus*. The results in this thesis could not resolve where *F. chalonii* originated from. However, two separate clusters were suggested.

The PcoA (Figure 15) revealed that *Fucus chalonii* from Cobarón grouped with *F. vesiculosus* from Muxía, and *F. chalonii* from Talaipe grouped with *F. spiralis* from Cobarón. The STRUCTURE analysis (Figure 16B) supported this by grouping *F. chalonii* from Talaipe with *F. spiralis*. However, the assumed number of clusters was four and *F. chalonii* from Cobarón appeared as a separate group. According to the comparison of pairwise F_{ST} (Table 12), high and significant genetic differentiation from *F. vesiculosus* was found. Small morphological variations between *F. chalonii* from Talaipe and *F. chalonii* from Cobarón were seen. *Fucus chalonii* from Talaipe appeared to be shorter and with smaller leaf width (Figure 12A), than specimens from Cobarón (12B-E). Since *F. chalonii* from Talaipe was collected approximately two months before the specimens from Cobarón (Table 2), seasonal variations could explain the size difference.

In summary, the results suggest little connection between *F. chalonii* and *F. vesiculosus*. However, there are indications that the two *F. chalonii* populations are not in the same genetic cluster. It might be the case that *F. chalonii* in this study was not a separate species, but two morphotypes with comparable morphology to *F. chalonii*. Considering the small number of samples and unclear results, the research question could not be fully resolved. Further studies involving more samples are desirable for better understanding of genetic relations between *F. chalonii* and *F. vesiculosus*.

4.1.4 Remarks on three closely related *Fucus* species

The STRUCTURE analysis (Figure 16A) suggested a large genetic variability for *F. spirals* from Norway. Comparison of pairwise F_{ST} (Table 12) showed that seven of 15 pairs were not significantly differentiated from each other. This indicates a high level of gene flow between the sites. All the *F. spiralis* samples from Norway are grouped together (Figure 14), except for one sample (N_YEGFs1). The results in this thesis could not reveal why this sample appeared as a separate group. However, low sampling numbers may have been impacting the results.

Fucus vesiculosus was also found with genetic variation within the individuals, but less than in *F. spiralis* (Figure 16A). Comparison of pairwise F_{ST} (Table 12) showed no significant genetic differentiation between *Fucus* sp. and nearby sampled *F. vesiculosus* (IEGFv1). This was supported by the STRUCTURE analysis (Figure 16A). Based on these findings, our results suggest that *Fucus* sp. is *F. vesiculosus*. Moreover, high level of inbreeding was seen among the *F. spiralis* and *F. guiryi* and not for *F. vesiculosus* (Table 11). Since *F. guiryi* and *F. spiralis* are hermaphrodites and *F. vesiculosus* are dioecious (Figure 2), different mating systems would impact the level of inbreeding. High levels of inbreeding for *F. guiryi* and *F. spiralis,* and not for *F. vesiculosus* are supported by other studies (Monteiro et al., 2012; Almeida et al., 2017).

4.2 Methodological issues

4.2.1 Fieldwork and sampling

Minor suggestions for improving fieldwork and sampling, involves increasing the sample size and more consistent sampling time. *Fucus spiralis* f. *nanus* was exclusively found in one location in Norway. Due to COVID-19 travel restrictions, sampling outside of Norway was not possible. The Spanish material was collected a few years earlier, and only parts of the material were available. Additional samples with the emphasis on *F. chalonii* and *F. spiralis* f. *nanus* would be desirable. Furthermore, morphological descriptions indicated seasonal variations among the sites. More consistent sampling time would be advised so morphological recordings could be carried out on all the Norwegian samples.

4.2.2 Laboratory work and statistics

Using NucleoMag® Plant (Macherey Nagel) for DNA extraction on *Fucus* species provided adequate DNA material for all the 306 individuals, and all the eight microsatellites were amplified successfully. Despite the many advantages of using microsatellite markers, a few issues were encountered.

Three loci (L38, Fsp2, L78) were removed from most of the analysis, due to the appearance of three alleles (Appendix III, A, Table A). Since most population genetic programs are designed for diploid individuals, they do not know how to handle three alleles (Duarte et al., 2015). Another issue related to three alleles, is possibilities for genome duplications or triploid species. Since very high observed heterozygosity was found, this may indicate that more individuals potentially had three alleles. However, they may have been "masked".

Linkage disequilibrium test (Table 10) suggested five pairs of loci being linked. If two loci on a chromosome are very close, they may transmit to the next generation as a pair, even if they are not linked (Selkoe & Toonen, 2006). Significant pairs were exclusively found in 11 pairs of *F. spiralis* (Appendix III, B, Table B), and not for the other taxa. Zardi et al. (2011) suggested that inbreeding and selfing could induce linkage and Hardy-Weinberg disequilibrium. Moreover, Perrin et al. (2007) found high number of significant linkage disequilibrium between pairs of loci for *F. spiralis*.

The STRUCTURE analysis for the Spanish samples (Figure 16B) revealed a very similar genetic structure between *F. spiralis* from Cobarón and *F. chalonii* from Talaipe. Since one individual from *F. chalonii* from Cobarón appeared in population seven and 17, a mix up during molecular work was suspected. However, photo documentation from the DNA extraction process and controlling the raw datafile (Appendix III, A, Table A), excluded the possibility of potential mix up in the lab. It could be argued that low sample numbers may have been a contributing factor. Certain statistical programs (such as MICRO-CHECKER, LOSITAN) were developed years ago and minor problems occurred during installations.

4.3 Future perspectives

Despite the arguments above, using microsatellite analysis for investigating the relationship of closely related *Fucus* taxa has proven to be an efficient method used for separating miniaturized species. Microsatellites have also been used in other studies trying to resolve the genetic affinity for species associated with the second lineage in the *Fucus* genus (Wallace et al., 2004; Coyer et al., 2006b; Neiva et al., 2012; Sjøtun et al., 2017). Moreover, contribution of genetics analysis in conservation biology is an important factor to counteract extinction of small sized populations (Frankham, 2003).

Relatively low allelic richness was found for all the miniaturized species, except *F. chalonii* sampled in Cobarón (Table 11). Since diversity is considered a key component for natural selection, a decrease in the allelic richness may challenge a population's adaptation potential regarding future environmental changes (Greenbaum et al., 2014). Also, the loss of genetic diversity increases the susceptibility of extinction (Frankham, 2003). As mentioned in section 1.1.4, there are several threats to the *Fucus* genus. It could be argued how important the three morphotypes in this study are for the intertidal communities. However, from a biodiversity aspect, the morphotypes are highly valuable. On the 9th of June, the Norwegian Nature Diversity Act was entered into force, and the third aspect of the Act states that biodiversity is the world's most important resource (Sørensen, 2010). Organizations such as the Norwegian Biodiversity Information Centre (NBIC) and the International Union for Conservation of Nature (IUCN) aim to preserve biological diversity both locally and globally. According to NBIC (2015), the conservation status of *F. cottonii* was categorized as "Near Threatened" in 2015. Revising the sites where *F. cottonii* was found and updating the status are suggested. Since one sample of *F. spiralis* f. *nanus* was included in this thesis, does not mean that the species is not found in other sites in Norway. However, *F. spiralis* f. *nanus* was not listed in NBIC. *Fucus chalonii* was also not listed in the IUCN red list. Limited information challenge to provide a complete overview of the distribution. Considering that *F. chalonii* is almost extinct, it may be too late to save this species. However, the data from studying vulnerable species can be useful for conservation of other species. Updated information regarding genetic structure, population size, distribution, and potential threats, are essential for appropriate conservation management and to safeguard species with the potential to become locally extinct.

To unwind the complexity of the *Fucus* genus it is suggested to further investigate the genetic affinity and origin of miniaturized *Fucus* species by continuing with morphological descriptions and genetic analysis. Although microsatellite markers are highly versatile, efficient, and affordable in genotyping analysis, other methods such as complete genome sequencing may be able to tackle problems that microsatellites cannot. Moreover, even though there has been increased focus on marine flora in the past years, this thesis highlights the need for regular assessments and conservation status updates concerning *Fucus* morphotypes in Norway and Spain.

Bibliography

Almeida, S. C., Nicastro, K. R., Zardi, G. I., Pearson, G. A., Valero, M., & Serrão, E. A. (2017). Reproductive strategies and population genetic structure of *Fucus* spp. across a northeast Atlantic biogeographic transition. *Aquatic Living Resources, 30*, 16.

Anderson, C., & Scott, G. (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.

Antao, T., Lopes, A., Lopes, R. J., Beja-Pereira, A., & Luikart, G. (2008). LOSITAN: a workbench to detect molecular adaptation based on a F_{ST}-outlier method. *BMC bioinformatics, 9*(1), 1-5.

Armstrong, R. A. (2014). When to use the Bonferroni correction. *Ophthalmic and Physiological Optics, 34*(5), 502-508.

Arrontes, J. (1993). Nature of the distributional boundary of *Fucus serratus* on the north shore of Spain. *Marine Ecology-Progress Series, 93*, 183-183.

Artsdatabanken. (2015). *Rodliste2015*. Norske rødliste for arter. Retrieved March 29, 2021 from https://artsdatabanken.no/Rodliste2015/rodliste2015/Norge/107207

Benfey, T. J., Dye, H. M., Solar, I. I., & Donaldson, E. M. (1989). The growth and reproductive endocrinology of adult triploid Pacific salmonids. *Fish physiology and biochemistry, 6*(2), 113-120.

Bergström, L., Tatarenkov, A., Johannesson, K., Jönsson, R. B., & Kautsky, L. (2005). Genetic and morphological identification of *Fucus radicans* sp. nov. (Fucales Phaeophyceae) in the brackish Baltic Sea. *Journal of Phycology, 41*(5), 1025-1038.

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*, 163-174.

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.

Bindoff, N. L., W.W.L. Cheung, J.G. Kairo, J. Arístegui, V.A. Guinder, R. Hallberg, N. Hilmi, N. Jiao, M.S. Karim, L. Levin, S. O'Donoghue, S.R. Purca Cuicapusa, B. Rinkevich, T. Suga, A. Tagliabue, & Williamson, P. (2019). *Changing Ocean, Marine Ecosystems, and Dependent Communities*. https://www.ipcc.ch/srocc/

Brawley, S. H. (1992). Fertilization in natural populations of the dioecious brown alga *Fucus ceranoides* and the importance of the polyspermy block. *Marine Biology, 113*(1), 145-157.

Bringloe, T. T., Starko, S., Wade, R. M., Vieira, C., Kawai, H., De Clerck, O., Cock, J. M., Coelho, S. M., Destombe, C., & Valero, M. (2020). Phylogeny and Evolution of the Brown Algae. *Critical Reviews in Plant Sciences, 39*(4), 281-321.

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), 371.

Carella, M., Agell, G., & Uriz, M. J. (2019). Asexual reproduction and heterozygote selection in an Antarctic demosponge (*Stylocordyla chupachus*, Suberitida). *Polar Biology, 42*(3), 475-483.

Cotton, A. (1912). A biological survey of Clare Island in the county of Mayo, Ireland. Marine algae. *Proc. R. Ir. Acad, 31*(15), 1-178.

Coyer, J., Hoarau, G., Costa, J., Hogerdijk, B., Serrão, E., Billard, E., Valero, M., Pearson, G., & Olsen, J. (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.

Coyer, J. A., Hoarau, G., Oudot-Le Secq, M.-P., Stam, W. T., & Olsen, J. L. (2006a). A mtDNA-based phylogeny of the brown algal genus *Fucus* (Heterokontophyta; Phaeophyta). *Molecular phylogenetics and evolution, 39*(1), 209-222.

Coyer, J. A., Hoarau, G., Pearson, G. A., Serrão, E. A., Stam, W. T., & Olsen, J. L. (2006b). Convergent adaptation to a marginal habitat by homoploid hybrids and polyploid ecads in the seaweed genus *Fucus*. *Biology letters, 2*(3), 405-408.

d'Avack, E., & Tyler-Walters, H. (2015). *Fucus serratus* and red seaweeds on moderately exposed lower eulittoral rock.

de Pedro, R. S., Fernández, A. N., García-Sánchez, M. J., Flores-Moya, A., & Bañares-España, E. (2019). Seasonal shifts in thermal resilience of multi-celled early-developmental stages of *Fucus guiryi*: Maternal acclimation vs. chronic stress.

Duarte, L., Rossi, F., Docal, C., & Viejo, R. M. (2015). Effects of alga *Fucus serratus* decline on benthic assemblages and trophic linkages at its retreating southern range edge. *Marine Ecology Progress Series, 527*, 87-103.

Dudgeon, S., & Petraitis, P. S. (2005). First year demography of the foundation species, *Ascophyllum nodosum*, and its community implications. *Oikos, 109*(2), 405-415.

Dufresne, F., Stift, M., Vergilino, R., & Mable, B. K. (2014). Recent progress and challenges in population genetics of polyploid organisms: an overview of current state-of-the-art molecular and statistical tools. *Molecular ecology, 23*(1), 40-69.

Earl, D. A. (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation genetics resources, 4*(2), 359-361.

Edelstein, T., Greenwell, M., Bird, C. J., & McLachlan, J. (1974). Investigations of the marine algae of Nova Scotia, X: Distribution of *Fucus serratus* L. and some other species of *Fucus* in the Maritime Provinces. *Proceedings of the Nova Scotian Institute of Science, 27*, 1971-1977.

Engel, C., Brawley, S., Edwards, K., & 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.

Engel, C., Daguin, C., & Serrão, E. (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.

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular ecology, 14*(8), 2611-2620.

Excoffier, L., Laval, G., & Schneider, S. (2005). Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evolutionary bioinformatics, 1*, 117693430500100003.

Feldmann, J. (1941). Sur une nouvelle espèce de *Fucus* de la côte basque: *Fucus Chalonii* nov. sp. *Bulletin de la Société Botanique de France, 88*(1), 143-147.

Fernández, C. (2016). Current status and multidecadal biogeographical changes in rocky intertidal algal assemblages: the northern Spanish coast. *Estuarine, Coastal and Shelf Science, 171*, 35-40.

Ferreira, R. M., Ramalho Ribeiro, A., Patinha, C., Silva, A., Cardoso, S. M., & Costa, R. (2019). Water extraction kinetics of bioactive compounds of *Fucus vesiculosus*. *Molecules, 24*(18), 3408.

Flint-Garcia, S. A., Thornsberry, J. M., & Buckler IV, E. S. (2003). Structure of linkage disequilibrium in plants. *Annual review of plant biology, 54*(1), 357-374.

Fort, A., Guiry, M. D., & Sulpice, R. (2018). Magnetic beads, a particularly effective novel method for extraction of NGS-ready DNA from macroalgae. *Algal research, 32*, 308-313.

Frankham, R. (2003). Genetics and conservation biology. *Comptes Rendus Biologies, 326*, 22-29.

Gómez-Garreta, A., Barceló, M., Gallardo, T., Pérez-Ruzafa, I., Ribera, M., & Rull, J. (2001). Flora Phycologica Iberica Vol. 1. Fucales. *Universidad de Murcia*.

