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Complex Kelp: novel approaches to genetic and morphological characterisation of kelp

by

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Summary

Kelp are large brown macroalgae in the order Laminariales that provide the foundation for some of the most productive habitats on earth. While historically considered to be plants, molecular evidence has placed kelp in a distantly related clade known as the Stramenopiles, which have independently evolved multicellularity compared to plants. While progress is being made, there are still many fundamental evolutionary, developmental, and ecological questions about kelp that remain unanswered including taxonomic delineation of closely related species and their population genetic. Additionally, the lack of fundamental research in kelp when compared to other groups such as plants leads to naturally arising biases in the literature, such as individual author preferences for geographical locations, study species, and methodological approach. This thesis attempts to bridge some of these disparities by addressing knowledge gaps, reconciling conflict regarding some of these theoretical assumptions, and identifying overarching trends in the research.

The first systematic review of kelp population genetics in the north-east Atlantic was conducted in Chapter 2, with an emphasis on identifying overarching patterns of biogeography between species as well as research biases and methodologies. This review identified that there is a small core of individuals who form strong co-authorship clusters and are authors on many of the papers identified, in addition to the existence of smaller unconnected clusters. There was substantial re-use of microsatellite markers across species and studies, as well increasing use of barcode markers and SNPs. This review provides a foundational basis for new researchers and collaboration opportunities alike, and addresses a fundamental gap in the path forward towards the recently proposed European Board of Macroalgal Genetic Resources (EBMGR).

In Chapter 3 and 4, the three most abundant species were studied, *Saccharina latissima*, *Laminaria digitata*, and *Laminaria hyperborea* using high quality Single Nucleotide Polymorphism (SNP) markers generated using Genotyping By Sequencing (GBS). These were sampled along projected glacial retreat patterns from the Last Glacial Maximum (LGM) in order to identify glacial refugia, as well as connectivity, genetic structure, and genetic diversity. Data herein indicates species-specific patterns unique to all three kelp, but evidence for glacial refugia was seen on the west coast (*S. latissima* and *L. digitata*) as well as the east coast (*L. digitata*) and in the north/east (*L. hyperborea*). Additionally, strong differentiation was seen in populations along the east coast across species, suggesting limited geneflow and/or small effective population sizes in east Ireland. Both *S. latissima* and *L. hyperborea* showed signs of

being highly diverse across Ireland, while *L. digitata* appeared comparatively impoverished. Population genetic structure of *L. digitata* and *L. hyperborea* suggest that prevailing south-west to north-east ocean currents may result in directional geneflow from the south northwards for *L. digitata*, but may effectively separate eastern and western populations in *L. hyperborea*. Populations of *L. digitata* approached Hardy-Weinberg equilibrium, while *L. hyperborea* and *S. latissima* both exhibited a substantial heterozygote excess, providing evidence for strong outbreeding tendencies in these two species. The underlying cause of this outbreeding is hypothesised to result, at least in part, from gametophytic persistence and fragmentation. The segregation of gametophyte sexes and opportunity for each haploid genotype to engage in multiple instances of recombination might uniquely affect interpretation of such population genetic statistics in haplo-diplontic organisms with dioecious haploid life stages.

An extensive morphometric analysis of *Laminaria* samples using traits typically used in species discrimination between *L. digitata* and *L. hyperborea* found that identification by morphology alone is unreliable for species identification in this genus in the north-east Atlantic. Some data to support low rates of interspecific hybridisation was also found, and directional introgression into *L. hyperborea* might help explain some of the observed genetic structure. While significant differences between commonly used traits (such as stipe flexibility and upper stipe roundness) were identified between genetically identified species, these tended to correlate heavily with both specimen age and depth of sampling, and showed a high degree of overlapping variance. Both depth and age also correlated with one another, suggesting ecological benefits of depth on sporophyte longevity. Notably, genetically confirmed *L. digitata* had a distinctly shallower depth of sampling, being entirely absent at 2m below Lowest Astronomical Tide (LAT). Moreover, *L. digitata* samples from the subtidal were more likely to be identified as *L. hyperborea*, while intertidal *L. hyperborea* were often identified as *L. digitata*, suggesting the morphologies typically associated with these species to largely be a function of depth. Sample sites also appeared to have strong influences on morphology, suggesting site-specific environmental factors may be more important in determining the phenotype of these species than species-specific genotype. Additionally, this observed niche segregation provides support for the increased heterozygote excess observed in *L. hyperborea,* when compared to *L. digitata,* being due to unrecognised gametophyte persistence and fragmentation in the environment which in turn impacts the population genetic structure of kelp. Hypotheses such as the MultiAnnual Delayed (MAD) gametophyte hypothesis rely on gametophyte persistence in less ideal conditions, typically low light, and the data in this thesis provides support for *L. digitata* and *L. hyperborea* being a promising future avenue to investigate this recently proposed hypothesis.

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List of Abbreviations

aff.	affinis
Ar	Allelic richness
BIC	Bayesian Information Criterion
bp	base pair
C	Celsius
cm	Centimetre
COI	Cytochrome C Oxidase subunit I gene
CTAB	Cetyltrimethylammonium bromide
DAPC	Discriminant Analysis of Principal Components
ddRADseq	Double Digest Restriction-Site Associated DNA Sequencing
Dest	Population differentiation
DNA	Deoxyribonucleic Acid
Dst	Gene diversity among samples
EBMGR	European Board of Macroalgal Genetic Resources
EST	Expressed Sequence Tag
Fis	Inbreeding coefficient
Fst	Fixation index
GBS	Genotyping By Sequencing
He	Expected heterozygosity

Ho	Observed heterozygosity
Hs	Within population gene diversity
Ht	Overall gene diversity
HWE	Hardy-Weinberg Equilibrium
IBD	Isolation By Distance
Ka	Thousand years
km	Kilometres
Lat	Latitude
LAT	Lowest Astronomical Tide
LGM	Last Glacial Maximum
Lon	Longitutde
m	Metres
MAD	MultiAnnual Delayed
mg	Milligram
MMD	Minimum Marine Distance
MYA	Million Years Ago
ng/uL	Nanograms per Microlitre
nGBS	normalised Genotyping By Sequencing
NGS	Next Generation Sequencing
nInd	Number of individuals corrected for sample size
NJ	Neighbour Joining
OUT	Operational Taxonomic Unit
Pa	Private alleles
PAR	Photosynthetically Active Radiation
PC	Principal Component
PCA	Principal Components Analysis
PCR	Polymerase Chain Reaction
PE-150	150 base pair paired-end
PhD	Doctor of Philosophy
PRISMA	Preferred Reporting Items for Systematic reviews and Meta-Analyses
PVPP	Polyvinylpolypyrrolidone
RLTP	Regions of Long-Term Persistence
SAR	Stramenopiles, Alveolates, Rhizaria
SCUBA	Self-Contained Underwater Breathing Apparatus
SNP	Single Nucleotide Polymorphism
SSR	Single Sequence Repeat
SST	Sea Surface Temperature
TE	Tris-Ethylenediaminetetraacetic acid
uHe	unbiased expected Heterozygosity
UK	United Kingdom

Chapter 1 Introduction

“A tree not good simile - endless piece of seaweed dividing"

– Charles Darwin, 1843

above a sketch of seaweed when developing his theory of evolution van Putten (2020)

Seaweeds have a long history of use by humans but are increasingly being recognised as providers of important and wide ranging ecosystem services more generally (Cotas et al., 2023). This is concomitant with an increasing interest in seaweed aquaculture for human use (Duarte et al., 2021; Hu et al., 2021) as well as interest in the potential of seaweeds for carbon sequestration (Canvin et al., 2025). Seaweed research however has lagged behind terrestrial research due to difficulty in access as well as lower historical economic interest. A scientometric analysis over a 48 year period (1975-2022) identified 48,286 studies on all species of macroalgae across all fields of study, with a rapid increase in publications in recent years (Segaran et al., 2024). By comparison, a similarly broad analysis of all publications just on oilseed rape (*Brassica napus*) over the course of 10 years (2011-2021) identified 7,617 studies (Zheng & Liu, 2022). Many of the major foci in this scientometric analysis appear to be related to ocean acidification, interactions within reef ecosystems, and studies on antioxidant properties of macroalgae. This mismatch of growing economic interest, increasing conservation concern, and lag in fundamental research of many algae requires systematic addressal. Principally, some of the species that provide the greatest overlap between ecosystem services and economic benefits, but for which much fundamental research is lacking, are kelps (Eger et al., 2023). Kelp can form complex structural habitats called “kelp forests”, typically within the temperate and polar regions of the northern hemisphere (Figure 1.1), which far exceed the productive limit of biomass density of any known plant (Creed et al., 2019). Addressing some of the fundamental knowledge gaps within this group of macroalgae not only provides outsized benefit to the habitats they create, but also provides an opportunity to increase the sustainability of their utilisation, via wild harvesting or through aquaculture.

Figure 1.1 Distribution of kelp forests of Laminaria*, and* Saccharina*, with lighter shades representing regions for which distribution estimations were not available. Modified from (Eger et al., 2023). Note the distinct northern coastal distribution, with Iceland only showing distribution for* Saccharina.

This thesis is focused on addressing knowledge gaps in the Atlantic species *Laminaria digitata* (Hudson) J.V.Lamouroux 1813*, Laminaria hyperborea* (Gunnerus) Foslie 1885, and *Saccharina latissima* (Linnaeus) C.E.Lane, C.Mayes, Druehl & G.W.Saunders 2006*,* all of which are brown seaweeds generally known as kelp (Figure 1.2). However, the definition of kelp is not always applied consistently. Historically "kelp" was an undifferentiated word used to describe the soda ash obtained from burning of large brown seaweeds (Chapman, 1980). This later became generalised to refer to the large brown macroalgae themselves, and later yet was refined to refer to members of the taxonomic order Laminariales in the early 20th century (Smith, 1938). This latter refinement however has been somewhat controversial among some researchers who argue that kelp should continue to refer to all large brown macroalgae (Fraser, 2012). While this approach is in line with common use of the term and could provide utility to ecological studies of "kelp forests" where macroalgal habitat structure is the prime motive for research, it raises some conflict about the utility of the term and what size or form a brown seaweed needs to be for it to be considered "kelp". In this thesis, the word "kelp" is used to exclusively refer to the order Laminariales, as this is the least ambiguous delineation. Furthermore, this is in line with terms such as "bug" which commonly refer to many small invertebrates across unrelated lineages, but for which also exists a specific taxonomic definition of "True bug" referring to the order of Hemiptera, with the suborder of Heteroptera being distinguished as

"Typical bugs". This approach strikes a reasonable balance between common and specific usage without sacrificing the communicative utility provided by the term kelp, with further contextual refinement allowing for clarification, as has been seen in the use of the word bug.

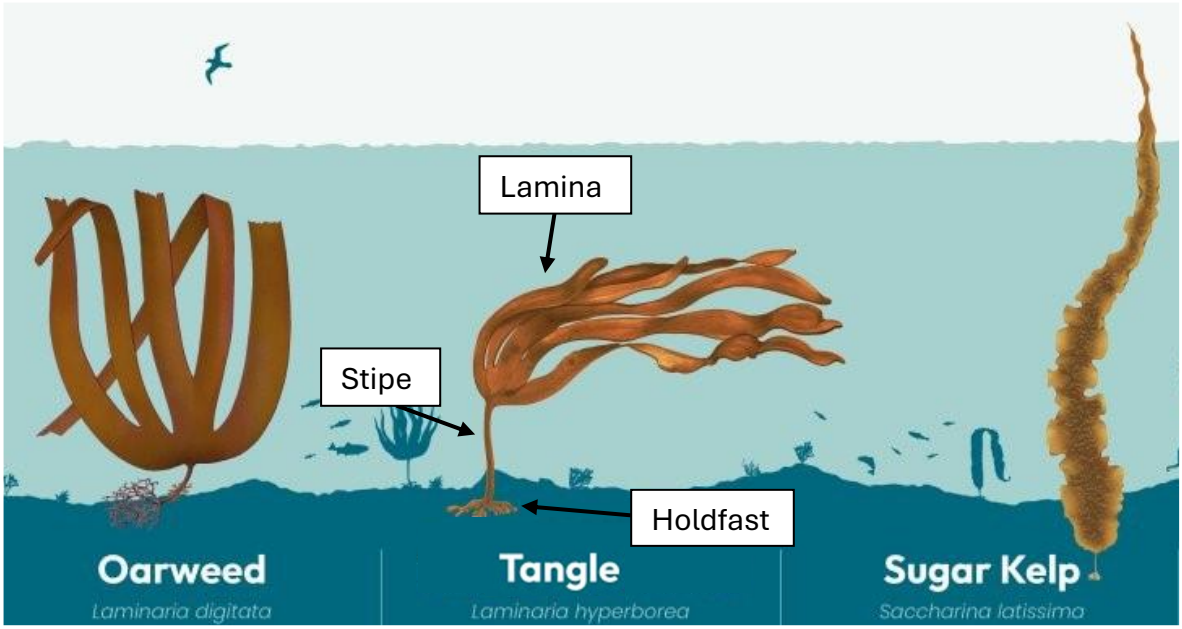

Figure 1.2. Species being studied in this thesis. Image modified and taken from Sussex kelp recovery project (https://sussexkelp.org.uk). All species consisting of a root-like hapteran holdfast, stipe, and lamina, which are labelled in the central L. hyperborea. S. latissima *typically described by a short stipe, small holdfast, and long simple lamina, occasionally bullate, and typically with undulating margins.* Laminaria *have split lamina, with* L. digitata *generally considered to have shallower holdfasts, and shorter more flexible stipes than* L. hyperborea. *Stipes on* L. hyperborea *are also typically considered to have greater presence of epiphytic growth.*

Kelp has an heteromorphic haplo-diplontic lifestyle, with large diploid sporophytes that produce spores that develop into microscopic dioecious haploid gametophytes, which in turn undergo syngamy to produce the sporophyte (Figure 1.3). These sporophytes and gametophytes occupy different ecological niches (Monteiro, Heinrich, et al., 2019), with progression between generations (via sporogenesis or gametogenesis) having additional environmental constraints, and which vary both with species as well as with regional ecotype (Veenhof et al., 2022).

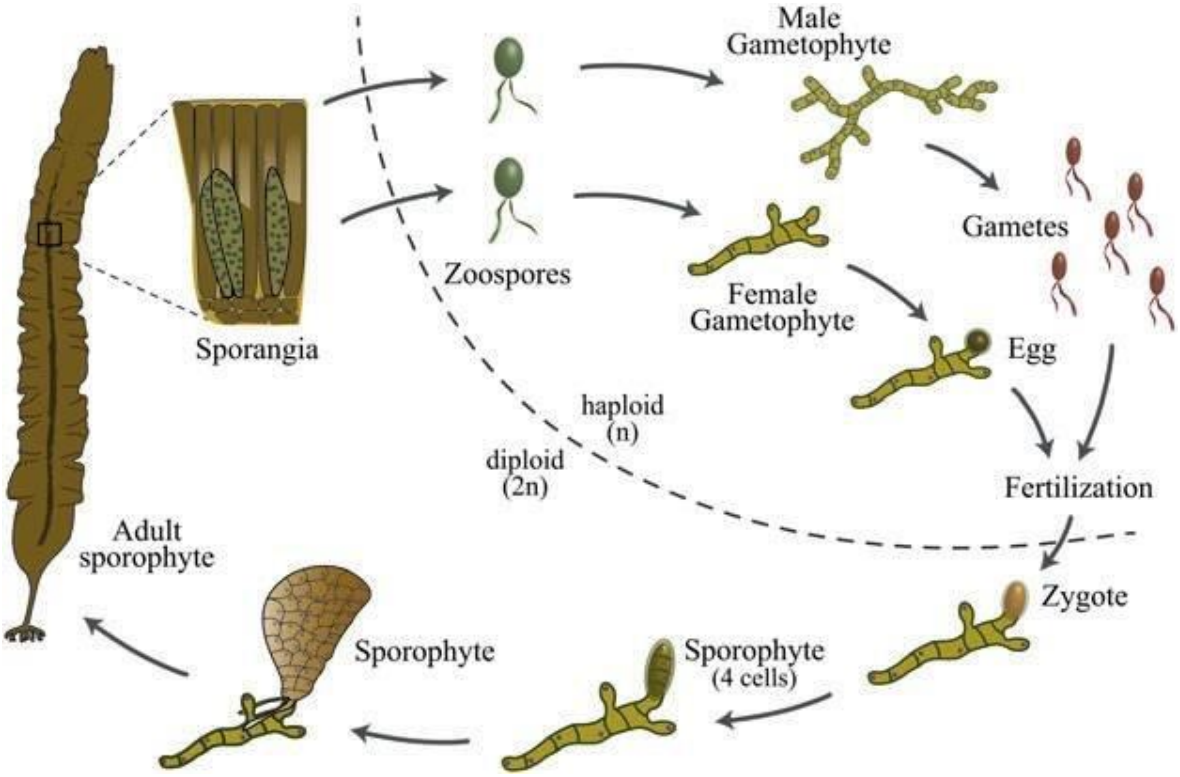

Figure 1.3. Typical life cycle of kelp, here representing S. latissima*, showing the segregation of sexes in the free-living microscopic haploid phase as well as the diploid macroscopic sporophyte. Taken from (Visch et al., 2019).*

1.1 From morphology to molecules: bridging the methodological gap in kelp research

Algae were initially described on basis of morphology alone and included among plants taxonomically (Linné, 1753). This taxonomic classification was later amended by French and Irish botanists to divide algae into four distinct groups, green algae, red algae, brown algae, and diatoms (Harvey, 1836; Lamouroux, 1813), which was the first use of biochemical criteria within the field of plant systematics. As improved microscopes and molecular tools became available, algae were seen to be highly polyphyletic, with the brown algae and diatoms being in a highly divergent clade to the Archaeplastida which include plants, red, and green algae (Keeling et al., 2005). Further research revealed the complex endosymbiotic nature of the stramenopile clade, which include diatoms and brown seaweeds (Khan et al., 2020). Stramenopiles are part of the SAR (Stramenopiles, Alveolates, Rhizaria) supergroup of eukaryotic life and only distantly related to plants and other multicellular eukaryotes, but estimated to account for around half of all eukaryotic species (Burki et al., 2020). Within this clade, multicellularity arose independently from the rest of the Archaeplastida (Cock et al., 2010), with kelp being physically the largest representatives of the entire supergroup (Figure 1.4).

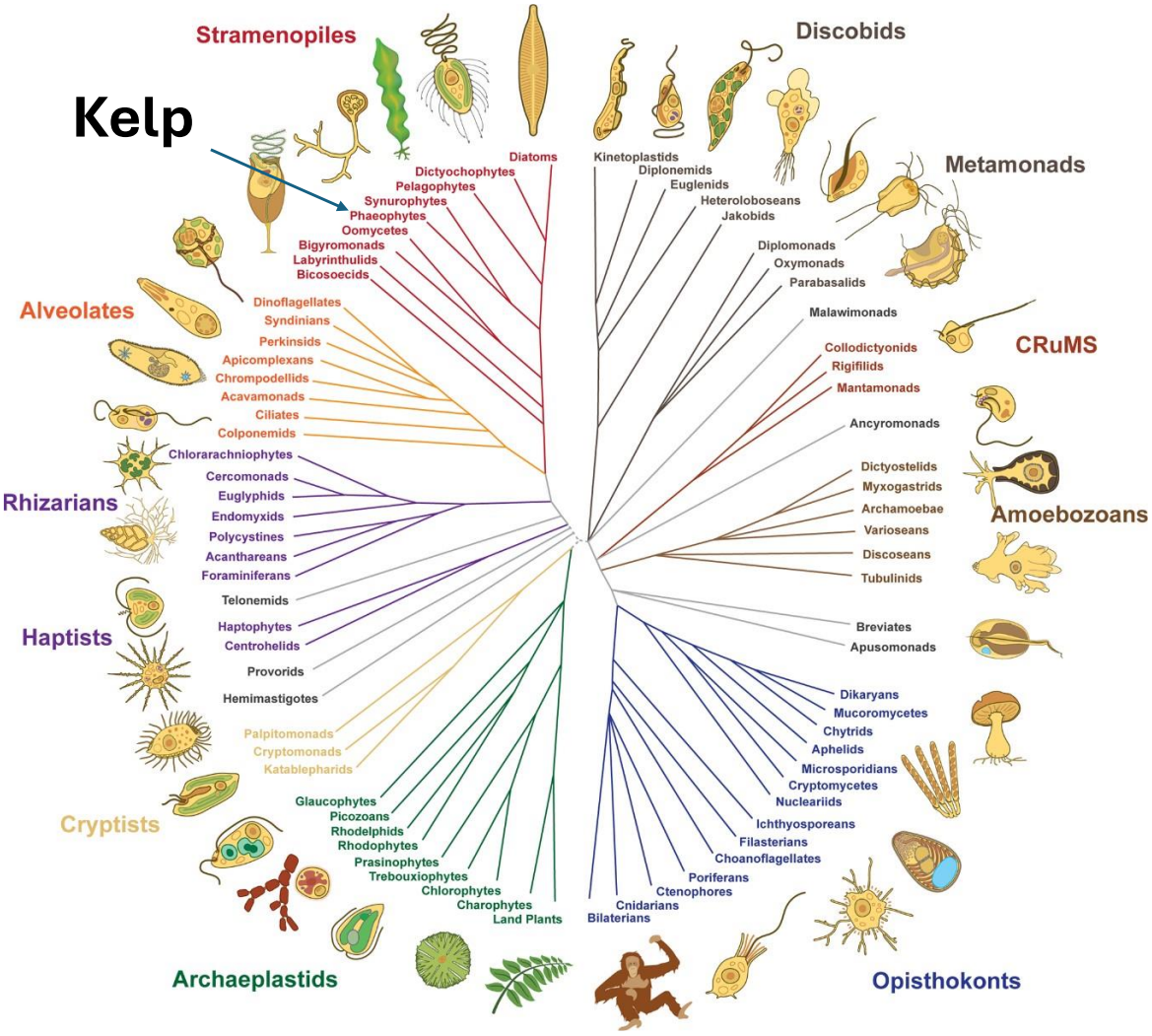

Figure 1.4. Schematic of lineages of Eukaryotic life modified to highlight position of kelp, used under Creative Commons (Keeling & Eglit, 2023).

As classification of kelp moved from systems based on sporophyte morphology, life cycle structure, and gametic traits towards systems based on molecular DNA data there has been a recontextualization of the evolutionary history of kelp, with several attempts at reconciling phenotype with phylogenetic data (Lane et al., 2006; Starko et al., 2019). Not only have these studies identified the recurrent evolution of complex traits such as branching, pneumatocysts, and upright stipes across the Laminariales, but these complex kelp are also closely related to the filamentous Ectocarpales, raising further questions about the determinative utility of morphological criteria in species identification and classification (Bringloe et al., 2020). This is a particularly important consideration when studying closely related species that may share similar habitat and morphology, as is the case for *Laminaria digitata* and *L. hyperborea* (Kain & Jones, 1975; Longtin & Saunders, 2015). These two species have been confirmed genetically as 'confusion species' in previous research (Fouqueau et al., 2024; Longtin & Saunders, 2015; Schübert, 2022) and recent molecular tools to rapidly screen them, such as the barcode

marker of the mitochondrial cytochrome c oxidase subunit I gene (COI), have been developed as a direct response (Mauger et al., 2021).

While this molecular work is an important step forward, it highlights the lack of traditional morphological work done on these species, much of which is limited in scope and sampling (Lund, 2014; Lüning, 1979). The limited data that pairs morphological and molecular data comparing *L. digitata* and *L. hyperborea* suggests strong phenotypic similarity as well as high phenotypic plasticity (Henry, 2018). This similar phenotype with high plasticity paired with species specific but overlapping ecological niches (Kain & Jones, 1975) paint a complex picture of species classification that is rarely addressed in the literature. This picture is further complicated by conflicting reports of hybridisation between these species (Schübert, 2022; tom Dieck, 1992), hybrids between the less closely related *L. digitata* and *L. pallida* (Martins et al., 2019), and even inter-familial hybrids within Laminariales (Murua et al., 2020). There are suggestions that there may also be reinforcement of species' boundaries in sympatric algal populations, but that recent contact zones may be more liable to exhibit hybridisation (Bringloe et al., 2020; Martins et al., 2019; Moalic et al., 2011; Rothman et al., 2017). This multi-factorial problem of classification underscores the necessity to take a holistic approach to species description, considering both phenotype and the ecophysiological variables that may impact it, as well as genotype and the possibility of interspecific hybridisation. The history of algal classification has repeatedly demonstrated cryptic complexity hidden in plain sight, but has also seen over-classification based on morphological data, with over 200 species and varieties being described in *Laminaria* since establishment of the genus which now only contains 20 accepted species (Guiry & Guiry, 2025; Lane et al., 2006). Many of the evolutionary assumptions carried over from other multicellular eukaryotes cannot be assumed to hold true for kelp, which are not only evolutionarily divergent but also fill an ecological niche that is incomparable to that of other clades. This highlights the need for further research that considers these clade-specific factors when defining macroalgal species (McCoy et al., 2020).

It is in this light that discrimination of species based on underpinning ecological traits may provide additional utility in discrimination of confusion species such as *L. digitata* and *L. hyperborea*. While the majority of kelp are shallow marine species with a preference for rocky substrates, there is strong evidence for the existence of ecotypic differentiation even within species, such as in *S. latissima* (Monteiro, Li, et al., 2019). While *S. latissima* may best be described as a species complex with circumboreal distribution (Bringloe et al., 2020; Mao et al., 2020; Neiva et al., 2018) with no known confusion species for the North East Atlantic, the same cannot be said for *Laminaria* and the accurate discrimination of the two sister species *L. digitata* and *L. hyperborea*. Historically ecological data on Atlantic *Laminaria* has focused on

succession, morphology, and biochemistry, with much of it being published by a few researchers focusing on *L. hyperborea* (Kain, 1971; Kain & Jones, 1963, 1964, 1965, 1969, 1975, 1977). Additionally, the local environmental variability associated with *Laminaria* appears to be equally as significant in the determination of morphology as the latitude of the population (Kain, 1967), but despite the recognition of this extreme morphological variability, to date there has been little effort to combine molecular confirmation of species with their morphological descriptions across sites. Quantifiable traits such as depth and age are complex proxies for metrics such as colonisation ability, sporophyte development, and inter-annual persistence rate, and comparing species across sites should help to disentangle how these traits segregate as different species and genotypes behave differently in differing environments. Traits such as stipe length may both have a genetic component to them as well as an environmental one, mediated by age and development. Such traits can be quantified either via standardisation of genetic or environmental variables, or through associative correlation using large datasets.

One paper that did compare the ecological impact of depth on the growth of *S, latissima*, *L. digitata* ¸and *L. hyperborea* found species specific behaviours that suggest that there is measurable differences in the functioning of these species (Lüning, 1979), however it is unclear if species identification was confirmed molecularly, or whether species-specific ecotypic variation of the source population may have had an impact on the findings, as the source of the kelp is not specified.

Typically *L. digitata* is described as having more of a shallow distribution than *L. hyperborea*, and being completely absent from deeper waters (<2m depth according to Lüning (1979)), but direct comparison of complex multidimensional morphological traits between species is extremely limited. Furthermore, population genetic studies rarely report on morphology, depth of collection, or whether they confirmed species identification using molecular methods, raising concern about the species identity and regional-specificity of many of these findings. This gap in the research can lead to a very disparate conception of species for phycologists focusing on ecological functioning and those focusing on evolutionary history, and such mismatches of species concepts has already been established as a known problem in algal research (McCoy et al., 2020). This lack of fundamental natural history data in recent decades means that the research landscape is reliant on literature that used a vastly different theoretical framework than we do nowadays, and as such demands careful examination to ensure the assumptions derived from such publications still hold true within the current research landscape. As such, one of the objectives of this thesis is to verify the veracity of long held beliefs that *Laminaria digitata* and *Laminaria hyperborea* are reliably distinguishable by

morphology alone, and does so by direct comparison in multiple different sites across the study area, with molecular confirmation of lineage (as per the macroalgal species conception posited by McCoy et al. (2020)).