Goudet, J. (2003). FSTAT (version 2.9. 4), a program (for Windows 95 and above) to estimate and test population genetics parameters. *Department of Ecology & Evolution, Lausanne University, Switzerland, 53*.

Graiff, A., Dankworth, M., Wahl, M., Karsten, U., & Bartsch, I. (2017). Seasonal variations of *Fucus vesiculosus* fertility under ocean acidification and warming in the western Baltic Sea. *Botanica Marina, 60*(3), 239-255.

Greenbaum, G., Templeton, A. R., Zarmi, Y., & Bar-David, S. (2014). Allelic richness following population founding events–a stochastic modeling framework incorporating gene flow and genetic drift. *PloS one, 9*(12), e115203.

Guiry, M. D. (2012). How many species of algae are there? *Journal of Phycology, 48*(5), 1057-1063.

Guiry, M. D., & Guiry, G. M. (2021). *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. Retrieved March 26, 2021 from https://www.algaebase.org

Hardy, F., Scott, G., Sisson, P., & Lightfoot, N. (1998). Pyrolysis mass spectrometry as a technique for studying inter-and intraspecific relationships in the genus *Fucus*. *Journal of the Marine Biological Association of the United Kingdom, 78*(1), 35-42.

Havforskningsinstituttet. (2021). *Klimaet ved kysten*. Retrieved June 14, 2021 from https://www.hi.no/hi/temasider/hav-og-kyst/klimaet-i-havet/klimastatus/kysten

Heesch, S., Serrano-Serrano, M., Luthringer, R., Peters, A. F., Destombe, C., Cock, J. M., Valero, M., Roze, D., Salamin, N., & Coelho, S. (2019). Evolution of life cycles and reproductive traits: insights from the brown algae. *bioRxiv*, 530477.

Husa, V., & Sjøtun, K. (2006). Vegetative reproduction in "*Heterosiphonia japonica*" (Dasyaceae, Ceramiales, Rhodophyta), an introduced red alga on European coasts.

Kautsky, L., Qvarfordt, S., & Schagerström, E. (2019). *Fucus vesiculosus* adapted to a life in the Baltic Sea: impacts on recruitment, growth, re-establishment and restoration. *Botanica Marina, 62*(1), 17-30.

Kautsky, N., Kautsky, H., Kautsky, U., & Waern, M. (1986). Decreased depth penetration of *Fucus vesiculosus* (L.) since the 1940's indicates eutrophication of the Baltic Sea. *Mar. Ecol. Prog. Ser, 28*(1-2), 1-8.

Kinnby, A., Jonsson, P. R., Ortega-Martinez, O., Töpel, M., Pavia, H., Pereyra, R. T., & Johannesson, K. (2020). Combining an Ecological Experiment and a Genome Scan Show Idiosyncratic Responses to Salinity Stress in Local Populations of a Seaweed. *Frontiers in Marine Science, 7*, 470.

Korpinen, S., Jormalainen, V., & Pettay, E. (2010). Nutrient availability modifies species abundance and community structure of *Fucus*-associated littoral benthic fauna. *Marine environmental research, 70*(3-4), 283-292.

Kucera, H., & Saunders, G. W. (2008). Assigning morphological variants of *Fucus* (Fucales, Phaeophyceae) in Canadian waters to recognized species using DNA barcoding. *Botany, 86*(9), 1065-1079.

Laughinghouse, H. D., Müller, K. M., Adey, W. H., Lara, Y., Young, R., & Johnson, G. (2015). Evolution of the northern rockweed, *Fucus distichus*, in a regime of glacial cycling: implications for benthic algal phylogenetics. *PloS one, 10*(12), e0143795.

Leberg, P. (2002). Estimating allelic richness: effects of sample size and bottlenecks. *Molecular ecology, 11*(11), 2445-2449.

Lima, F. P., Ribeiro, P. A., Queiroz, N., Hawkins, S. J., & Santos, A. M. (2007). Do distributional shifts of northern and southern species of algae match the warming pattern? *Global change biology, 13*(12), 2592-2604.

Loiseaux-de Goër, S., & Noailles, M.-C. (2008). *Algues de roscoff*. Éd. de la Station biologique de Roscoff.

Lubchenco, J. (1980). Algal zonation in the New England rocky intertidal community: an experimental analysis. *Ecology, 61*(2), 333-344.

Lüning, K. (1984). Temperature tolerance and biogeography of seaweeds: the marine algal flora of Helgoland (North Sea) as an example. *Helgoländer Meeresuntersuchungen, 38*(2), 305-317.

Lüning, K. (1990). *Seaweeds: their environment, biogeography, and ecophysiology*. John Wiley & Sons.

Maier, I., & Muller, D. (1986). Sexual pheromones in algae. *The Biological Bulletin, 170*(2), 145-175.

Mathieson, A. C., Dawes, C. J., Anderson, M. L., & Hehre, E. J. (2001). Seaweeds of the Brave Boat Harbor salt marsh and adjacent open coast of southern Maine. *Rhodora*, 1-46.

Mathieson, A. C., Dawes, C. J., Wallace, A. L., & Klein, A. S. (2006). Distribution, morphology, and genetic affinities of dwarf embedded *Fucus* populations from the Northwest Atlantic Ocean. *Botanica Marina, 49*(4), 283-303.

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), e35978.

Moussa, H., Hassoun, M., Salhi, G., Zbakh, H., & Riadi, H. (2018). Checklist of seaweeds of Al-Hoceima National Park of Morocco (Mediterranean Marine Protected Area).

Müller, D., & Gassmann, G. (1985). Sexual reproduction and the role of sperm attractants in monoecious species of the brown algae Order Fucales (*Fucus, Hesperophycus, Pelvetia,* and *Pelvetiopsis*). *Journal of plant physiology, 118*(5), 401-408.

Neiva, J., Hansen, G. I., Pearson, G. A., Vliet, M. S. V. D., Maggs, C. A., & Serrão, E. A. (2012). *Fucus cottonii* (Fucales, Phaeophyceae) is not a single genetic entity but a convergent salt-marsh morphotype with multiple independent origins. *European journal of phycology, 47*(4), 461-468.

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.

Nicastro, K. R., Zardi, G. I., Teixeira, S., Neiva, J., Serrão, E. A., & Pearson, G. A. (2013). 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), 1-13.

Norton, T. A. (1991). Conflicting constraints on the form of intertidal algae. *British Phycological Journal, 26*(3), 203-218.

Næss, T. (2019). Population genetics of *Saccharina latissima* (sugar kelp) in Norway. Master thesis, University of Bergen.

Peakall, R., & Smouse, P. E. (2006). GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes, 6*(1), 288-295.

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*, 211-223.

Peck, J. R., Yearsley, J. M., & Waxman, D. (1998). Explaining the geographic distributions of sexual and asexual populations. *Nature, 391*(6670), 889-892.

Pereyra, R., Huenchuñir, C., Johansson, D., Forslund, H., Kautsky, L., Jonsson, P., & Johannesson, K. (2013). Parallel speciation or long-distance dispersal? Lessons from seaweeds (*Fucus*) in the Baltic Sea. *Journal of Evolutionary Biology, 26*(8), 1727-1737.

Pereyra, R. T., Bergström, L., Kautsky, L., & Johannesson, K. (2009). Rapid speciation in a newly opened postglacial marine environment, the Baltic Sea. *BMC evolutionary biology, 9*(1), 1-9.

Perrin, C., Daguin, C., Vliet, M. V. D., 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.

Powell, H. (1963). New records of *Fucus distichus* subspecies for the Shetland and Orkney Islands. *British Phycological Bulletin, 2*(4), 247-254.

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics, 155*(2), 945-959.

Rinne, H., Björkman, U., Sjöqvist, C., Salovius-Laurén, S., & Mattila, J. (2018). Morphological and genetic variation of *Fucus* in the eastern Gulf of Bothnia, northern Baltic Sea. *European journal of phycology, 53*(3), 369-380.

Rousset, F. (2008). genepop'007: a complete re-implementation of the genepop software for Windows and Linux. *Molecular Ecology Resources, 8*(1), 103-106.

Rueness, J. (1977). *Norsk algeflora*. Universitetsforl.

Ruiz, G. M., Fofonoff, P. W., Carlton, J. T., Wonham, M. J., & Hines, A. H. (2000). Invasion of coastal marine communities in North America: apparent patterns, processes, and biases. *Annual review of Ecology and Systematics, 31*(1), 481-531.

Ruuskanen, A., & Bäck, S. (2002). Morphological changes in submerged *Fucus vesiculosus* (L)(Phaeophyta) along the salinity gradient of the River Keret estuary, Russia. *Sarsia, 87*(2), 185-188.

Råberg, S., & Kautsky, L. (2007). A comparative biodiversity study of the associated fauna of perennial fucoids and filamentous algae. *Estuarine, Coastal and Shelf Science, 73*(1-2), 249-258.

Sahla, M., Tolvanen, H., Ruuskanen, A., & Kurvinen, L. (2020). Assessing long term change of *Fucus* spp. communities in the northern Baltic Sea using monitoring data and spatial modeling. *Estuarine, Coastal and Shelf Science, 245*, 107023.

Saraiva, S., Meier, H. M., Andersson, H., Höglund, A., Dieterich, C., Gröger, M., Hordoir, R., & Eilola, K. (2019). Baltic Sea ecosystem response to various nutrient load scenarios in present and future climates. *Climate Dynamics, 52*(5), 3369-3387.

Schonbeck, M., & Norton, T. A. (1978). Factors controlling the upper limits of fucoid algae on the shore. *Journal of Experimental Marine Biology and Ecology, 31*(3), 303-313.

Scott, G., Hull, S., Hornby, S., Hardy, F. G., & Owens, N. (2001). Phenotypic variation in *Fucus spiralis* (Phaeophyceae): morphology, chemical phenotype and their relationship to the environment. *European journal of phycology, 36*(1), 43-50.

Scott, G., Shaw, J., Hull, S., Pickaert, C., & Burlak, A. (2000). Some implications of plant size in monotypic and polytypic populations of *Fucus spiralis*. *Journal of the Marine Biological Association of the United Kingdom, 80*(2), 359-360.

Selkoe, K. A., & Toonen, R. J. (2006). Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. *Ecology letters, 9*(5), 615-629.

Serrão, E. A., Alice, L. A., & Brawley, S. H. (1999a). Evolution of the Fucaceae (Phaeophyceae) inferred from nrDNA-ITS. *Journal of Phycology, 35*(2), 382-394.

Serrão, E. A., Brawley, S. H., Hedman, J., Kautsky, L., & Samuelsson, G. (1999b). Reproductive success of *Fucus vesiculosus* (Phaeophyceae) in the Baltic Sea. *Journal of Phycology, 35*(2), 254-269.

Serrão, E. A., Pearson, G., Kautsky, L., & Brawley, S. H. (1996). Successful external fertilization in turbulent environments. *Proceedings of the National Academy of Sciences, 93*(11), 5286-5290.

Sjøtun, K., Heesch, S., Rull Lluch, J., Martín Martín, R., Gómez Garreta, A., Brysting, A. K., & Coyer, J. A. (2017). Unravelling the complexity of salt marsh '*Fucus cottonii*'forms (Phaeophyceae, Fucales). *European journal of phycology, 52*(3), 360-370.

Steele, J. H., Brink, K. H., & Scott, B. E. (2019). Comparison of marine and terrestrial ecosystems: suggestions of an evolutionary perspective influenced by environmental variation. *ICES Journal of Marine Science, 76*(1), 50-59.

Steinberg, P. D. (2019). Geographical variation in the interaction between marine herbivores and brown algal secondary metabolites. In *Ecological roles of marine natural products* (pp. 51-92). Cornell University Press.

Steneck, R. S., Graham, M. H., Bourque, B. J., Corbett, D., Erlandson, J. M., Estes, J. A., & Tegner, M. J. (2002). Kelp forest ecosystems: biodiversity, stability, resilience and future. *Environmental conservation*, 436-459.

Sørensen, H. (2010). *The Norwegian Nature Diversity Act*. Retrieved June 23, 2021 from https://www.regjeringen.no/globalassets/upload/md/vedlegg/taler_artikler/the_new_approach_the_norwegian_nature_diversity_act_sorensen_lange_100603.pdf

Tatarenkov, A., Bergström, L., Jönsson, R. B., Serrão, E. A., Kautsky, L., & Johannesson, K. (2005). Intriguing asexual life in marginal populations of the brown seaweed *Fucus vesiculosus*. *Molecular ecology, 14*(2), 647-651.

Tatarenkov, A., Jönsson, R. B., Kautsky, L., & Johannesson, K. (2007). Genetic structure in populations of *Fucus vesiculosus* (Phaeophyceae) over spatial scales from 10 m to 800 km 1. *Journal of Phycology, 43*(4), 675-685.

Thibaut, T., Blanfuné, A., Boudouresque, C.-F., & Verlaque, M. (2015). Decline and local extinction of Fucales in French Riviera: the harbinger of future extinctions? *Mediterranean marine science, 16*(1), 206-224.

Torn, K., Krause-Jensen, D., & Martin, G. (2006). Present and past depth distribution of bladderwrack (*Fucus vesiculosus*) in the Baltic Sea. *Aquatic Botany, 84*(1), 53-62.

Torres, M. D., Kraan, S., & Dominguez, H. (2020). *Sustainable Seaweed Technologies: Cultivation, Biorefinery, and Applications*. Elsevier.

Valero, M., Engel, C., Billot, C., Kloareg, B., & Destombe, C. (2001). 5. Concept and issues of population genetics in seaweeds. *Cahiers de Biologie Marine, 42*(1/2), 53-62.

Van Oosterhout, C., Hutchinson, W. F., Wills, D. P., & Shipley, P. (2004). MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes, 4*(3), 535-538.

Verlaque, M., Boudouresque, C.-F., & Perret-Boudouresque, M. (2019). Mediterranean seaweeds listed as threatened under the Barcelona Convention: A critical analysis. *Scientific Reports. Port-Cros natl. Park, 33*, 179-214.

Vernet, P., & Harper, J. (1980). The costs of sex in seaweeds. *Biological Journal of the Linnean Society, 13*(2), 129-138.

Wahl, M., Jormalainen, V., Eriksson, B. K., Coyer, J. A., Molis, M., Schubert, H., Dethier, M., Karez, R., Kruse, I., & Lenz, M. (2011). Stress ecology in *Fucus*: abiotic, biotic and genetic interactions. *Advances in marine biology, 59*, 37-105.

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.

Wendel, J. F. (2000). Genome evolution in polyploids. *Plant molecular evolution*, 225-249.

Wernberg, T., Krumhansl, K., Filbee-Dexter, K., & Pedersen, M. F. (2019). Status and trends for the world's kelp forests. In *World seas: An environmental evaluation* (pp. 57-78). Elsevier.

Wigginton, J. E., Cutler, D. J., & Abecasis, G. R. (2005). A note on exact tests of Hardy-Weinberg equilibrium. *The American Journal of Human Genetics, 76*(5), 887-893.

Wikström, S. A., & Kautsky, L. (2007). Structure and diversity of invertebrate communities in the presence and absence of canopy-forming *Fucus vesiculosus* in the Baltic Sea. *Estuarine, Coastal and Shelf Science, 72*(1-2), 168-176.