1.2 Moving with the tides: the influence of historical biogeography on kelp diversity

Kelp are thought to have evolved in the Pacific and diversified 31.5 MYA at the Eocene-Oligocene boundary and crossed to the Atlantic at least four separate times (Starko et al., 2019). These movements into the Atlantic likely coincided with interglacial periods that allowed kelp to move through the Arctic via the Bering Strait, with the cycling of glacial periods providing species-pump dynamics through iterative isolation and secondary contact between closely related lineages (Neiva et al., 2018; Neiva et al., 2020). This was followed by more recent radiation in the Atlantic, with some species crossing the equator on at least two occasions < 2 MYA (Rothman et al., 2017). This close association of kelp diversity with glacial cycles becomes apparent in figure 1.1, where both the east and west Pacific coasts were iteratively isolated from one another as well as from the Atlantic due to glaciation of the Bering strait and Barents Sea respectively, leading to contemporary distributions of kelp forests across the northern hemisphere. Several studies have looked at individual kelp species biogeography over a large range in the Atlantic (Assis, Serrão, et al., 2018; Guzinski et al., 2020; Jaugeon et al., 2025; Neiva et al., 2020), with many others looking at smaller regional populations (Brennan et al., 2014; Evankow et al., 2019; Reynes et al., 2021), often looking at patterns of isolation and signs that glacial refugia that persisted through these interglacial cycles. This pattern of glacial vicariance has led to highly diverse and locally adapted populations which may variably be described as incipient species or species complexes depending on the kelp (Neiva et al., 2018). While models of glacial extent have varied throughout time, recent data suggests that western glacial coverage in Europe left parts of the south of Ireland uncovered during the Last Glacial Maximum (Clark et al., 2022). Furthermore, the retreat of these ice sheets over the course of ~10,000 years may allow for the identification of recolonisation pathways along both the Atlantic coast of Ireland as well as the more sheltered Irish sea coast. As these glaciers retreated they likely altered habitat suitability for kelp along the coasts, due to factors such as salinity, sedimentation, nutrient availability, substrate availability, and temperature. Therefore examination of these retreat pathways may uncover glacial refuges that exist in locations that may otherwise be considered unlikely in modern environments, such as the sediment-rich east coast of Ireland.

Interestingly, one of the least sampled regions for kelp in the Atlantic that consistently appears to be highly diverse is around Ireland (Bringloe et al., 2022; Jaugeon et al., 2025; Neiva et al., 2020; Schoenrock et al., 2020). The Celtic Sea and southern half of Ireland is thought to contain glacial refugia (Assis, Araujo, et al., 2018), and the shallow seabed around Ireland during glacial periods when sea level was lower could have potentially provided ideal habitat for kelp (Becker et al., 2015). These refugial populations are typically inferred by comparison of private diversity identified through molecular work, sometimes through enzymatics, but typically through sequencing of genetic material. While multiple metrics of private allele quantification are available, they all suffer from methodological limitations. Limitations such as non-infinite sampling are inherent to these kinds of biodiversity metrics, but these are compounded by selective sampling and erroneous sampling, which may include only sampling a small region or inclusion of closely related but distinct species. This places Ireland in a particularly interesting position to study Atlantic kelp diversity, as its relatively stable climatic conditions and location at the hypothesised glacial boundary suggest the presence of high diversity, for which the limited genetic studies seem to provide supporting evidence. Failure to account for the possibility of including misidentified *Laminaria*, especially when using lower resolution markers such as microsatellites, could give rise to a false signal of refugia, by introduction of divergent genotypes into the dataset by being misidentified or hybridised.

1.3 Research outline and thesis structure

The aim of this thesis is to identify and address the previously outlined knowledge gaps arising from a mismatch of theoretical frameworks across ecological and molecular phycological research, as well as to determine the biogeographic knowledge gaps in kelp research in the north-east Atlantic. It contains three data chapters, the specific objectives of which are outlined in more detail in the respective sections on each chapter below.

There have been no systematic reviews of kelp population genetic research in this region, with disparate research being conducted across institutions. In light of this disparate research environment across species and researchers, chapter 2 consists of a systematic review of north-east Atlantic kelp population genetics, following PRISMA guidelines, that aims to find similarities in kelp biogeography across species, as well as identify knowledge gaps that require additional resource allocation. This data chapter is framed in the context of kelp aquaculture and wild-harvesting in an attempt to provide a roadmap for allocation of future resources towards both kelp conservation and sustainable exploitation. It also explores geographic and institutional research clusters using species-specific heatmaps, as well as the

connectivity between these via co-authorship analysis, in an effort to allow researchers to identify effective and collaborative kelp population genetic projects in the future.

There has been no cohesive study identifying the diversity of *S. latissima* in Ireland to date, despite being one of the most extensively studied kelps in Europe, and previous studies suggesting high levels of diversity in this region. The aim of chapter 3 is to use Next Generation Sequencing (NGS) methods to examine Irish *Saccharina latissima* population genetics in light of existing biogeographic research and the geographical knowledge gaps that exist for this region and species. This was done to provide a high number of high resolution SNP markers. Samples were collected from four primary sites in the north, south, east, and west of Ireland at ~30 samples per site, which allowed for the comparison of population diversity metrics between sites. Some samples were also stochastically collected to allow for better geographic coverage and identification of meta-population dynamics, and to help increase the reliability of metrics such as private allele count that rely on good geographic sampling coverage. The mix of population diversity metrics and structure allows this chapter to summarise the current state of *S. latissima* across Ireland for the first time, and is a timely addressal of one of the research gaps identified in Chapter 2.

The previously identified lack of recent natural history data that aligns with modern theoretical frameworks of algal species concepts, particularly for closely related *Laminaria*, has the potential to confound much of the modern research on these species. Chapter 4 builds on the approach outlined in chapter 3 for *Laminaria digitata* and *Laminaria hyperborea*, and adds comprehensive morphological data to the analysis, allowing for high resolution comparisons between phenotype and genotype within and between species, and across sites. This chapter attempts to resolve questions of species delineation, identification, and hybridisation within this genus. Specifically, it tests the reliability of gross morphology as an identification method for molecular species using high resolution SNP markers, aims to identify which (if any) morphological characteristics can be used to discriminate between species, and whether the genetic structure and diversity of these species differs across the study area.

A final discussion chapter (Chapter 5) aims to synthesize knowledge gained throughout the thesis, identify shortcomings, and make recommendations for further work.

Chapter 2 European kelp genetic resources for sustainable aquaculture: A systematic review.

2.1 Abstract

This systematic review identified all original published population genetics journal articles on *Laminariales* in the north-east Atlantic, using the Scopus and Web of Science databases, in English, and which reported metrics of population genetic diversity, structure, or connectivity, in line with Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines. In total, 29 articles were included for review across six species, and these were analysed by species, with the limitation that these were all screened and selected for by a single author. Additionally, authorship analysis was performed to identify the frequency of authorship as well as co-authorship clusters. This review identified strong preference in the literature for studying *Saccharina latissima* and *Laminaria digitata*, as well as frequent use of microsatellites as genetic markers. Authorship was heavily skewed towards a few authors, with strong co-authorship research clusters identified in France and Portugal. Increased research focus on *Laminaria hyperborea* and *Laminaria ochroleuca* for conservation purposes as well as on *Alaria esculenta* for aquacultural uses would help close the identified knowledge gaps. Increased use of SNPs and barcode markers where possible would also help to increase the interpretative robustness of the existing data. General trends across species indicated a north/south divide in Europe, with populations near the Celtic sea and Ireland frequently being identified as particularly diverse. Recommendations arising from this review are for greater inter-institutional collaboration to increase geographic scope of sampling, allowing for direct comparison of populations across the European range, and for more effective reuse of existing data. Additionally, this review can act as a starting point for new research into European kelp population and conservation genetics, thereby facilitating researchers to systematically address gaps in the literature.

2.2 Introduction

Kelp are brown macroalgae, defined here to be in the order Laminariales, that have long been utilised for food, fertiliser, medicine, as well as providing many valuable ecosystem services (Eger et al., 2023). Kelp are particularly important in many East Asian cultures, often as a food, and have been cultivated for about 100 years with increasing intensity (Hu et al., 2021). An increasing global demand for kelp and kelp-derived products has stimulated efforts to increase aquacultural output of farmed kelp in other parts of the world (Duarte et al., 2021), in part to prevent wild-harvested kelp from meeting demand unsustainably. Therefore it is prudent to identify the issues already seen in parts of Asia where kelp aquaculture has been

well established and apply the lessons learned to burgeoning industries in other parts of the world. China in particular has a well-established kelp industry and provides a good case study to identify potential issues that might hinder sustainable kelp aquacultural production elsewhere.

One aspect that is important early on in the utilisation of a new agricultural crop is that of crop genetic diversity, and the wild genetic resources available to modify that crop in the future (Hofmann et al., 2025). Many land crops have a long history of domestication since the last ice age and have a diverse gene pool from which to select from. This diversity safeguards against the risks of crop homogeneity, especially in a climatically uncertain future (Gao et al., 2023). Previous research in China has identified a homogenising effect of cultivation and domestication on the diversity of kelps (Liu et al., 2012; Shan et al., 2017; Zhang et al., 2017), and similar patterns have recently been identified in Europe (Jaugeon et al., 2025). Additional risks may exist for kelps, because they are important habitat-forming species, which is typically not the case for land based crops (Ceausu et al., 2025; Pimentel et al., 2012). This creates a dual incentive for wild kelp conservation for both its habitat value and its potential as a genetic resource for kelp aquaculture. Poor management of wild stocks paired with short-term economic perspectives on cultivation could lead to a loss of wild genetic resources and risk over-reliance of the developing kelp industries on a stock of kelp that struggles with long term viability. This is increasingly important as wild harvesting may increase to meet demand in the absence of a competitive aquaculture industry. The number of enterprises cultivating and harvesting brown seaweeds more broadly in Europe is increasing (Canvin et al., 2025), and the impact of these activities on wild kelp populations will differ by species and cultivation/harvesting method, but important information is lacking to predict the extent of this impact. Kelp are the most frequently cultivated and harvested brown macroalgae in Europe (Figure 2.1), making the existing knowledge gaps more consequential both economically and environmentally speaking.

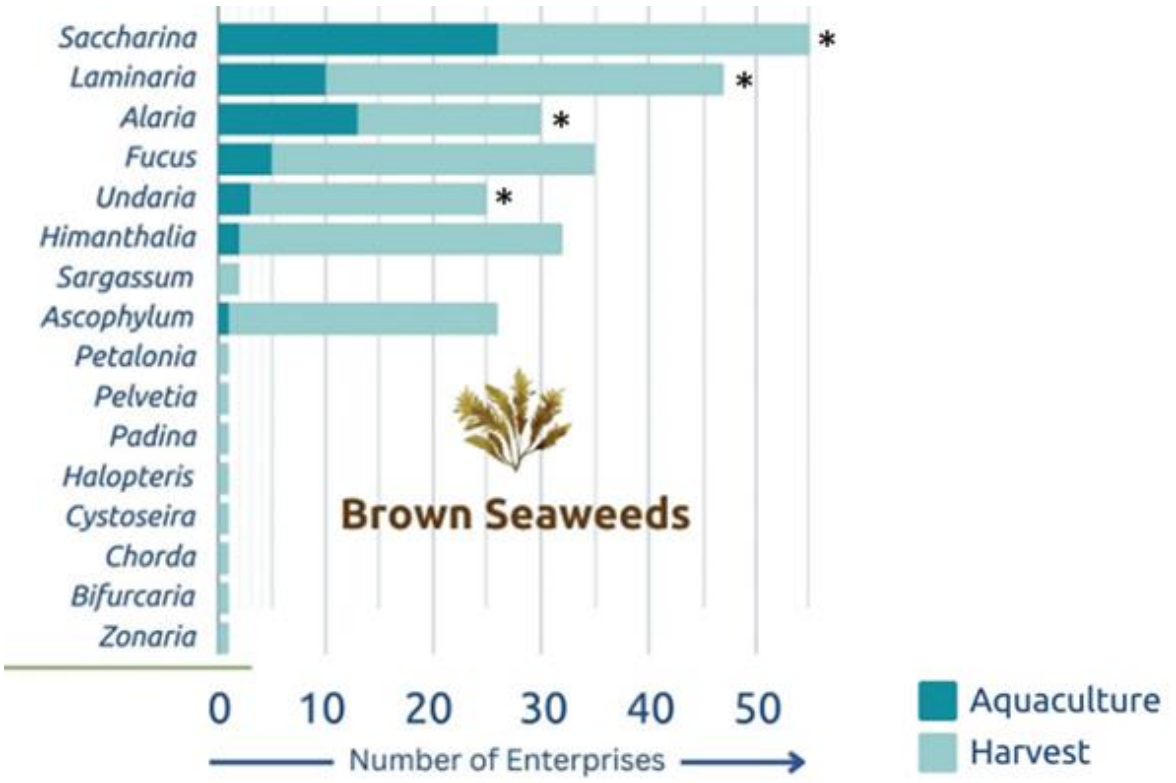

*Figure 2.1. The number of enterprises cultivating and harvesting brown macroalgae in Europe and the relative proportion of each genera that is cultivated or wild harvested, adapted from Hofmann et al. (2025). * Denotes genera of kelp.*

To better align and balance the interests of kelp-based industry and wild kelp conservationists with the increased economic demand, it is imperative to first identify the extent of the available resources as well as the quality of the standing stock. This is in line with a recent opinion paper arising from a SeaStrains Workshop supported by the Global Seaweed Coalition that outlined the need to biobank macroalgal strains and wild genetic resources in Europe, but for which there exists no comprehensive overview of the currently collected data (Hofmann et al., 2025). The following systematic review addresses this knowledge gap directly by synthesising the current state of knowledge and assessing progress made towards understanding the available intraspecific genetic diversity of all kelps in Europe and the North East Atlantic. The review follows the most recent PRISMA guidelines (Page et al., 2021) to increase reporting quality, as well as the Ecology and Evolutionary biology extension to the PRISMA format (O'Dea et al., 2021). This includes reporting on screening criteria and rationale, search terms, data bases, and Boolean operators used, and gaps in the available evidence amongst others relevant to a systematic review. The goal is to synthesize information and identify biases in sampling location, species sampling, and molecular tools used, and then make recommendations at a European scale for effective apportioning of future research resources. Specifically, this review collates diversity data across studies for each kelp species, identifies broad structural trends

across the entire study area, and identifies gaps in sampling efforts which should be prioritised, such as unsampled glacial refugia. Additionally, this review will examine the authorships and co-authorship clusters in an effort to promote greater inter-institutional collaboration and therefore a more integrated research landscape for European kelp population genetics. Identifying critical nexus points within the authorship networks allows for future researchers to make use of the existing expertise, as well as identify research clusters that may have local or technical expertise for future collaboration.

2.3 Methods

This systematic review was conducted using Web of Science and Scopus in line with the PRISMA (Page et al., 2021) statement and Eco-Evo extension (O'Dea et al., 2021). SCOPUS was comprehensively searched using Title, Abstract, and Key words on 11th of August 2025 and the Web of Science core collection was searched in "All fields" for all search term combinations on September 2nd 2025. Search terms were limited to all native Laminariales species within the target distributional range (north-east Atlantic/European waters), considering alternative taxonomic synonyms, and without a publication year limitation. The Boolean operator "AND" was used to join genus and species names, as well as "DNA" and the wildcard term "gene*" in order to capture all genetic studies on these species. The Boolean operator "OR" was used to include alternative species names in conjunction with the genus name, and to separate "gene*" and "DNA" as third search terms (Table 2.1). For example "Hedophyllum AND (nigripes OR bongardianum) AND (gene* OR DNA)", for a total of 34 search combinations. Searches were filtered to be research articles in English, and selection criteria were defined *a-priori* (Table 2.1).

Table 2.1. Search terms used for database search. Genus column AND Species column AND Term column, with rows being separated by OR, and bold lines delineating search term groups. Species names in bold are depreciated taxonomic synonyms that were included for search completeness.

Genus	Species	Term
Alaria	esculenta	gene*
Laminaria	digitata	
	hyperborea	
	ochroleuca	
	rodriguezii	
	solidungula	
	saccharina	

	nigripes	
	bongardiana	
Saccharina	latissima	DNA
	nigripes	
	groenlandica	
	bongardiana	
Hedophyllum	nigripes	
	bongardianum	
Agarum	clathrum	

Table 2.2. Selection criteria and rationale used for screening of identified studies.

Criteria	Rationale
Must be original research/new data	Reviews or papers using the same data does not add to the existing coverage of kelp population diversity
Must be of the selected species	Papers that do not principally deal with the selected species are outside the scope of this review
Must be within the selected distributional range	Some species (e.g. *Saccharina latissima*) have distributional ranges outside of the scope of this study (North-east Atlantic/European) and those studies were excluded
Must quantify intraspecific genetic diversity of wild populations	To assess genetic resources a metric of diversity or population genetic structure needs to be included for genotype comparison between geographic locations

All studies obtained (*n* = 1,728) from the raw search were processed for screening and inclusion using Covidence software (Veritas Health Innovation), resulting in the inclusion of 29 studies for the final review (Figure 2.2). After duplicates were removed, 1,040 were identified for screening via abstracts, with 980 of these being removed due to being deemed as firmly outside the scope of this study (e.g. Nutrition, cosmetics, or microbiome research involving kelp). Of the 60 remaining studies, six were removed due to being outside the geographic scope of this review, and 25 were removed due to study designs that prohibited extraction of population diversity metrics.

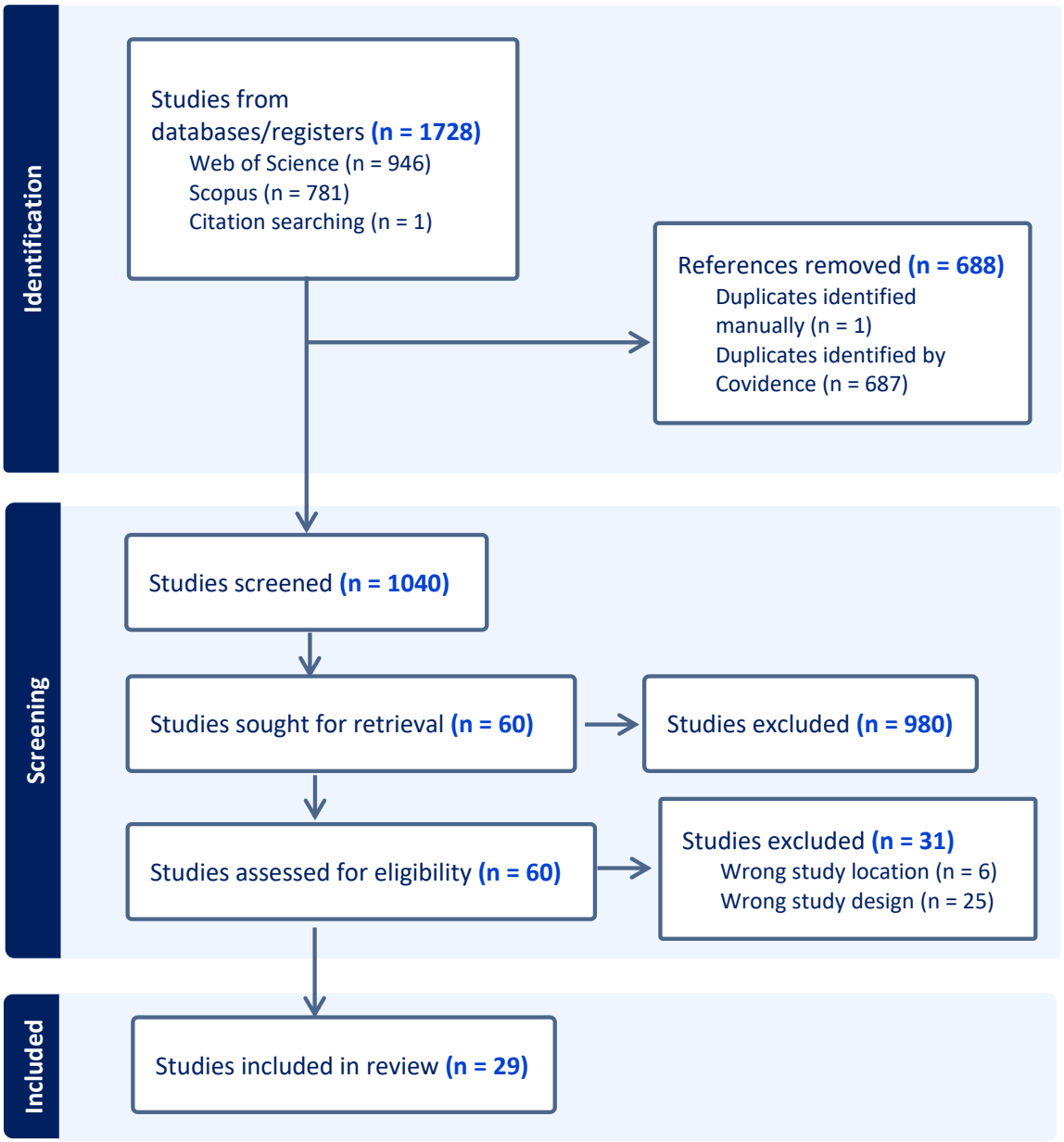

Figure 2.2. PRISMA informed identification and screening of studies for inclusion using Covidence.

Location of sampling sites was extracted from included studies and processed using QGIS (QGIS Development Team, 2009), and figures were created using the "ggplot" package (Wickham, 2016) in R (R Core Team, 2024). Network analysis was done using the package "WGCNA" (Langfelder & Horvath, 2008, 2012), "igraph" (Antonov et al., 2023) and "ComplexHeatmap" (Gu et al., 2016) in R.

2.4 Results and Discussion

2.4.1 Publication analysis

Species and marker bias

Of the 29 included studies, there were none that that included the genera *Hedophyllum* or *Agarum*. *Laminaria digitata* was included in 13 studies (44.8%), *Saccharina latissima* was included in 12 studies (41.4%), *L. hyperborea* and *Alaria esculenta* were each included in three studies (10.3%), *L. ochroleuca* in two (6.9%), and *L. rodriguezii* was only included in a single study (3.5%). Four studies included multiple species (Bringloe & Saunders, 2018; Evankow et al., 2019; Reynes et al., 2021; Robuchon et al., 2014), two compared different types of markers (Guzinski et al., 2020; Luttikhuizen et al., 2018), and a single study (Bringloe et al., 2022) compared markers (SNPs) from different genomes (mitochondrial, chloroplast, and nuclear).

When the total number of unique sites sampled (*n* = 343) was analysed by species, it was found that *S. latissima* had the most sampled sites in Europe at 132 sites (38.5%), *L. digitata* was sampled at 129 sites (37.6%), *L. hyperborea* at 37 sites (10.8%), *L. ochroleuca* at 25 sites (7.3%), *A. esculenta* at 16 sites (4.7%), and *L. rodriguezii* at 4 sites (1.2%) (Figure 2.3a). The 29 studies used three categories of markers, with some studies using multiple markers, or the same markers on multiple species. Most commonly Single Sequence Repeats (SSRs) in the form of microsatellites (*n* = 26, or 66.7%) were used, followed by Single Nucleotide Polymorphisms (SNPs) (*n* = 8, or 20.5%), and finally the Cytochrome C Oxidase subunit I gene barcode (COI) (*n* = 5, 12.8%) (Figure 2.3b).

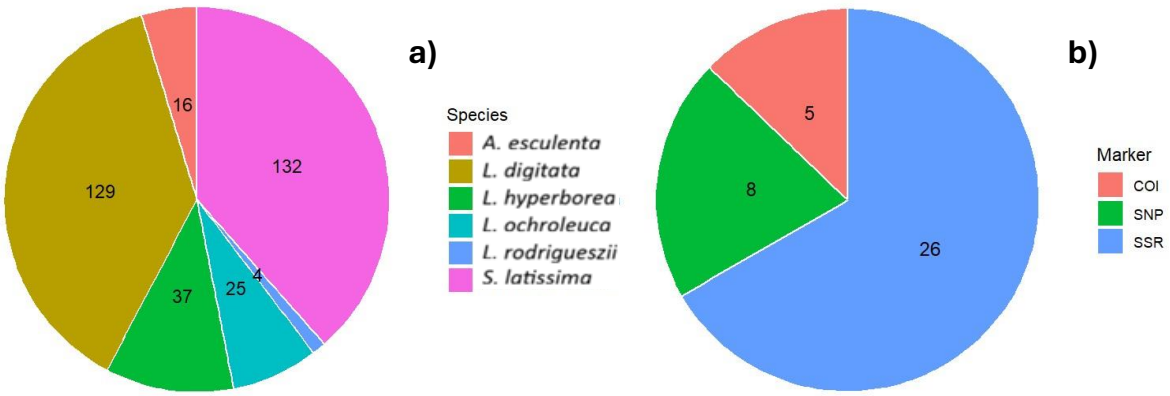

Figure 2.3. a) Number and proportion of total of all unique sites sampled per species. b) Number and relative proportion of marker types used in all studies included in this review.

Interestingly, SSRs are the most utilised marker type and have the longest history of use (Figure 2.4), with the first study using them to analyse *L. digitata* (Billot et al., 2003). In 2018 there were two studies that used COI barcodes located on the mitochondrial genome, but no others since have used barcodes (Bringloe & Saunders, 2018; Luttikhuizen et al., 2018). One of these studies (Bringloe & Saunders, 2018) used the COI barcode markers to compare biogeographic patterns of different macroalgae (incl. *A. esculenta*, *L. digitata*, and *S. latissima*), while the other used COI in conjunction with SSR markers (Luttikhuizen et al., 2018) to help tease apart the complex patterns of recolonisation and differentiation often seen in kelp. Notably, SNPs are first used in 2020 with *S. latissima* (Guzinski et al., 2020), but have since been used on *L. digitata*, *L. rodriguezii* (Reynes et al., 2021), *A. esculenta* (Bringloe et al., 2022), as well as on *L. digitata* (Reynes et al., 2024) and *S. latissima* (Bråtelund et al., 2024) again. It is notable that Bringloe et al. (2022) has been the only study to use high density SNPs that compare nuclear and plastid (mitochondrial and chloroplast) genome (Figure 2.4).

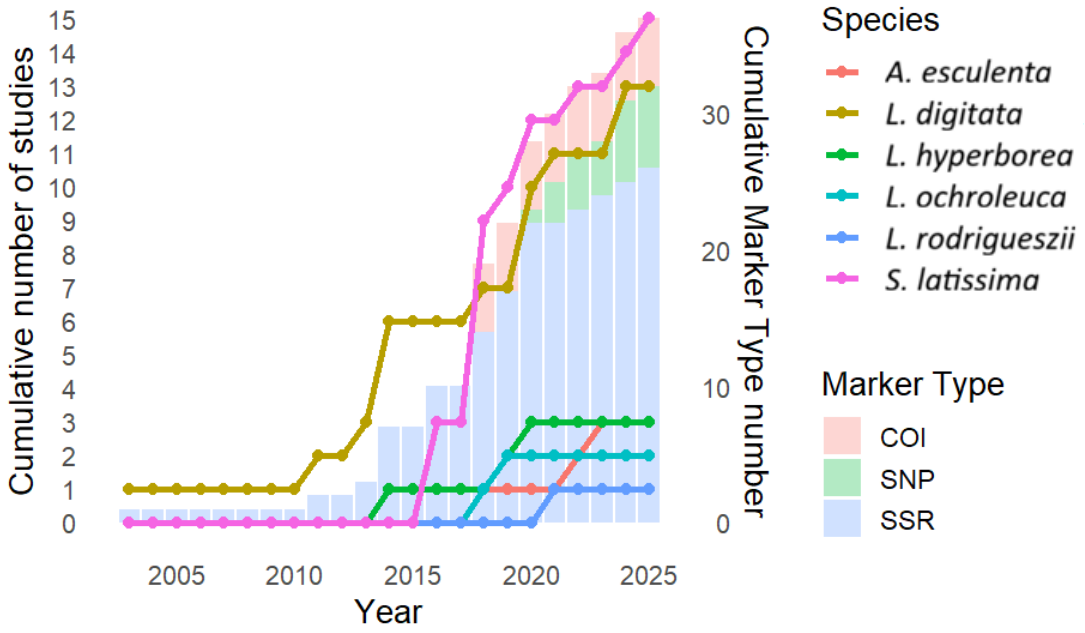

Figure 2.4. Cumulative number of studies including each of six species and marker type used in studies over time. Note that within studies that used multiple markers for the same species, each unique combination of study, marker, and species counts towards additional species numbers.

While using high-density, species-specific SNPs from multiple genomes (nuclear, mitochondrial, and chloroplast) might be considered the most reliable method of analysing population genetic diversity in kelp, it also is very expensive and relies on much more technical expertise (e.g. bioinformatics pipelines). This likely explains the early proliferation of SSR use, as these tend to be cheaper, reusable between species, and require less complex analysis. They do however have a lower resolution than SNPs, and may suffer from null alleles

and cross-amplification artefacts if species identification is uncertain, possibly leading to false conclusions. Use of barcode markers is cheap and provides utility at a large scale. They may help identify major demographic shifts in populations over large geographic regions and time periods, but also lacks resolution to identify more recent and fine-scale geographic structure. The greater access to molecular tools and the development of new methodologies likely underlies the increase in marker type diversification since 2018 (Figure 2.4), and is an encouraging trend for the reliable production of robust kelp population genetic research.

Co-authorship network analysis

When authorship of the studies was analysed using network analysis, using the "cluster_optimal" function from the package "igraph" as per Brandes et al. (2008) it was found that the papers could be divided into six distinct groups, one large and interconnected primary network (Figure 2.5a) and five small and minimally connected communities of authors (Figure 2.5b). Three of these networks consisted of a single paper with no authors shared to any other paper in this review (Bråtelund et al., 2024; King, McKeown, et al., 2020; Luttikhuizen et al., 2018), consisting of a Norwegian, Dutch, and English research group respectively. The other two consisted of two papers each, with one being connected by a single author (S. Fredriksen) in a Norwegian cluster (Evankow et al., 2019; Ribeiro et al., 2022), and the second network cluster (Brennan et al., 2014; Mooney et al., 2018) having four coauthors in common (L. Kregting, G.E. Beatty, B. Elsasser, and J. Provan) and consisting of a Northern Irish research group.

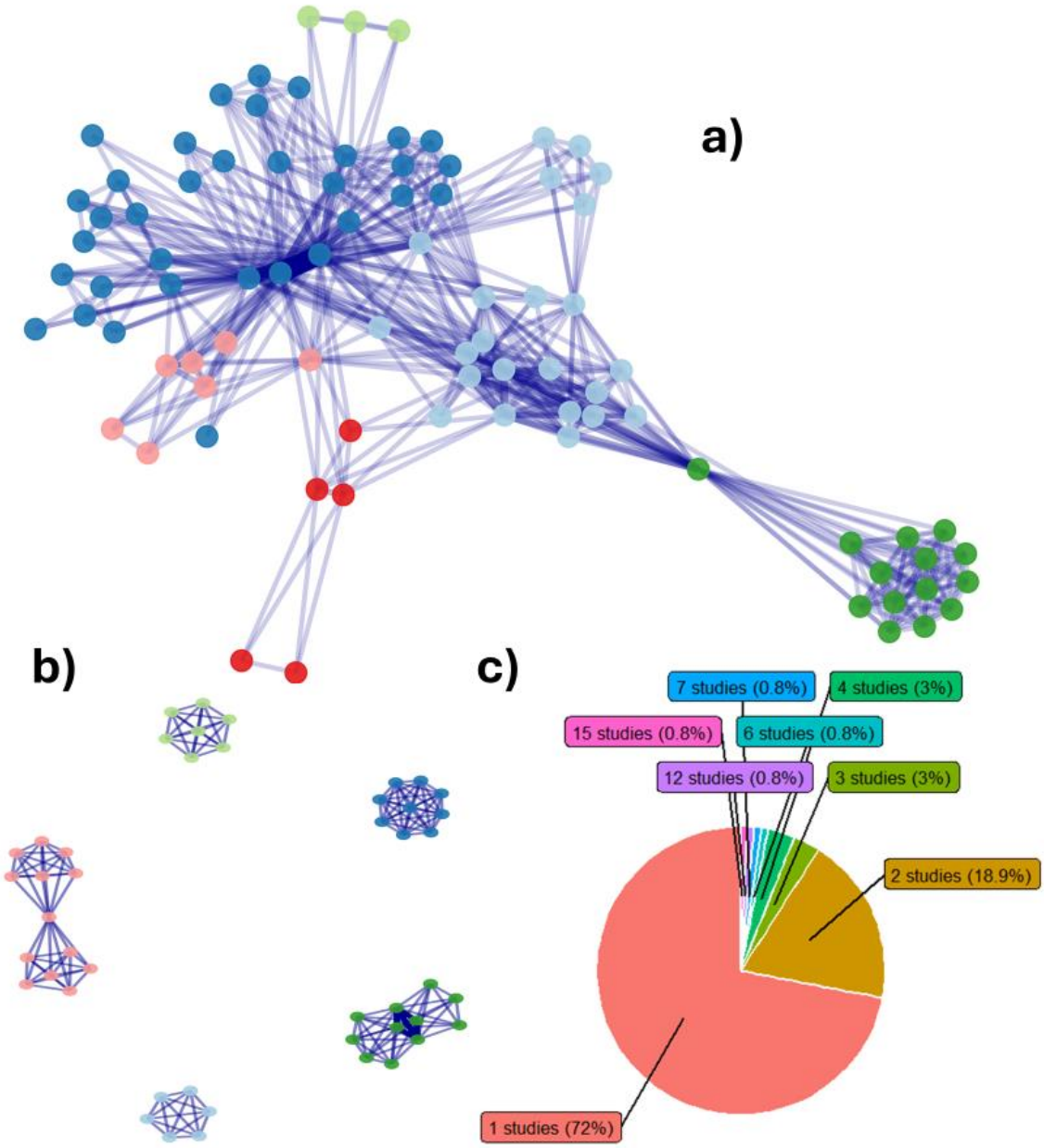

Figure 2.5. a) Primary co-authorship network identified, coloured according to cluster using maximal modularity clustering. b) Secondary co-authorship networks that were either not connected to any other paper, or a single other paper. c) The total of number of authors according to how many studies each has published. Nodes in a and b represent unique authors, with edges representing instances of collaboration on publications, weighted by number of instances of co-authorship.

When analysing the primary network (Figure 2.5a), six communities of collaborators emerge. Interestingly, three of these groups can be described best by papers with a specific primary authorship, namely an Ireland associated community in pink (Schoenrock et al., 2019; Schoenrock et al., 2020), an international community (Canada, Australia, Ireland, Japan, Denmark, and Faroe islands) in light green (Bringloe et al., 2022; Bringloe & Saunders, 2018), and a more eco-physiologically focused community with authors from Germany, France, Norway, Philippines and Portugal in red (Liesner et al., 2020).

The remaining large and well connected clusters represents a number of papers and authors associated primarily with France and Portugal, specifically the Station Biologique de Roscoff and University of Algarve. The dark green community can be best described by papers authored by associates of the Station Biologique de Roscoff, but with collaborators in Université de Toulon and the Muséum National d'Histoire Naturelle amongst others (Fouqueau et al., 2024; Reynes et al., 2024; Reynes et al., 2021). The light blue community is primarily associated with University of Algarve (Assis, Serrão, et al., 2018; Moller Nielsen et al., 2016; Neiva et al., 2018; Neiva et al., 2020; Paulino et al., 2016), but has some international collaboration, with a single author acting as a collaborative nexus point between the light blue Portuguese community and light green international community (D. Krause-Jensen). The dark blue community is perhaps the most complex, and can be best described as the core research community from Station Biologique de Roscoff along with their collaborators (Billot et al., 2003; Couceiro et al., 2013; Guzinski et al., 2016; Guzinski et al., 2020; Jaugeon et al., 2025; Mauger et al., 2023; Oppliger et al., 2014; Robuchon et al., 2014; Valero et al., 2011), many of whom are authors of many of the papers identified in this review (Figure 2.6).

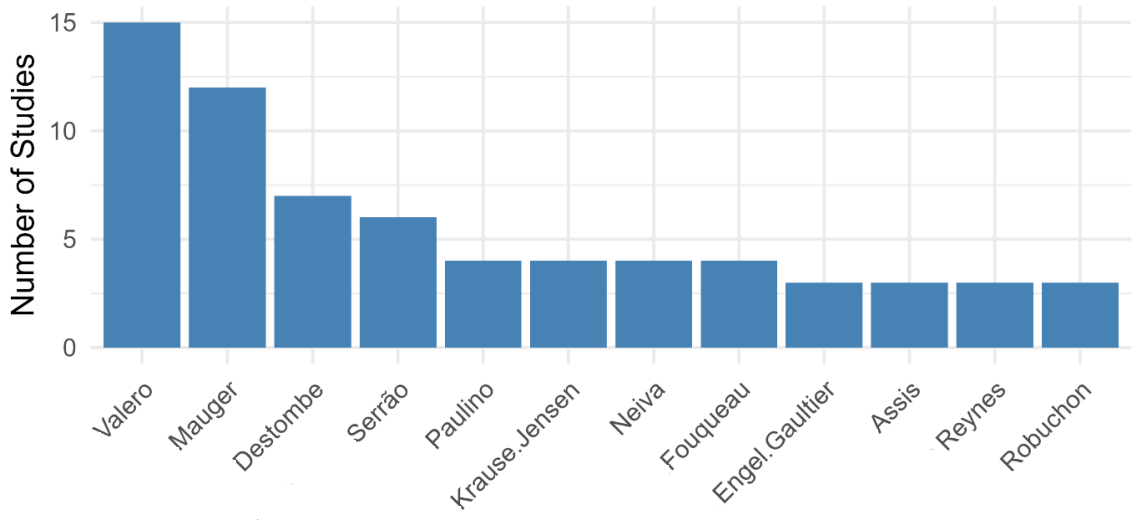

Figure 2.6. All authors that published more than two papers included in this review.

When this primary community identified in the network analysis is plotted on a heatmap (Appendix B) according to co-authorship, it becomes apparent that a small minority of authors tend to collaborate very frequently, but that most authors only work on a single paper. This is likely in part explainable due to principal research investigators whose focus is in kelp working with early career researchers, such as PhD researchers, who have since not published anything in this review. One notable cluster is of the three most published authors, Valero, Mauger, and Destombe, all associated with Station Biologique de Roscoff, and whom

often co-author on papers. Another notable cluster of co-authors is the University of Algarve cluster consisting of Serrão, Paulino, and Neiva and their collaborators including Krause-Jensen from Aarhus University, Denmark.

Geographic sampling clusters

The geographic distribution of all sampling sites extracted in this review (Figure 2.7) shows hotspots of activity near Brittany (France), Bergen (Norway), and Co. Down (Northern Ireland). The Norwegian hotspot can largely be attributed to regional sampling of *S. latissima* at two major fjords (Ribeiro et al., 2022) to identify Isolation By Distance (IBD) and local isolation of populations. The northern Irish cluster can be attributed to the Northern Irish community that studied *L. digitata* (Brennan et al., 2014) and *S. latissima* (Mooney et al., 2018) in Strangford Narrows, a channel that links a sea lough (Strangford Lough) to the Irish Sea, in an effort to link complex hydrodynamic modelling of currents to genetic structure. The hotspot in Brittany is the most well sampled and composed of a mixture of *S. latissima* and *L. hyperborea*, but with the majority of sites being sampled for *L. digitata*. This Brittany cluster can be explained by the presence of the major research station at Roscoff (Station Biologique de Roscoff), which is associated with both the dark blue and dark green communities identified in network analysis (Figure 2.5a) (Billot et al., 2003; Couceiro et al., 2013; Fouqueau et al., 2024; Oppliger et al., 2014; Robuchon et al., 2014; Valero et al., 2011). This suggests that researchers frequently study populations nearby their research institution, and that this can skew the research landscape as a whole, as it doesn't necessarily reflect the true distribution of these species.

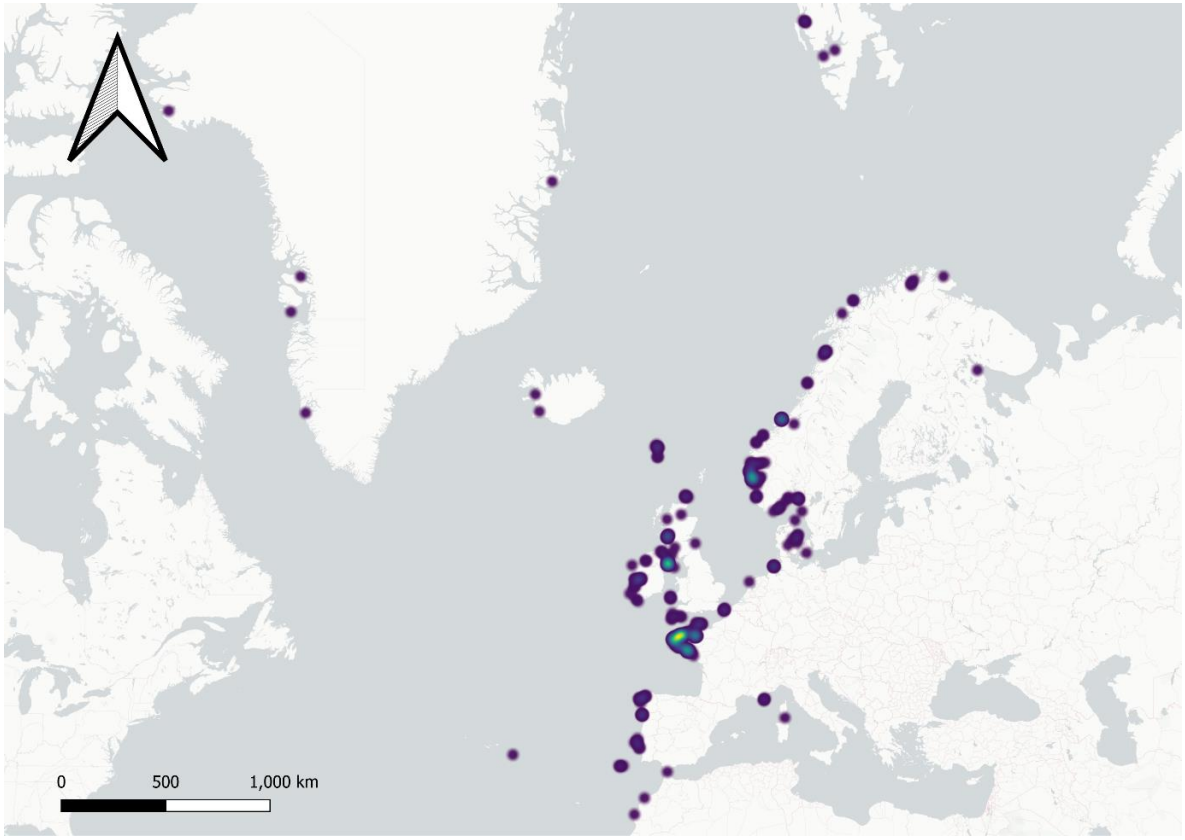

Figure 2.7. Heat map of all unique kelp sampling sites included in papers identified in this review. Species included are Alaria esculenta, Laminaria digitata, Laminaria hyperborea, Laminaria ochroleuca, Laminaria rodriguezii, *and* Saccharina latissima. Colour gradient ranges from blue (low density sampling) to red (high density) along an increasingly warm spectrum of colours.

2.4.2 Species diversity and population genetic structure

Each kelp species identified in this study was assessed for broad patterns of genetic diversity and population genetic structure across Europe and the north-east Atlantic, and where possible compared to the modelling of (Assis, Araujo, et al., 2018), who identified regions of long-term persistence (RLTP) throughout glacial cycles. This was not possible for *L. rodriguezii* for which there were no RLTP data available for this species.

2.4.3 Saccharina latissima

Overview

Saccharina latissima was included in 41.3% of the reviewed studies and accounted for 38.5% of the sampled sites. The majority of studies on *S. latissima* used SSRs (7/12), while a couple combined COI barcodes and SSRs (2/12) (Luttikhuizen et al., 2018; Neiva et al., 2018), one combined SSRs and SNPs (Guzinski et al., 2020), and one each used only COI barcodes

(Bringloe & Saunders, 2018) and SNPs (Bråtelund et al., 2024). Most of these were genomic SSRs developed by (Paulino et al., 2016), with expressed sequence tag (EST) derived SSRs by Guzinski et al. (2016) being the next most commonly used, which are cheaper to develop but show less polymorphism due to higher conservation of transcribed regions. Additional SSRs were developed by Mooney et al. (2018) which were genomic (including one adapted for use from *S. japonica*) and Luttikhuizen et al. (2018) which were transcriptomic, but do not appear to have been reused elsewhere, while Evankow et al. (2019) cross amplified an additional three SSRs (two EST derived, one genomic) from closely related species (Wang et al., 2011; Zhang et al., 2015).

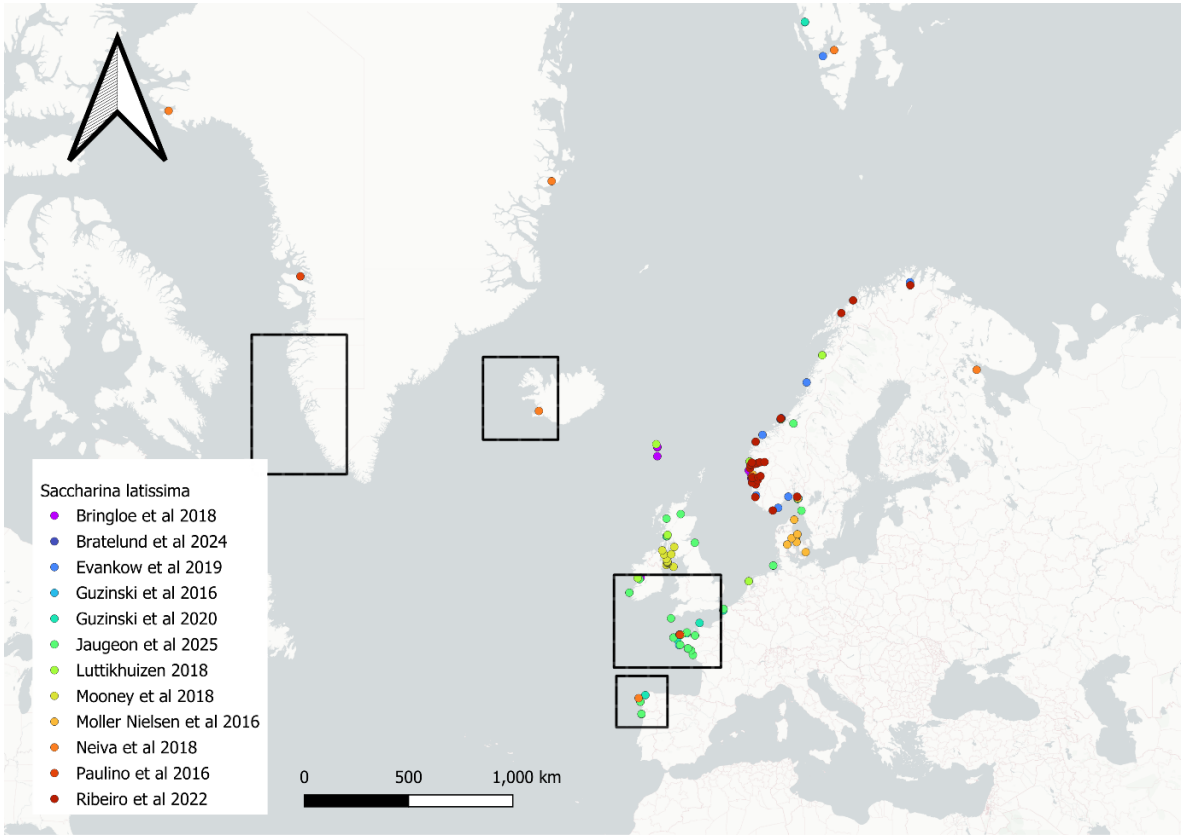

Figure 2.8. Map of Saccharina latissima *sites with each colour representing a different study. Black quadrilaterals represent projected regions of long-term persistence following Assis, Araujo, et al. (2018).*

Genetic structure

European *S. latissima* appears to broadly be structured into two main clusters according to a recent and extensive SSR study, a northern cluster that includes Ireland, Scotland, Norway, and Sweden, and a southern cluster that ranges from Helgoland to Portugal, including the southern coasts of the UK (Jaugeon et al., 2025). A COI haplotype study found a similar north/south divide and pattern of diversity where samples from Denmark, Ireland, and southern and northern Norway were undifferentiated, compared to more differentiated

haplotypes in France and the Netherlands (Luttikhuizen et al., 2018). A further study using COI haplotypes and SSRs indicated a similar divide between southern and northern populations, but this included genotypes from Iceland, Russia, and Svalbard in the Arctic Circle (Neiva et al., 2018).

The northern cluster further divides into two groups, one consisting of Norway and Sweden and one that includes Scotland and Ireland (Jaugeon et al., 2025). A study that only used COI but sampled Faroe Islands in addition to Norway and Ireland found a dominant haplotype across all three sites, but with a unique haplotype in the Faroe Islands (Bringloe & Saunders, 2018). This is corroborated by another study that used both COI and SSR, which found shared haplotypes in Faroe Islands and Scotland (Luttikhuizen et al., 2018). Additional sub-structuring within the northern cluster may be seen in Norway, where in addition to the Skagerrak and south Norway clusters, there is a noticeably distinct cluster in the Barents Sea (Evankow et al., 2019). Interestingly, other studies found that there was significant differentiation between sites in south Norway and Sweden and the Skagerrak region, suggesting there may be more genetic subdivision in this particular region(Guzinski et al., 2016; Moller Nielsen et al., 2016). Further results from SSR data add to the structural patterns in Norway, as another study found some differentiation between samples from the Skagerrak region and coastal Norway, but also from samples towards the Barents sea (Ribeiro et al., 2022). Additionally, it identified Lofoten archipelago as a possible boundary that decreases geneflow, possibly helping explain the sub-structuring previously observed in northern Norwegian sites (Evankow et al., 2019).

A finer scale study found that Northern Irish sites had noticeable population genetic structure that was dependant on hydrodynamic conditions, and were distinguishable from west Scottish sites, as well as from a site in the Isle of Man (Mooney et al., 2018). Interestingly, a small scale study using high density SNPs and read-backed haplotypes in Norway identified that a lot of genetic structure depends heavily on hydrodynamics, and that populations can show significant sub-structuring even at less than 2 km apart (Bråtelund et al., 2024). The first study to use SNPs (in conjunction with SSRs) found that the Scottish site formed a cluster of its own that was substantially different from one at Svalbard (Guzinski et al., 2020). It also differentiated sites in the southern cluster, one consisting of sites from the Iberian Peninsula, but more surprisingly also one sample on the south of Brittany as its own group, the three sites at the tip of Brittany as another, and sites west of Brittany until Helgoland as the final cluster.

Geneflow and Connectivity

While a majority of studies that looked at models of Isolation By Distance (IBD) in *S. latissima* found significant IBD results (Bråtelund et al., 2024; Evankow et al., 2019; Guzinski et al., 2020; Moller Nielsen et al., 2016; Mooney et al., 2018; Ribeiro et al., 2022), one study did not (Guzinski et al., 2016), which may in part be due to geographic distance being measured "as the crow flies" instead of as minimum marine distance or coastal distance. One study compared logarithmic and linear models and found logarithmic to be a better fit (Moller Nielsen et al., 2016). Many other studies included caveats for IBD models with Mooney et al. (2018) and Ribeiro et al. (2022) both finding regional exceptions to the general pattern of IBD (in and around lough and within a fjord respectively). This suggests that more complex hydrodynamic influences play a larger role at smaller scales and with higher resolution sampling. Bråtelund et al. (2024) improved the fit of their IBD model from ~15% to ~27% when they changed to an Isolation By Oceanography model that considered spore dispersal ability by currents. Interestingly, Guzinski et al. (2020) found a significant pattern of IBD for both SSRs and SNPs, but SNPs appeared to have more significance and higher explanatory power than SSRs when a geographically distant site was removed from analysis.

Genetic diversity

Many studies on *S. latissima* were not designed to adequately analyse population genetic diversity because they used COI barcoding (Bringloe & Saunders, 2018; Luttikhuizen et al., 2018), found no clear pattern of allelic richness or observed/expected heterozygosity (Guzinski et al., 2016; Ribeiro et al., 2022), or were on small regional scales that are difficult to extrapolate to larger geographical regions (Bråtelund et al., 2024; Mooney et al., 2018). Additionally, comparison of values obtained across studies using different markers, data processing, and data analysis is not likely to provide sound results, as has been demonstrated for SSRs (Schoenrock et al., 2020). In the most recent study however, Jaugeon et al. (2025), provides a good geographic sampling range and can act as a baseline for further investigation. They found that generally the northern cluster was more diverse than the southern cluster, having higher rarefied allelic richness (Ar) (8.67 vs 7.6 ± 0.13), expected heterozygosity (He) (0.48 vs 0.31), and private alleles (Pa) (54.57 ± 1.79 vs 32.16 ± 2.02). The southern cluster interestingly seemed to have higher Ar and He in the clusters around Brittany compared to south North Sea and Iberian Peninsula clusters (which corresponds well to Guzinski et al. (2020), but the Iberian Peninsula exhibited the highest Pa in the southern cluster (11.14 ± 1.51). Notably, the northern cluster only separated into two clusters in this analysis, an Irish/Scottish cluster and Norwegian/Swedish one. The latter had Pa and Ar comparable to the southern cluster, but a notably elevated He. The Irish/Scottish cluster however exhibited the highest Ar

(6.17 ± 0.22), He (0.47), and Pa (21.75 ± 3.13) of all sub-clusters, making it the most diverse based on all metrics. This finding may additionally help explain how Mooney et al. (2018) identified four highly distinct genetic clusters in Northern Ireland, Scotland, and Isle of Man despite the small geographic range and use of only six SSR markers, which have a relatively low resolution at smaller geographic scales.

Recommendations and conclusions

These studies suggest that there is a major divide in the northern and southern populations of *S. latissima*, with the boundary between these clusters being south of Ireland, and possibly bisecting southern England, until west Sweden, the southern tip of Norway, or near Denmark. This northern cluster further seems to be divided into a southern (i.e. Skagerrak), mid, and arctic Norwegian cluster, as well as an Irish cluster, and Scottish/Faroe Islands cluster. This suggests that more complete sampling around Ireland, Scotland, and the North Sea could provide valuable information on the population structure of the northern cluster. The Irish and Scottish populations additionally show signs of being highly diverse with large effective population sizes and are possible locations of glacial refugia. Ideally future studies should include samples from Iceland, Faroe Islands, east Greenland, and Svalbard, as evidence for Arctic refugial recolonisation may be uncovered by inclusion of these regions.

Sampling around the southern coastline of the North Sea could also help increase the resolution of the southern cluster, in particular around the Netherlands, Belgium, and south-east England. The North Sea is a potential contact zone between these two clusters, and understanding the geneflow in this region could help frame larger geographical findings in wider context, although populations in this area are probably fragmented owing to habitat discontinuities. The southern cluster appears to be more complex in terms of structure, with groups existing in the southern North Sea, Celtic Sea, Bay of Biscay, and along the Portuguese coastline. Further sampling in southern Portugal, the Spanish coasts of the Bay of Biscay and around the southern coasts of England and Ireland could help to further refine the boundaries and structure of the southern cluster, because sampling across studies have focused heavily on Brittany. Similar fragmented habitats may be seen in the Bay of Biscay as in the North Sea, and increased surveying of wild kelp populations in these regions is advisable.

S. latissima was sampled the most extensively across its distribution compared to the other kelp species in this review and showed hotspots of sampling near Roscoff, Strangford Lough, Bergen, and in the eastern North Sea more generally (Figure 2.8). Interestingly, the only RLTP identified that has been extensively sampled is within the southern coastlines of the English Channel region, although the Iberian RLTP has had several sites. This leaves much of Ireland

and England, as well as Wales entirely unsampled despite indications of being regions of higher diversity, and potentially containing key populations that may help explain post-glacial population dynamics in this area. The northernly regions in Greenland and Iceland have been almost entirely unsampled apart from a single site that appeared to suggest moderate diversity in this region, similar to sites from Bergen and Russia (Neiva et al., 2018).

Future studies should aim to sample sites from a broad geographic scale, similar to Jaugeon et al. (2025) and include more sites from Ireland, England, Iceland, Greenland, and from the Barents Sea where possible for direct comparison. Additionally, haplotype mapping using markers such as COI can be relatively cheap and should be done in conjunction with SSR or SNP analysis, especially when analysing on a large geographic scale. SSR markers are best used on larger geographic scales or particularly diverse/differentiated populations, while SNPs might provide more utility for fine scale regional studies.

2.4.4 Laminaria digitata

Overview

Laminaria digitata was included in 44.8% of the studies and accounted for 37.6% of the sampled sites. The majority of studies (10/13) used SSRs, while two used SNPs (Reynes et al., 2024; Reynes et al., 2021), and one used COI barcodes (Bringloe & Saunders, 2018). The first study that developed genomic SSRs became key to later work (Billot et al., 1998), with many studies using the same 7 SSR markers for their analyses (Billot et al., 2003; Couceiro et al., 2013; Oppliger et al., 2014; Valero et al., 2011). These SSRs were also used in combination with EST derived SSRs by Brennan et al. (2014) and in conjunction with SSRs cross amplified from an *L. ochroleuca* study (Coelho et al., 2014), which was the most common approach among all *L. digitata* studies (Fouqueau et al., 2024; King, McKeown, et al., 2020; Liesner et al., 2020; Neiva et al., 2020; Robuchon et al., 2014).