Wynne, M., & Magne, F. (1991). Concerning the name *Fucus* muscoides (Cotton) J. Feldmann et Magne. *Cryptogamie. Algologie, 12*(1), 55-65.

Wynne, M. J., & Bold, H. (1985). *Introduction to the Algae: Structure and Reproduction*. Prentice-Hall, Incorporated.

Zardi, G. I., Nicastro, K. R., Canovas, F., Costa, J. F., Serrão, E. A., & Pearson, G. A. (2011). Adaptive traits are maintained on steep selective gradients despite gene flow and hybridization in the intertidal zone. *PloS one, 6*(6), e19402.

Appendix I – Fieldwork

A. Fieldwork Norway

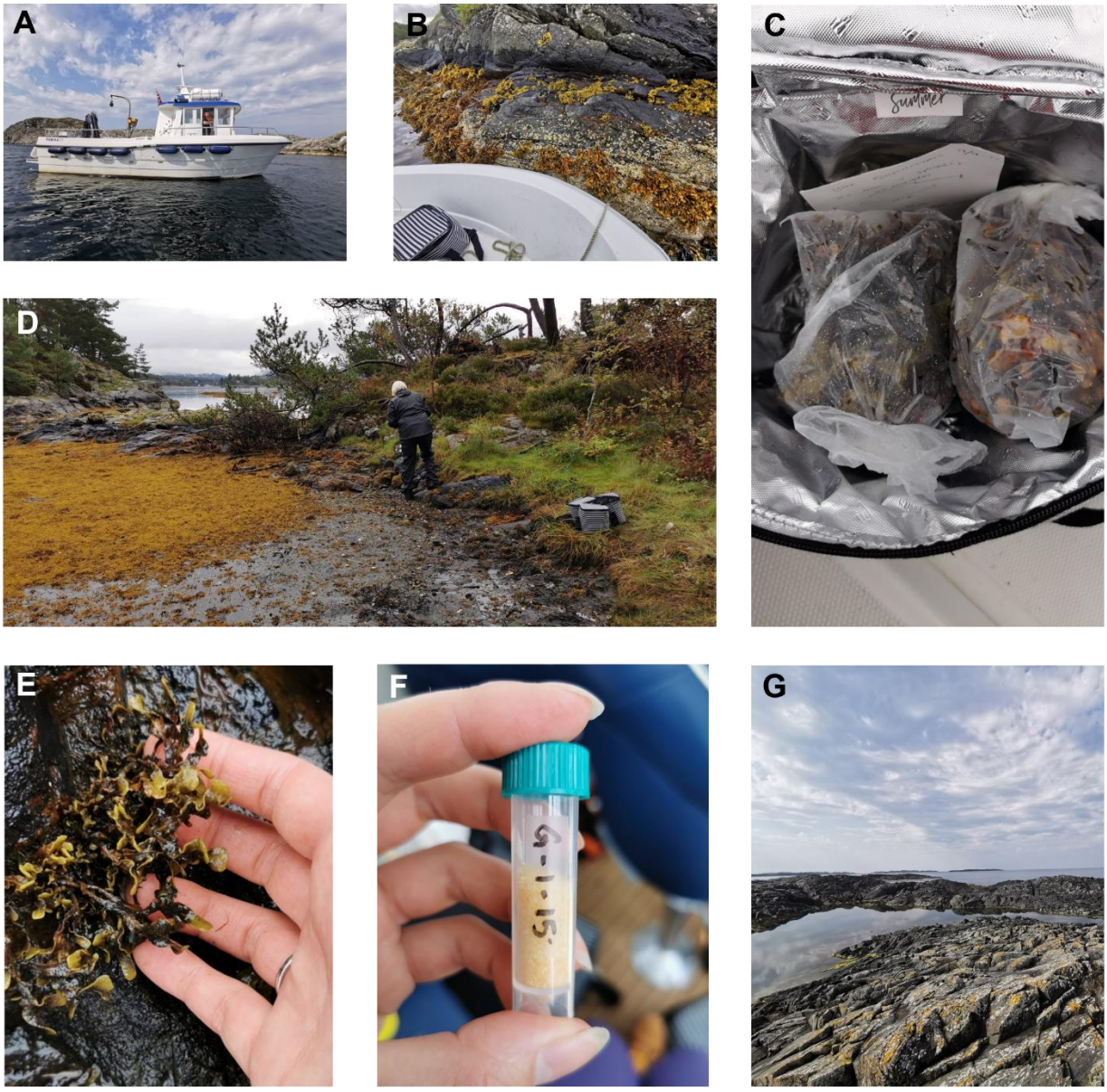


Figure I. Fieldwork carried out in Bømlo and Eggholmane by Kjersti Sjøtun and Frida Knoop between August and September 2020. **A.** The boat (Emiliana Huxley) was used to access the sampling areas in Bømlo. **B**. Sampling *F. spiralis* from a smaller motorboat in Ytre Eggholmane. **C.** Seaweed was collected in plastic bags and stored cool during transportation back to the University. **D.** Collection of *F. Cottonii* on the muddy substrate from Indre Eggholmane. **E.** *Fucus spiralis* attached to the rock in Ytre Eggholmane. **F.** Tissue samples were placed into screw capped tubes with silica gel. **G.** Wave exposed sampling area in Ytre Gulo, where *F. spiralis* f. *nanus* was found.

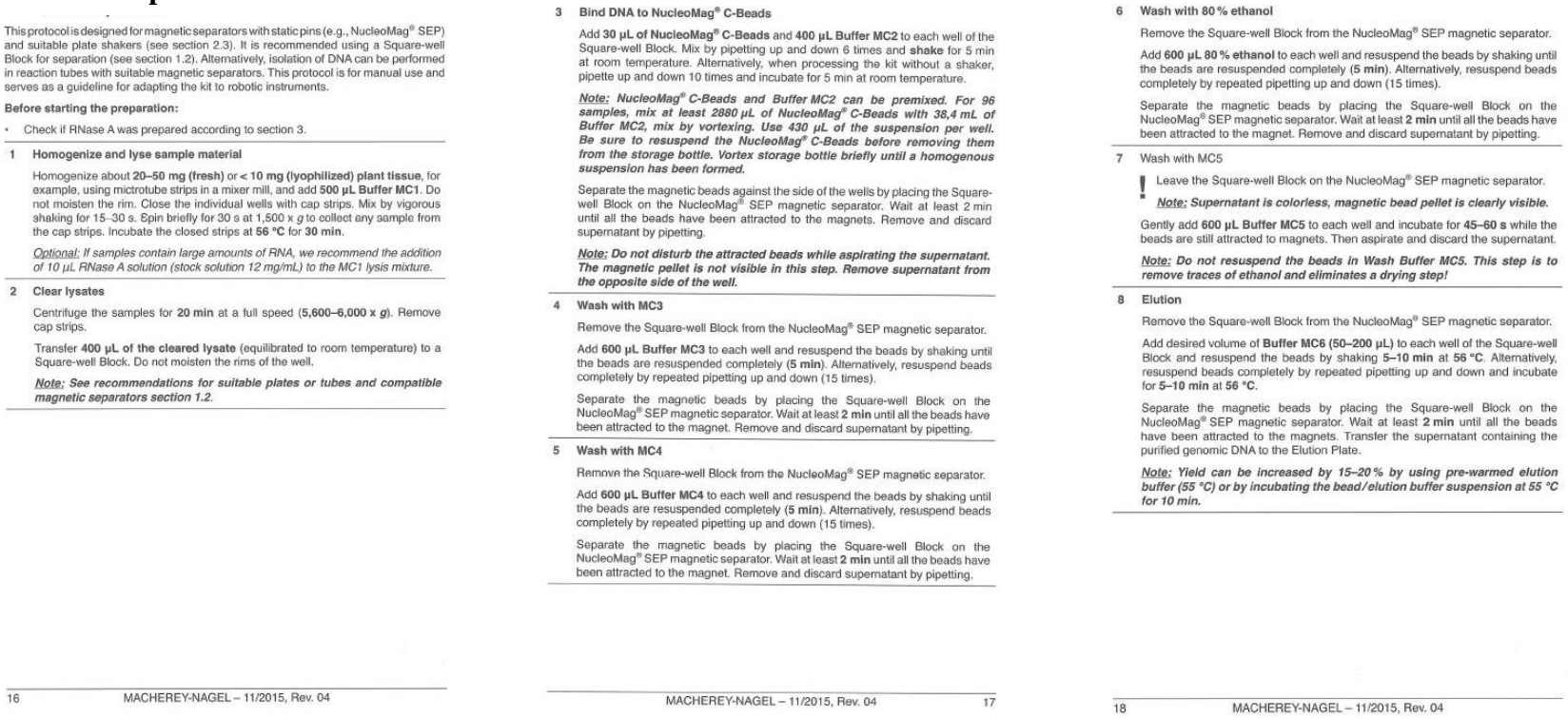
B. The Norwegian herbarium

Figure II. Photographs of the 50 individuals that were used for morphometric recordings. Images are not to scale. **A.** Six *F. spiralis* individuals sampled from Indre Eggholmane, 2020. **B.** Three *F. spiralis* individuals sampled from Ytre Eggholmane, 2019 **C.** Nine *F. spiralis* individuals sampled from Ytre Eggholmane, 2020. **D.** Five *F. spiralis* individuals sampled from Indre Gulo, 2020. **E.** Five *F. spiralis* individuals sampled in Indre Toska, 2020.

Figure II. Continued. **F.** Five *F. spiralis* individuals sampled from Lygra, 2020. **G.** Five *F. spiralis* forma *nanus* individuals sampled from Ytre Gulo, 2020. **H.** Five *F. cottonii* individuals sampled from Indre Eggholmane, 2020. **I.** Six *F. cottonii* individuals sampled from Lygra, 2020.
J. Two *Fucus* sp. individuals sampled from Indre Eggholmane, 2020.

Appendix II - Labwork

A. Detailed protocol



This protocol is designed for magnetic separators with static pins (e.g., NucleoMag® SEP) and suitable plate shakers (see section 2.3). It is recommended using a Square-well Block for separation (see section 1.2). Alternatively, isolation of DNA can be performed in reaction tubes with suitable magnetic separators. This protocol is for manual use and serves as a guideline for adapting the kit to robotic instruments.

Before starting the preparation:

- Check if RNase A was prepared according to section 3.

1 Homogenize and lyse sample material

Homogenize about **20–50 mg (fresh)** or **< 10 mg (lyophilized) plant tissue**, for example, using mictrotube strips in a mixer mill, and add **500 µL Buffer MC1**. Do not moisten the rim. Close the individual wells with cap strips. Mix by vigorous shaking for 15–30 s. Spin briefly for 30 s at 1,500 x *g* to collect any sample from the cap strips. Incubate the closed strips at **56 °C** for **30 min**.

Optional: If samples contain large amounts of RNA, we recommend the addition of 10 µL RNase A solution (stock solution 12 mg/mL) to the MC1 lysis mixture.

2 Clear lysates

Centrifuge the samples for **20 min** at a full speed (**5,600–6,000 x *g***). Remove cap strips.

Transfer **400 µL of the cleared lysate** (equilibrated to room temperature) to a Square-well Block. Do not moisten the rims of the well.

Note: See recommendations for suitable plates or tubes and compatible magnetic separators section 1.2.

16 MACHEREY-NAGEL – 11/2015, Rev. 04

3 Bind DNA to NucleoMag® C-Beads

Add **30 µL of NucleoMag® C-Beads** and **400 µL Buffer MC2** to each well of the Square-well Block. Mix by pipetting up and down 6 times and **shake** for 5 min at room temperature. Alternatively, when processing the kit without a shaker, pipette up and down 10 times and incubate for 5 min at room temperature.

Note: NucleoMag® C-Beads and Buffer MC2 can be premixed. For 96 samples, mix at least 2880 µL of NucleoMag® C-Beads with 38,4 mL of Buffer MC2, mix by vortexing. Use 430 µL of the suspension per well. Be sure to resuspend the NucleoMag® C-Beads before removing them from the storage bottle. Vortex storage bottle briefly until a homogenous suspension has been formed.

Separate the magnetic beads against the side of the wells by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least 2 min until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

Note: Do not disturb the attracted beads while aspirating the supernatant. The magnetic pellet is not visible in this step. Remove supernatant from the opposite side of the well.

4 Wash with MC3

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add **600 µL Buffer MC3** to each well and resuspend the beads by shaking until the beads are resuspended completely (**5 min**). Alternatively, resuspend beads completely by repeated pipetting up and down (15 times).

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

5 Wash with MC4

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add **600 µL Buffer MC4** to each well and resuspend the beads by shaking until the beads are resuspended completely (**5 min**). Alternatively, resuspend beads completely by repeated pipetting up and down (15 times).

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

MACHEREY-NAGEL – 11/2015, Rev. 04 17

6 Wash with 80 % ethanol

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add **600 µL 80 % ethanol** to each well and resuspend the beads by shaking until the beads are resuspended completely (**5 min**). Alternatively, resuspend beads completely by repeated pipetting up and down (15 times).

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

7 Wash with MC5

Leave the Square-well Block on the NucleoMag® SEP magnetic separator.

Note: Supernatant is colorless, magnetic bead pellet is clearly visible.

Gently add **600 µL Buffer MC5** to each well and incubate for **45–60 s** while the beads are still attracted to magnets. Then aspirate and discard the supernatant.

Note: Do not resuspend the beads in Wash Buffer MC5. This step is to remove traces of ethanol and eliminates a drying step!

8 Elution

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add desired volume of **Buffer MC6 (50–200 µL)** to each well of the Square-well Block and resuspend the beads by shaking **5–10 min** at **56 °C**. Alternatively, resuspend beads completely by repeated pipetting up and down and incubate for **5–10 min** at **56 °C**.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Transfer the supernatant containing the purified genomic DNA to the Elution Plate.

Note: Yield can be increased by 15–20 % by using pre-warmed elution buffer (55 °C) or by incubating the bead/elution buffer suspension at 55 °C for 10 min.

18 MACHEREY-NAGEL – 11/2015, Rev. 04

Figure III. In the user manual of NucleoMag® Plant (Macherey Nagel), the detailed protocol was followed with the following modifications. In step 1, the tissue samples were homogenized dry instead of with buffer 1, and proteinase K and RNase A were added to buffer MC1. For master mix 1, 520 µl were distributed into the wells, instead of 523 µl, because the Repetman® is limited to 0.5 decimals. The incubation time was 2h instead of 30min at 56°C. In step 2, the samples were centrifuged for 20min at 4°C instead of at room temperature. For mixing, an automatic 12-pipette was used to pipette up and down 15 times. In step 8, 100 µl of buffer MC6 was added and then incubated in the oven for 10min at 100 rpm.