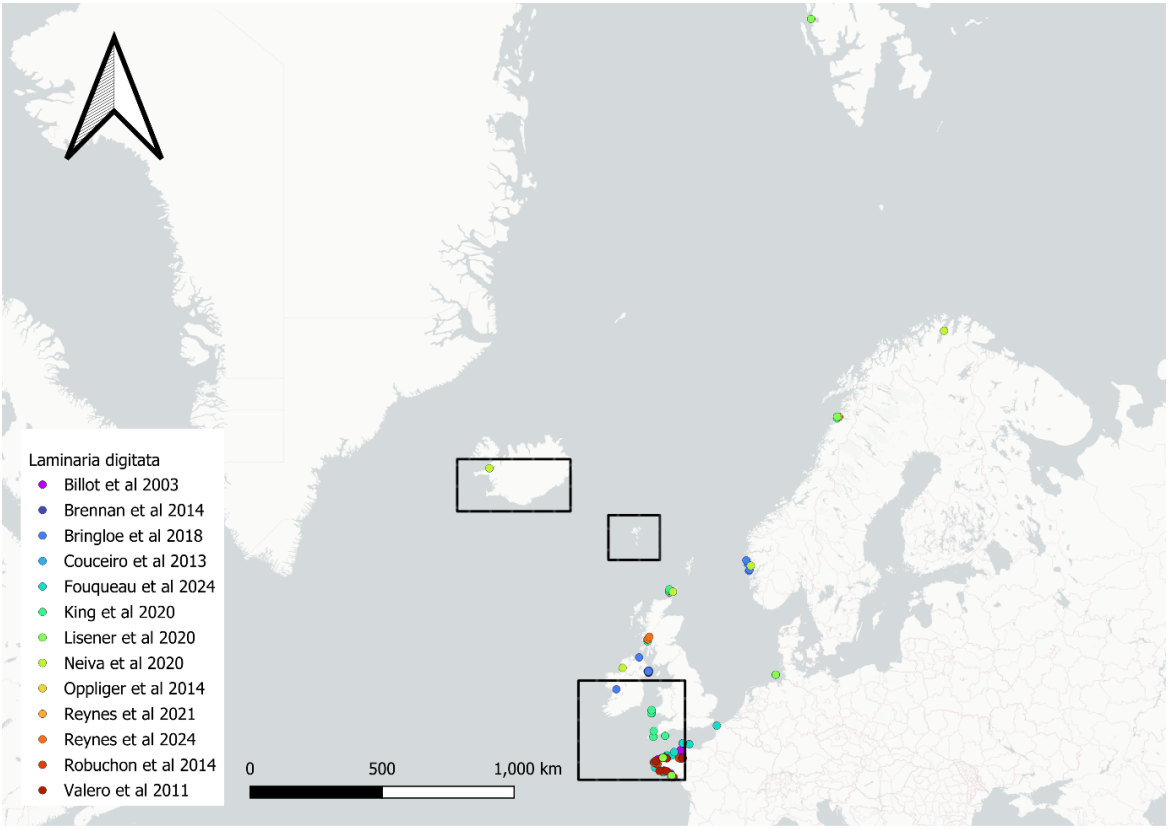

Figure 2.9. Laminaria digitata *sampling sites with each colour representing a different study. Black quadrilaterals represent projected regions of long-term persistence following Assis, Araujo, et al. (2018).*

Genetic structure

The study by Neiva et al. (2020) is the most geographically extensive on *L. digitata* in Europe to date, and identifies a north-south divide between populations from Brittany (Robuchon et al., 2014) and those from Ireland, Scotland, Iceland, and Norway. Further sub-structuring in this study separates out Iceland and Ireland as another cluster in the north and the most eastern population of France in the English channel as distinct. At the finest level of structure analysed in this study the northern cluster consists of Ireland and Iceland as independent groups, while Orkney in Scotland groups with the south Norwegian cluster (both in the North Sea), and the two remaining northernly Norwegian sites cluster together. The southern group in Brittany shows high levels of admixture in the central sites at this level, but the most southernly and easternly sites both separate relatively distinctly. Some of these broadscale patterns can be somewhat corroborated by Liesner et al. (2020) who found a north/south divide between sites from Quiberon, Roscoff, and Helgoland in the south and the northern Norwegian samples, with the most northernly site being located in Spitzbergen (Figure 2.9). Interestingly, when samples from sites in north Norway, Orkney, west Scotland, Wales, and south-west England were included, a clear distinction between clusters from Scotland, Norway, and south-west UK was

found (King, McKeown, et al., 2020). This can be compared to sites in west Scotland, Roscoff, Quiberon, and Helgoland, which similarly found sub-structuring that separated out west Scotland as being a separate group, further indicating a north/south divide (Reynes et al., 2024).

Therefore, the evidence to date suggests that the southern *L. digitata* cluster may broadly include sites along the English Channel (including the south of the UK) and southern North Sea as far as Helgoland. It is unclear from these data to what extent samples in the northern cluster are connected by geneflow, and further sampling that includes sites from Scotland, Ireland, Faroe Islands, Iceland, and along the Norwegian coast would help increase resolution. There appears to be identifiable structure in the northern cluster (King, McKeown, et al., 2020; Neiva et al., 2020; Reynes et al., 2024), suggesting a potential east/west divide within this northern cluster (possibly between the North Sea and Atlantic Ocean) but further studies are needed to confirm this. While sites in Ireland, Northern Ireland, and southern Norway which could have helped to resolve this were sampled, the use of COI barcode markers resulted in only a single haplotype, which is unsuitable for identification of any population genetic structure (Bringloe & Saunders, 2018).

The remaining studies only sampled *L. digitata* in limited regions, including Northern Ireland (Brennan et al., 2014), but mostly Brittany and other regions of France (Billot et al., 2003; Couceiro et al., 2013; Fouqueau et al., 2024; Robuchon et al., 2014; Valero et al., 2011). No appreciable structure was found within Strangford Lough in Northern Ireland, probably because of the small geographic scale of the study (<10 km) and the complex local hydrodynamic environment (Brennan et al., 2014). Contrastingly, there has been substantial work done to identify the structure around Brittany and Normandy at similar scales. For example, (Fouqueau et al., 2024) aimed to build on previous studies in this region and included the entire range previously sampled. In their analysis of 32 sites they identified 8 clusters; in south Brittany, south-west Brittany, north and west Brittany, Bay of St Malo, Normandy, and isolated individual populations at the farthest eastern Wissant, the island of Roche-Douvres, and near the Bay of Locquirec. Interestingly, there were variable levels of clustering, with k = 2, k = 3, and k = 12 all considered. At k = 2 there was a notable differentiation of southern Brittany and Bay of St-Malo, with southern Brittany clearly separating out as its own cluster at k = 3. The k = 12 clustering separated out the above 8 clusters, but with substantial admixture in the western and northern population that reduced the interpreted cluster amount from 12 to 8. This high level of admixture detected in many of the sites is in contrast to many of the previous studies (Billot et al., 2003; Robuchon et al., 2014; Valero et al., 2011), and was hypothesised by the authors to be in part a function of discrete sampling regimes. They apply similar caveats

to the sites they sampled at the Bay of St Malo and Normandy, but suggest Wissant may not be affected by this because of its geographic isolation from nearby populations. This in-depth analysis of a region and comparison of previous findings and sites indicates the necessity of collating data in order to identify sampling sites that may represent divergent genetic groupings for direct comparison. This approach helps identify whether *L. digitata* populations are truly divergent or whether they represent regions of admixture with nearby unsampled populations. It also allows for direct comparison of the magnitude of divergence of different groups by providing an internal statistical quantification of structure, such as via the Bayesian Information Criterion (BIC).

Genetic diversity

Several studies sampled *L. digitata* extensively and allow for comparisons of populations across Europe (King, McKeown, et al., 2020; Liesner et al., 2020; Neiva et al., 2020; Reynes et al., 2024; Reynes et al., 2021). Others, however, were not suitable for broad comparison of genetic diversity of *L. digitata*, because they used COI barcode markers that only had a single European haplotype (Bringloe & Saunders, 2018) or were based on a very limited spatial scale (Brennan et al., 2014).

Genetic diversity of *L. digitata*, as measured by standardised allelic richness and unbiased gene diversity, has been shown to be greater in the northern European cluster consisting of sites in Ireland, Scotland, and Norway (A69 = 8.473 ± 0.262, He = 0.645) than in the southern Brittany cluster (A69 = 6.854 ± 0.32, He = 0.520). Additionally, there was a signal of decreasing diversity in both of these metrics with increasing latitude within the northern European cluster. The population in Ireland was the most diverse for both metrics of all sites sampled, while Iceland had comparatively low diversity in all measured metrics for the northern cluster, despite being in a hypothetical RLTP (Neiva et al., 2020). Similarly, northern sites (west Scotland and Orkney) were more diverse (as measured by He and Ar) than southern sites (Wales and south-west England) as well as less related (King, McKeown, et al., 2020). Comparison with data from Robuchon et al. (2014), indicated that the northern sites in Scotland were still more diverse, while the southern sites showed similarity to Brittany populations (King, McKeown, et al., 2020). It is important to note, however, that no inter-lab calibration appears to have been conducted to standardise results for comparison as in other studies (Schoenrock et al., 2020).

Another study by Reynes et al. (2021) included *L. digitata* only for comparison purposes to *L. rodriguezii* and was limited in analysis, but most of these sites (with the exception of the Norwegian site) were analysed by the authors in another study (Reynes et al., 2024). (Reynes

et al., 2024) tested for effects of temporal changes in populations in west Scotland, Quiberon, Roscoff and Helgoland and concluded that sites in Scotland were more diverse (He = ~0.106) than any other site, with Roscoff being the next most diverse (He = ~0.067) and Helgoland being the least diverse (He = ~0.014). These findings can be compared a study that sampled Quiberon, Roscoff, and Helgoland as well, but also three sites in Norway, as far as Spitzbergen and concluded that the lowest genetic diversity was always seen in Helgoland (standardised Ar = 2.594 ± 0.422, mean Pa per locus = 0.083 ± 0.083, He = 0.306 ± 0.076) while the highest (standardised Ar = 4.875 ± 0.786, mean Pa per locus = 0.583 ± 0.229, He = 0.480 ± 0.082) was always in Roscoff (Liesner et al., 2020). This also aligns with similar findings from this region (Reynes et al., 2024). Interestingly the Norwegian sites were relatively diverse, being more comparable to Quiberon samples in their Ar, Pa, and He than to Helgoland. Notably, the northern (Spitzbergen) and southern (Bodø) sites in Norway were more diverse than the central site (Trømso). Another study perhaps best encapsulates the complex diversity patterns in Brittany, and shows similar patterns to those already discussed, which finds that Quiberon and the southern Brittany cluster are less diverse (as measured by Ar and He) than Roscoff and the north and west Brittany cluster (Fouqueau et al., 2024). Interestingly, this study also identifies the south-west Brittany cluster and Normandy cluster as somewhat diverse, while it finds that the sheltered Bay of St Malo sites and Wissant near the strait of Dover had low diversity (Fouqueau et al., 2024).

Geneflow and Connectivity

The four studies that specifically tested for IBD in *L. digitata* (Billot et al., 2003; Brennan et al., 2014; King, McKeown, et al., 2020; Robuchon et al., 2014) all had significant results, with some additionally showing insignificant IBD for specific sites/regions. Billot et al. (2003) found a slight pattern of IBD to exist among all sites (R^2 = 0.034, pMantel = 0.016), but this explanatory power and significance increased when only including sites continuous unbroken stands of kelp (R^2 = 0.504, pMantel = 0.006). Interestingly Brennan et al. (2014) found a somewhat similar pattern of IBD at a smaller scale (R^2 = 0.4738, p = 0.002), which held true when removing a somewhat hydrodynamically isolated site, but reduced in both power and significance (R^2 = 0.111, p = 0.015). When a less extensive hierarchical sampling scheme was used in Brittany similar to Billot et al. (2003) by Robuchon et al. (2014), a strong and significant signal of IBD was found (p = 0.002, R^2 = 0.867). This did not hold true for all regions within the study, however, with the more marginal regions in southern Brittany (p = 0.182, R^2 = 0.831) and St Malo Bay (p = 0.299, R^2 = 0.192) showing insignificance, and the core populations at Iroise Sea (p = 0.008, R^2 = 0.728) and Morlaix Bay (p = 0.032, R^2 = 0.526) showing both significance and relatively high explanatory power. The study by King, Moore, et al. (2020) simply states a highly significant

pattern of IBD ($p < 0.001$) between samples from west Scotland, Orkney, Wales, south-west England, and Norway alongside a graph. Another study identified a significant IBD across Europe ($R^2 = 0.5148$, $p = 0.001$), which is significant when analysing the northern European and southern Brittany clusters (north-east: $R^2 = 0.4581$, $p = 0.006$; Brittany: $R^2 = 0.8646$, $p = 0.008$) independently (Neiva et al., 2020). This is notable because the Brittany cluster is sampled over a much smaller distance but shows higher explanatory power, which may in part be a function of decreasing diversity with increasing latitude in the northern cluster lowering resolution.

Perhaps most interestingly, Fouqueau et al. (2024) attempted to apportion the explanatory power of geographic distance (equivalent to IBD), Oceanographic distance (based on hydrodynamic modelling), habitat continuity, and Sea Surface Temperature (SST) to the genetic differentiation of *L. digitata* observed across all sites, regional sites (Brittany), and sites of continuous kelp stands. At the overall scale they found that geographic distance explained the greatest amount of variance ($R^2 = 0.475$), followed by mean and minimum SST ($R^2 = 0.165$), and lastly oceanographic distance ($R^2 = 0.073$). When more distant sites (> 600 km from the rest of the sites) were removed from this analysis it was found to increase the explanatory power of oceanographic connectivity ($R^2 = 0.489$) to similar levels as geographic distance ($R^2 = 0.485$), while SST (mean and min.) also became more explanatory ($R^2 = 0.273$). When only continuous stands were analysed (excluding sites in St Malo Bay) oceanographic connectivity ($R^2 = 0.794$) explained most of the variance, followed by geographic distance ($R^2 = 0.513$) and SST (max.) ($R^2 = 0.179$). While authors could not draw any firm conclusions about SST or habitat continuity from this study, it was apparent that the populations at St Malo Bay were more isolated due to geographic distance than due to oceanographic currents, in contrast to suggestions from previous studies (Billot et al., 2003; Robuchon et al., 2014). Evidence for the effect of minor currents on genetic structure at a local level similar to Brennan et al. (2014) was found, in addition to the effect of major currents, helping explain the robust divergence of south and north Brittany populations, possibly via the Ushant front.

While overall IBD seems to be a good explanatory model for genetic differentiation of *L. digitata* over a large distance, the extent to which this is true is highly dependent on the scale at which this is being analysed. Furthermore, the finer the scale becomes, the more important oceanographic features become in determining the connectivity between sites. Additionally, the use of SSRs in all of the studies looking at IBD may have an impact on the resolution of these models, and finer geographic scales may benefit from use of high-density SNPs in order to examine sites that are hypothesised to be relatively well connected by geneflow.

Recommendations and conclusion

On a European scale it appears that there is a general north/south divide in *L. digitata*, with the northern cluster ranging from Ireland to Norway, and a boundary probably existing somewhere on the coastline of Great Britain. Further sampling along the coastlines of the Irish Sea as well as around the coastlines of the North Sea would help resolve the extent of this divide. Comparison of the well sampled coastlines of Brittany to other coastlines in the Celtic Sea may identify additional structure and unsampled diversity as tidal currents, and the Ushant and Irish Sea front add additional pressures to oceanographic connectivity. The data from these studies is consistent with modelling studies that the predicted that the Celtic Sea was a potential region of high diversity because of to long-term persistence, however, it also identifies Iceland and the Faroe Islands as additional regions of interest (Assis, Araujo, et al., 2018). There have been no populations sampled from the Faroe Islands yet, but the single site from Iceland found low diversity and no private alleles when compared to other northern cluster sites, which contrasts with this hypothesis (Neiva et al., 2020). The lack of comprehensive sampling along the coast of Norway, and the comparable diversity of Norwegian populations to regions of the southern cluster, could suggest additional diversity existing in the north (King, McKeown, et al., 2020; Liesner et al., 2020; Neiva et al., 2020). Comparisons of more Norwegian sites are needed to identify finer sub-structuring within this northern cluster.

2.4.5 Alaria esculenta

Overview

Alaria esculenta was included in 10.3% of the studies and accounted for 4.7% of the sampled sites, with eight being in Norway, three in the Faroe Islands, two sites each in Greenland and France, and one in Ireland. While this is a relatively broad distribution considering the low number of sites sampled, seven of the eight sites in Norway were centred around the Bergen region (Bringloe & Saunders, 2018). Three of the five identified regions of long-term persistence were sampled despite this relatively low number of sites (West Greenland, English Channel/South Ireland, and Faroe Islands).

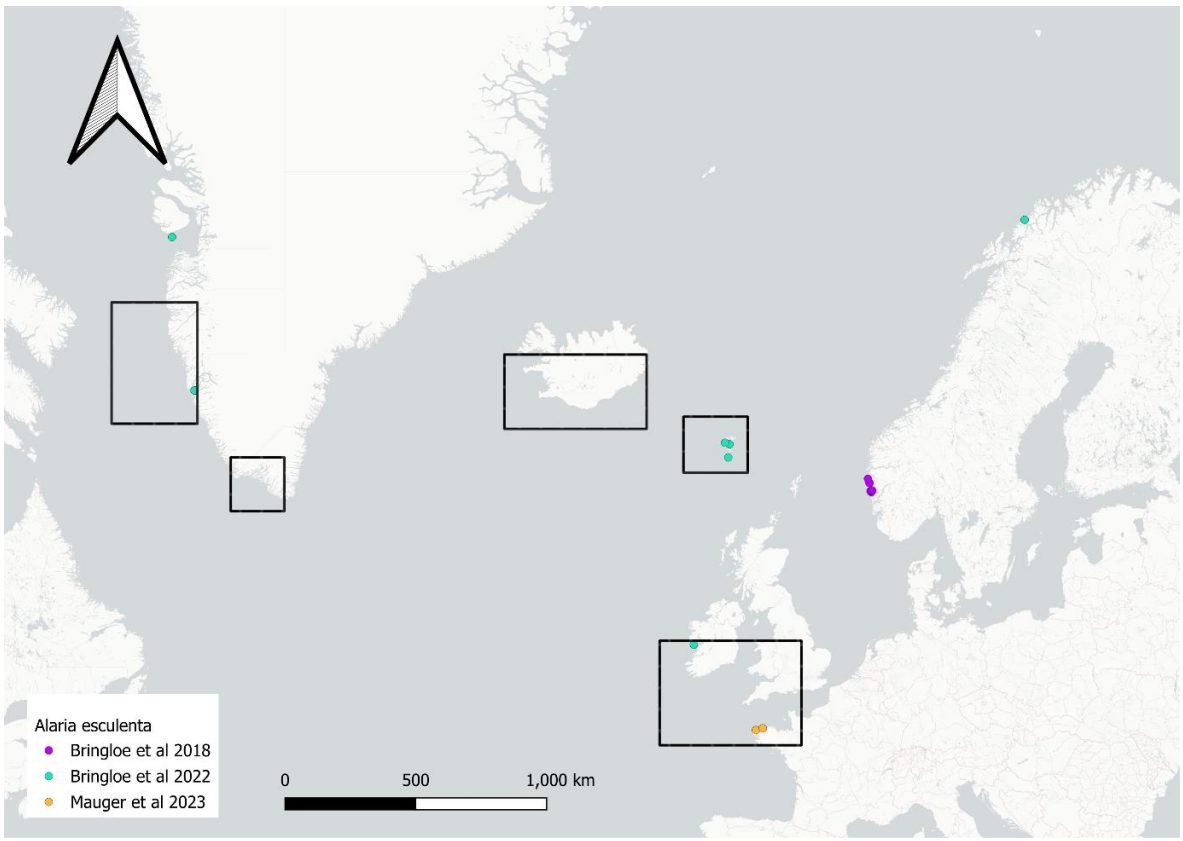

Figure 2.10. Alaria esculenta *sites sampled with each colour representing a different study. Black quadrilaterals represent projected regions of long-term persistence following Assis, Araujo, et al. (2018).*

Genetic structure

Bringloe et al. (2022) conducted the most comprehensive study of *A. esculenta* populations to date, sampling seven sites across Europe, two from Greenland, three from the Faroe Islands, and one each from Norway and the west of Ireland using SNPs from the nuclear genome (172,544), mitochondrial genome (605), and chloroplast genome (698). It found an optimal k = 4 (including very differentiated samples from the north-west Atlantic, not included in this review) and identified clusters in Greenland, Norway, and Ireland. It also noted that samples from the Faroe Islands appeared to be an admixture of Greenland and Norway genotypes. Interestingly, they discuss the potential for a glacial refuge in the European north (in addition to the one identified in Greenland), and this may be why Bringloe and Saunders (2018) observed a second minor haplotype in their study despite only sampling a small number of individuals from a very small range in Norway. The only other study suggests that there may be significant unsampled population genetic structure in the southern range of this species in

Europe, because they found significant levels of differentiation using SSR markers ($p < 0.001$, Fst = 0.136) over a very short geographic distance (Mauger et al., 2023).

Genetic diversity

Bringloe et al. (2022) found that *Alaria esculenta* in Ireland and Norway both exhibited high levels of nucleotide diversity, moderate levels of heterozygosity, high frequency of minor alleles at lower counts, and rapid linkage disequilibrium decay, suggesting stable and persistent populations with large population sizes. Meanwhile, Greenland showed lower nucleotide diversity but an excess of heterozygosity, potentially indicating small marginal refugia in this region that experienced drift followed by interglacial admixture. Faroe Islands, as a region of admixture, seemed to exhibit high levels of nucleotide diversity (which included organellar diversity), but had the lowest levels of heterozygosity in their study. This apparent contradiction in signals was reconciled by the hypothesis that the founding populations of the Faroe Islands were small and that inbreeding and/or selfing lead to homozygous allele segregation despite the high overall nucleotide diversity because it is a region of recent admixture. The more western French site (Plouguerneau) was more heterozygous than the site at Roscoff (He = 0.566; He = 0.505 respectively), while standardised allelic richness showed a contrasting pattern (4.24 ± 0.15, 3.32 ± 0.10 respectively) of diversity (Mauger et al., 2023). This points to additional complexity in the southern range of Europe for *A. esculenta* that has not been studied yet.

Geneflow and Connectivity

There were no specific analyses that measured connectivity in *A. esculenta* directly, because the study designs either looked at historical population dynamics over large geographic scales or small regional patterns of diversity.

Recommendations and conclusion

Additional studies are needed to identify the structure and diversity hotspots of *A. esculenta* in Europe, which is a key species for human exploitation despite being relatively understudied (Figure. 2.3a, 2.4). Sampling from populations in Ireland, south-west UK, Iceland, and Brittany seem particularly important based on modelling studies but there is a lack of data for comparative studies among sites. Additional sampling on the Norwegian coast is needed to place existing data into wider context, and samples from Scotland may further elucidate the dynamics of geneflow indicated by the admixture identified in Faroe Islands (Bringloe et al., 2022). The development of new SSR markers (Mauger et al., 2023) and construction of an annotated reference genome (Bringloe et al., 2022), provide a promising foundation for further research into this species in Europe. Iceland and the southern tip of Greenland are both

regions that may benefit from more sampling, but greater sampling around Southern Ireland and the south east UK could also identify diverse populations at the southern range edge for this species. Further work to identify geneflow patterns in contemporary populations of *A. esculenta* in Europe is of vital importance because there are fundamental differences in their reproductive physiology compared to other kelp (Starko et al., 2019) species (e.g. they carry spores on the basal sporophylls rather than distally on the lamina as is common in other kelps).

A. esculenta is lacking in both primary research and those studies that exist are geographically limited. *A. esculenta* is of prime economic interest, for both cultivation (Hofmann et al., 2025) and wild harvesting (Figure 2.1). To facilitate a more sustainable European kelp industry research on this species in particular must be increased. This is of vital importance because its aquacultural production has the potential to economically compete with the invasive non-native *Undaria pinnatifida*, with which it shares many gastronomic qualities (Rhatigan, 2009).

2.4.6 Laminaria hyperborea

Overview

Laminaria hyperborea was included in 10.3% of the studies and accounted for 10.8% of the sampled sites. Notably, each of the studies for this species focused on a single country, with French (Robuchon et al., 2014) and Irish (Schoenrock et al., 2020) sites being located in a region of long-term persistence, in contrast to the Norwegian samples (Evankow et al., 2019). Schoenrock et al. (2020) however compared their findings to those of Robuchon et al. (2014) by calibrating microsatellite raw allele calls, which was not possible for the Norwegian sites because of the lack of raw allele call availability. Such inter-study comparisons can greatly increase the utility of the data collected, but can often be difficult to conduct accurately because of the use of different markers as well as a myriad of bioinformatic and methodological approaches that can give rise to inconsistencies among studies (Schoenrock et al., 2020). All studies identified in this review used cross-amplified SSR markers from *L. digitata* (Billot et al., 1998) and *L. ochroleuca* (Coelho et al., 2014), with one study (Evankow et al., 2019) additionally cross amplifying a single marker from *Saccharina* (Wang et al., 2011).

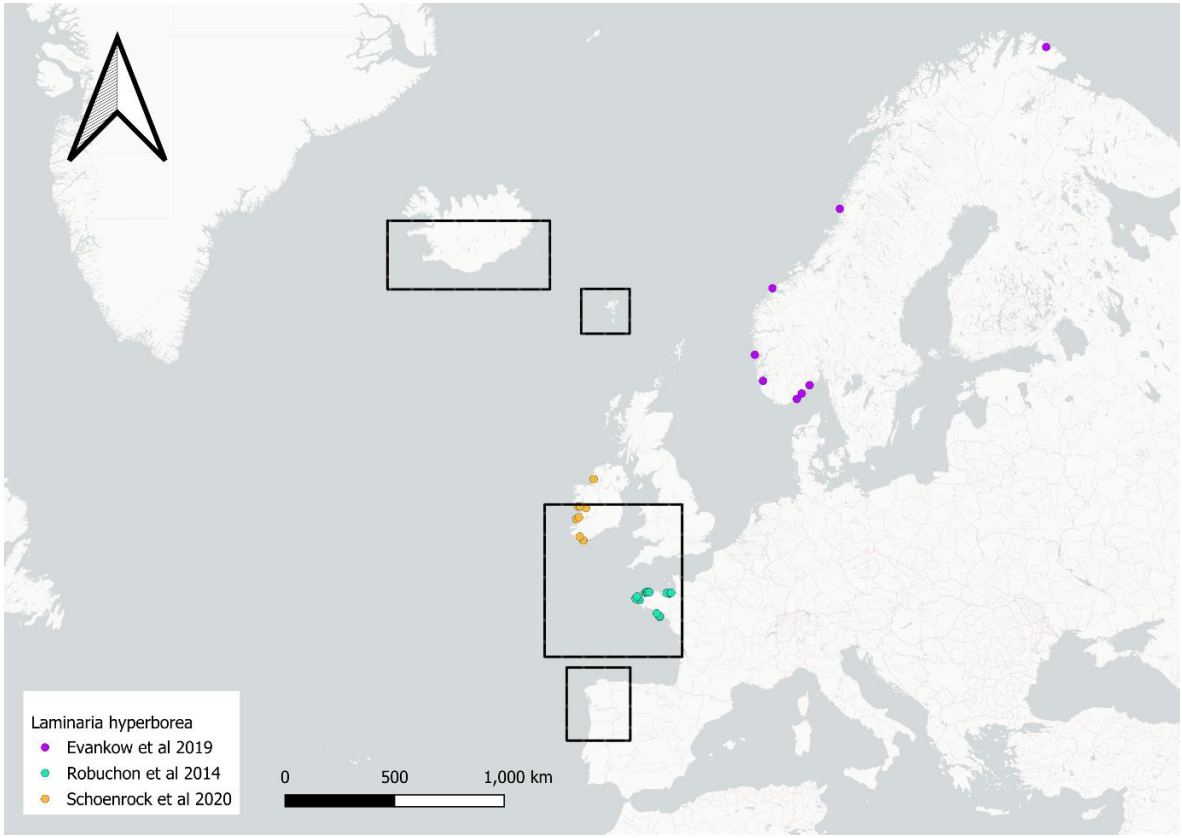

Figure 2.11. Laminaria hyperborea sites with each colour representing a different study. Black quadrilaterals represent projected regions of long-term persistence following Assis, Araujo, et al. (2018).

Genetic structure

All studies on *L. hyperborea* to date were regional in nature, making it difficult to establish population genetic structure of this species at a European scale. Evankow et al. (2019) sampled across the Norwegian coast and found four distinct clusters, corresponding each to the Skagerrak region, North Sea, Norwegian Sea, and Barents Sea. Interestingly, Schoenrock et al. (2020) found an optimal k = 6, but resolved five groupings, one in the north-west of Ireland, one in the west, one in the south-west, and two in the south. This might be greater than anticipated considering the small geographic distance that was sampled compared to (Evankow et al., 2019). Robuchon et al. (2014) did not explicitly include cluster-analysis, but a Principal Components Analysis (PCA) shows that they identified three major groupings, one in west Brittany, one in south Brittany, and one larger cluster consisting of the core populations at the tip of Brittany. Furthermore, when Irish data was compared to the data from Brittany using Discriminant Analysis of Principal Components (DAPC), they found that the Irish populations were not only much more structured, but also much more diverse (Schoenrock et al., 2020).

Genetic diversity

Robuchon et al. (2014) found that diversity of *L. hyperborea* was highest in the Iroise Sea, but that there was generally high diversity from south Brittany until Morlaix Bay, with St Malo Bay in the south-western English Channel being the only region displaying lower diversity. Interestingly, Evankow et al. (2019) found that samples from the Skagerrak region had low diversity, while the neighbouring region in the Norwegian North Sea had increased levels of diversity. They also identified increased diversity from the site in the Barents Sea, while the Norwegian Sea showed reduced diversity, hinting towards a complex pattern of increased diversity in both the north and south of Europe (possibly owing to refugia). Comparison of the Norwegian data to the Brittany data from that the Norwegian sites were generally less diverse than those from Brittany (Evankow et al., 2019). Schoenrock et al. (2020) found particularly high diversity in Lough Hyne, a marine lough in the south of Ireland, which was more diverse than any of the other sites found in Brittany (Robuchon et al., 2014). It is notable, however, that in a DAPC used to compare these data sets, all regions in Ireland appeared more diverse than those found in Brittany, indicating that Ireland might be a diversity hotspot for this species. These findings align with modelling studies that identified the Celtic Sea as a region of long-term persistence, but it also indicates that the diversity may be more concentrated to the north of the Celtic Sea rather than to the south near Brittany.

Geneflow and Connectivity

All studies tested for and found a significant pattern of IBD for *L. hyperborea*. French populations showed significant and strong IBD for all sites ($p = 0.002$, MantelR = 0.693), but the strength of this model increased when removing the most divergent site ($p = 0.002$, mantel-R = 0.881) at St Malo Bay (Robuchon et al., 2014). Norwegian populations identified significant IBD for both genetic distance ($p < 0.0001$, $R^2 = 0.72$) and Fst ($p = 0.0050$, $R^2 = 0.59$) based metrics, but that the disparity between these metrics in both significance and power could lead to alternative results for some datasets depending on the methodology used (Evankow et al., 2019). The Irish populations reported a significant pattern of IBD ($p = 0.001$, MantelR = 0.650) that is also aligned with previous studies (Schoenrock et al., 2020).

Recommendations and conclusion

Further sampling of this species in Europe is vital because it is one of Europe's most abundant kelp species and provides perennial habitat in the form of kelp forests, in addition to being one of the primary wild-harvested species. All genetic studies on *L. hyperborea* have been regional in nature, with only limited comparisons being available among studies. The increased connectivity of this species when compared to *L. digitata* (Robuchon et al., 2014) but stronger

population genetic structure compared to *S. latissima* (Evankow et al., 2019) indicate complex meta-population dynamics that are distinct from other species in the north-east Atlantic. Furthermore, the lack of sampling in regions of long-term persistence in Iceland, the Faroe Islands, and the Iberian Peninsula suggest that there is a lot of unsampled diversity yet to be identified (Assis, Araujo, et al., 2018). Further research should include samples from across the range of this species, as well as diversify use of markers, such as barcodes (for deeper phylogenetic analysis) and SNPs, as all studies so far have only used SSRs that were designed for use in other species. While this species is potentially less useful for cultivation, it is of prime conservation concern because it is a relatively long-lived foundation species that is frequently targeted for wild-harvesting. To be able to make a decision about the sustainability of wild harvesting of this species, it is imperative to first understand the population genetic structure of this species both on a regional and European scale, as it may otherwise be lost and thus jeopardise the habitats they help to create. This mirrors recent calls to prioritise preservation of non-commercial kelp species that act as ecosystem engineers (Hofmann et al., 2025). Future sampling efforts might consider sampling sites in the RLTP at Portugal, the Faroe Islands, and Iceland to identify diversity hotspots and better understand north-east Atlantic populations. Additionally, more sampling around the diverse populations identified in Ireland may further refine our understanding of what appear to be extremely diverse and refugial populations, and thus may be of prime conservation concern.

2.4.7 Laminaria ochroleuca

Overview

Laminaria ochroleuca was included in 6.9% of the studies and accounted for 7.3% of the sampled sites. The majority (24 of 25 sites) were sampled by a single study (Assis, Serrão, et al., 2018) with one additional single site being described by an additional study (Schoenrock et al., 2019) as an example of northward expansion of the species. The majority of the regions identified as regions of long term stability by modelling have been sampled and sampling sites are spread across the hypothesised range of *L. ochroleuca,* likely owing to the primary population genetic study on *L. ochroleuca* having two of the same authors as the modelling study (namely Assis and Serrão). Both studies used the same markers (Coelho et al., 2014) but had variable success with amplification and presence of null alleles.

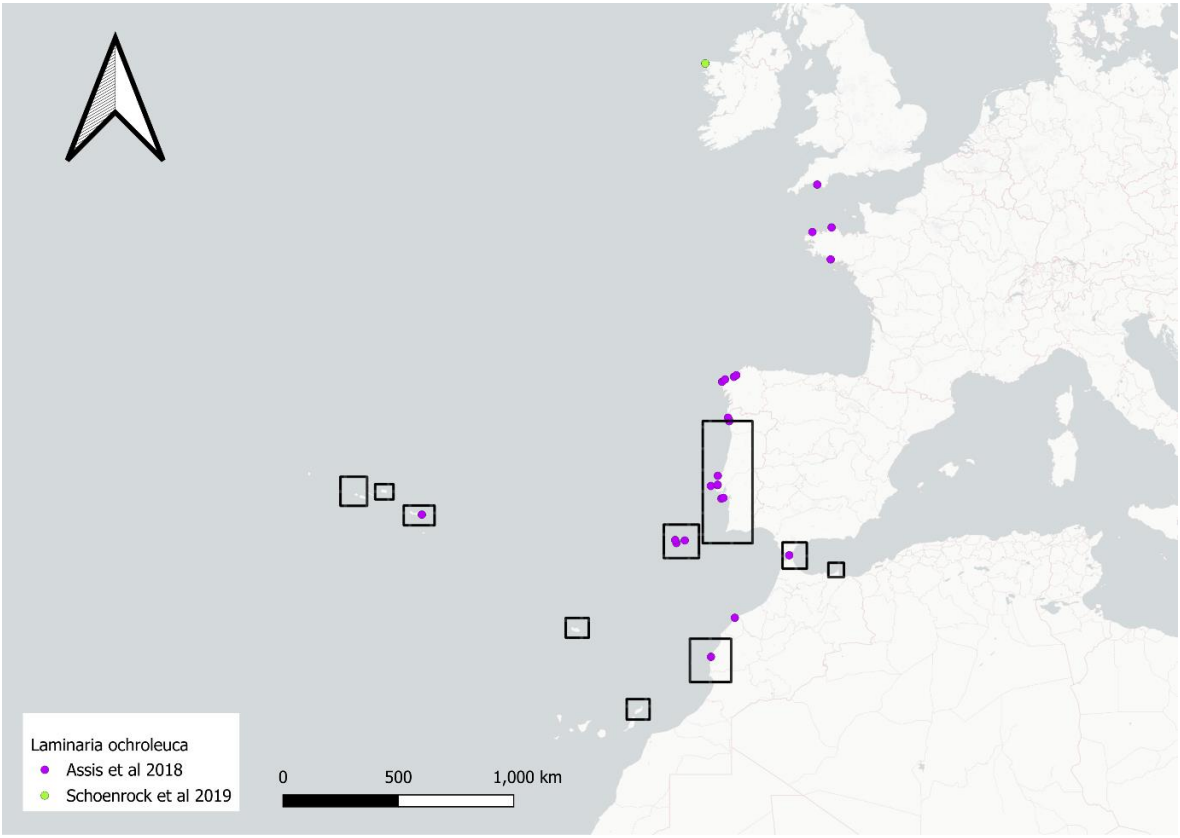

Figure 2.12. Laminaria ochroleuca *sites with each colour representing a different study. Black quadrilaterals represent projected regions of long-term persistence following Assis, Araujo, et al. (2018).*

Genetic structure

Assis, Serrão, et al. (2018) studied 24 sites across the entire known range of *L. ochroleuca* and broadly identified two major clusters, a more northernly consisting of sites from Iberia to the English Channel, and a southernly one consisting of sites near Morocco and the Azores. The northernly cluster substructures into sites from the English Channel, sites from western Iberia, and the Portuguese seamount populations, while the southern cluster subdivides into their individual sites located along the Moroccan coast and Azores. Interestingly, when the single site found in north west Ireland was compared to these populations, it differed significantly from its geographically nearest and most closely related population (Jost D = 0.331 ± 0.057) in Plymouth, south-west England (Schoenrock et al., 2019). This suggests that there is additional structure in the northern population that is unsampled but also unexpected because this site was deemed to fall outside the thermal niche of *L. ochroleuca* (Schoenrock et al., 2019).

Genetic diversity

Assis, Serrão, et al. (2018) identified an increasing pattern of diversity in *L. ochroleuca* towards the south of the range, noting that southern regions likely to be refugia had ~2 times more allelic richness and ~7 times the private allelic richness when compared to northern populations in the English Channel, which are thought to be post Last Glacial Maximum (LGM) settlements. Surprisingly, the site in Ireland was substantially more northernly than the previously identified sites, were more similar in diversity (allelic richness, private alleles, and expected heterozygosity) to populations near Iberia than those that were more geographically proximate (Schoenrock et al., 2019). This suggests that there may be unidentified cryptic populations of *L. ochroleuca* around the Irish coast, which is corroborated by the heterozygote excess and population structure observed.

Geneflow and Connectivity

A pattern of stepping stone dispersal was identified in *L. ochroleuca* in addition to oceanographic isolation (Assis, Serrão, et al., 2018). It was also noted that many of the more diverse southern sites were located near cold upwellings or deeper pockets of habitat and did not appear to contribute towards post-LGM recolonisation in the north. This suggests that, unless refugia are well connected in an interglacial cycle, they may not contribute to the recolonisation of habitat.

Recommendations and conclusion

More studies using different markers may help further refine the results found here for *L. ochroleuca*, such as plastid-associated barcodes to look at maternally inherited haplotypes and SNPs for higher resolution bi-parental inheritance. While Assis, Serrão, et al. (2018) was possibly the most extensive population genetics study on any kelp species within Europe, the identification of another population outside the range highlights the limitations of our knowledge on European kelps (Schoenrock et al., 2019). Assumptions made about species distribution and thermal niche are often based on limited natural history data, which in turn can bias sampling efforts, niche modelling, and interpreted biogeographic dynamics. Direct comparison of samples from Morocco, Iberia, the English Channel and Ireland using mitochondrial (e.g. COI) and plastid (e.g. *rbcL,* or whole plastid genome) markers, as well as nuclear SNPs could establish whether the population found in Ireland is a function of admixture from other unsampled populations or represents a refugial population. Additionally, this highlights the importance of greater geographical sampling effort and distributional recording of kelp along European coasts, as a poor distributional resolution can influence interpretation of molecular data, and thus undermine conservation efforts.

2.4.8 Laminaria rodriguezii

Overview

Laminaria rodriguezii was included in 3.5% of the studies and accounted for 1.2% of the sampled sites. It was only analysed at four sites in a single study as it is comparatively rare and possibly endangered (Reynes et al., 2021). This species however, unlike the others in this review, has an exclusively Mediterranean distribution, and is the only deep-water kelp in this study, which has severely limited research capacity. It is also one of the few stoloniferous kelp, and the only one included in this review, making population genetic interpretations for this species somewhat different than for primarily sexually reproducing species. The single study of this species used Double Digest Restriction-Site Associated DNA Sequencing (ddRADseq) and an as-of-then unpublished *L. digitata* reference genome to identify 4,077 putatively neutral SNPs.

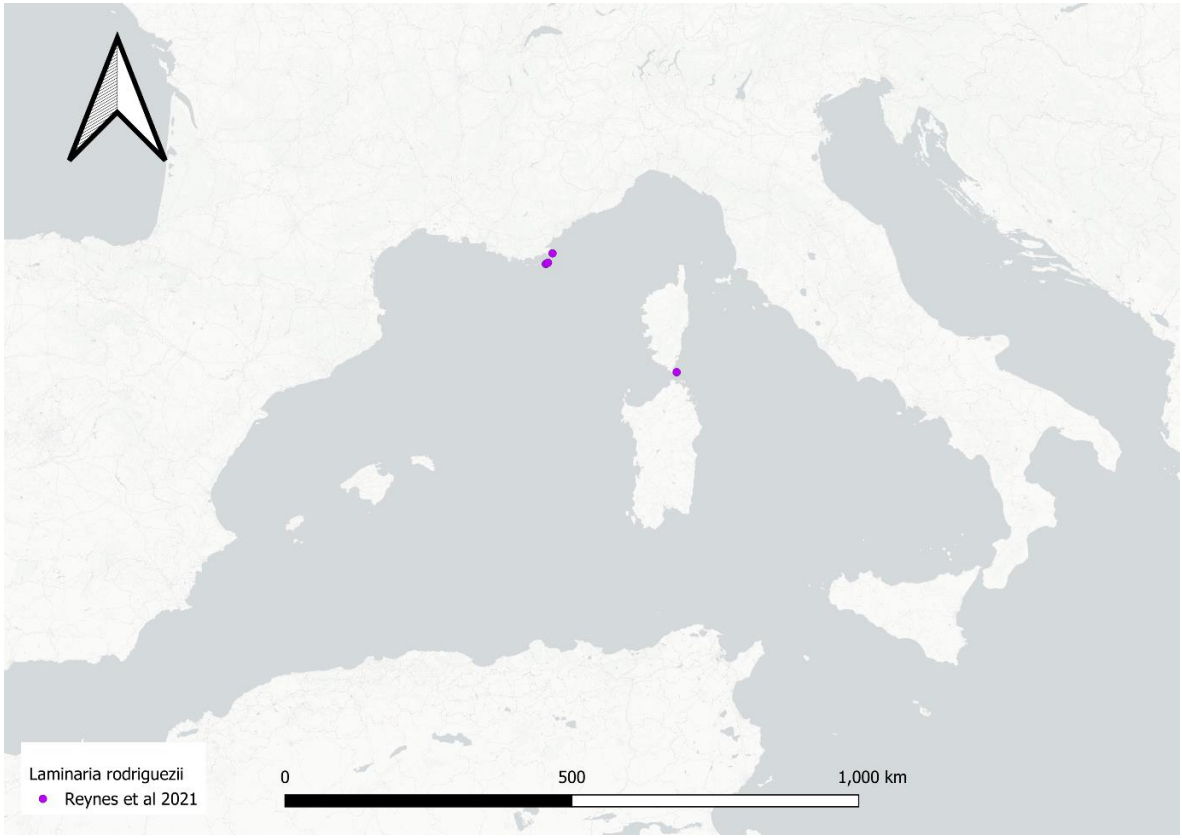

Figure 2.13. Map of Laminaria rodriguezii *sites with each colour representing a different study. Black quadrilaterals represent projected regions of long-term persistence following Assis, Araujo, et al. (2018).*

Genetic structure

Owing to the limited distribution and abundance of *L. rodriguezii* and the low geographical sampling of previous studies, it is difficult to assess spatial structure (Reynes et al., 2021). There was, however, significant structure identified among populations, with global Fst = 0.28 and Fst = 0.18 after clone correction. Banc Magaud near Levant Island in the south-east French Mediterranean in particular appeared to be more structured, but the authors suggest this species' structure is primarily a function of its patchy habitat and limited dispersal.

Genetic diversity

There was a moderate level of clonality identified, with the site in Corsica being identified as having the highest clonality, both in terms of repeated multi-locus lineages as well as individual clones (Reynes et al., 2021). Despite this, there was an average within population He of 0.14, with some outliers being identified that showed ~2 times the locus heterozygosity and up to 20 times the Pa of non-outliers. These outliers were hypothesised to be the result of ancient clonal lineages which may have accrued mutations, or hybrids of ancient lineages of clones. This is because clonality can continue to keep individual heterozygosity high, but more extensive sampling would help to resolve this as it may also be due to unsampled variation. There was an excess of heterozygotes (mean Fis = -0.14), but most of the sites had an Fis ≤ -0.05, except for Corsica, which had an Fis of -0.47. After clone correction only the population in Corsica had a significant Fis (-0.26), possibly due to higher levels of clonality at this site (Reynes et al., 2021).

Geneflow and Connectivity

Only the second site at Banc Magaud had data to model IBD in *L. rodriguezii*, where a significant differentiation with distance was identified (MantelR = 0.40, $p < 0.001$) and that all clonal individuals were < 1 m away from one another (Reynes et al., 2021).

Recommendations and conclusion

Future research on *L. rodriguezii* should focus on sampling more sites within the Mediterranean range of this species (Reynes et al., 2021). There has been a significant reduction in population size in parts of its range (Zuljevic et al., 2016), not only making this goal more important but also making it more difficult. While of limited aquacultural interest, it is plausible that this deep water kelp might provide insight into genetic and physiological adaptations that could be used

in the breeding and management of related aquacultural species, such as *L. digitata* and *L. ochroleuca*.

2.5 Overall Conclusion

This review identified that the authorship of studies on European kelp is often fragmented between countries and research institutions, and that a minority of researchers publish extensively while the majority (> 70%) are only authors on a single paper. While this likely reflects early career researchers working with established researchers in the field, it also highlights that there is potential for greater retention of human capital within kelp genetic research, which would help develop institutional knowledge generation across studies. Additionally, the most published authors and most well connected community of authors appear to be associated with a minority of institutions, primarily Station Biologique de Roscoff and to a lesser extent the University of Algarve.

The most commonly used genetic marker type was SSRs, with many studies using the same set of markers or cross amplifying them between species. The addition of COI barcodes since 2018 and SNPs since 2020 indicate a promising diversification of methods that can help provide higher resolution and novel data in the future. The comparison of SNPs from plastid and nuclear genomes (Bringloe et al., 2022) is particularly informative, but requires additional resources and expertise when compared to ddRAD-seq derived SNPs. This bias of using specific marker types for specific species can be both a product of hysteretic inertia as well as through research clusters having preferences for both species and analytical methods, and as such must be identified and consciously considered to avoid biased research trajectories. Future research should carefully consider the limitations of the markers used in the existing research for their species of interest, and aim to generate hypotheses that disprove the limitations of previous markers using alternative markers to hedge systematic error.

There appeared to be a strong geographic sampling bias across all kelp that favoured sites near Brittany where Station Biologique de Roscoff is located, except for species whose distribution falls outside this range, such as *L. rodriguezii*. While this could in part be due to the distribution of kelp, the geographic under sampling within the known distribution of European kelp may cause a skew in our understanding of these populations. When population genetic structure and diversity of *S. latissima*, *L. digitata*, and *L. hyperborea* were collated, it appeared that samples from around Ireland often appeared to be more diverse and differentiated than in Brittany, but this has not been as extensively highlighted in these papers. This is probably a

function of sampling discrepancies between these regions, and the Celtic Sea generally being identified as a region of long-term persistence and thus a likely interglacial refuge. Interestingly however, this trend seems to hold up for *L. ochroleuca* too, despite Ireland having been considered to be well outside its natural distributional range, indicating that the lack of data for many of these species may be severely impacting the interpretation of wild kelp distribution in Europe as a whole. There also seemed to be a common pattern of north/south European divergence among these species, but the lack of comprehensive sampling along the Celtic, Irish, and west North Seas makes delineation of these converging biogeographic signals difficult. Refugial sites appeared more commonly on the Atlantic fringes of Europe, in particular near the Celtic Sea. Populations appeared generally to be somewhat distinct, but there was noticeable structural differences, particularly where north and south clusters were identified. This highlights the increased need for inter-institutional collaboration across Europe to better understand kelp diversity and population dynamics. This is particularly poignant in light of recent calls to establish a European Board of Macroalgal Genetic Resources (EBMGR), which is somewhat analogous to the previously proposed East Asian Kelp Consortium (Hofmann et al., 2025; Hu et al., 2021). The EBMGR would not only help coordinate inter-institutional collaboration but could also bank genotypes from regions identified as having both economic and conservation interest, helping promote regional aquacultural production in the process.

The complete absence of studies of *Hedophyllum nigripes* may be both a function of being limited to the Arctic Circle of Europe and Greenland, and the frequent misidentification of species within the split-lamina Laminariaceae (Mauger et al., 2021). Similarly, *Agarum clathrum* is primarily located on the western Atlantic, with its only European distribution being in Greenland.

Additionally, this review highlighted the severe lack of population genetic research on the prime aquacultural species *A. esculenta* when compared to species such as *L. digitata* and *S. latissima*, as well as one of the most important habitat forming species, *L. hyperborea*, that should be conserved. This is likely influenced by the ruderal and cosmopolitan nature of *S. latissima* and *L. digitata*, as well as their more intertidal habitat preference, making them more easy to study than the species which have a distinctly subtidal preference. While *S. latissima* is typically seen as an ideal candidate for aquaculture due to its close relationship to *S. japonica* and other kelps commonly grown in Asia, *L. digitata* is often wild harvested for sale as ‘kombu’ (soup stock ingredient), and *A. esculenta* has gastronomic qualities that make it suitable for eating whole (e.g. thin and epiphyte-free lamina) (Rhatigan, 2009). This suggests

that future research into *A. esculenta* could provide an outsized economic benefit to kelp growers compared to other species of native kelp.

Future distributional recording of *L. rodriguezii* as well as conservation of the existing known populations appear critical for this species, but it is unclear what the ecological importance of this species is in comparison, and further baseline data is needed. Similarly, *L. ochroleuca* has shown a greater distributional range than previously thought, and increased resources into basic distributional recording in Europe (particularly around Ireland and the UK) is vital for conservation in a climatically uncertain future.

Overall this review identifies a recent boom in kelp population genetic research in the North-east Atlantic, but that this research is spread unevenly across institutions, geographic locations, and species. There appear to be strong methodological biases across studies, likely due to ease of use and cost of SSR markers, and this risks blind spots in the research as each methodology comes with its interpretative limitations. It is hoped that this review may allow researchers to avoid some of these blind spots and biases through their identification, as well as provide a comprehensive resource for future researchers, thereby lowering the bar for entry into this specific field of research. The establishment of the EBMGR would theoretically provide this service for future researchers, and as such, allow them to generate more robust data to support both the conservation of wild species as well as ensure the sustainable production of kelp in aquacultural settings.

Chapter 3 The genetic diversity of *Saccharina latissima* populations and implications for sustainable cultivation and management.

3.1 Abstract

Kelp aquaculture is expanding rapidly in Europe, where production of sugar kelp, *Saccharina latissima*, is predicted to increase to reach growing market demands. An increase in cultivation increases the risk of genetic pollution within local populations and maladaptive drift via an influx of spores from farms. To mitigate negative impact on native populations, it is imperative to first establish a baseline for how differentiated and diverse these populations are, and the extent of geneflow among them. This was the first study to examine *S. latissima* populations across the full range of the Irish coastline and analysed 105 sporophytes, following predicted glacial retreat patterns from the Last Glacial Maximum (LGM). We used Genotyping By Sequencing (GBS) to generate 5,128 highly informative biallelic SNPs, which were subsequently used to assess population differentiation, presence of glacial refugia, and the extent of Isolation By Distance (IBD). We found that populations in Ireland were highly differentiated and diverse, with populations in the south and west both showing signals of glacial refugia. There was a significant (p = 0.001) signal of IBD observed (R^2 = 0.509) and strong evidence of outbreeding (Global Fis = -0.68). Based on these results we recommend that movement of sugar kelp for cultivation is limited as much as possible among sites to retain the locally adapted population genetic structure. Practices that reduce the loss of heterozygosity in cultivated kelp material and minimise the movement of propagules from aquaculture into local populations should be encouraged.

3.2 Introduction

Aquaculture is a growing global industry with all algae reaching 36.5 million tonnes annual production in 2022, an increase of 4.1% since 2020 (FAO, 2024), with a significant portion of these being macroalgae, mostly kelp. Historically kelp aquaculture has been centred in East Asia, with China, Japan, and Korea accounting for the majority of its production and much of the associated aquacultural research (Hu et al., 2021). *Saccharina japonica* has accounted for the majority of this kelp production followed closely by *Undaria pinnatifida* (Hu et al., 2021). This large-scale and long-term history of aquacultural production of *S. japonica* in Asia provides insight into the potential problems that may arise when establishing macroalgal aquaculture industries in Europe. This would apply particularly to the closely related and economically significant kelp species *Saccharina latissima*, which is native to the north-east Atlantic region.

As Europe is projected to increase kelp aquaculture production (Vincent et al., 2020), it is vital that this be done in a manner that minimises the risks of native population gene pool pollution, reduces decline in the diversity of cultivated kelp germplasms, and maximises the genotype diversity from which production stock can be produced. This would minimise the negative consequences witnessed in Chinese *S. japonica* cultivar development (Hu et al., 2021). The introduction of non-native aquacultural species, which may become invasive, such as *U. pinnatifida* (Epstein & Smale, 2017), is also a concern that should be avoided in the establishment of a larger European aquaculture industry. Future research into the genetic structure of *S. latissima* populations in order to aid in development of sustainable aquaculture have already been identified as one of the major knowledge gaps within the cultivation of this species (Sæther et al., 2024), as it has been neglected in the literature and is foundational to crop breeding and long-term crop integrity.

The existence of interspecific *Saccharina* hybrids (*S. japonica x S. longissima*; *S. japonica x S. latissima*) could add further complication because established Chinese cultivars of *S. japonica* have complex patterns of introgression as a result of heterosis breeding and selection for agronomic traits (Hu et al., 2021). Movement of genotypes into new populations during aquaculture may be desirable from an economic production perspective but may endanger the long-term viability of local industries by compromising locally adapted gene pools (Goecke et al., 2020; Nepper-Davidsen et al., 2021). Relying on elite cultivars for production similarly risks homogenisation of production (Li et al., 2025) as seen in terrestrial agriculture, which has experienced erosion of crop genetic diversity through breeding, replacement of landraces and scaling up of production (Khoury et al., 2022).

Patterns of local adaptation and the ease of interspecific hybridisation can be explained by the amphiboreal distribution of the genus *Saccharina*, which originated in the Pacific, and has been subjected to interglacial species pump dynamics (Neiva et al., 2018). These cycles of glaciation have led to its speciation and radiation (Neiva et al., 2018) and genomic evidence suggests that population equilibrium in the Atlantic has still not been achieved (Luttikhuizen et al., 2018). In light of these findings, it is imperative to preserve locally adapted diversity where possible and minimise homogenising effects. This is particularly important for the north-east Atlantic, notably around the coastal regions of Ireland, where habitat suitability models have predicted both historical and future habitat suitability despite projected climate change scenarios (Assis, Araujo, et al., 2018). This region of relative habitat stability with modelled population persistence during interglacial cycles, would predictably contain more diverse gene pools. Indeed, recent comparison of some Irish populations of *S. latissima* to populations across the European range has noted an exceptionally high level of expected

heterozygosity (He), allelic richness (Ar) and private alleles (Pa) within the Irish-Scottish region studied (Jaugeon et al., 2025). This is consistent with biogeographic history and highlights the need for increased research in these centres of diversity for European, and thus Atlantic *Saccharina* (Neiva et al., 2018).

Irish kelp aquaculture is placed in a prime position to develop a sustainable industry using native species while simultaneously avoiding many of the complications observed from almost a century of Asian kelp aquaculture. It has been noted that despite high potential for kelp aquaculture in Ireland that the Irish market is unusually reliant on wild-harvesting, and that there is a concerted effort to promote sustainable kelp aquaculture in Ireland, with only 0.14% of total Irish seaweed biomass being sourced from aquaculture rather than wild harvesting (BIM, 2023). To fully utilise this natural potential, there is a need for basic research on the natural history, distribution, and diversity of Irish kelp. To date there have been no major diversity studies of Irish *Saccharina* populations, although small regional studies have looked at more fine scale population dynamics (Mooney et al., 2018). European research on kelp has typically focused on other kelp species and/or been conducted on populations from France, Norway, the Iberian peninsula or other continental coasts (see Chapter 2). Limited existing data suggest that some Irish populations exhibit higher diversity compared to more well studied populations in France and the North Sea (Jaugeon et al., 2025), which in turn showed higher diversity metrics than the north-west Atlantic populations on American coasts (Mao et al., 2020). This is consistent with Isolation By Distance (IBD) models that show strong spatial structuring in *S. latissima* with geographic distance (Evankow et al., 2019; Mao et al., 2020; Mooney et al., 2018) as well as the presence of north-east Atlantic glacial refugia (Neiva et al., 2020). While IBD models are relatively simple, they tend to ignore geographic features like dispersal barriers unlike Isolation By Resistance models, but function well for large scale marine studies and are computationally simple. Furthermore, similar glacial refugia were predicted for *S. japonica* in the Pacific region (Zhang et al., 2019) resulting from the same glacial cycles.