B. Preparing for DNA extraction

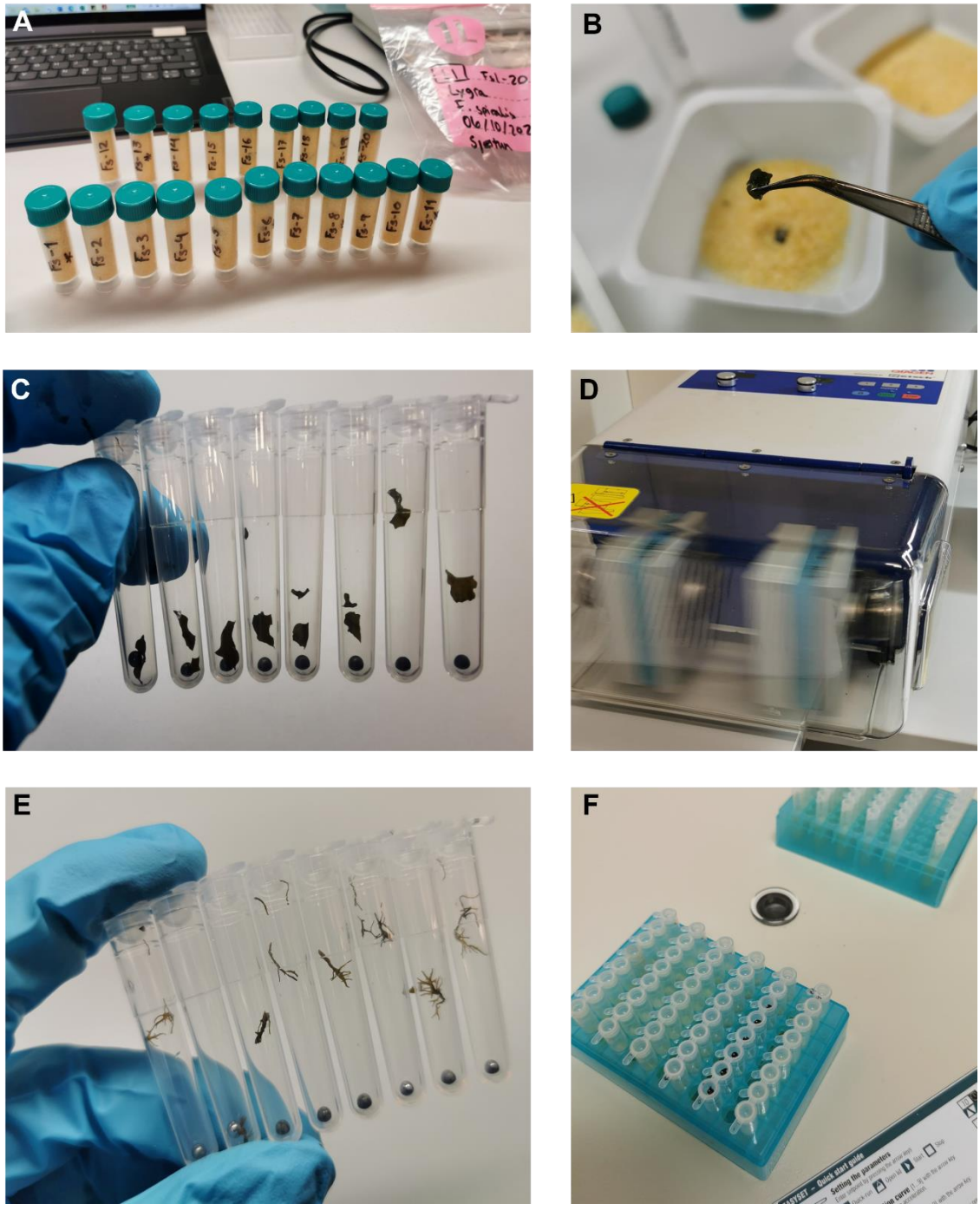


Figure IV. A. Tissue samples in a screw capped container with silica gel. **B.** Transferring subsample of the tissue into 96-cassette wells using a tweezers that was sterilized between each sample to avoid contamination. **C.** *Fucus spiralis* subsample in 8-tube wells with the 3mm tungsten ball. **D.** TissueLyser machine in process of grinding the subsamples. **E.** Less tissue in the subsample from *F. cottonii* due to the small size. **F.** Homogenized subsample ready for DNA extraction.

C. DNA extraction stage

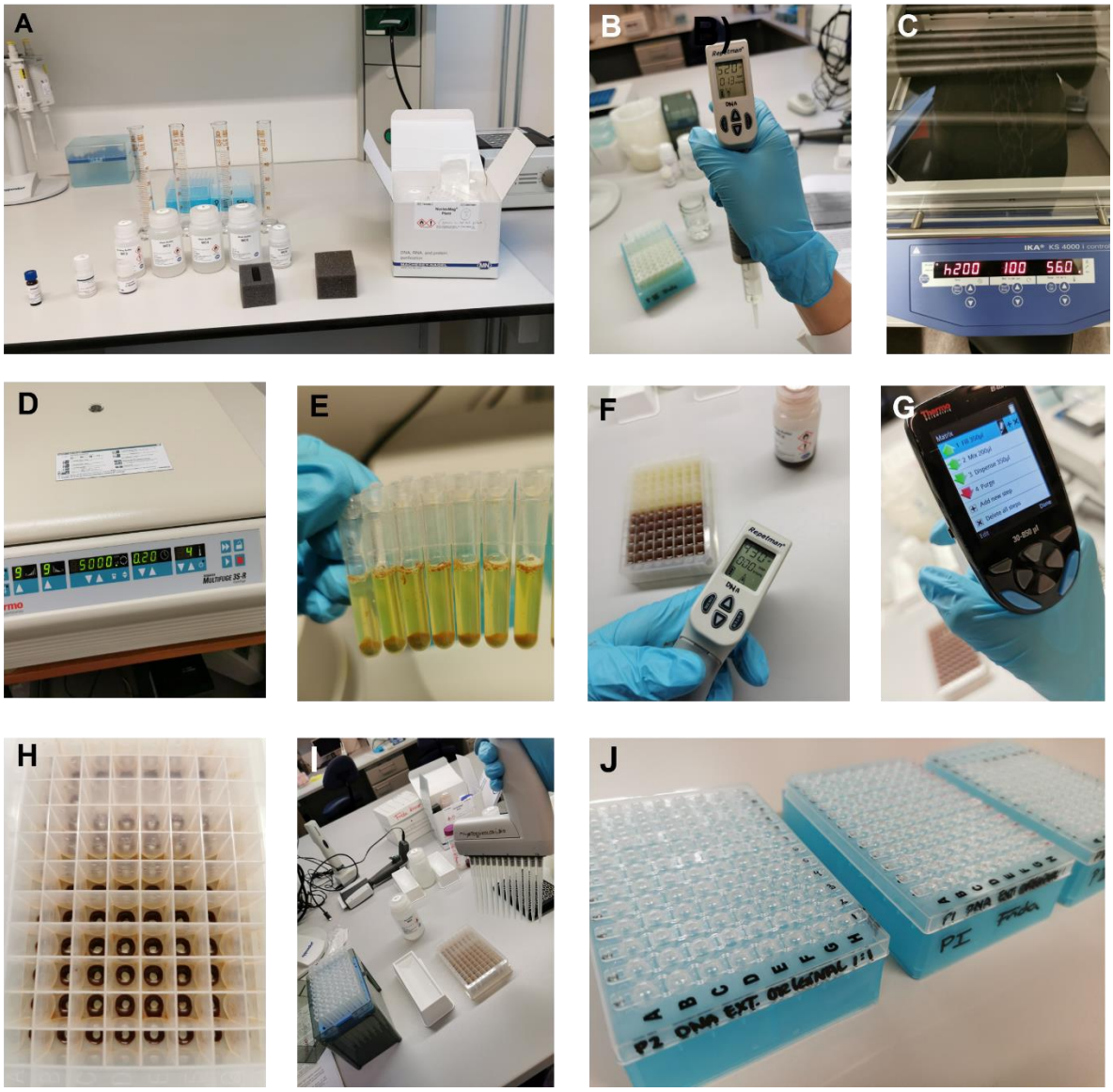


Figure V. **A.** All components of the NucleoMag® Plant Kit were sorted in chronological order and additional tools such as measuring cylinders were prepared. **B.** In step 1, the reagents were mixed into a 60 ml glass container and distributed out into the wells with Repetman®. **C.** The IKA® KS 4000 i Control was used for incubation at 56°C for two hours. **D.** After incubation, the samples were centrifuged for 20min at 4°C. **E.** After centrifugation, 400 µl of cleared lysate were transferred into a square-well block. **F.** The Repetman® were used to distribute the C-beads mix into the Square-well Block with the cleared lysate. **G.** An automatic 12-pipette was used to mix by pipetting up and down 15 times. **H.** When the Square-well Block was placed on the NucleMag® SEP, the beads were attracted to the magnet which formed a ring with the beads and DNA. **I.** The reagent was transferred into disposable reagent reservoirs when using the multiple 12-pipette. **J.** Approximately 100 µl of purified DNA was transferred into the 96-cassette.

D. Technical Data Sheet

Sigma-Aldrich.

Technical Data Sheet

SALES ORDER NO: 8815457299
CUSTOMER NO: 0000002053
SHIPMENT DATE: 03/09/2020

INSTITUTE: UNIVERSITETET I BERGEN
RESEARCHER: UNIVERSITETET I BERGEN
PURCHASE ORDER NO: PM2029065

Batch #	Oligo Name	Oligo #	Len	Pur	Scale	MW	Tm°	µg/OD	OD	µg	nmol	Epsilon 1/(mMcm)	Dimer	2ndry	GC %	µl for 100µM *	Sequence(5'-3')
HA14159501	Fsp1_Fwd	8815457299-10/0	37	DST	0.025	11481	85.0	31.0	15.44	478.7	41.7	370.3	No	Moderate	54.0	416	TGTAAAACGACGGCCAGTTCAAAAGCCAGCAGGGGTG
HA14159502	Fsp1_Rev	8815457299-20/0	24	DST	0.025	7383	61.5	31.7	9.92	315.2	42.7	232.3	No	None	41.6	427	TCTTCTGGGAGCTGTAAAATAGTC
HA14159503	Fsp2_Fwd	8815457299-30/0	41	DST	0.025	12582	84.4	32.9	7.68	252.6	20.1	382.4	No	Strong	48.7	200	TGTAAAACGACGGCCAGTGCATCTGGTGTCATTCCTTGTTC
HA14159504	Fsp2_Rev	8815457299-40/0	20	DST	0.025	6090	66.2	34.4	12.16	419.3	68.9	176.6	No	None	50	688	TTGTTTGAGTGCCACCTTGC
HA14159505	Fsp4_Fwd	8815457299-50/0	36	DST	0.025	11135	86.5	31.8	16.08	512.3	46.0	349.5	No	Very Strong	58.3	460	TGTAAAACGACGGCCAGTATGACCGGGCCGGATTGC
HA14159506	Fsp4_Rev	8815457299-60/0	24	DST	0.025	7244	70.7	36.3	12.36	449.4	62.0	199.2	No	None	54.1	620	GTGCTTCCCCTCCTTGTTCTGTTG
HA14159507	L20-Fwd	8815457299-70/0	38	DST	0.025	11648	82.6	32.2	15.32	493.4	42.4	361.6	No	Weak	52.6	423	TGTAAAACGACGGCCAGTACTCCATGCTGCGAGACTTC
HA14159508	L20-Rev	8815457299-80/0	20	DST	0.025	6077	65.1	32.0	10.64	341.3	56.2	189.4	No	Weak	50	561	CCTCGGTGATCAGCAATCAT
HA14159509	L58-Fwd	8815457299-90/0	38	DST	0.025	11787	81.6	29.9	16.36	489.1	41.5	394.2	No	Strong	44.7	415	TGTAAAACGACGGCCAGTAAACGAAAATGGCACAGTGA
HA14159510	L58-Rev	8815457299-100/0	20	DST	0.025	6182	63.4	31.4	12.16	382.9	61.9	196.3	No	Weak	55	619	CCTTGCATGTAGGAGGGAAC
HA14159511	L94-Fwd	8815457299-110/0	36	DST	0.025	11254	83.6	31.0	15.32	475.6	42.3	362.5	No	Moderate	52.7	422	TGTAAAACGACGGCCAGTTTAGGAATGGGCGGGATG
HA14159512	L94-Rev	8815457299-120/0	20	DST	0.025	6179	65.2	32.3	11.56	373.3	60.4	191.3	No	Very Weak	50	604	GATTTCGTGAGGCTGGTTCA
HA14159513	L38_Fwd	8815457299-130/0	38	DST	0.025	11701	83.2	33.2	15.96	530.3	45.3	352.1	No	Weak	52.6	453	TGTAAAACGACGGCCAGTTGCTAGCTGCTCTTGTGTGC
HA14159514	L38_Rev	8815457299-140/0	19	DST	0.025	5789	67.2	32.5	9.6	312.0	53.9	178.1	No	Weak	57.8	539	TAACCTGTCGGTCGCAACG
HA14159515	L78_Fwd	8815457299-150/0	36	DST	0.025	11199	84.5	31.1	16.36	509.7	45.5	359.4	No	Strong	55.5	455	TGTAAAACGACGGCCAGTCGTGAGGGCAGGAATGTC
HA14159516	L78_Rev	8815457299-160/0	20	DST	0.025	6052	64.1	32.1	11.36	364.9	60.3	188.4	No	Weak	45	602	GATTTCCGGCATCATCAATC

Figure VI. Technical data sheet (Sigma-Aldrich) with specifications for stock solutions.

E. PCR process

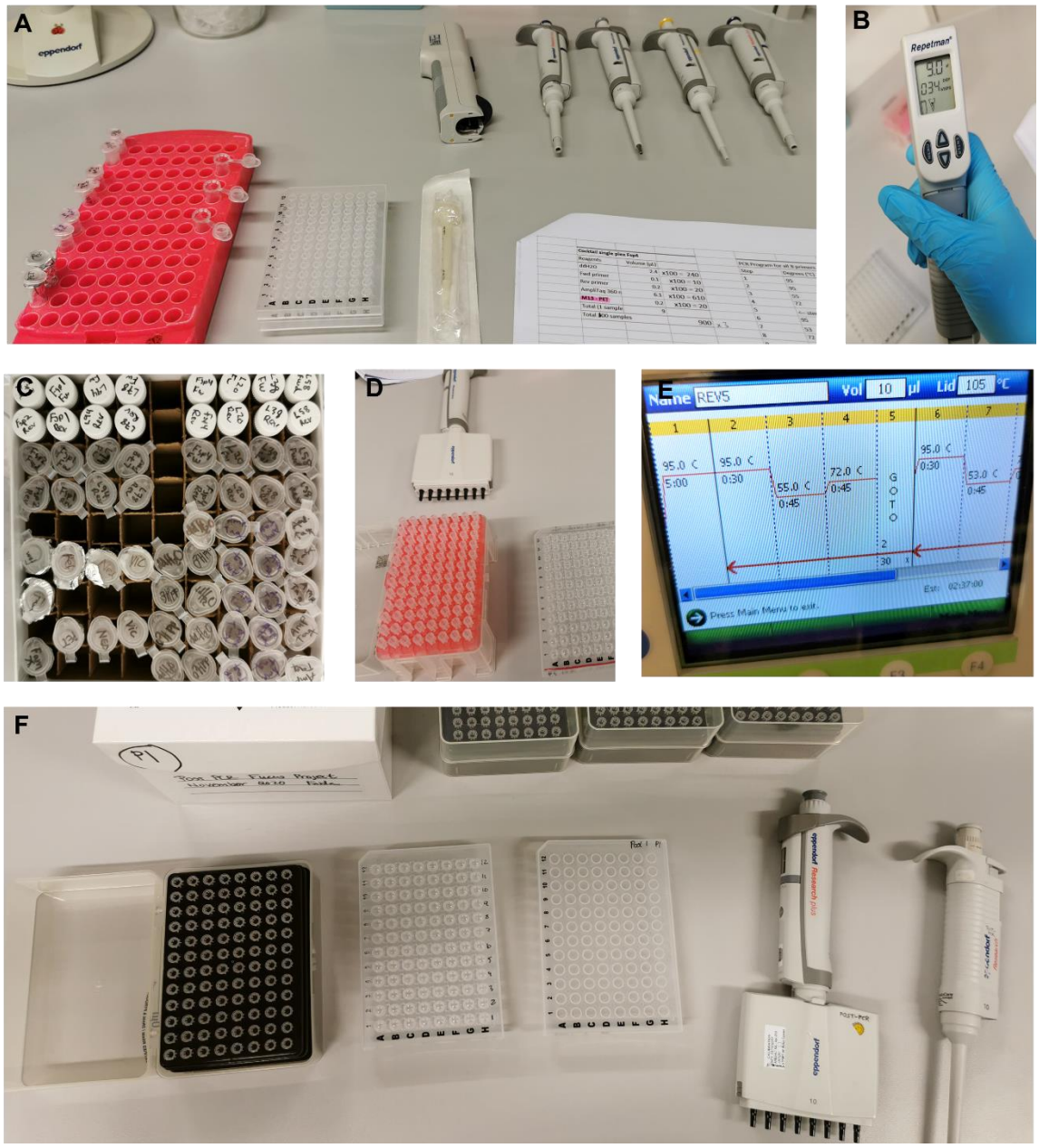


Figure VII. A. PCR components were assembled while reagents were thawing in an Eppendorf tube rack. Due to AmpliTaq 360 mix (Applied Biosystems) hot-start enzymes the work could be carried out at room temperature. **B.** The Repetman® with a 500 µl pipette tip was used to distribute 9 µl of the cocktail mix into an Axygen 96-plate. **C.** Reagents for the PCR process were stored in a Sarstedt box (1.5-2.0 ml tubes) in the freezer -18°C. **D.** 1 µl of 1:2 diluted DNA were added to the cocktail mix. **E.** A total of 10 µl PCR mix was used in the C1000 Thermal Cycler (Bio-Rad). **F.** Multiple 8-pipette were used to transfer 2 µl post-PCR products into a new 96-cassette.

Appendix III – Data

A. Raw data

Table A. Microsatellite genotyping results for all 304 individuals at eight loci. The individuals are assigned a specific ID. The first capitalized letter describes the sample region (N = Norway, S = Spain), the following three capitalized letters indicates sample location (LYG = Lygra, IEG = Indre Eggholmane, YEG = Ytre Eggholmane, IGU = Indre Gulo, YGU = Ytre Gulo, ITO = Indre Toska, thereafter two letters describes name of the species (Fch = *Fucus chalonii*, Fc = *Fucus cottonii*, Fg = *Fucus guiryi*, Fsfn = *Fucus spiralis* forma *nanus*, Fs = *Fucus spiralis*, Fv = *Fucus vesiculosus*), and the two number on the end describes the number of individual sampled.

Fucus																
Fsp4																
L20																
L38																
L58																
Fsp1																
Fsp2																
L78																
L94																
pop	Fsp4		L20		L38		L58		Fsp1		Fsp2		L78		L94	
N_LYGFs01	150	176	179	182	205	226	141	141	161	167	212	212	156	156	180	180
N_LYGFs02	168	176	182	188	205	205	141	143	161	167	212	212	156	201	180	201
N_LYGFs03	174	174	182	182	205	205	141	141	161	161	174	174	156	156	180	180
N_LYGFs04	150	176	179	182	205	235	141	145	161	165	176	182	156	186	180	195
N_LYGFs05	174	174	182	182	205	205	141	141	161	161	212	212	156	156	180	180
N_LYGFs06	174	174	182	182	205	205	141	141	161	161	184	184	156	156	180	180
N_LYGFs07	156	172	182	191	205	226	141	145	161	173	182	182	156	195	180	195
N_LYGFs08	156	176	182	182	205	205	141	143	161	171	182	212	156	156	180	189
N_LYGFs09	176	176	182	182	205	205	141	141	161	161	212	212	156	156	180	180
N_LYGFs10	176	176	182	182	205	205	141	141	161	161	212	212	156	156	180	180
N_LYGFs11	174	174	182	182	205	205	141	141	161	161	212	212	156	156	180	180
N_LYGFs12	176	176	182	182	205	205	141	141	161	161	212	212	156	156	180	180
N_LYGFs13	176	176	182	182	205	205	141	141	161	161	212	212	156	156	180	180
N_LYGFs14	176	176	182	182	205	205	141	141	161	161	174	212	156	156	180	180

N_LYGFs15	174	174	182	182	205	205	141	141	161	161	212	212	156	156	180	180
N_LYGFs16	174	174	182	182	205	205	141	141	161	161	174	174	156	156	180	180
N_LYGFs17	174	174	182	182	205	205	141	141	161	161	174	174	156	156	180	180
N_LYGFs18	176	176	182	182	205	205	141	141	161	161	212	212	156	156	180	180
N_LYGFs19	174	174	182	182	205	205	141	141	161	161	212	212	156	156	180	180
N_LYGFs20	176	176	182	182	205	205	141	141	161	161	212	212	156	156	180	180
N_IEGFs01	176	176	182	182	205	205	141	141	161	161	182	182	156	156	180	180
N_IEGFs02	172	176	182	182	205	205	141	141	161	161	174	184	156	156	180	180
N_IEGFs03	172	172	182	182	205	205	141	141	161	161	184	184	156	156	180	180
N_IEGFs04	176	176	182	182	205	205	141	141	161	161	182	182	156	156	180	180
N_IEGFs05	150	150	173	179	235	235	143	147	167	173	176	182	189	195	189	189
N_IEGFs06	172	172	182	182	205	205	141	141	161	161	184	184	156	156	180	180
N_IEGFs07	172	172	182	182	205	205	141	141	161	161	184	184	156	156	180	180
N_IEGFs08	176	176	182	182	205	205	141	141	161	161	182	182	156	156	180	180
N_IEGFs09	174	174	182	182	205	205	141	141	161	161	210	210	156	156	180	180
N_IEGFs10	150	156	173	191	205	226	151	151	171	171	172	178	177	195	189	201
N_IEGFs11	172	172	182	182	205	205	141	141	161	161	184	184	156	156	180	180
N_IEGFs12	174	174	182	182	205	205	141	141	161	161	184	184	156	156	180	180
N_IEGFs13	176	176	182	182	205	205	141	141	161	161	182	182	156	156	180	180
N_IEGFs14	148	154	176	188	226	226	141	141	161	177	182	208	183	186	189	189
N_IEGFs15	174	174	182	182	205	205	141	141	161	161	210	210	156	156	180	180
N_IEGFs16	150	150	173	179	235	235	143	147	167	173	176	182	189	195	189	189
N_IEGFs17	172	172	182	182	205	205	141	141	161	161	184	184	156	156	180	180
N_IEGFs18	172	172	182	182	205	205	141	141	161	161	184	184	156	156	180	180
N_IEGFs19	176	176	182	182	205	205	141	141	161	161	174	174	156	156	180	180
N_IEGFs20	174	174	182	182	205	205	141	141	161	161	210	210	156	156	180	180
N_YEGFs01	174	174	182	182	205	205	141	141	161	161	210	210	156	156	180	180
N_YEGFs02	174	174	182	182	205	205	141	141	161	161	210	210	156	156	180	180
N_YEGFs03	174	174	182	182	205	205	141	141	161	161	210	210	156	156	180	180
N_YEGFs04	174	174	182	182	205	205	141	141	161	161	210	210	156	156	180	180
N_YEGFs05	174	174	182	182	205	205	141	141	161	161	210	210	156	156	180	180
N_YEGFs06	174	174	182	182	205	205	141	141	161	161	210	210	156	156	180	180
N_YEGFs07	176	176	182	182	205	205	141	141	161	161	210	210	156	156	180	180
N_YEGFs08	174	174	182	182	205	205	141	141	161	161	210	210	156	156	180	180
N_YEGFs09	174	174	182	182	205	205	141	141	161	161	210	210	156	156	180	180

N_YEGFs10	172	172	182	182	205	205	141	141	161	161	182	182	156	156	180	180
N_YEGFs11	176	176	182	182	205	205	141	141	161	161	182	182	156	156	180	180
N_YEGFs12	174	174	182	182	205	205	141	141	161	161	210	210	156	156	180	180
N_YEGFs13	172	172	182	182	205	205	141	141	161	161	182	182	156	156	180	180
N_YEGFs14	172	172	182	182	205	205	141	141	161	161	210	210	156	156	180	180
N_YEGFs15	174	174	182	182	205	205	141	141	161	161	210	210	156	156	180	180
N_YEGFs16	172	172	182	182	205	205	141	141	161	161	182	182	156	156	180	180
N_YEGFs17	176	176	182	182	205	205	141	141	161	161	182	182	156	156	180	180
N_YEGFs18	172	172	182	182	205	205	141	141	161	161	182	182	156	156	180	180
N_YEGFs19	172	172	182	182	205	205	141	141	161	161	182	182	156	156	180	180
N_YEGFs20	174	174	182	182	205	205	141	141	161	161	182	182	156	156	180	180
N_YEGFs21	172	172	182	182	205	205	141	141	161	161	210	210	156	156	180	180
N_YEGFs22	176	176	182	182	205	205	141	141	161	161	210	210	156	156	180	180
N_YEGFs23	172	172	182	182	205	205	141	141	161	161	182	182	156	156	180	180
N_YEGFs24	174	174	182	182	205	205	141	141	161	161	182	182	156	156	180	180
N_YEGFs25	172	172	182	182	205	205	141	141	161	161	210	210	156	156	180	180
N_YEGFs26	172	176	182	182	205	205	141	141	161	161	182	182	156	156	180	180
N_YEGFs27	172	172	182	182	205	205	141	141	161	161	184	184	156	156	180	180
N_YEGFs28	174	174	182	182	205	205	141	141	161	161	210	210	156	156	180	180
N_YEGFs29	172	172	182	182	205	205	141	141	161	161	182	182	156	156	180	180
N_YEGFs30	176	176	182	182	205	205	141	141	161	161	210	210	156	156	180	180
N_IGUFs01	172	172	182	182	205	205	141	141	161	161	184	184	156	156	180	180
N_IGUFs02	172	172	182	182	205	205	141	141	161	161	210	210	156	156	180	180
N_IGUFs03	172	172	182	182	205	205	141	141	161	161	184	184	156	156	180	180
N_IGUFs04	172	172	182	182	205	205	141	141	161	161	210	210	156	156	180	180
N_IGUFs05	172	172	182	182	205	205	141	141	161	161	184	184	156	156	180	180
N_IGUFs06	172	172	182	182	205	205	141	141	161	161	184	184	156	156	180	180
N_IGUFs07	172	172	182	182	205	205	141	141	161	161	210	210	156	156	180	180
N_IGUFs08	172	174	182	182	205	205	141	141	161	161	184	184	156	156	180	180
N_IGUFs09	172	172	182	182	205	205	141	141	161	161	210	210	156	156	180	180
N_IGUFs10	172	172	182	182	205	205	141	141	161	161	210	210	156	156	180	180
N_IGUFs11	172	172	182	182	205	205	141	141	161	161	184	184	156	156	180	180
N_IGUFs12	172	172	182	182	205	205	141	141	161	161	184	184	156	156	180	180
N_IGUFs13	172	172	182	182	205	205	141	141	161	161	210	210	156	156	180	180
N_IGUFs14	172	172	182	182	205	205	141	141	161	161	210	210	156	156	180	180

N_IGUFs15	172	172	182	182	205	205	141	141	161	161	210	210	156	156	180	180
N_IGUFs16	174	174	182	182	205	205	141	141	161	161	184	210	156	156	180	180
N_IGUFs17	172	172	182	182	205	205	141	141	161	161	210	210	156	156	180	180
N_IGUFs18	172	172	182	182	205	205	141	141	161	161	210	210	156	156	180	180
N_IGUFs19	172	172	182	182	205	205	141	141	161	161	210	210	156	156	180	180
N_IGUFs20	172	172	182	182	205	205	141	141	161	161	184	184	156	156	180	180
N_IGUFs21	172	172	182	182	205	205	141	141	161	161	210	210	156	156	180	180
N_IGUFs22	172	172	182	182	205	205	141	141	161	161	210	210	156	156	180	180
N_IGUFs23	172	172	182	182	205	205	141	141	161	161	210	210	156	156	180	180
N_IGUFs24	172	172	182	182	205	205	141	141	161	161	210	210	156	156	180	180
N_IGUFs25	172	176	182	182	205	235	141	141	161	161	210	216	156	198	180	189
N_IGUFs26	172	172	182	182	205	205	141	141	161	161	210	210	156	156	180	180
N_IGUFs27	172	172	182	182	205	205	141	141	161	161	184	184	156	156	180	180
N_IGUFs28	174	174	182	182	205	205	141	141	161	161	184	184	156	156	180	180
N_IGUFs29	172	172	182	182	205	205	141	141	161	161	210	210	156	156	180	180
N_IGUFs30	172	172	182	182	205	205	141	141	161	161	184	184	156	156	180	180
N_ITOFs01	176	176	182	182	205	205	141	141	161	161	174	174	156	156	180	180
N_ITOFs02	172	172	182	182	205	205	141	141	161	161	184	184	156	156	180	180
N_ITOFs03	172	172	182	182	205	205	141	141	161	161	184	184	156	156	180	180
N_ITOFs04	172	172	182	182	205	205	141	141	161	161	182	182	156	156	180	180
N_ITOFs05	172	172	182	182	205	205	141	141	161	161	184	184	156	156	180	180
N_ITOFs06	172	176	182	182	205	205	141	141	161	161	174	184	156	156	180	180
N_ITOFs07	176	176	182	182	205	205	141	141	161	161	174	174	156	156	180	180
N_ITOFs08	176	176	182	182	205	205	141	141	161	161	174	174	156	156	180	180
N_ITOFs09	172	176	182	182	205	205	141	141	161	161	174	182	156	156	180	180
N_ITOFs10	172	172	182	182	205	205	141	141	161	161	184	184	156	156	180	180
N_ITOFs11	176	176	182	182	205	205	141	141	161	161	174	174	156	156	180	180
N_ITOFs12	172	172	182	182	205	205	141	141	161	161	184	184	156	156	180	180
N_ITOFs13	176	176	182	182	205	205	141	141	161	161	174	174	156	156	180	180
N_ITOFs14	176	176	182	182	205	205	141	141	161	161	184	184	156	156	180	180
N_ITOFs15	172	172	182	182	205	205	141	141	161	161	174	174	156	156	180	180
N_ITOFs16	176	176	182	182	205	205	141	141	161	161	174	174	156	156	180	180
N_ITOFs17	172	172	182	182	205	205	141	141	161	161	184	184	156	156	180	180
N_ITOFs18	176	176	182	182	205	205	141	141	161	161	174	174	156	156	180	180
N_ITOFs19	172	172	182	182	205	205	141	141	161	161	174	174	156	156	180	180