This study aimed to establish a foundational base for Irish *Saccharina* diversity, which will complement existing research on *S. latissima* in its broader amphiboreal and more specifically, European range. To achieve this, the research questions focused on identifying patterns of diversity of *S. latissima* around the coast of Ireland with consideration to historical biogeography of the species in the region using Genotyping By Sequencing (GBS) SNP data. Samples were collected from four primary populations around the Irish coast to capture regional differences, following glacial retreat patterns. Sampling included major populations on the north, south, east, and west coasts of Ireland to identify local diversity as well as minor

populations to obtain a more regionally complete structural overview. More specifically, we tested whether: 1) *Saccharina latissima* populations exhibit regional differentiation around Ireland; 2) potential glacial refugia could be identified; and 3) dispersal with a pattern of IBD could be shown. Specific recommendations relating to future potential aquaculture practices in Ireland are made on the basis of these findings and detailed analysis of the natural history of this species.

3.3 Methods

3.3.1 Sampling

Saccharina latissima was collected from primary and minor sites to construct the dataset. Primary populations were selected to capture population-level differentiation and private alleles, as well as intra-population diversity in line with long-term population dynamics and biogeographic patterns, and included ~30 samples per site. Minor populations were sampled to account for potential stepping-stone dynamics and better capture connectivity, dispersal, and admixture of unsampled diversity *at* 3-4 samples per site. Samples were haphazardly sampled from within primary sites, with sites being located in the north (Port na Happle), south (Galley Cove), east (Sandycove), and west (Trá Dhireáin Huston) coasts of Ireland using SCUBA to construct four primary populations. Sites were selected based on presence of hard substratum comprised of habitat suitable for kelp populations and were accessible by land. The sites for sampling primary populations were selected on the basis of the most recent and reliable suggested glacial retreat patterns from the Last Glacial Maximum (LGM) (Figure 3.1) across Ireland and the UK. This was to test the populations along the glacial retreat route for signals of recolonisation and refugia, which should correlate with these glacial models. Additional minor sites were sampled from the shore, at Killary (a fjord/fjard on the western coast), a south-eastern site in a region of major habitat discontinuities (Bagginbun), and a north-eastern site that is relatively close to Scotland and the Isle of Man and in a high-density area for marine traffic (Groomsport) (Figure 3.1).

All study sites were selected to include suitable rocky habitat (estimated by satellite imagery using Google® Earth) and avoid proximity to aquacultural sites, licensed for macroalgal cultivation of *S. latissima,* to reduce any potential gene pool contamination. Additionally, all samples were selected to avoid clonality, the same parent gametophytes giving rise to multiple sporophytes, and rafting of sporophytes by selecting specimens that were from different clusters (> 50 cm apart) and securely attached to the substratum in a somewhat purposive sampling methodology. All primary samples were collected during August 1-15th 2022, while minor populations were sampled over the spring and summer of 2023, and a total 128 individuals were sampled. Due to the lack of comprehensive diversity data for this species in

Ireland, sites were chosen to facilitate ease of collection and provide geographical coverage across glacial retreat patterns, in an effort to establish a baseline from which future studies can generate more site-specific hypotheses. A section (10 x 10 cm^2) of meristem was excised from near the base of each sample and epiphytes were removed prior to drying using silica gel for subsequent extraction of genetic material.

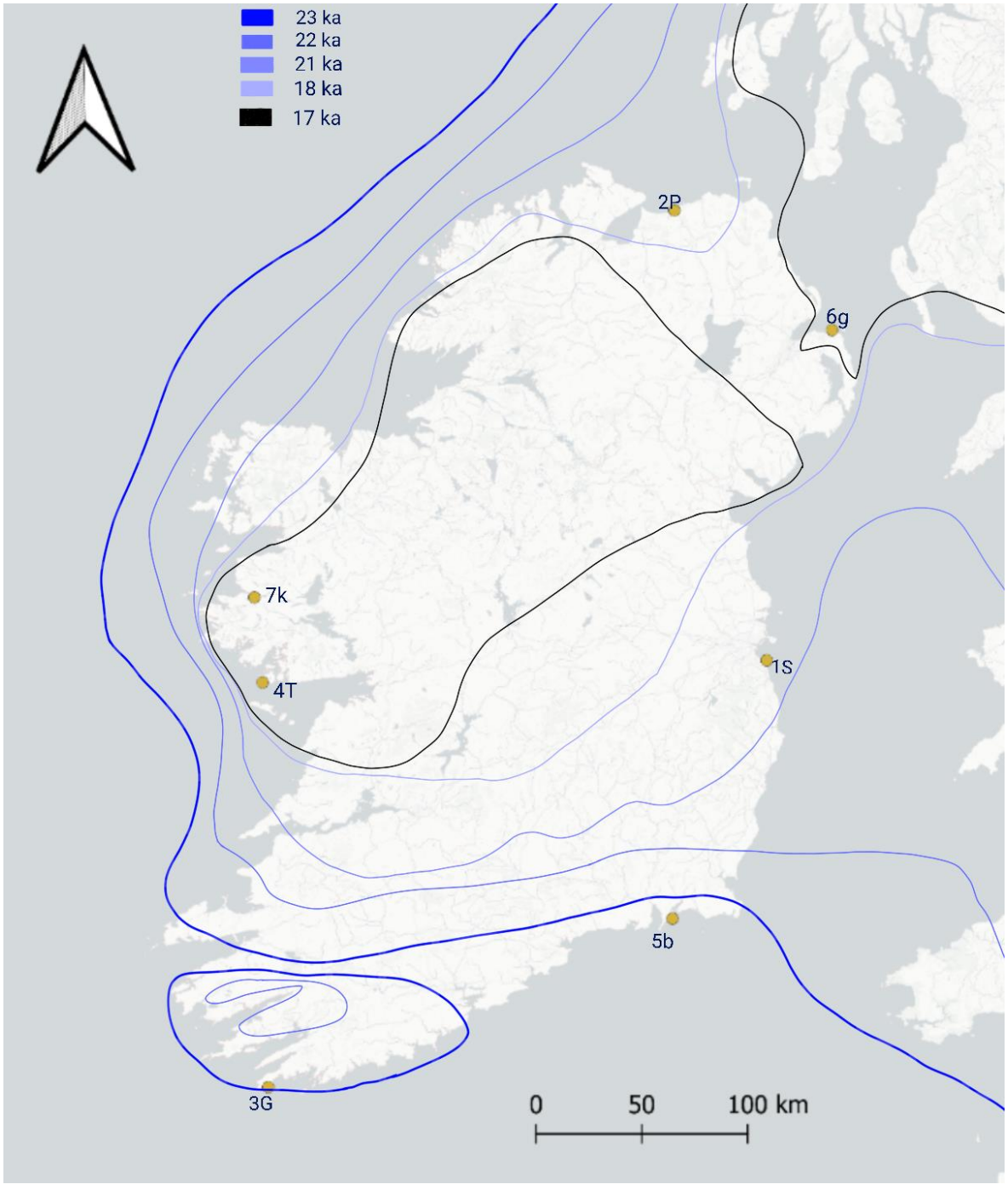

Figure 3.1. Map of Saccharina latissima *sample sites with glacial isochrons adapted from Clark et al. (2022) from older (dark blue) to younger (lighter blue, black). Primary sites (1-4) and minor sites (5-7). 1S = Sandycove, 2P = Port na Happle, 3G = Galley Cove, 4T = Trá Dhireáin Huston, 5b = Bagginbun, 6g = Groomsport, 7k = Killary. Ka= Thousands of years ago.*

3.3.2 DNA extraction

DNA extraction followed a modified Cetyltrimethylammonium bromide (CTAB) Protocol (Hodkinson et al., 2007), and used approximately 150 mg dried tissue per extraction with the addition of Polyvinylpolypyrrolidone (PVPP) to the extraction buffer to minimise effects of kelp polyphenols on genomic DNA quality. Crude extracts were cleaned from polysaccharides, single-stranded nucleotides, and other impurities using PureLink™ PCR Purification Kit. Samples were quality controlled in duplicate using a Nanodrop lite to assess A260:A280 ratio and give a broad indication of DNA concentration. DNA concentration was then more accurately assessed using a Qubit fluorometer (Invitrogen) and broad spectrum B2 buffer. Samples needed an A260:A280 ratio of 1.5-1.99 on the Nanodrop as well as a DNA concentration of at least 10ng/uL as measured by the Qubit for GBS sequencing. Clean samples that had low DNA concentration were concentrated using an excess of isopropanol (100 %) to induce precipitation (minimum twice the sample volume), ethanol washing (70 %) and resuspension in the desired volume of TE buffer. Samples were stored at -70° C until shipment to LGC Genomics (Berlin, Germany).

3.3.3 GBS, data pre-processing and SNP calling

Library preparation and GBS sequencing was processed by LGC Genomics using their normalised GBS (nGBS) approach (Arvidsson et al., 2016), including use of PstI-MseI double enzyme restriction, adapter ligation, Polymerase Chain Reaction (PCR) amplification, molecular normalisation, size selection, and 150 bp paired-end (PE-150) Illumina sequencing on a NovaSeq instrument as in Bråtelund et al. (2024). Samples were aligned to the available reference genome for *S. latissima* (GCA_034768055.1) from GenBank using BWA-MEM v0.7.12.

Variant discovery and genotyping of samples was done with Freebayes v1.2.0, with a minimum base quality of 10, minimum supporting allele qsum of 10, read mismatch-limit of 3, minimum coverage of 5, no-indels, minimum alternate count of 4, exclusion of unobserved genotypes and multi-nucleotide polymorphisms, and mismatched base quality of 10. Using bcftools, variants were filtered for a minimum of 9 reads per locus, genotypes must have been observed in at least 10 % of samples, and minimum allele frequency across all samples must be at least 5 %.

3.3.4 Data analyses

The resulting SNP variants were iteratively filtered in R 4.3.1 (R Core Team, 2024) using the SNPfiltR (DeRaad, 2023) and vcfR (Knaus & Grünwald, 2017) packages for quality and

completeness. Samples were filtered for a minimum depth of 5, minimum genotype quality of 20 and a maximum depth of 60. An allele balance of 0.25-0.75 was imposed, and a SNP missingness threshold of 0.5 and sample missingness threshold of 0.9 applied. After filtering there were 106 samples with 5,128 newly identified SNPs retained and 31.87% missing data.

3.3.5 Population diversity statistics and refugia

Global diversity statistics across the populations were calculated using the basic.stats function in Hierfstat (Goudet & Jombart, 2022). Population-level diversity statistics were generated using the function gl.report.heterozygosity in the program dartR (Gruber et al., 2018). Pa was calculated using the private_alleles function in Poppr (Kamvar et al., 2014), and mean Ar was calculated using the package PopGenReport (Gruber et al., 2015).

3.3.6 Population Connectivity

The multivariate clustering method of Discriminant Analysis of Principle Components (DAPC) (Jombart et al., 2010) was conducted on *a-priori* populations to examine population-level structure. To examine genetic structure, DAPC was conducted using k-means clusters as *a-priori* groupings. To identify genotype affinities of individuals to sampled populations, posterior-membership probability compoplots were constructed using these DAPC results for each site. K-means clusters from 2-7 were explored to identify substructure within the sampled populations using the adegenet (Jombart & Ahmed, 2011) function “find.clusters”. Membership to each optimal k-means cluster was visually mapped to site using the package “mapmixture” (Jenkins, 2024) along with posterior membership assignment.

Pairwise population FST was calculated for each population using Weir-Cockerham distance (Weir & Cockerham, 1984) in the package Hierfstat (Goudet & Jombart, 2022) to assess comparative population divergence. Each population was used to construct a dendrogram using prevosti distance and a neighbour-joining algorithm in the “aboot” function in Poppr (Kamvar et al., 2015; Kamvar et al., 2014), and visualised using plot.phylo in ape (Paradis & Schliep, 2019), with a bootstrap sample number of 1000.

3.3.7 Isolation By Distance

For IBD calculations, genetic differentiation was measured using scaled Euclidean distance using the function ”gl.dist.ind” in the package “dartR” (Gruber et al., 2018). Geographic distance between sampling sites was measured using the measure distance function in Google® Maps and a matrix of minimum marine distance along the coastline between sites was constructed. Mantel tests were performed at 10,000 permutations using “gl.ibd” in “dartR”

(Gruber et al., 2018). Genetic distance was transformed as dist/(1-dist) and plotted against transformed geographic distance of log(dist+1) (Rousset, 1997).

3.4 Results

3.4.1 Diversity statistics

Global observed heterozygosity (Ho) was 0.45 compared to the expected within population gene diversity (Hs) of 0.27, which results in a highly negative inbreeding coefficient (Fis) of -0.68 (Table 1). This negative Fis indicates deviation from Hardy-Weinberg Equilibrium (HWE) suggesting that allele combinations are observed less frequently than under HWE assumptions, or the presence of an excess of heterozygotes. Overall gene diversity (Ht) was relatively high at 0.28, while gene diversity among samples (Dst) was only 0.01. Global fixation index (Fst) was low to intermediate at 0.04, while population differentiation (Dest) was 0.02 (Table 3.1).

Table 3.1. Summary statistics of the genetic diversity and differentiation for all populations.

	Definition	***Saccharina latissima* (*n* = 105)**
Ho	Observed heterozygosity	0.45
Hs	Within population gene diversity	0.27
Ht	Overall gene diversity	0.28
Dst	Gene diversity among samples	0.01
Fst	Fixation index	0.04
Fis	Inbreeding coefficient	-0.68
Dest*	Population differentiation	0.02

**(Jost, 2008)*

Similar levels of Ho were recorded in each primary population (0.460-0.485) compared to the global average (Table 3.2). Expected heterozygosity (He) was similarly low for each of these same populations compared to global Ht (0.268-0.288). These values were somewhat lower in the minor populations, but notably the Bagginbun population showed a particularly low Ho and He. All reported Fis values are negative, ranging in the primary populations from -0.471 in Sandycove to -0.503 in Trá Dhireáin Huston, and in the minor populations from -0.291 in Killary to -0.442 in Bagginbun.

Primary populations had similar mean allelic richness ranging from 1.224 in Sandycove to 1.248 in Trá Dhireáin Huston. Two of the minor populations had a similar range for Ar (Groomsport = 1.241, Killary = 1.252) while the Bagginbun samples only had an Ar of 1.182. Private alleles were identified in all populations, ranging from 240 (Sandycove) to 459 (Trá Dhireáin Huston) for the primary populations, and 6 (Groomsport) to 38 (Bagginbun) in minor populations.

Table 3.2. Population genetic diversity statistics. nInd = Number of individuals (corrected for sample size) used to calculate Ho, He, and FIS, Lat. = latitude, Lon. = longitude, Ho = Observed Heterzygosity, He = Expected Heterozygosity, FIS = Inbreeding coefficient, Pa = Private allele, Mean Ar = Mean Allelic richness (for biallelic SNPs).

***S. latissima* (105)**	**nInd**	**Lat.**	**Lon.**	**Ho**	**He**	**FIS**	**Pa**	**Mean Ar**
Sandycove (24)	14.809	53.28821	-6.12029	0.460	0.268	-0.503	240	1.224
Port na Happle (25)	16.903	55.1806	-6.72414	0.471	0.278	-0.487	307	1.232
Galley Cove (23)	13.111	51.46144	-9.73986	0.481	0.281	-0.486	368	1.245
Trá Dhireáin Huston (27)	17.726	53.23125	-9.73373	0.485	0.288	-0.471	459	1.248
Killary (2)	1.604	53.59706	-9.77032	0.453	0.238	-0.291	14	1.252
Groomsport (1)	1.000	54.67536	-5.61267	0.419	0.210	0.000	6	1.241
Bagginbun (3)	2.324	52.17668	-6.82917	0.323	0.172	-0.442	38	1.182

3.4.2 Population connectivity

A dendrogram was produced (Figure 3.2), using the neighbour-joining method and Provesti distance genetic distances between samples, which recreated much of the population structure. Samples were annotated according to population colour (Figure 3.2), showing that the northern Port na Happle, eastern Sandycove, and western Trá Dhireáin Huston populations are relatively distinct. The southern Galley Cove population showed the poorest resolution of all primary populations, with one major grouping and five samples that were distributed across the other population groups (notably, except the eastern populations). All Killary samples formed an outgroup to Trá Dhireáin Huston, but were still sister to the major Galley Cove group. Similarly, Bagginbun formed a single distinct group, but this was nested within samples from Sandycove. This suggests a south-west/north-east divide and strong population differentiation.

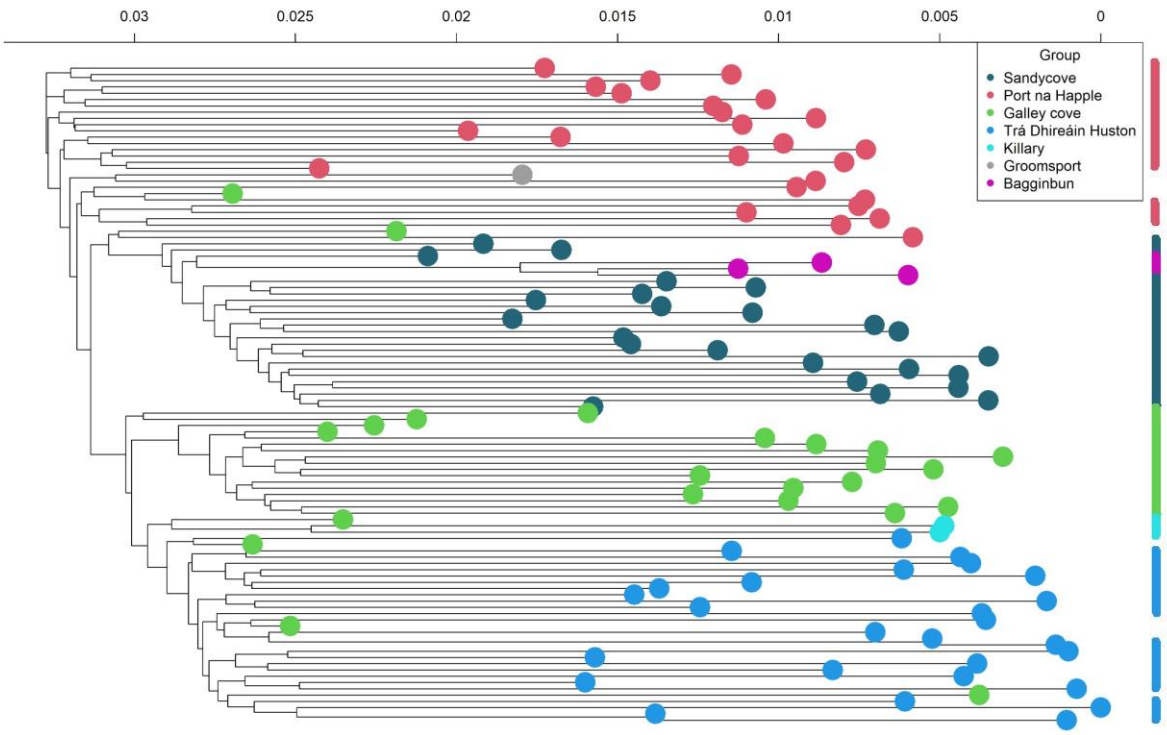

Figure 3.2. Neighbour-joining tree of samples using Prevosti genetic distance, annotated by population, showing relatively consistent clustering of primary populations of S. latissima. *Groups in monophylly indicated by population colour bar on right.*

DAPC (Figure 3.3) indicates that samples from the west (Trá Dhireáin Huston) and south (Galley Cove) show similarity to one another, but still have very distinct posterior membership reassignments, with only one sample from Galley Cove showing a minor assignment proportion to the Trá Dhireáin Huston population. Populations on the east (Sandycove) and north (Port na Happle) coasts are substantially differentiated along the primary axes. The minor population at Killary (west) clustered very closely with Galley Cove and Trá Dhireáin Huston samples (south/west), while Groomsport (north-east) was intermediate to all populations. Interestingly, the three samples from the minor population at Bagginbun (south-east) showed an extremely high level of divergence from the remaining samples in the DAPC (Figure 3.3).

Figure 3.3. DAPC of all S. latissima *SNPs (N = 105), 5128 biallelic SNPs, 1000 cross validation, 12 PCs retained. Coloured bars on the right show the posterior membership probabilities for each sample derived from DAPC, indicating the likelihood of each sample (horizontal bar) being assigned to a population based on geographically-naïve data structure. All samples had a near perfect reassignment to their sampled population, except for a single Galley cove sample that had a small proportion of assignment to Trá Dhireáin Huston. The eigenvalue plots show the amount of genetic information retained by the PCA (on the right) and the discriminant function (DA, on the left).*

When k-means clustering from k = 2-7 was examined to look at population structure (Figure 3.4), the previously identified south-west/north-east divide can be seen to potentially be more of a west/east divide. Galley Cove has a strong affinity to the west (k = 2 and 3), as do the northern sites (k = 2). The south-east (Bagginbun) and east (Sandycove) populations cluster together early, while the northern clusters (Port na Happle and Groomsport) both group with the western cluster (k = 2 and 3) before segregating into their own genotypes by k = 5. Notably, internal structure was seen in the west (Trá Dhireáin Huston) and north (Port na Happle) in k = 6 and k = 7 respectively. The second clusters observed at these primary sites clustered with nearby minor sites (Killary and Groomsport). Notably Bagginbun in the south-east fell into its own cluster at k = 5, and while it is more distant from primary sites than the other minor populations, this further supports the hypothesis that there is a substantially divergent population in the south-east despite only having three samples in this study. This appears consistent with models of glacial retreat (Figure 3.1), which indicate early ice free regions near Galley Cove and Bagginbun, progressively opening up to the west and east respectively. Further sampling on the southern coasts might be expected to show high levels of population

structure, as the limited sampling here showed strong refugial signals that bolster models of glacial retreat.

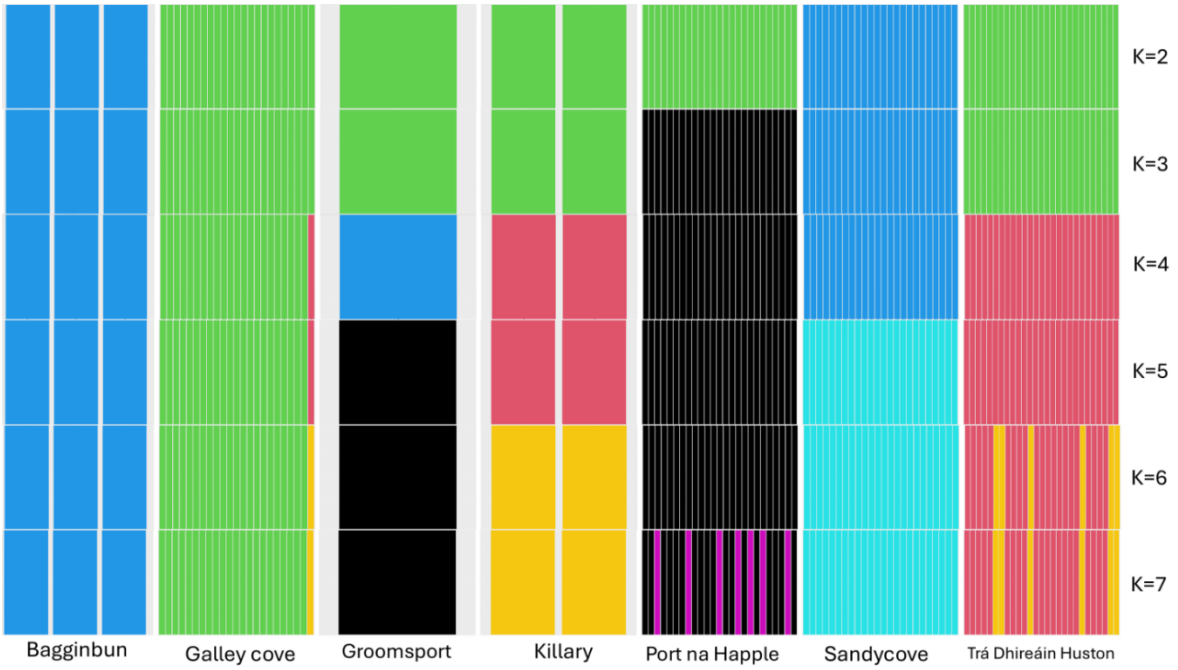

*Figure 3.4. Posterior membership probability, according to DAPC, using 5,128 biallelic SNPs according to k = 2-7 (*n *= 105). Retained PCs 1000x cross validated for each k-means cluster. Each column is an individual and the proportion of the column in each colour represents the affinity of that individual to said group.*

Optimal k-means clustering (k = 4) was mapped (Figure 3.5), with diversity statistics (Ar, Pa, uHe, and Fis) calculated for each cluster. This found that western cluster had the highest Pa (642), followed by the eastern cluster (398), supporting the hypothesis that the western cluster in particular is distinct owing to the presence of glacial refugia. The western cluster also had the highest Ar and unbiased expected Heterozygosity (uHe), suggesting it is highly diverse and may have a large population size that has persisted for a long time, corresponding well to Pa data that suggest the presence of a refuge. Interestingly the eastern cluster had the lowest uHe (0.274) and Ar (1.216), indicating low population size and low connectivity despite refugial signals, possibly due to lower substratum suitability. The northern cluster was somewhat intermediate in all metrics, except that it had the lowest amount of private alleles (307). This, along with the population sub-structuring (Figure 3.4, k = 7) point towards possible admixture and/or founder effects, which is consistent with expectations from modelled patterns of glacial retreat. The relatively low Fis (-0.482) suggests that more recent admixture is plausible here.

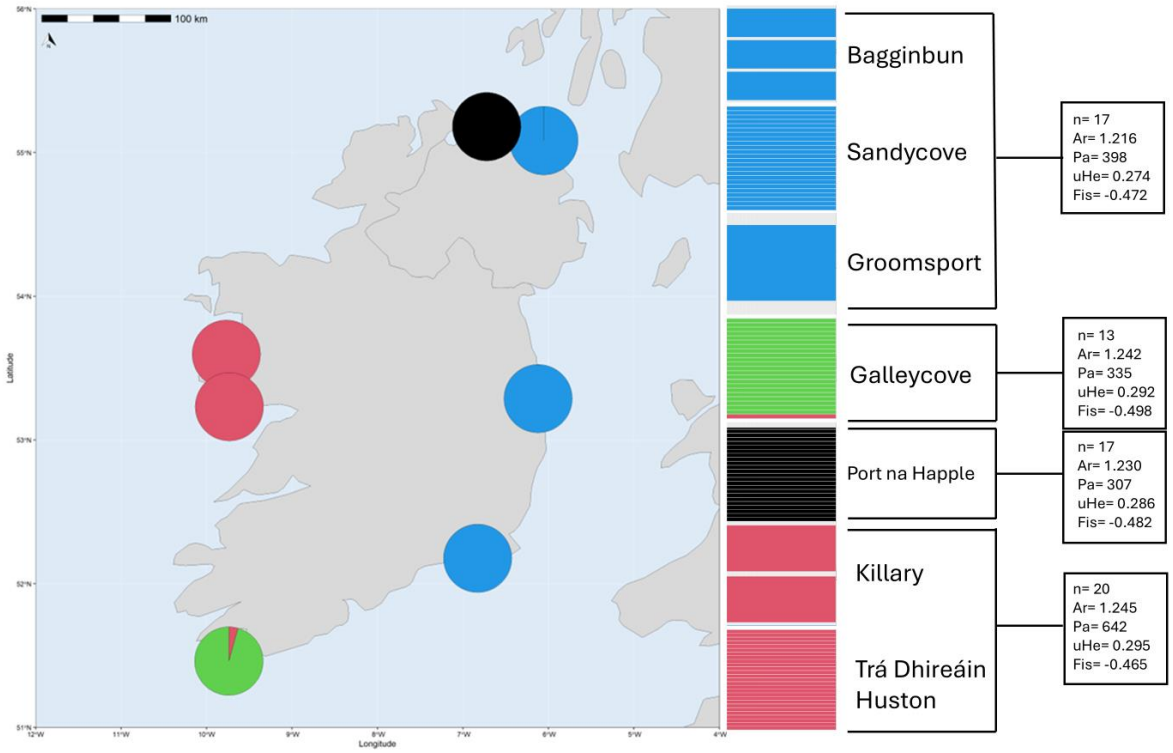

Figure 3.5. Optimal k-means cluster assignments (k = 4) as a proportion of cluster assignment by site. Optimal k-cluster posterior membership assignments on right, with sample number (n) corrected for sample size according to Nei (1978) used for uHe and Fis calculations, rarefied allelic richness (Ar), total private alleles (Pa), unbiased expected Heterozygosity (uHe), and fixation index (Fis) for each cluster.