N_ITOFs20	172	172	182	182	205	205	141	141	161	161	184	184	156	156	180	180
N_ITOFs21	172	172	182	182	205	205	141	141	161	161	184	184	156	156	180	180
N_ITOFs22	176	176	182	182	205	205	141	141	161	161	174	174	156	156	180	180
N_ITOFs23	176	176	182	182	205	205	141	141	161	161	174	174	156	156	180	180
N_ITOFs24	176	176	182	182	205	205	141	141	161	161	182	182	156	156	180	180
N_ITOFs25	176	176	182	182	205	205	141	141	161	161	174	174	156	156	180	180
N_ITOFs26	176	176	182	182	205	205	141	141	161	161	174	174	156	156	180	180
N_ITOFs27	176	176	182	182	205	205	141	141	161	161	174	174	156	156	180	180
N_ITOFs28	172	172	182	182	205	205	141	141	161	161	174	174	156	156	180	180
N_ITOFs29	176	176	182	182	205	205	141	141	161	161	174	174	156	156	180	180
N_ITOFs30	176	176	182	182	205	205	141	141	161	161	174	174	156	156	180	180
S_COBFs01	168	168	155	155	205	205	141	141	161	161	174	174	171	171	171	171
S_COBFs02	168	168	155	155	205	205	141	141	161	161	174	174	171	171	171	171
S_COBFs03	168	168	155	155	205	205	141	141	161	161	174	174	171	171	171	171
S_COBFs04	168	168	155	155	205	205	141	141	161	161	174	174	171	171	171	171
S_COBFs05	168	168	155	155	205	205	141	141	161	161	174	174	171	171	171	171
S_COBFs06	176	178	179	179	226	226	145	145	165	173	178	178	183	186	195	195
S_COBFs07	168	168	155	155	205	205	141	141	161	161	174	174	171	171	171	171
S_COBFs08	168	168	155	155	205	205	141	141	161	161	174	174	171	171	171	171
N_YGUFsfn01	172	172	182	182	205	205	141	141	161	161	182	182	156	156	180	180
N_YGUFsfn02	172	172	182	182	205	205	141	141	161	161	182	182	156	156	180	180
N_YGUFsfn03	172	172	182	182	205	205	141	141	161	161	182	182	156	156	180	180
N_YGUFsfn04	170	170	182	182	205	205	141	141	161	161	182	182	156	156	180	180
N_YGUFsfn05	172	172	182	182	205	205	141	141	161	161	182	182	156	156	180	180
N_YGUFsfn06	172	172	182	182	205	205	141	141	161	161	182	182	156	156	180	180
N_YGUFsfn07	172	172	182	182	205	205	141	141	161	161	182	182	156	156	180	180
N_YGUFsfn08	172	172	182	182	205	205	141	141	161	161	182	182	156	156	180	180
N_YGUFsfn09	172	172	182	182	205	205	141	141	161	161	182	182	156	156	180	180
N_YGUFsfn10	172	172	182	182	205	205	141	141	161	161	182	182	156	156	180	180
N_YGUFsfn11	172	172	182	182	205	205	141	141	161	161	182	182	156	156	180	180
N_YGUFsfn12	172	172	182	182	205	205	141	141	161	161	182	182	156	156	180	180
N_YGUFsfn13	172	172	182	182	205	205	141	141	161	161	182	182	156	156	180	180
N_YGUFsfn14	172	172	182	182	205	205	141	141	161	161	182	182	156	156	180	180
N_YGUFsfn15	172	172	182	182	205	205	141	141	161	161	182	182	156	156	180	180
N_YGUFsfn16	172	172	182	182	205	205	141	141	161	161	182	182	156	156	180	180

N_YGUFsfn17	172	172	182	182	205	205	141	141	161	161	182	182	156	156		180	180
N_YGUFsfn18	172	172	182	182	205	205	141	141	161	161	182	182	156	156		180	180
N_YGUFsfn19	172	172	182	182	205	205	141	141	161	161	182	182	156	156		180	180
N_YGUFsfn20	172	172	182	182	205	205	141	141	161	161	182	182	156	156		180	180
N_YGUFsfn21	172	172	182	182	205	205	141	141	161	161	182	182	156	156		180	180
N_YGUFsfn22	172	172	182	182	205	205	141	141	161	161	182	182	156	156		180	180
N_YGUFsfn23	172	172	182	182	205	205	141	141	161	161	182	182	156	156		180	180
N_YGUFsfn24	172	172	182	182	205	205	141	141	161	161	182	182	156	156		180	180
N_YGUFsfn25	172	172	182	182	205	205	141	141	161	161	182	182	156	156		180	180
N_YGUFsfn26	172	172	182	182	205	205	141	141	161	161	182	182	156	156		180	180
N_YGUFsfn27	172	172	182	182	205	205	141	141	161	161	182	182	156	156		180	180
N_YGUFsfn28	172	172	182	182	205	205	141	141	161	161	182	182	156	156		180	180
N_YGUFsfn29	172	172	182	182	205	205	141	141	161	161	182	182	156	156		180	180
N_YGUFsfn30	172	172	182	182	205	205	141	141	161	161	182	182	156	156		180	180
S_BAKFg01	188	188	167	167	205	205	141	141	161	161	182	182	156	156		180	180
S_BAKFg02	182	182	167	167	205	205	141	141	161	161	174	174	156	156		180	180
S_BAKFg03	188	188	167	167	205	205	141	141	161	161	182	182	156	156		180	180
S_BAKFg04	000	000	167	167	205	205	141	141	161	161	174	210	156	156		180	180
S_BAKFg05	188	188	167	167	205	205	141	141	161	161	182	182	156	156		180	180
S_BAKFg06	182	182	167	167	205	205	141	141	161	161	174	174	156	156		180	180
S_BAKFg07	188	188	167	167	205	205	141	141	161	161	182	182	156	156		180	180
S_BAKFg08	188	188	167	167	205	205	141	141	161	161	182	182	156	156		180	180
N_LYGFv01	150	150	182	191	226	226	141	143	169	189	174	182	186	186		195	204
N_LYGFv02	150	150	173	179	226	241	153	153	167	167	182	182	177	183	186	189	195
N_LYGFv03	150	156	164	182	226	226	141	145	167	169	174	176	186	195		189	195
N_LYGFv04	156	168	173	179	205	241	143	145	161	171	174	174	186	195		177	195
N_LYGFv05	150	168	179	191	205	235	145	145	167	173	174	182	186	195		189	189
N_LYGFv06	156	156	173	176	226	235	141	145	161	161	172	228	183	189	201	189	204
N_LYGFv07	150	168	179	188	235	235	141	145	167	171	174	174	186	195		204	209
N_LYGFv08	150	156	173	179	226	229	145	145	167	183	174	212	177	201		189	195
N_LYGFv09	150	176	179	179	226	235	143	143	171	175	174	182	186	207		189	195
N_LYGFv10	150	156	188	188	226	229	141	141	167	167	174	182	195	201		189	201
N_LYGFv11	156	184	173	173	205	205	141	145	171	179	182	208	186	210		189	195
N_LYGFv12	150	156	179	191	226	235	145	145	169	171	172	182	201	210		189	204
N_LYGFv13	138	150	164	173	226	241	145	145	167	169	174	182	198	207		189	195

N_LYGFv14	150	150	173	173	205	226	141	145	167	167	172	176	182	186	195		189	195
N_LYGFv15	152	152	179	191	205	205	141	145	163	183	174	182		189	195		189	189
N_LYGFv16	150	150	173	188	226	235	145	145	167	167	174	182		195	201		189	195
N_LYGFv17	150	168	164	188	205	205	145	145	167	171	174	228		186	195		189	195
N_LYGFv18	150	176	191	191	205	235	145	145	167	175	182	182		183	186	207	189	195
N_IEGFv01	152	158	176	191	226	226	141	145	165	181	182	216		171	201		195	195
N_IEGFv02	150	150	179	188	205	217	141	145	165	167	174	176		195	195		189	201
N_IEGFv03	152	158	173	176	226	235	141	145	161	161	176	208		171	201		195	195
N_IEGFv04	144	144	173	173	205	226	143	145	165	175	000	000		186	201		195	204
N_IEGFv05	150	152	173	185	205	226	143	143	169	171	172	176		186	201		189	189
N_IEGFv06	150	150	179	188	205	217	141	145	167	167	174	176		195	195		189	201
N_IEGFv07	148	150	173	176	217	226	143	145	169	173	176	212		195	198		189	195
N_IEGFv08	150	150	173	173	207	207	143	143	167	171	182	182		186	201		183	189
N_IEGFv09	144	148	173	182	205	226	145	145	161	165	174	176		186	195		204	204
N_IEGFv10	152	158	176	191	226	226	141	145	165	181	182	182		171	201		195	195
N_IEGFv11	150	150	179	179	205	205	143	143	167	171	172	174		186	186		189	195
N_IEGFv12	156	172	188	188	226	226	141	147	165	169	176	176		186	186		189	195
N_IEGFv13	150	150	173	173	205	235	143	143	161	171	172	172		186	195		174	195
N_IEGFv14	144	156	170	191	226	226	145	145	163	173	174	182		177	201		189	201
N_IEGFv15	150	154	188	188	205	205	145	145	167	167	174	182		186	195		195	204
N_IEGFv16	150	150	188	188	226	226	143	151	167	175	182	182		183	186		189	195
N_IEGFv17	144	144	176	188	205	205	143	145	167	175	176	182		195	201		189	204
N_IEGFv18	144	156	176	179	205	226	143	143	171	175	172	182		186	201		204	204
N_IEGFv19	150	180	176	176	205	226	141	141	163	169	176	176		195	195		189	204
N_IEGFv20	150	156	179	188	226	226	145	145	171	171	174	182		186	201		204	204
S_MUXFv01	150	150	179	197	205	226	145	145	161	167	174	180		171	183		195	195
S_MUXFv02	150	150	179	206	205	205	145	145	161	167	178	196		171	183	186	189	189
S_MUXFv03	150	150	197	206	205	205	145	145	167	169	196	196		183	186		189	189
S_MUXFv04	150	150	179	206	205	205	145	145	161	167	178	180		171	183	186	189	195
S_MUXFv05	150	150	179	188	205	205	145	145	161	167	180	196		171	183		189	189
S_MUXFv06	150	150	194	194	205	205	145	145	167	183	178	180		171	183	186	189	189
S_MUXFv07	150	166	194	197	205	205	145	145	161	183	174	180		171	183	189	189	189
S_MUXFv08	150	150	173	179	205	226	145	145	167	167	178	180		171	183		183	189
S_MUXFv09	150	150	173	194	226	226	145	145	161	167	178	196		171	183		189	189
S_MUXFv10	150	150	206	206	205	205	143	145	167	167	196	196		171	183	186	183	189

S_MUXFv11	150	150	173	194	205	205		145	145	161	161	180	196		171	183	186	189	189
S_MUXFv12	150	150	197	206	226	244		145	145	161	161	178	180		171	183		189	189
S_MUXFv13	150	166	173	206	205	205		145	149	161	167	178	178		171	183		189	189
S_MUXFv14	166	166	179	197	205	205		145	149	167	167	180	196		171	183	186	189	195
S_MUXFv15	176	176	173	194	205	205		145	145	161	161	178	180		171	183	186	189	189
S_MUXFv16	150	166	194	206	205	205		141	145	161	167	180	196		183	186		189	189
S_MUXFv17	150	150	173	179	205	205		145	145	161	167	178	178		171	183		189	195
S_MUXFv18	150	150	194	197	205	226		145	149	167	167	178	196		171	183		189	189
N_LYGFc01	150	156	188	188	205	226		141	145	161	165	176	182		186	189		195	198
N_LYGFc02	150	156	188	188	205	226		141	145	161	165	176	182		186	189		195	198
N_LYGFc03	150	156	188	188	205	226		141	145	161	165	176	182		186	189		195	198
N_LYGFc04	150	156	188	188	205	226		141	145	161	165	176	182		186	189		195	198
N_LYGFc05	150	156	188	188	205	226		141	145	161	165	176	182		186	189		195	198
N_LYGFc06	150	156	188	188	205	226		141	145	161	165	176	182		186	189		195	198
N_LYGFc07	150	156	188	188	205	226		141	145	161	165	176	182		186	189		195	198
N_LYGFc08	150	156	188	188	205	226		141	145	161	165	176	182		186	189		195	198
N_LYGFc09	150	156	188	188	205	226		141	145	161	165	176	182		186	189		195	198
N_LYGFc10	150	156	188	188	205	226		141	145	161	165	176	182		186	189		195	198
N_LYGFc11	150	156	188	188	205	226		141	145	161	165	176	182		186	189		195	198
N_LYGFc12	150	156	188	188	205	226		141	145	161	165	176	182		186	189		195	198
N_LYGFc13	150	156	188	188	205	226		141	145	161	165	176	182		186	189		195	198
N_LYGFc14	150	156	188	188	205	226		141	145	161	165	176	182		186	189		195	198
N_LYGFc15	150	156	188	188	205	226		141	145	161	165	176	182		186	189		195	198
N_LYGFc16	150	156	188	188	205	226		141	145	161	165	176	182		186	189		195	198
N_LYGFc17	150	156	188	188	205	226		141	145	161	165	176	182		186	189		195	198
N_LYGFc18	150	156	188	188	205	226		141	145	161	165	176	182		186	189		195	198
N_LYGFc19	150	156	188	188	205	226		141	145	161	165	176	182		186	189		195	198
N_LYGFc20	150	156	188	188	205	226		141	145	161	165	176	182		186	189		195	198
N_IEGFc01	146	152	182	191	205	226	235	141	145	159	167	174	208	210	165	195		189	209
N_IEGFc02	146	152	182	191	205	226	235	141	145	159	167	174	208	210	165	195		189	209
N_IEGFc03	146	152	182	191	205	226	235	141	145	159	167	174	208	210	165	195		189	209
N_IEGFc04	146	152	182	191	205	226	235	141	145	159	167	174	208	210	165	195		189	209
N_IEGFc05	146	152	182	191	205	226	235	141	145	159	167	174	208	210	165	195		189	209
N_IEGFc06	146	152	182	191	205	226	235	141	145	159	167	174	208	210	165	195		189	209
N_IEGFc07	146	152	182	191	205	226	235	141	145	159	167	174	208	210	165	195		189	209

N_IEGFc08	146	152	182	191	205	226	235	141	145	159	167	174	208	210	165	195		189	209
N_IEGFc09	146	152	182	191	205	226	235	141	145	159	167	174	208	210	165	195		189	209
N_IEGFc10	146	152	182	191	205	226	235	141	145	159	167	174	208	210	165	195		189	209
N_IEGFc11	146	152	182	191	205	226	235	141	145	159	167	174	208	210	165	195		189	209
N_IEGFc12	146	152	182	191	205	226	235	141	145	159	167	174	208	210	165	195		189	209
N_IEGFc13	146	152	182	191	205	226	235	141	145	159	167	174	208	210	165	195		189	209
N_IEGFc14	146	152	182	191	205	235	000	141	145	159	167	174	208	210	165	195		189	209
N_IEGFc15	146	152	182	191	205	226	235	141	145	159	167	174	208	210	165	195		189	209
N_IEGFc16	146	152	182	191	205	226	235	141	145	159	167	174	208	210	165	195		189	209
N_IEGFc17	146	152	182	191	205	226	235	141	145	159	167	174	208	210	165	195		189	209
N_IEGFc18	146	152	182	191	205	226	235	141	145	159	167	174	208	210	165	195		189	209
N_IEGFc19	146	152	182	191	205	226	235	141	145	159	167	174	208	210	165	195		189	209
N_IEGFc20	146	152	182	191	205	226	235	141	145	159	167	174	208	210	165	195		189	209
N_IEGFc21	146	152	182	191	205	226	235	141	145	159	167	174	208	210	165	195		189	209
N_IEGFc22	000	000	182	191	205	226	235	141	145	159	167	174	208	210	165	195		189	209
N_IEGFc23	146	152	182	191	205	226	235	141	145	159	167	174	208	210	165	195		189	209
N_IEGFc24	146	152	182	191	205	226	235	141	145	159	167	174	208	210	165	195		189	209
N_IEGFc25	146	152	182	191	205	226	235	141	145	159	167	174	208	210	165	195		189	209
N_IEGFc26	146	152	182	191	205	226	235	141	145	159	167	174	208	210	165	195		189	209
N_IEGFc27	146	152	182	191	205	226	235	141	141	159	167	174	208	210	165	195		189	209
N_IEGFc28	146	152	182	191	205	226	235	141	145	159	167	174	208	210	165	195		189	209
N_IEGFc29	146	152	182	191	205	226	235	141	145	159	167	174	208	210	165	195		189	209
N_IEGFc30	146	152	182	191	205	226	235	141	145	159	167	174	208	210	165	195		189	209
S_COBFch01	150	176	170	179	205	205		145	145	159	159	178	178		186	189		195	195
S_COBFch02	150	178	170	179	205	205		143	145	161	167	178	178		183	186		195	195
S_COBFch03	150	168	179	185	205	205		143	145	171	173	172	178		183	189		195	195
S_COBFch04	150	178	170	179	205	226		145	145	159	165	178	182		171	186		195	195
S_COBFch05	168	176	179	179	205	226		145	145	173	173	178	178		186	189		195	195
S_COBFch06	176	178	170	179	226	226		143	143	165	165	178	178		183	189		195	195
S_COBFch07	150	180	179	179	205	226		145	145	159	165	178	182		171	186		195	195
S_TALFch01	168	168	155	155	205	205		141	141	161	161	174	174		171	171		171	171
S_TALFch02	168	168	155	155	205	205		141	141	161	161	174	174		171	171		180	180
S_TALFch03	168	168	155	155	205	205		141	141	161	161	174	174		171	171		180	180
S_TALFch04	168	168	155	155	205	205		141	141	161	161	174	174		171	171		171	171
S_TALFch05	000	000	179	179	226	226		155	155	173	173	178	178		186	186		000	000

S_TALFch06	168	168	155	155	205	205	141	141	161	161	174	174	171	171	171	171
S_TALFch07	168	168	155	155	205	205	141	141	161	161	168	168	171	171	171	171
N_IEGFsp01	150	156	173	179	217	235	143	143	171	173	176	176	189	195	189	189
N_IEGFsp02	150	150	173	179	235	235	143	147	167	173	176	182	189	195	189	189
N_IEGFsp03	150	150	173	176	226	235	145	147	167	167	176	182	186	195	189	189
N_IEGFsp04	150	150	173	176	226	235	145	147	167	167	176	182	186	195	189	189
N_IEGFsp05	150	150	173	179	235	235	143	147	167	173	176	182	189	195	189	189
N_IEGFsp06	150	150	173	179	235	235	143	147	167	173	176	182	189	195	189	189
N_IEGFsp07	150	150	173	179	235	235	143	147	167	173	176	182	189	195	189	189
N_IEGFsp08	150	150	173	179	235	235	143	147	167	173	176	182	189	195	189	189

B. Linkage disequilibrium

Table B. P-values and standard error (S.E.) for 16 samples and pairs of loci. *Fucus cottonii* (N_IEGFc, N_LYGFc) was removed since there is no connection with the other samples due to asexual reproduction (clones). Asterisk indicates significant P-values after Bonferroni correction; $\alpha = 0.05$; $P \leq 0.0003$).

Sample	Locus#1	Locus#2	P-Value	S.E.
N_LYGFs	Fsp4	L20	0.0000*	0.0000
N_LYGFs	Fsp4	L58	0.0015	0.0006
N_LYGFs	L20	L58	0.0128	0.0018
N_LYGFs	Fsp4	Fsp1	0.0000*	0.0000
N_LYGFs	L20	Fsp1	0.0001*	0.0001
N_LYGFs	L58	Fsp1	0.0006	0.0003
N_LYGFs	Fsp4	L94	0.0003*	0.0002
N_LYGFs	L20	L94	0.0139	0.0032
N_LYGFs	L58	L94	0.0004	0.0003
N_LYGFs	Fsp1	L94	0.0007	0.0006
N_IEGFs	Fsp4	L20	0.0001*	0.0001
N_IEGFs	Fsp4	L58	0.0022	0.0006
N_IEGFs	L20	L58	0.0002	0.0001
N_IEGFs	Fsp4	Fsp1	0.0001*	0.0001
N_IEGFs	L20	Fsp1	0.0000*	0.0000
N_IEGFs	L58	Fsp1	0.0001*	0.0001
N_IEGFs	Fsp4	L94	0.0001*	0.0001
N_IEGFs	L20	L94	0.0002*	0.0002
N_IEGFs	L58	L94	0.0017	0.0005
N_IEGFs	Fsp1	L94	0.0000*	0.0000
N_YEGFs1	Fsp4	L20	-	-
N_YEGFs1	Fsp4	L58	-	-
N_YEGFs1	L20	L58	-	-
N_YEGFs1	Fsp4	Fsp1	-	-
N_YEGFs1	L20	Fsp1	-	-
N_YEGFs1	L58	Fsp1	-	-
N_YEGFs1	Fsp4	L94	-	-
N_YEGFs1	L20	L94	-	-
N_YEGFs1	L58	L94	-	-
N_YEGFs1	Fsp1	L94	-	-
N_YEGFs2	Fsp4	L20	-	-
N_YEGFs2	Fsp4	L58	-	-
N_YEGFs2	L20	L58	-	-
N_YEGFs2	Fsp4	Fsp1	-	-
N_YEGFs2	L20	Fsp1	-	-
N_YEGFs2	L58	Fsp1	-	-
N_YEGFs2	Fsp4	L94	-	-
N_YEGFs2	L20	L94	-	-

N_YEGFs2	L58	L94	-	-
N_YEGFs2	Fsp1	L94	-	-
N_IGUFs	Fsp4	L20	-	-
N_IGUFs	Fsp4	L58	-	-
N_IGUFs	L20	L58	-	-
N_IGUFs	Fsp4	Fsp1	-	-
N_IGUFs	L20	Fsp1	-	-
N_IGUFs	L58	Fsp1	-	-
N_IGUFs	Fsp4	L94	0.0625	0.0026
N_IGUFs	L20	L94	-	-
N_IGUFs	L58	L94	-	-
N_IGUFs	Fsp1	L94	-	-
N_ITOFs	Fsp4	L20	-	-
N_ITOFs	Fsp4	L58	-	-
N_ITOFs	L20	L58	-	-
N_ITOFs	Fsp4	Fsp1	-	-
N_ITOFs	L20	Fsp1	-	-
N_ITOFs	L58	Fsp1	-	-
N_ITOFs	Fsp4	L94	-	-
N_ITOFs	L20	L94	-	-
N_ITOFs	L58	L94	-	-
N_ITOFs	Fsp1	L94	-	-
S_COBFs	Fsp4	L20	0.1246	0.0018
S_COBFs	Fsp4	L58	0.1215	0.0014
S_COBFs	L20	L58	0.1215	0.0015
S_COBFs	Fsp4	Fsp1	0.1259	0.0018
S_COBFs	L20	Fsp1	0.1255	0.0016
S_COBFs	L58	Fsp1	0.1237	0.0016
S_COBFs	Fsp4	L94	0.1267	0.0016
S_COBFs	L20	L94	0.1255	0.0016
S_COBFs	L58	L94	0.1236	0.0018
S_COBFs	Fsp1	L94	0.1241	0.0016
N_YGUFsfn	Fsp4	L20	-	-
N_YGUFsfn	Fsp4	L58	-	-
N_YGUFsfn	L20	L58	-	-
N_YGUFsfn	Fsp4	Fsp1	-	-
N_YGUFsfn	L20	Fsp1	-	-
N_YGUFsfn	L58	Fsp1	-	-
N_YGUFsfn	Fsp4	L94	-	-
N_YGUFsfn	L20	L94	-	-
N_YGUFsfn	L58	L94	-	-
N_YGUFsfn	Fsp1	L94	-	-
S_BAKFg	Fsp4	L20	-	-
S_BAKFg	Fsp4	L58	-	-
S_BAKFg	L20	L58	-	-

S_BAKFg	Fsp4	Fsp1	-	-
S_BAKFg	L20	Fsp1	-	-
S_BAKFg	L58	Fsp1	-	-
S_BAKFg	Fsp4	L94	-	-
S_BAKFg	L20	L94	-	-
S_BAKFg	L58	L94	-	-
S_BAKFg	Fsp1	L94	-	-
N_LYGFv	Fsp4	L20	1.0000	0.0000
N_LYGFv	Fsp4	L58	0.9735	0.0051
N_LYGFv	L20	L58	0.4886	0.0298
N_LYGFv	Fsp4	Fsp1	0.0046	0.0025
N_LYGFv	L20	Fsp1	1.0000	0.0000
N_LYGFv	L58	Fsp1	1.0000	0.0000
N_LYGFv	Fsp4	L94	0.5666	0.0245
N_LYGFv	L20	L94	0.3379	0.0292
N_LYGFv	L58	L94	0.3244	0.0212
N_LYGFv	Fsp1	L94	0.2325	0.0278
N_IEGFv1	Fsp4	L20	0.0718	0.0073
N_IEGFv1	Fsp4	L58	0.0708	0.0059
N_IEGFv1	L20	L58	0.2493	0.0080
N_IEGFv1	Fsp4	Fsp1	0.1296	0.0163
N_IEGFv1	L20	Fsp1	0.0788	0.0116
N_IEGFv1	L58	Fsp1	0.2772	0.0123
N_IEGFv1	Fsp4	L94	0.0012	0.0007
N_IEGFv1	L20	L94	0.0297	0.0058
N_IEGFv1	L58	L94	0.0078	0.0020
N_IEGFv1	Fsp1	L94	0.0959	0.0175
N_IEGFv2	Fsp4	L20	1.0000	0.0000
N_IEGFv2	Fsp4	L58	0.4956	0.0175
N_IEGFv2	L20	L58	1.0000	0.0000
N_IEGFv2	Fsp4	Fsp1	1.0000	0.0000
N_IEGFv2	L20	Fsp1	1.0000	0.0000
N_IEGFv2	L58	Fsp1	1.0000	0.0000
N_IEGFv2	Fsp4	L94	0.3948	0.0180
N_IEGFv2	L20	L94	0.2895	0.0184
N_IEGFv2	L58	L94	1.0000	0.0000
N_IEGFv2	Fsp1	L94	1.0000	0.0000
S_MUXFv	Fsp4	L20	0.6581	0.0152
S_MUXFv	Fsp4	L58	0.1289	0.0081
S_MUXFv	L20	L58	0.1473	0.0111
S_MUXFv	Fsp4	Fsp1	0.3630	0.0120
S_MUXFv	L20	Fsp1	0.6378	0.0220
S_MUXFv	L58	Fsp1	0.7133	0.0109
S_MUXFv	Fsp4	L94	0.4924	0.0095
S_MUXFv	L20	L94	0.2218	0.0139

S_MUXFv	L58	L94	0.6870	0.0098
S_MUXFv	Fsp1	L94	0.6102	0.0114
S_COBFch	Fsp4	L20	0.3392	0.0095
S_COBFch	Fsp4	L58	1.0000	0.0000
S_COBFch	L20	L58	0.7712	0.0043
S_COBFch	Fsp4	Fsp1	1.0000	0.0000
S_COBFch	L20	Fsp1	1.0000	0.0000
S_COBFch	L58	Fsp1	0.3318	0.0096
S_COBFch	Fsp4	L94	-	-
S_COBFch	L20	L94	-	-
S_COBFch	L58	L94	-	-
S_COBFch	Fsp1	L94	-	-
S_TALFch	Fsp4	L20	-	-
S_TALFch	Fsp4	L58	-	-
S_TALFch	L20	L58	0.1412	0.0017
S_TALFch	Fsp4	Fsp1	-	-
S_TALFch	L20	Fsp1	0.1421	0.0018
S_TALFch	L58	Fsp1	0.1432	0.0018
S_TALFch	Fsp4	L94	-	-
S_TALFch	L20	L94	-	-
S_TALFch	L58	L94	-	-
S_TALFch	Fsp1	L94	-	-
N_IEGFsp	Fsp4	L20	1.0000	0.0000
N_IEGFsp	Fsp4	L58	0.1251	0.0021
N_IEGFsp	L20	L58	0.0376	0.0014
N_IEGFsp	Fsp4	Fsp1	0.1208	0.0023
N_IEGFsp	L20	Fsp1	0.0345	0.0015
N_IEGFsp	L58	Fsp1	0.0070	0.0010
N_IEGFsp	Fsp4	L94	-	-
N_IEGFsp	L20	L94	-	-
N_IEGFsp	L58	L94	-	-
N_IEGFsp	Fsp1	L94	-	-

C. Summary statistics

Table C. Estimates of the genetic diversity for the complete dataset (per sample and per loci). Number of individuals (N), number of alleles (*Na*), allelic richness (*Ar*), observed heterozygosity (H_O), expected heterozygosity (H_E), standard deviation (S.D), inbreeding coefficient (F_{IS}). Asterisk indicates significant P-values after Bonferroni correction; $\alpha = 0.05$; $P \leq 0.0028$. NA = monomorphic.