Pairwise Fst was calculated at the population level (Figure 3.6). The most divergent population is Bagginbun ranging from a value of 0.088 with Sandycove to 0.142 with Killary (mean of 0.107). This south-east population at Bagginbun showed the highest pairwise Fst for all combinations of populations. The lowest degree of differentiation according to pairwise Fst was found between Killary and Trá Dhireáin Huston (0.025), while Trá Dhireáin Huston was most divergent from Sandycove on the east (other than Bagginbun) at 0.048. Overall, mean pairwise Fst values ranged from 0.045 (Trá Dhireáin Huston) to 0.107 (Bagginbun), but was highest in Sandycove (0.055) among the primary populations. The comparatively high Fst values for the minor populations can partly be explained by the lower sampling density at these sites, yet despite this signal bias, Bagginbun stands out as particularly divergent with a mean pairwise Fst approximately twice as large as all the other populations (0.107).

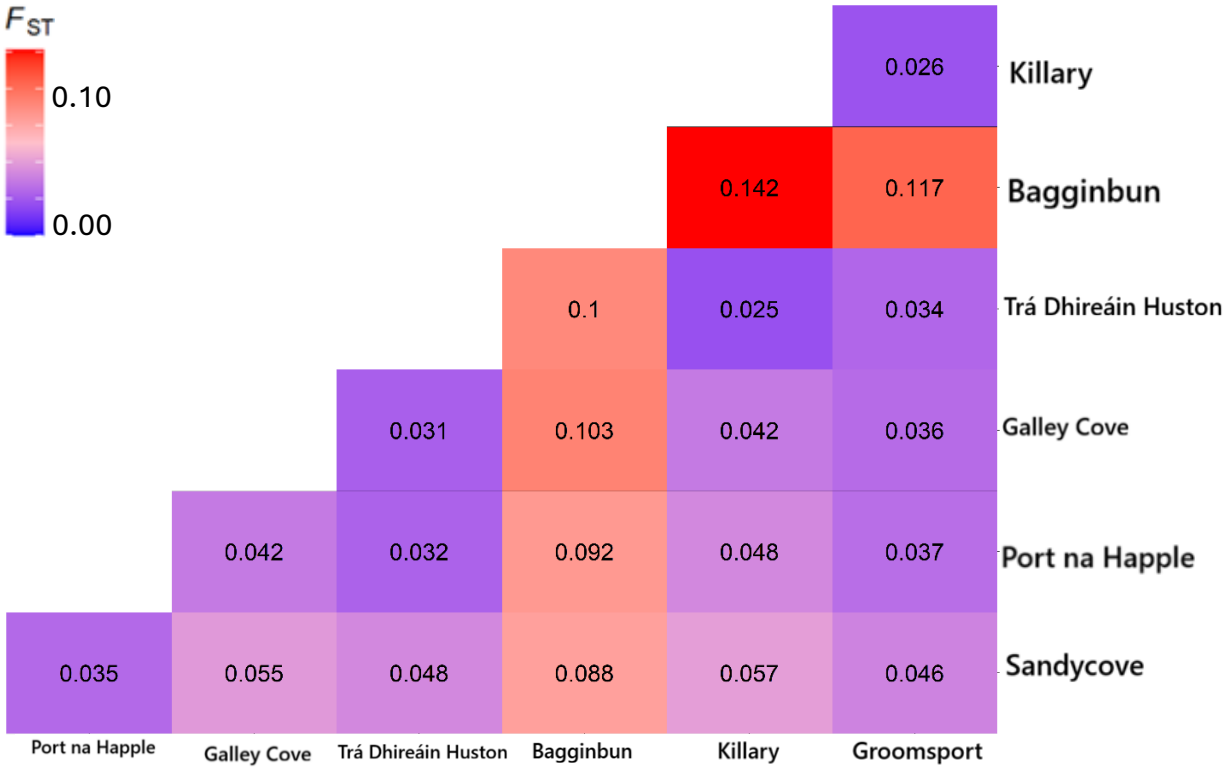

Figure 3.6. Pairwise Fst of all populations. Mean pairwise Fst: Mean Pairwise Fst: Trá Dhireáin Huston (0.045); Port na Happle (0.048); Galley Cove (0.052); Sandycove (0.055); Groomsport (0.049); Killary (0.057); Bagginbun (0.107). The higher the number, the greater the pairwise differentiation.

3.4.3 Isolation by Distance

An IBD model was constructed using minimum marine distance along the coast (in km) between sample populations and the transformed Euclidean genetic distance to best describe marine habitats as per Rousset (1997) between individuals (Figure 3.7). This model using minimum distance explained approximately half of the variance recorded among populations (R^2 = 0.509) and was highly significant (p = 0.001). These results suggest that individual-level diversity is extremely high (x = 0, y = 0.26; resulting in mean individual Fst of ~0.206) and also that an IBD model describes the pattern of geneflow among these populations reasonably well.

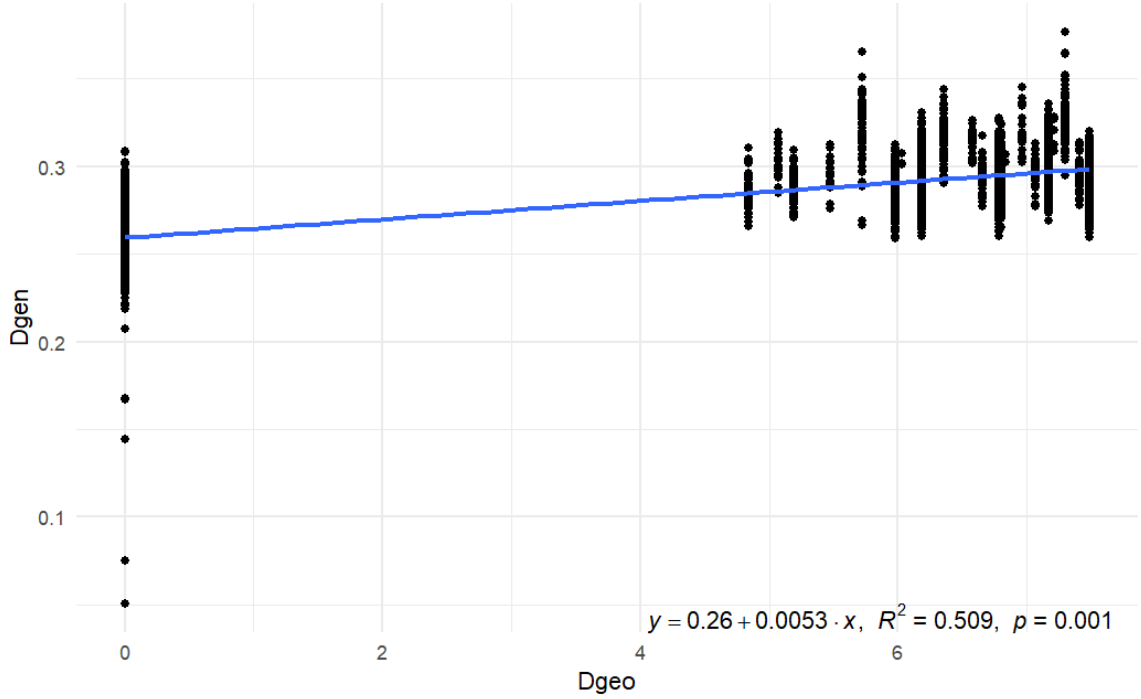

Figure 3.7. IBD model of populations. Dgeo = log(minimum marine distance+1), and Dgen = (Pairwise Fst/1-pairwise Fst). Fst according to Weir and Cockerham (1984). Equation of the line given at the bottom, along with R^2 (explanatory power) and p (significance).

3.5 Discussion

All populations displayed a high degree of diversity, with sites on west and south coast exhibiting refugial signals that corresponds well with previous modelling data (Assis, Araujo, et al., 2018). Populations were distinct, with strong resolution in the phylogenetic tree (Figure 3.2) and perfect posterior population reassignment (Figure 3.3). An east/west divide was seen in the major branching in the phylogenetic tree which was recreated in the DAPC (Figure 3.3) as well as the k-assignments (Figure 3.4). These additionally showed that the populations in the south and west had greater similarity to one another while the east and north were more divergent. Differentiation was notably stronger in the minor population in the south-east, in addition to comparatively high Pa and lower Ar, suggesting a small but refugial population. There was a strong and highly significant pattern of Isolation By Distance ($R^2 = 0.509$, $p = 0.001$).

3.5.1 Population structure

Most populations exhibited a high degree of monophylly in the NJ tree (Figure 3.2) with the exception of the southern population at Galley Cove. This population had several samples nested among other populations, indicating that this may be a source population from which glacial recolonisation occurred. This is consistent with the prevailing ocean current flow, which

is predominantly from the south-west to the north-east, as well as the glacial retreat models (Figure 3.1).

When DAPC is observed (Figure 3.3), it can be seen that the populations at Trá Dhireáin Huston and Galley Cove are relatively similar to one another, but also with high intra-population diversity. Samples from Killary show affinity with these south and west coast populations. Populations in Sandycove on the east coast and Port na Happle on the north coast are comparatively more divergent from other populations, showing high separation to other populations along both principal component axes. Bagginbun is relatively distinct, showing major divergence and low affinity to any primary population, indicating that further sampling on the south-east coast of Ireland may be necessary to get a full picture of the connectivity of Irish populations. The posterior membership assignment compoplot using *a-priori* defined populations (Figure 3.3) had close to perfect levels of reassignment success, with only one sample in Galley Cove showing partial assignment to Trá Dhireáin Huston. This reassignment success indicates that these populations are relatively distinct from one another.

From posterior membership plots using k-means clustering from k = 2-7 (Figure 3.4) it can be seen that the east and south-east populations are relatively divergent from the other populations (k = 2). This is likely a function of habitat suitability and population sizes, as eastern sites tend to be less diverse as well (Figure 3.5). Interestingly, north and west populations show more internal sub-structuring (k = 6/7), with one of the clusters in each of these primary populations showing affinity to neighbouring minor populations. This suggests there is additional unsampled variation, and that finer scale sampling between the northern and western sites might reveal additional sub-structuring that places these populations in a more complete context.

Pairwise Fst values (Figure 3.6) indicated the most divergent primary population was at Sandycove in the east, followed by Galley Cove in the south. The population at Port na Happle in the north showed higher mean Fst values than the population in the west at Trá Dhireáin Huston, but these populations showed comparatively low pairwise-Fst to one another, indicating potential geneflow between the west and north. This may indicate that these are sites of greater admixture, which may explain the sub-structuring observed in Figure 3.4. The lower pairwise Fst values between the southern, western, and northern primary populations when compared to the eastern population might indicate preferential gene-flow along the west coast, likely being a function of dispersal via currents and habitat suitability. Interestingly, pairwise Fst is relatively low between the eastern and northern primary populations, suggesting that there is primarily an increased resistance to geneflow between the southern

and eastern populations, which correlates well to the location of the Celtic Sea front. Notably the population at Bagginbun in the south-east was highly divergent when compared to all other populations, further indicating low gene-flow here, possibly due to habitat discontinuities or influx of unsampled and divergent genotypes from across the Irish/Celtic Sea. Interestingly, the population at Bagginbun is located near the Celtic Sea front, which may limit geneflow from both the Irish and Celtic Sea populations, and a more fine scale regional study here may uncover important geneflow dynamics (See Chapter 2, section 2.4.3). The population at Killary showed relatively high divergence and this is likely due to its relative isolation and possibly founder effects. The findings from this chapter further bolster findings from chapter 2 which indicate a broad North/South divide in European *S. latissima* populations despite the consistently high diversity of these populations in Ireland.

This suggests that the sampled populations show a high level of regional differentiation, and that the east coast in particular appears relatively isolated and differentiated. It also suggests geneflow may preferentially flow from the southern population to the north via the west coast, but interestingly, that there may be some geneflow between the northern and eastern populations. This aligns well with findings in Chapter 2 that suggest higher diversity in the Irish and Scottish populations (Jaugeon et al., 2025), but also highlights that there may be unexpected diversity of this species in the Celtic sea, and not just the Atlantic coastal fringe. Future research comparing multiple Irish populations to other European populations might elucidate whether Irish *S. lastissima* are a source population for postglacial European recolonisation, or whether the underlying forces structuring European populations (such as major water currents) merely influence the dispersal and structure of contemporary populations.

3.5.2 Diversity statistics

Private alleles (Table 3.2) are typically indicative of long-term population persistence and may be a sign of glacial refugia, with the primary populations showing the largest presence of Pa in the west (459), followed by Galley Cove in the south (368), Port na Happle in the north (307), and finally Sandycove in the east. Minor populations had the highest Pa in Bagginbun in the south-east, (38), followed by the isolated western population in Killary (14) and then the north-east site in Groomsport (6). While the major populations had private alleles that were consistent with patterns seen in Ar, comparison of the minor populations show that the south-east site at Bagginbun displayed both the highest Pa and lowest Ar and uHe, suggesting there may be a refuge here but that the effective population size and connectivity size may be small (Eugenia Barrandeguy & Victoria García, 2021). This indicates a high likelihood of refugia existing along the west coast of Ireland, but also hint at refugia on the south and south-east

coast. This broadly follows projected regions of glacial retreat (Figure 3.1; (Clark et al., 2022)) and is consistent with previous reports for *Laminaria hyperborea* in Ireland (Schoenrock, 2022). It is, however, important to note that more data from south-east coast populations are required as sample size here was small. Additionally, more extensive sampling along the Irish coast as well as from the coast of Great Britain and Brittany, France, may uncover more complex patterns of refugial signals and admixture, as geneflow from divergent populations across the Irish and Celtic Seas may appear as refugial signals in this analysis.

Allelic richness can be biased by unequal sample number among populations as sampling effort and Ar exhibit a non-linear relationship (Leberg, 2002). This makes direct comparison of minor and primary populations difficult despite rarefaction, however, comparison within these sampling regimes should avoid some of this bias and provide qualitative interpretive power. Ar was seen to be roughly similar in the primary populations, with a slight increase identified in the south and west as compared to the north and east. A similar pattern can be observed for the minor populations with decreasing diversity from the west to north to south-east being observed. It is worth noting that the south-east site at Bagginbun had an Ar that was somewhat lower than the other sites even when compared to minor sites, indicating that this may be reflective of a true pattern of lower Ar at this site despite low sampling. These findings suggest that populations in the west and south coasts of Ireland are more allele rich while those on the north and east coast appear comparatively impoverished, possibly because of lower overall availability of rocky substrata, greater habitat discontinuities, and thus smaller population sizes and lower opportunity for geneflow to these populations (Greenbaum et al., 2014). These line up well with the findings from Chapter 2, which suggest strong refugial signals in Ireland, particularly in the west, where we expect refugial habitat to have existed according to glacial modelling during the LGM (Clark et al., 2022). Interestingly, the Ar values in this study did not vary as much as might be anticipated from other studies covered in Chapter 2, section 2.4.3. While Ar is not directly intercomparable between studies, the high Ar across populations in Ireland suggests that the previously identified diversity in Irish populations (e.g. in Jaugeon et al. (2025)

Heterozygosity was determined using biallelic SNPs, filtering for some missing data, and with uneven population sizes measured together. This approach has recently been shown to potentially introduce bias into data (Sopniewski & Catullo, 2024), however, in combination with other metrics, such as F-statistics, Ar, and Pa is still informative. As such, both population-level He and cluster-level uHe broadly correlated with Ar and Pa, showing greater diversity in the west on the Atlantic coast, followed by the populations near the Celtic Sea in the south, the northern Irish Sea, and finally the east coast.

3.5.3 Population connectivity

A strong and significant pattern of IBD was found to exist among these populations despite the high individual level diversity. These data could be improved upon by including more populations from around the coast to reduce inter-population geographic distance and clustering of genetic data points at the same distances (Landguth et al., 2011).

Previous studies on brown macroalgae using SNPs (Bråtelund et al., 2024; Vranken et al., 2021) and microsatellites (Evankow et al., 2019; Schoenrock et al., 2020) have both indicated that models of IBD explain a significant portion of genetic variance (often $R^2 > 0.5$). Sampling of more loci and use of more variable loci better capture spatial variation in contrast to lower allele count/variability per locus (Landguth et al., 2011), which highlights a trade-off between using a few highly variable microsatellites and many biallelic SNP loci. The increased loci count sequenced in GBS here may help offset this lower variability of locus alleles, but also highlights the benefits of sampling more extensively when using high density biallelic SNPs and GBS filtering regimes. This variability makes comparison of both markers across studies difficult but both types of markers can be used effectively to test this hypothesis (Guzinski et al., 2020).

3.5.4 Mating system and inferred genetic dynamics

The highly negative Fis values exhibited by all populations indicate high outbreeding tendencies in regard to HWE (heterozygote excess), potentially due to underlying outbreeding mechanisms of sugar kelp, such as kinship recognition (Camus et al., 2021) or their diecious free-living haploid gametophyte. Killary notably has the highest Fis, and this might be because of its geological structure, as a fjord/fjard, which is more likely to exhibit reduced geneflow and thus higher levels of inbreeding (Evankow et al., 2019). Similar patterns of highly negative Fis in *S. latissima* using both SNPs and SSRs has been observed in Europe (Bråtelund et al., 2024), but the values in this study are notably even more negative than previous reports. While inter-study comparison of these diversity metrics can be unreliable, the use of similar SNP markers and filtering regimes indicate that this is a biological signal, and thus that *S. latissima* on the edges of the north-east Atlantic show substantial heterozygote excess. This contrasts to many of the other studies in chapter 2, with the most extensive study (using SSRs) reporting positive Fis values for all populations across Europe, with half of these values being statistically significant (Jaugeon et al., 2025). Interestingly, this study found more outbreeding tendencies in areas of lower diversity for primary populations, which may be a statistical function of smaller and less diverse populations and the stochastic effects this can have for a haplodiplontic organism such as kelp with segregated sexes. This may also be a function of purifying selection at the gametophyte stage (Cervantes et al., 2023) or negative assortative

mating, as heterozygote advantage and local adaptation is widely observed in many kelp taxa (Hu et al., 2021; Solas et al., 2023).

Notably absent from previous studies, however, is a consideration for how the haplo-diplontic heteromorphic life cycle impacts kelp at a population level, particularly as it relates to their genetic structure. A study comparing phylogenetically diverse plants found lower Fis values associated with woody, long-lived, high-stature, abiotically-pollinated plants (Duminil et al., 2009). While many of these traits are not particularly transferable to kelp traits, the tendency of long-lived organisms to have lower Fis values points towards a bias in partitioning of diversity toward the individual compared to the subpopulation. This "long-lived" phase is typically interpreted as referring to the sporophyte in seed plants but may also apply to persistent populations of gametophytes in haplo-diplontic organisms with free-living gametophyte stages such as kelp (or ferns), which can be viewed as partially analogous to the concept of "seed banks" in terrestrial seed plants (Edwards, 2022). Additionally, the division of sexes between gametophytes indicates that when populations are small, genotypes will statistically be distributed among the sexes unevenly (Luikart & Cornuet, 1998). This combination of gametophyte persistence, sex segregation in the haploid phase, and the ability of single long-lived haploid genotypes to engage in fragmentation and multiple instances of sexual recombination (and thus sporophyte production) (Ebbing et al., 2025) all probably contribute to this signal of outbreeding that has been identified. Heterozygote excess has previously been identified in another kelp species due to its semi-clonal habit during its sporophyte stage (Reynes et al., 2021), as well as an asexually reproducing fern gametophyte (Pelosi et al., 2025), which further suggest that the gametophyte stage in kelp may be behaving similarly here. This may be more apparent in the Irish population than elsewhere in Europe because of its long term habitat suitability resulting in increased standing diversity driven by both refugial populations and admixture. Interglacial species-pump dynamics can lead to increased heterozygosity (Alcala et al., 2013) and this may increase the outlined effects via purifying selection at the gametophyte stage, leading to lower Fis values. Alternatively there could be a strong selection for heterosis in Irish populations, as has previously been suggested for the presence of unexpected heterozygote excess in *L. ochroleuca* in Ireland (Schoenrock et al., 2019). Further research using SNPs and comparing them to SSRs might clarify the extent of which these signals reflect a difference in methodological approach or are truly reflective of the Irish kelp populations.

3.5.5 Recommendations

We show that *S. latissima* populations around the coast of Ireland are highly diverse and distinct and identified little evidence for geneflow between populations, with more distant

populations being more distinct. Currently it is estimated that <1% of Irish seaweed is cultivated (BIM, 2020), with hand and mechanical wild harvesting predominating in the industry (Vance et al., 2023). Due to the kelp aquaculture industry being in its infancy in Ireland, there are few hatcheries, and the majority do not keep gametophyte cultures interannually, but rather collect fresh material every year as needed for their production. This highlights the current reliance of kelp aquaculture on wild populations for production, and also emphasises the utility of establishing a baseline of kelp genetic diversity in these populations currently being utilised as a genetic resource for farmed kelp.

Based on these results, it is recommended that movement of genotypes around the Irish coast (e.g. for use in aquaculture), or from non-Irish localities, is not permitted. While management practices, such as harvesting prior to sporulation, can help mitigate some of the risks of gene pool pollution it is unlikely to be an effective long-term solution when used in isolation. This is due to the high fecundity of *S. latissima*, which has been previously shown to release 11×10^3 to 1.2×10^6 spores cm^{-2} sori under natural conditions, and which can produce some sori throughout the growing season (Boderskov et al., 2021). Furthermore, during the aquacultural process it is inevitable that some sporophytes detach, and there is little known about the persistence of gametophytes on the cultivation line after sporogenesis, allowing for the possibility of gametophytic fragmentation of cultivated strains into natural populations. Recent development in kelp breeding has seen the production of triploids (Tian et al., 2025), which should be sterile and thus not interbreed with local populations, however the precise mechanics of triploid production and breeding of *S. latissima* cultivars are still poorly understood (Bråtelund et al., 2026). Further studies on these triploids is required because it remains uncertain how polyploidy and diploidisation may contribute to a return to fertility in kelp, which may be exacerbated by the high rates of meiotic division necessary for spore production in kelp, and which may persist vegetatively as gametophytes regardless of non-lethal aneuploidy. Additionally, male gametophytes may undergo apogamous sporogenesis (Bartsch et al., 2008), further risking introgression of genetic material into wild populations. This seems particularly important for sites located in the south and west coasts, which exhibit the highest standing diversity and private alleles found in this study.

Owing to the high local diversity identified in these populations, we suggest that local populations should be utilised instead of importing elite genotypes developed elsewhere. This high diversity, however, may be affected by large aquacultural sites that swamp local gene pools with a subset of local genes, possibly leading to stronger genetic drift in the local population. To avoid this genetic homogenisation it is recommended to use multiple local sporophytes for gametophyte production, which can then be utilised for sporophyte

aquaculture (Li et al., 2025). Additionally, it is recommended to gather fertile sporophyte material on an annual basis to help avoid temporal Wahlund effects that can reduce heterozygosity in local populations, as a reduction in He can be seen as soon as the F2 generation (Bråtelund et al., 2026). The production of landraces in terrestrial agriculture appears to strike a reasonable balance between production and biodiversity (Gao et al., 2023) and is likely a good candidate approach to addressing the tension between aquacultural production and gene pool pollution. This may involve selection of a few farmed sporophytes that showed superior traits of interest in the previous year and allowing them to reach sexual maturity, harvesting them for gametophyte production, and using these gametophytes to improve the wild-collected local stock for the following season. This minimises spore release on large scales into the local environment, minimises drift away from locally adapted genotypes in the farmed population, and improves the production characteristics of the crop over time.

Our findings also indicate that there is higher degrees of isolation among populations on the east coast compared to the rest of Ireland. This corresponds to discontinuous habitat suitability, possibly providing opportunity for aquaculture at sites where escaped spores are unlikely to settle. Typically in Europe, kelp is grown on long lines, which allows for production regardless of underlying substrata, and which might be ideal for these areas of low natural kelp habitat suitability. This however complicates sourcing of local genotypes, which may not be very diverse, or may be entirely absent. More extensive sampling, for population genetic studies, along the east and south-east coast is recommended because the data from populations sampled in the south-east coast at Bagginbun suggest that there may be glacial refugia along this coastline that are extremely isolated and divergent when compared to other populations and may be worth conserving. This also means that any gene pool pollution at these sites may have a disproportionately negative effect on the wild populations because they are likely to be smaller and more locally adapted owing to their isolation and the IBD pattern of diversity identified in this study.

Perhaps most significantly, these results indicate that clear-cut harvesting, such as by mechanical means (Greenhill et al., 2021), is likely to reduce the long-term diversity of these populations because it removes rare and private alleles from the population. While the persistence of a hypothetical gametophyte “seedbank” may allow for short-term rebounding of sporophyte populations after such wild harvesting as suggested for *Laminaria hyperborea* (Schoenrock, 2022), it is likely that this has a homogenising effect on local population-level diversity and may lead to maladaptive drift and lower adaptation potential. Additionally, the

highly negative Fis suggests an outbreeding nature in this species, leading to high levels of individual diversity, which may be lost following such activity.

3.6 Conclusion

The kelp *S. latissima* shows high levels of diversity and population-level differentiation, particularly on the south and west coasts of Ireland. These populations are indicative of glacial refugia with further refugia potentially existing on the south-east coast. While the minor populations in the west and south-east both showed signs of population genetic bottlenecks, the western population showed greater signs of inbreeding (possibly because of its isolated location), while the south-east site showed refugial signals. There was a strong and significant signal of IBD, corroborating signals of population differentiation. It is likely that the current Irish populations result from populations that persisted through interglacial cycles on coasts bordering the Atlantic ocean to the west and Celtic Sea to the south, owing to stable long-term habitat suitability, leading to high levels of standing variation. The lower levels of diversity observed on the east and north coasts may be due to glacial recolonisation and founder effects, but the north populations also show signs of secondary contact.

Future research should focus both on finer scale studies of *S. latissima* in Ireland as well as placing Irish populations in context of their broader European range, particularly on the eastern coasts where admixture and habitat discontinuities may exist. It likely that there is much more unsampled standing Irish diversity that may have contributed towards the glacial recolonisation of many of the modern European populations in the north, particularly in the south-west. This greater mapping of population differentiation and diversity would additionally facilitate sustainable and native kelp aquaculture because Ireland is anticipated to become a hotspot for kelp aquaculture in Europe (Canvin et al., 2025; Collins et al., 2022; Knell et al., 2024). Funding into the establishment of kelp hatcheries that are equipped to manage kelp genotypes from multiple different aquacultural sites across Ireland is vital for this industry to minimise its impact on the native kelp forests and facilitate a long-term sustainable kelp aquaculture industry. Such a hatchery should aim to act as a living repository of local Irish strains as well as a facilitate the production of germplasm for the Irish aquacultural sector.

Chapter 4 Cryptic algal diversity: A study on *Laminaria*.

4.1 Abstract

Historically algae have been described primarily by their morphology and their habitat. In more recent years however molecular tools have identified cryptic species within previously established taxa. This study uses morphometrics to analyse *Laminaria digitata* and *L. hyperborea* according to the morphological species concept and cross validates this with Single Nucleotide Polymorphisms (SNPs) generated using a Genotyping By Sequencing (GBS) approach in populations sampled across the coasts of Ireland. These species are then compared in their population diversity, structure, and refugial signals. It was found that morphology is not a good determinant of genetic species, and that age and depth effects are mostly responsible for divergence in morphotype. Depth of sampling also differed between genetic species, with *L. digitata aff.* having a shallower niche preference. Diversity as measured by Allelic richness (Ar), Observed Heterozygosity (Ho), and Unbiased expected Heterozygosity (uHe) was substantially higher in *L. hyperborea aff.*, and showed a much higher excess of heterozygotes. Refugial signals were observed for both species on the west coast, but *L. digitata aff.* showed strong signals in the east as well, while *L. hyperborea aff.* showed some northernly refugial signals. Population structure across both species suggest that south-west to north-east currents play a major role in the population connectivity and diversity of *Laminaria* across Ireland.

4.2 Introduction

Kelp are brown macroalgae in the order Laminariales, and are foundation species in temperate coastal habitats (Efird & Konar, 2013; Teagle et al., 2017; Udy, 2019). Kelp are increasingly important as a source of alginates and other biomolecules, such as laminarin, in addition to their direct use as a primary food source (Ertesvag, 2015; Goecke et al., 2020; Meng et al., 2021; Peteiro, 2018; Purcell-Meyerink et al., 2021). In Ireland, it is estimated that 3,010 km out of the 7,524 km of national shoreline are dominated by kelp, typically in rocky and exposed habitats (Schoenrock, 2022).