Sample	**Fsp4**	**L20**	**L58**	**Fsp1**	**L94**
N_LYGFs					
N	20	20	20	20	20
Na	6	4	3	5	4
Ar	3.6300	2.1150	2.0310	2.4150	2.1150
H_O	0.2500	0.2000	0.2000	0.2500	0.2000
H_E	0.6579	0.1908	0.1895	0.2355	0.1908
P-value	0.0000*	1.0000	1.0000	1.0000	1.0000
S.D	0.0000	0.0000	0.0000	0.0000	0.0000
F_{IS}	0.6200	-0.0480	-0.0560	-0.0610	-0.0480
N_IEGFs					
N	20	20	20	20	20
Na	7	6	4	5	3
Ar	4.6980	3.0840	2.5460	2.8460	2.2360
H_O	0.1500	0.2000	0.1000	0.1500	0.0500
H_E	0.7974	0.3632	0.2816	0.3237	0.3447
P-value	0.0000*	0.0000*	0.0001*	0.0003	0.0001*
S.D	0.0000	0.0000	00000	0.0000	0.0000
F_{IS}	0.8120	0.4490	0.6450	0.5370	0.8550
N_YEGFs1					
N	10	10	10	10	10
Na	3	1	1	1	1
Ar	2.7050	1.0000	1.0000	1.0000	1.0000
H_O	0.0000	0.0000	0.0000	0.0000	0.0000
H_E	0.3778	0.0000	0.0000	0.0000	0.0000
P-value	0.0030	NA	NA	NA	NA
S.D	0.0001	NA	NA	NA	NA
F_{IS}	1.0000	NA	NA	NA	NA
N_YEGFs2					
N	20	20	20	20	20
Na	3	1	1	1	1
Ar	2.9590	1.0000	1.0000	1.0000	1.0000
H_O	0.0500	0.0000	0.0000	0.0000	0.0000
H_E	0.6421	0.0000	0.0000	0.0000	0.0000
P-value	0.0000*	NA	NA	NA	NA
S.D	0.0000	NA	NA	NA	NA
F_{IS}	0.9220	NA	NA	NA	NA

N_IGUFs					
N	30	30	30	30	30
Na	3	1	1	1	2
Ar	1.8860	1.0000	1.0000	1.0000	1.2000
H_O	0.0667	0.0000	0.0000	0.0000	0.0333
H_E	0.1879	0.0000	0.0000	0.0000	0.0333
P-value	0.0042	NA	NA	NA	1.0000
S.D	0.0001	NA	NA	NA	0.0000
F_{IS}	0.6450	NA	NA	NA	0.0000
N_ITOFs					
N	30	30	30	30	30
Na	2	1	1	1	1
Ar	2.0000	1.0000	1.0000	1.0000	1.0000
H_O	0.0667	0.0000	0.0000	0.0000	0.0000
H_E	0.5069	0.0000	0.0000	0.0000	0.0000
P-value	0.0000*	NA	NA	NA	NA
S.D	0.0000	NA	NA	NA	NA
F_{IS}	08680	NA	NA	NA	NA
S_COBFs					
N	8	8	8	8	8
Na	3	2	2	3	2
Ar	2.5000	1.0000	1.9500	2.5000	1.9500
H_O	0.1250	0.0000	0.0000	0.1250	0.0000
H_E	0.2500	0.2500	0.2500	0.2500	0.2500
P-value	0.0652	0.0662	0.0672	0.0662	0.0664
S.D	0.0003	0.0003	0.0002	0.0002	0.0002
F_{IS}	0.5000	1.0000	1.0000	0.5000	1.0000
N_YGUFsfn					
N	30	30	30	30	30
Na	2	1	1	1	1
Ar	1.3630	1.0000	1.0000	1.0000	1.0000
H_O	0.0000	0.0000	0.0000	0.0000	0.0000
H_E	0.0667	0.0000	0.0000	0.0000	0.0000
P-value	0.0174	NA	NA	NA	NA
S.D	0.0001	NA	NA	NA	NA
F_{IS}	1.0000	NA	NA	NA	NA
S_BAKFg					
N	7	8	8	8	8
Na	2	1	1	1	1
Ar	2.0000	1.0000	1.0000	1.0000	1.0000
H_O	0.0000	0.0000	0.0000	0.0000	0.0000
H_E	0.4762	0.0000	0.0000	0.0000	0.0000
P-value	0.0211	NA	NA	NA	NA
S.D	0.0001	NA	NA	NA	NA

F_{IS}	1.0000	NA	NA	NA	NA
N_LYGFv					
N	18	18	18	18	18
Na	7	7	4	10	6
Ar	4.5860	54080	3.3680	5.9240	3.8170
H_O	0.6667	0.7222	0.4444	0.7222	0.8889
H_E	0.7010	0.8284	0.6029	0.7941	0.6634
P-value	0.4427	0.3348	0.0384	0.3974	0.0682
S.D	0.0004	0.0005	0.0002	0.0004	0.0003
F_{IS}	0.0490	0.1280	0.2630	0.0910	-0.3400
N_IEGFv1					
N	10	10	10	10	10
Na	5	7	3	8	5
Ar	4.7400	5.7430	2.9960	6.6910	4.4030
H_O	0.6000	0.8000	0.7000	0.8000	0.5000
H_E	0.7944	0.8056	0.6778	0.8889	0.7667
P-value	0.0120	0.1270	0.0825	0.3518	0.0928
S.D	0.0001	0.0003	0.0003	0.0003	0.0003
F_{IS}	0.2450	0.0070	-0.0330	0.1000	03480
N_IEGFv2					
N	10	10	10	10	10
Na	6	6	5	8	5
Ar	4.7710	5.0240	4.1510	6.4490	4.1960
H_O	0.6000	0.4000	0.3000	0.8000	0.8000
H_E	0.7556	0.8056	0.7500	0.8722	0.7556
P-value	0.2085	0.0197	0.0063	0.4733	0.4799
S.D	0.0005	0.0001	0.0001	0.0004	0.0005
F_{IS}	0.2060	0,5030	0.6000	0.0830	-0.0590
S_MUXFv					
N	18	18	18	18	18
Na	3	6	4	4	3
Ar	2.4490	5.1050	2.3830	2.8950	2.4490
H_O	0.1667	0.8889	0.2778	0.6111	0.2778
H_E	0.3431	0.8284	0.2565	0.5882	0.3399
P-value	0.0103	0.7090	1.0000	1.0000	0.5133
S.D	0.0001	0.0004	0.0000	0.0000	0.0005
F_{IS}	0.5140	-0.0730	-0.0830	-0.0390	0.1830
N_LYGFc					
N	20	20	20	20	20
Na	2	1	2	2	2
Ar	2.0000	1.0000	2.0000	2.0000	2.0000
H_O	1.0000	0.0000	1.0000	1.0000	1.0000
H_E	0.5000	0.0000	0.5000	0.5000	0.5000
P-value	0.0000*	NA	0,0000*	0.0000*	0.0000*

S.D	0.0000	NA	0,0000	0.0000	0.0000
F_{IS}	-1.0000	NA	-1,0000	-1.0000	-1.0000
N_IEGFc					
N	29	30	30	30	30
Na	2	2	2	2	2
Ar	2.0000	2.0000	2.0000	2.0000	2.0000
H_O	1.0000	1.0000	0.9667	1.0000	1.0000
H_E	0.5000	0.5000	0.5000	0.5000	0.5000
P-value	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*
S.D	0.0000	0.0000	0.0000	0.0000	0.0000
F_{IS}	-1.0000	-1.0000	-0.9330	-1.0000	-1.0000
S_COBFch					
N	7	7	7	7	7
Na	5	3	2	6	1
Ar	4.8460	2.8570	2,0000	5.5710	1.0000
H_O	1.0000	0.7143	0.2857	0.5714	0.0000
H_E	0.7976	0.5238	0.4524	0.8571	0.0000
P-value	1.0000	1.0000	0.4415	0.0453	NA
S.D	0.0000	0.0000	0.0005	0.0002	NA
F_{IS}	-0.2540	-0.3640	0.3680	0.3330	NA
S_TALFch					
N	6	7	7	7	6
Na	1	2	2	2	2
Ar	1.0000	1.9890	1.9890	1.9890	2.0000
H_O	0.0000	0.0000	0.0000	0.0000	0.0000
H_E	0.0000	0.2857	0.2857	0.2857	0.5333
P-value	NA	0.0767	0.0773	0.0767	0.0299
S.D	NA	0.0002	0.0003	0.0003	0.0001
F_{IS}	NA	1.0000	1.0000	1.0000	1.0000
N_IEGFsp					
N	8	8	8	8	8
Na	2	3	3	3	1
Ar	1.7500	2.9500	2.9500	2.7500	1.0000
H_O	0.125.0	1.0000	0.8750	0.7500	0.0000
H_E	0.1250	0.6071	0.6250	0.5625	0.0000
P-value	1.0000	0.0587	0.2844	0.2732	NA
S.D	0.0000	0.0002	0.0005	0.0004	NA
F_{IS}	0.0000	-0.6470	-0.4000	-0.3330	NA

Table D. Summary statistics per loci for all samples. Asterisk indicates significant P-values after Bonferroni correction; $\alpha = 0.05$; $P \leq 0.01$. Significant values were observed for Fsp4 ($P = 0.0000$).

Locus	***Na***	***Ar***	H_O	H_s/H_E	F_{IS}	F_{ST}	**P-value**	**S.E.**
Fsp4	64	6.252	0.326	0.472	0.310	0.457	0.0000*	0.0000
L20	43	4.916	0.329	0.330	0.003	0.581	0.4648	0.0121
L58	42	2.828	0.286	0.295	0.030	0.464	0.0513	0.0028
Fsp1	64	3.947	0.377	0.366	-0.030	0.405	0.5193	0.0152
L94	43	4.305	0.264	0.268	0.015	0.632	0.8709	0.0056

D. Allele frequency

The allele frequency distribution for *F. cottonii* (Figure VIII, A) showed different allele sizes between specimens from Eggholmane (N_IEGFc) and Lygra (N_LYGFc), in all loci except L58. *Fucus spiralis* f. *nanus* (N_YGUFsfn) allele frequency distribution was almost identical to *F. spiralis* (N_IGUFs, N_ITOFs), for all loci except Fsp4 (Figure VIII, B). For the Spanish samples (Figure VIII, C), there is more variation in the allele frequency distribution between the taxa. However, *F. chalonii* from Talaipe (S_TALFch) appear as more like *F. spiralis* from Cobarón (S_COBFs), and *F. chalonii* from Cobarón (S_COBFch) is presented as more similar to *F. vesiculosus* sampled in Muxía (S_MUXFv).

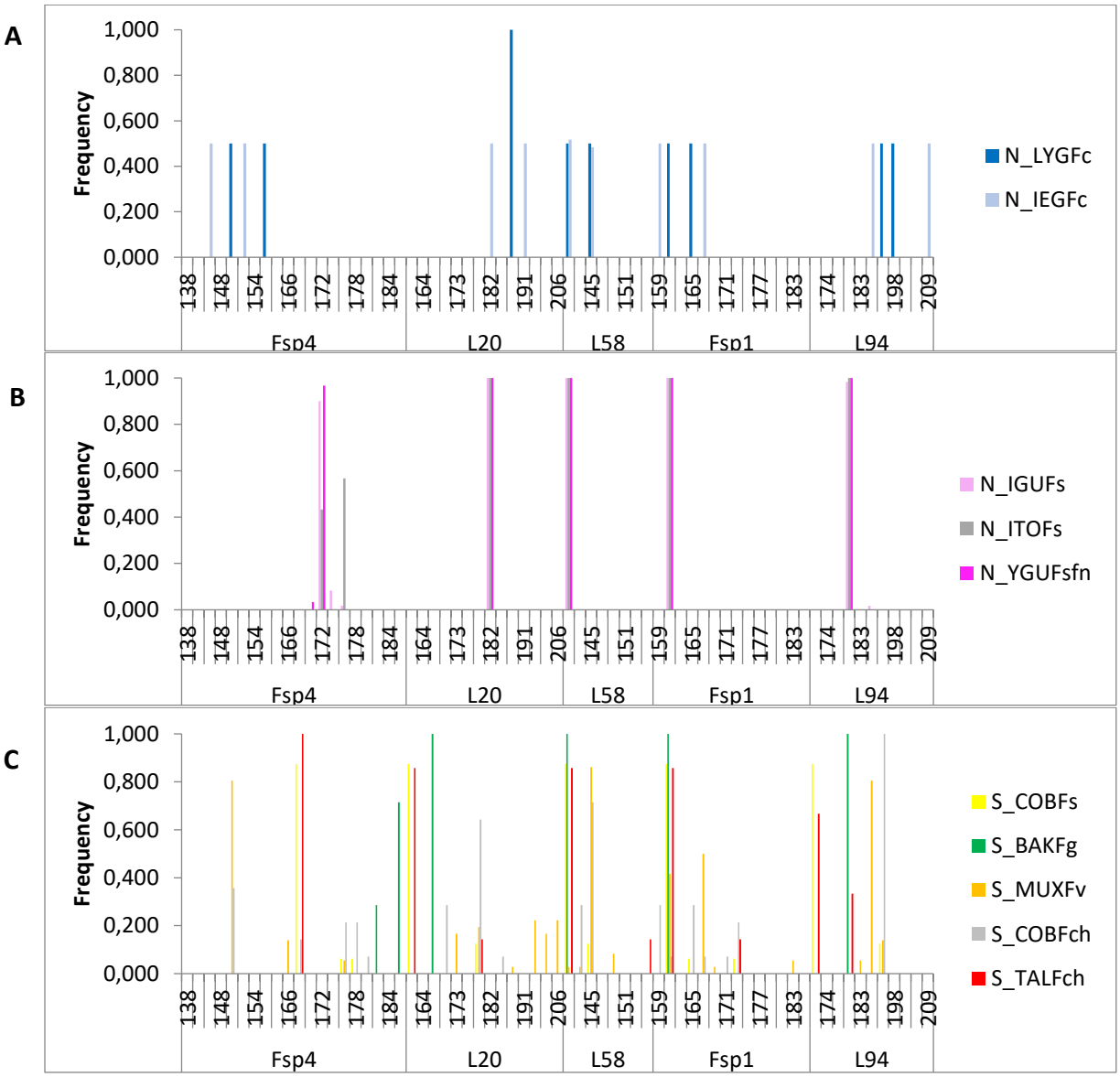


Figure VIII. Bar graph with allele frequencies at five microsatellite loci. **A.** *Fucus cottonii* from Indre Eggholmane and Lygra. **B.** *Fucus spiralis* f. *nanus* and *F. spiralis*, from Bømlo, Norway. **C.** *Fucus chalonii*, *F. guiryi*, *F, spiralis* and *F. vesiculosus* from Spain.

E. Evanno table outputs

Table E. Analysis of the Norwegian samples from Structure Harvester suggesting K = 4.

K	Reps	Mean	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
2	10	-2132.6700	0.5143	NA	NA	NA
3	10	-1912.7200	75.1375	219.950000	6.060000	0.080652
4	10	-1686.7100	97.1389	226.010000	81.280000	0.836740
5	10	-1541.9800	93.4626	144.730000	NA	NA

Table F. Analysis of the Spanish samples from Structure Harvester suggesting K = 4.

K	Reps	Mean	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
2	10	-452.0200	0.1874	NA	NA	NA
3	10	-377.1900	jan.19	74.830000	0.940000	0.733297
4	10	-303.3000	0.1700	73.890000	95.440000	561.519718
5	10	-324.8500	1.0835	-21.550000	NA	NA