Accurate natural history data for kelp has been difficult to collect prior to widespread access to molecular tools (Valero et al., 2011). Kelp are characterised by extreme phenotypic plasticity that can vary throughout the year, among sites, and intra-specifically amongst individuals (Lund, 2014). This has made identification and delineation of species complicated because key identifying traits of species may overlap heavily with one another and are often

context dependent (Bartsch et al., 2008; McCoy et al., 2020). The advent of new molecular techniques has helped to disentangle and identify many algal species but has also challenged our understanding of the morphological species concept and its applicability towards macroalgae (Diaz-Tapia et al., 2018; Gonzalez et al., 2012; Lane et al., 2007; Lane et al., 2006). Brown macroalgae such as kelp have evolved multicellularity independent of Archaeplastida (e.g. plants and red algae) and Opisthokonta (e.g. animals and fungi) are the only known complex multicellular representatives in the SAR Supergroup of Eukaryotic life (Cock et al., 2010), thus, it is important to challenge the evolutionary assumptions held true for other multicellular Eukarya. Some of these challenges can be seen when attempting to quantify *Saccharina latissima s.l.* diversity, which has been historically over-characterised as distinct species due emphasis on biogeography and morphological traits, and for which evidence now exists that indicates it may better be described as an amphi-boreal species complex rather than discrete species (Neiva et al., 2018).

It has been shown that macro-algal dispersal is often limited to a few kilometres despite stochastic long distance dispersal events, with signals of Isolation By Distance (IBD), indicating that meta-population structure and habitat discontinuities may play important roles in algal biogeography and evolution (Assis et al., 2022; Brennan et al., 2014; Robuchon et al., 2014; Vranken et al., 2021). Current evidence suggests that macroalgal glacial refugia exist in parts of the Atlantic with both *Laminaria hyperborea* (Schoenrock, O'Callaghan, et al., 2021) and *Fucus serratus* having glacial refugia in Southwest Ireland (Hoarau et al., 2007). More recently, other fucoid algal glacial refugia were identified in the north-east Atlantic along the Brittany-Irish coastline during the Last Glacial Maximum (LGM) (Neiva et al., 2016), with multiple similar refugial patterns displayed by different species depending on their biogeographic history and thermal tolerance ranges (Chimura et al., 2022; Coyer et al., 2003). Similarly, the red alga, *Palmaria palmata*, which often grows epiphytically on the kelp *L. hyperborea*, has been shown to potentially have refugial populations located in what is now Hurds Deep (English Channel) and Galway Bay (Irish west coast) (Assis, Araujo, et al., 2018; Provan, 2013; Provan & Bennett, 2008). This suggests that refugial marine rocky habitats existed off the south coast of Ireland during the LGM, and that recolonisation from refugia on the south-west coast, and potentially from the south-east coast near Brittany, could explain current diversity patterns observed in Irish kelp. The evidence for these refugia are seen not only in algal species, but also other benthic marine organisms (Maggs et al., 2008), however, extensive research that spans the distribution of these species is scarce.

Species delineation and identity are often uncertain and both intergeneric and interfamilial hybridisation have been reported within the order Laminariales (Murua et al., 2020).

Hybridisation as well as species misidentification may appear as false positive signals of refugial populations when using microsatellites in the form of private alleles or relatively highly divergent genotypes, particularly when studying more recently diverged species which show high degrees of marker cross amplification. Plastid and single gene markers may be impacted by these considerations too, but can also help identify potential hybridisation and introgression by comparison of maternally inherited plastids in contrast to bi-parental nuclear signals, which may be diminished via introgression. Without extensive sampling, lower resolution markers also run a higher risk of falsely interpreting secondary contact zones owing to refugial recolonisation with refugia themselves because of the comparatively higher gene diversity (Coyer et al., 2003). This can be mitigated by using more loci or more complete sampling regimes. Algae also often lack a good quality reference genome for alignment for genomic sequencing techniques such as Genotyping By Sequencing (GBS), potentially diminishing the resolution of the data, but these can still reproduce true evolutionary relationships (Loureiro et al., 2020). These limitations can be reduced by interpreting multiple data types to identify convergence of underlying signals, such as biogeography, morphology, habitat modelling, biochemistry, and reciprocal crossing experiments amongst others, increasing the robustness and meaningfulness of findings. Other alternatives for confirming species identification include the development of rapid tests based on genomic data that can be implemented relatively cheaply and quickly (Mauger et al., 2021). While these rapid tests help to quickly identify specimens, they may be confounded in areas where complex introgression occurs between species.

While it has previously been recognised that there is substantial variation in morphology of *Laminaria (Bartsch et al., 2008; Kain & Jones, 1977)*, more recent work that has used molecular confirmation of species has raised serious doubt about the reliability of many morphological characteristics typically used in the discrimination of these two species due to significant site effects on morphology (Lund, 2014). This molecularly confirmed morphological analysis however was low in sample size and lacked depth and age data, which raises further doubt about the reliability of these findings across depth profiles and age ranges. Notably, these same molecularly confirmed samples were later analysed more in detail, and further doubt on the utility of these morphological traits as reliable identifying features for species delineation was raised, as these seemed to be more affected by season than species identity when controlled for site (Henry, 2018).

Given the importance of understanding local and regional patterns of gene flow for the conservation and management of kelp species, this study aimed to identify and compare the diversity of two of the most abundant kelp species in the north-east Atlantic, *Laminaria digitata*

and *L. hyperborea*. Both species are located in regions thought to be LGM refugia on the Irish coast, and to establish the extent of these populations' connectivity across Ireland, a phylogenetic approach was paired with morphological analysis. Depth and age data were also collected for each specimen, as it is established these can have an effect on the phenotype of *Laminaria (Kain & Jones, 1977; Sjøtun & Fredriksen, 1995)*, which in turn may lead to species-ambiguous morphologies. Previous research in Brittany has mapped and quantified depth effects on both *Laminaria* (Bajjouk et al., 2015), identifying a distinct effect of depth on the presence of both species, particularly *L. hyperborea*. The effect of age has been even less studied, with most studies focusing on *L. hyperborea (Kain, 1971; Kain & Jones, 1963, 1977; Sjøtun & Fredriksen, 1995)* due to easier age estimation, which can reach up to ~20 years, and very little being reported for *L. digitata* (Chapman, 1993). Here Chapman identifies a mean age of ~5 years for the *L. digitata* with a maximum of 9 years in age in their study, but also cautions that the data from this research "can only be applied to the population of *Laminaria digitata* at Abbott's Harbour in Nova Scotia" which was studied. Gunnarsson (1991) in particular identifies that both species tend to have older individuals at greater depths, but that *L. hyperborea* appears to be more subtidally distributed on average.

Despite these data to suggest distinct age and depth preferences for each species, the consistent lack of reported molecular species confirmation in these studies may suggest that there is a conflation of depth-related morphologies with species identity in these studies. This can be seen in a recent paper that identified 12 *L. hyperborea* in a dataset of 908 presumed *L. digitata*, giving an approximate 1.3 % false positive identification rate *(Fouqueau et al., 2024)*. This study was conducted by many of the leading authors in the field, and account of 5/12 of the most published researchers in European kelp population genetics (Figure 2.6), including the two most published researchers, and as such highlights the critical need for molecular tests for accurate species identification. The other ecological studies, such as those looking at habitat-scale and community ecology typically rely on the natural history data collected ~50 years ago, when theoretical frameworks for species identification lacked the ability to molecularly confirm species, and thus over relied on gross morphology and ecological factors such as depth. As such, it is vital to revisit these base assumptions of morphospecies identification being consistent with phylogenetic species concepts, as to address the stochastically identified inconsistencies in the literature previously discussed.

This holistic approach incorporates genomic and morphological data to distinguish and characterise the inter- and intra-species diversity of these kelp species with the overall aim of providing evidence-based recommendations for the management of wild populations and future research. This is the first study to comprehensively include an examination of both

species to test for coherence between morpho- and phylogenetic species concepts. It does this by testing whether commonly used traits such as epiphyte coverage and stipe flexibility are merely correlative to major developmental factors such as depth and age, or truly reflective of an underlying consistent lineage with its own evolutionary trajectory. Specifically, the research questions were: Are Irish *L. digitata* and *L. hyperborea*, as typically described by morphology, distinct phylogenetic species? What morphological differences can be identified between sampled *Laminaria* species? Is there evidence of hybridisation between *L. digitata* and *L. hyperborea?* Is there evidence for Glacial refugia in Irish *Laminaria* populations? Are the sampled populations of *Laminaria* distinct, and can a pattern of Isolation By Distance be seen in *L. digitata* and *L. hyperborea* populations in Ireland? The application of both molecular and morphometric data is a timely and robust approach to understanding the diversity and evolution of these species.

4.3 Materials and Methods

4.3.1 Sampling design

Similarly to the methodology in chapter 3, to characterise populations of *Laminaria* and whether morphospecies description is consistent with phylogenetic species, samples of both *L. digitata* and *L. hyperborea* were collected from primary and minor sites to construct the dataset. Primary populations were selected to capture population-level differentiation and private alleles, as well as intra-population diversity in line with long-term population dynamics and biogeographic patterns, and included ~30 samples per site. Minor populations were sampled to account for potential stepping-stone dynamics and better capture connectivity, dispersal, and admixture of unsampled diversity *at* 3-4 samples per site. Samples were haphazardly sampled from four primary sites on the north, south, east, and west coasts of Ireland based on glacial retreat patterns of the LGM across the island using SCUBA (Figure 4.1). Primary sites were selected to be well sampled and representative of the populations living there. These samples were measured morphologically, had depth of sample recorded, and were included in the genetic dataset (~30 samples per species per site). To better test for population connectivity, Isolation By Distance, and capture rare alleles, additional minor sites were sampled at lower rates (3-4 per species per site), but were not measured for morphology. To minimise clonality or sampling of multiple sporophytes arising from the same female gametophyte, samples were collected from > 50cm distances from one another and specimens that were detached from the substrate were avoided. Juvenile-appearing samples were not sampled as to avoid confusion between species identification, as it is reported that juvenile *Laminaria* can be difficult to identify (Mauger et al., 2021). Samples were identified by two scientifically trained divers and the primary author of this thesis based on species

previously published descriptions (Henry, 2018; Lund, 2014; Lüning, 1979), primarily focusing on traits such as stipe texture, epiphyte growth, holdfast size and shape, and stipe rigidity.

Primary sites were sampled between August 1-15th 2022 and minor populations were sampled over the Spring and Summer of 2023. A section (10 x 10cm^2) of meristem was excised from near the base of each sample and epiphytes were removed prior to drying using silica for genetic extraction.

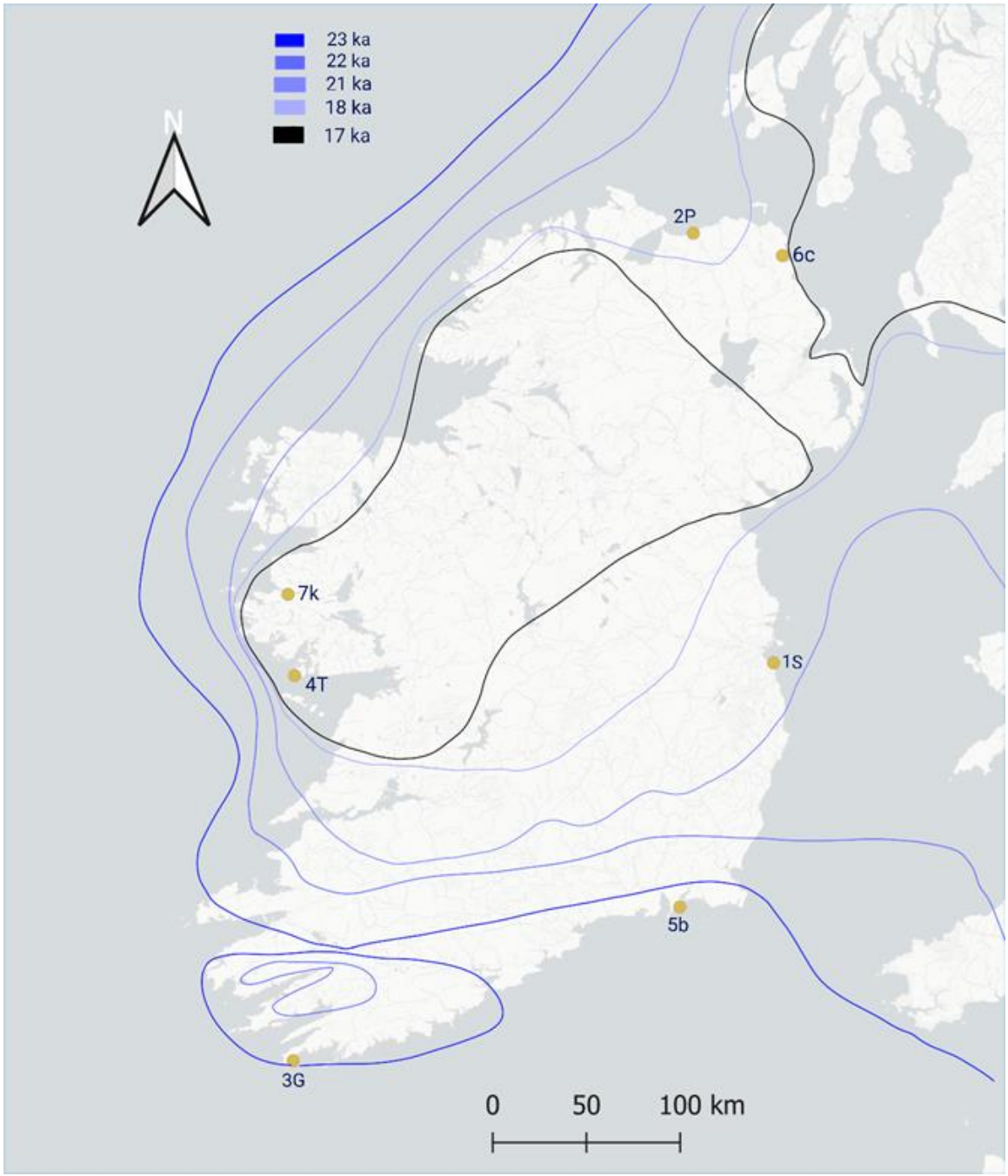

Figure 4.1. Map of sample sites with glacial isochrons adapted from Clark et al. (2022) from older (dark blue) to younger (lighter blue, black). Primary sites (1-4) and minor sites (5-7). 1S = Sandycove, 2P = Port na Happle, 3G = Galley cove, 4T = Trá Dhireáin Huston, 5b = Bagginbun, 6c = Cushendall, 7k = Killary. Ka = thousands of years ago.

4.3.2 Morphological measurements

Morphological measurements were taken for each *Laminaria* specimen collected at the four primary sampling sites (Figure 4.1), based on previous research of potentially useful discriminating traits (Henry, 2018; Hill, 2008; Lund, 2014; Svendsen & Kain, 1971; Tyler-Walters, 2007). These traits were selected based on established *Laminaria* species definition, which have traditionally focused on morphology, and thus would be considered morphospecies.

The measured morphological traits included lamina traits (lamina length and meristem thickness); stipe traits (stipe length, stipe flexibility, stipe surface texture, stipe width taken twice cross-sectionally at the base, mid-section, and upper portion of the stipe and expressed as a "roundness ratio" for each section by division of the smaller width by the larger one, and epiphyte coverage measured using Braun-Blanquet scale); holdfast traits (height, width taken twice cross-sectionally, volume); as well as an approximation of age using a type of hapteran and lower stipe "growth rings" caused by differential season growth, as described by (Kain & Jones, 1963); and finally the depth the specimen was sampled (adjusted for Lowest Astronomical Tide to normalise data between sites and sampling times). Qualitative traits such as stipe surface texture were scored as smooth, bumpy, or rugose and used for morphospecies identification, but excluded from quantitative analysis. Due to limitations relating to quantification of stipe rigidity, an internal metric of comparative flexibility was constructed by cutting the longest straight portion of stipe from the sample (length in cm = x), clamping this horizontally to a retort stand, and measuring the distance the stipe bends under its own weight (distance from 0° in cm = y). A standardised weight (86g) was suspended from the free end of the stipe, and the new distance from 0° was measured in cm (= z). This was standardised into a metric from 0-1, with 0 being completely rigid (no downward stipe deflection under the added weight) and 1 being absolute rigidity (complete downward deflection under standardised weight, i.e. the stipe is at a 90° angle from the point of clamping). When this methodology was tested in a pilot dataset it was found that the location from which the straight piece of stipe was excised had no noticeable systematic biasing effect on this metric. This metric can be calculated using the formula:

$$\frac{z - y}{x}$$

There is high phenotypic plasticity within *Laminaria* for many of these traits (Henry, 2018; Kain & Jones, 1977; Lund, 2014) and therefore it is vital that these traits are truly reflective of an underlying biological species if they are to be used for reliable identification, even by experts.

It is important to note that it is known that site effects can impact morphology of *Laminaria*, and this was partially controlled for by estimating age, recording depth of sample, and through sampling of many individuals across several sites. As discussed in section 1.1 however, much of this literature lacks comprehensive theoretical species concepts, and confounding variables in ecology, morphological species identity, and genetic species identity have not been examined in the literature. As such, site effects on morphology of each genetically confirmed species was tested in tandem (see *Morphological analysis* in section 4.3.5 for more information).

4.3.3 DNA extraction

DNA extraction followed the same protocol as Chapter 2 (section 3.3.2).

4.3.4 Data pre-processing, GBS, and SNP calling

Data was preprocessed as for the previous chapte, section 3.3.3, except that samples were aligned to the outgroup reference genome for *Laminaria ephemera* (GCA_036873875.1) available on GenBank as a single species-naïve group, as no reference genome for either study species was available at the time of analysis, using BWA-MEM v0.7.12.

4.3.5 Data analyses

SNP Filtering

The resulting SNP variants were iteratively filtered in R 4.3.1 (R Core Team, 2024) using the SNPfiltR (DeRaad, 2023) and vcfR (Knaus & Grünwald, 2017) packages for quality and completeness.

Samples were filtered for a minimum depth of = 5, minimum genotype quality of = 20 and a maximum depth of 60. An allele balance of 0.25-0.75 was imposed, as well as a SNP missingness threshold of 0.6 and sample missingness of 0.95. After filtering there were 193 samples with 719 SNPs retained and 26.21% missing data.

Genetic exploratory analysis and Hybrid identification

A phylogenetic tree was created using packages “poppr” and “ape” (Kamvar et al., 2015; Kamvar et al., 2014; Paradis & Schliep, 2019), and annotated according to morphologically

identified *Laminaria* species. The tree followed an UPGMA algorithm using bitwise distance with the "aboot" function and identified two major breeding clades within the *Laminaria* sampled. Discriminant Analysis of Principal Components (DAPC) was performed using *a-priori* k-means clustering assignment (k = 2), which used Bayesian Information Criteria (BIC) and the Elbow method using the R package Adegenet (Jombart & Ahmed, 2011), and which corresponded to the two major groups identified in the phylogenetic tree.

The larger of these groups was labelled "*Laminaria digitata affinis"* and the smaller "*Laminaria hyperborea affinis"* based on observed morphological tendencies that broadly corresponded to the typical morphospecies description for these species. These may also be referred to as Operational Taxonomic Units (OTUs). Samples that had a k = 2 DAPC posterior membership reassignment success of lower than 90% to either identified species were deemed to be hybrids, of which there was one sample.

Morphological analysis

To identify structure within the naive *Laminaria* morphospace a Principal Components Analysis (PCA) was performed on the morphological data. This PCA included normalised depth at which the specimen was sampled as well as approximate age to avoid depth and age effects on morphology being interpreted as being due to morphology alone. To visualise the PCA, identify the 5 most descriptive eigenvectors, and annotate morphospecies and k-means assignment, the packages FactoExtra and ggplot2 (Kassambara & Mundt, 2020; Wickham et al., 2019) were used. This PCA was annotated by both by genetic species/morphospecies to allow for comparison of the two contrasting species concepts, but also by genetic species/site to establish whether morphology is more reflective of site effects or genetic lineage.

To identify allometric differences in morphological traits between these two groups a correlogram was created for the morphological traits of each OTU identified using the package corrplot (Wei & Simko, 2024).

Population diversity statistics

To asses for differences between identified OTUs, global diversity statistics were calculated using the basic.stats function in Hierfstat (Goudet & Jombart, 2022). To asses population differentiation and diversity within each OTU, diversity statistics were generated using the function "gl.report.heterozygosity" in the program dartR (Gruber et al., 2018). Private alleles (Pa) were calculated using the "private_alleles" function in Poppr (Kamvar et al., 2014), and Mean Allelic richness was calculated using the package PopGenReport (Gruber et al., 2015).

Population Connectivity and Structure of *Laminaria* species

In order to identify genetic groups within each identified *Laminaria* OTU, k-means clustering analysis was performed on each group individually with optimal k-means clustering being selected according to BIC and the Elbow method of k selection using the R package Adegenet (Jombart & Ahmed, 2011).

To examine genetic structure, DAPC was conducted using both k-means clusters and site as *a-priori* groupings. To identify genotype affinities of individuals to sampled populations, posterior-membership probability compoplots were constructed using these DAPC results. Membership to each cluster was then visually mapped to site using the package "mapmixture" (Jenkins, 2024).

To assess comparative population divergence, pairwise population Fst was calculated for each population using Weir-Cockerham distance (Weir & Cockerham, 1984) in the package Hierfstat (Goudet & Jombart, 2022).

Isolation By Distance

Genetic differentiation was measured using scaled Euclidean distance using the function "gl.dist.ind" in the package "dartR" (Gruber et al., 2018). Geographic distance between sampling sites was measured using the measure distance function in Google® Maps and a matrix of minimum marine distance along the coast between sites was constructed. Mantel tests were performed at 10,000 permutations using "gl.ibd" in "dartR" (Gruber et al., 2018). Genetic distance was transformed as dist/(1-dist) against transformed geographic distance of log(dist+1) (Rousset, 1997).

4.4 Results

4.4.1 Are Laminaria digitata *and* L. hyperborea *distinct species?*

Two distinct genetic groups emerge in the phylogenetic tree and k-means clustering analysis of the sampled Irish *Laminaria* which broadly correlate to *Laminaria digitata* and *Laminaria hyperborea* when comparing morphology. It is important to note however that in-field identification based on accepted morphospecies definition did not reliably map onto these genetic divisions (Figure 4.2), leading to the conclusion that *Laminaria digitata* and *Laminaria hyperborea* species segregation as identified by commonly accepted morphology alone is inconsistent.

Due to poorly defined species boundaries in terms of morphospecies identification being inconsistent with phylogenetic species, these two OTUs will be referred to as *L. digitata aff.* and *L. hyperborea aff.* in the rest of this manuscript.

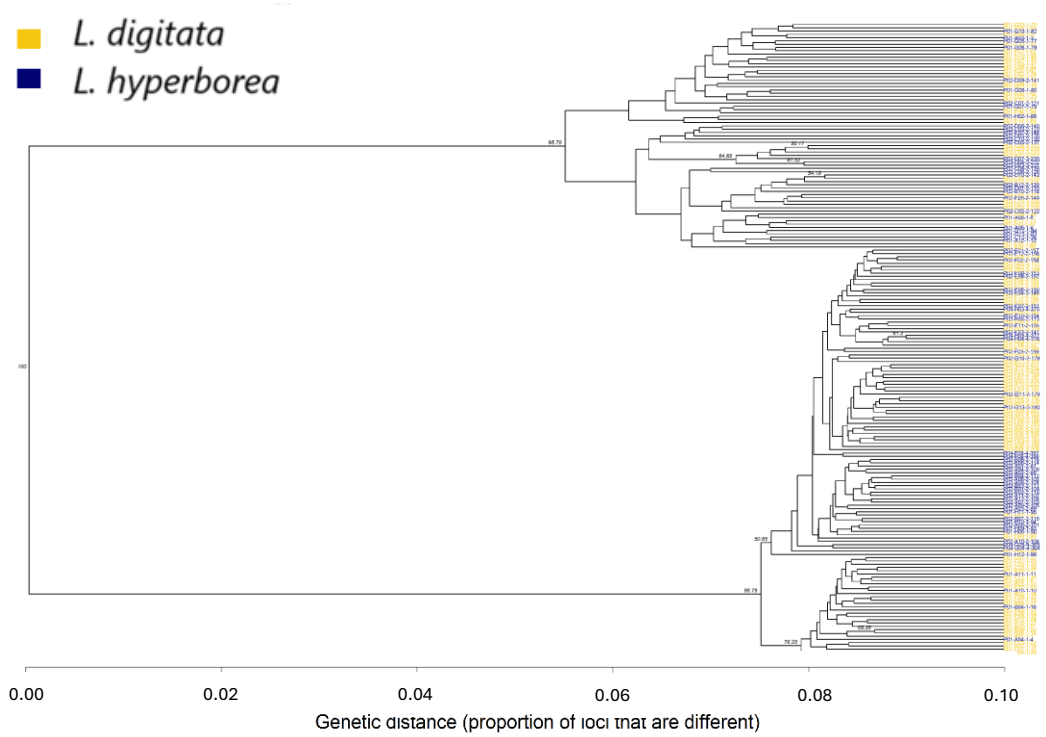

Figure 4.2. Phylogenetic tree of all Laminaria *(n = 194). Morphospecies ID annotated by colour. K = 2 optimal cluster assignment corresponds precisely to the diverging breeding groups visible in this tree, defining the genetic aff. groups. Bootstrap support indicated at nodes (10,000 permutations). Note the distribution of morphospecies in both clades.*

4.4.2 Are Laminaria species morphologically distinguishable?

To identify the differences in morphological traits between traditional morphospecies and identified genetic species, a PCA was constructed using all quantitatively measured traits and annotated by field-identified morphospecies and genetically identified OTU (Figure 4.3). The five most descriptive eigenvectors were shown to identify underlying drivers in the observed structure. No consistent relationship between quantitatively measured traits, morphospecies identification, and genetic species was observed.

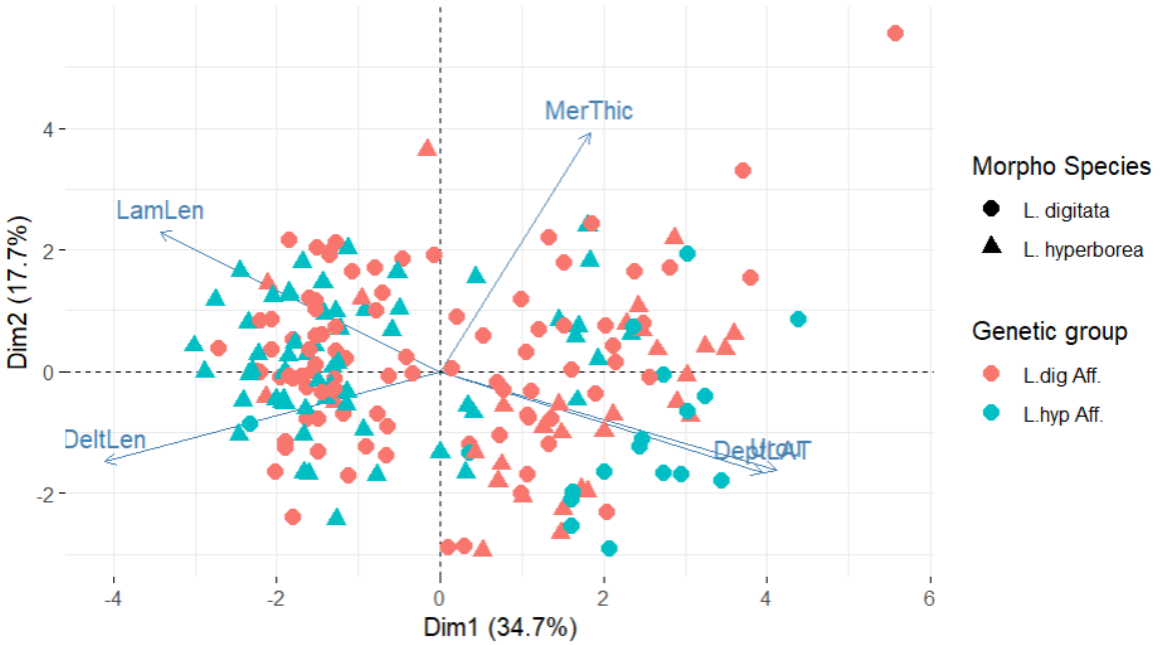

Figure 4.3. PCA of all quantitative morphological traits, including age and depth, with the top 5 contributing eigenvectors labelled. Morphospecies marked by shape and genetic group identified by k-means cluster analysis marked by colour. (N=194). LamLen = Lamina length, MerThic = Meristem thickness, DeltLen = Stipe flexibility, Urou = Upper stipe cross-sectional roundness, DeptLAT = Depth of sample at Lowest Astronomical Tide. Dim1 explains 34.7% of the variation, while Dim 2 explains 17.7%. Note no clear structure between morphospecies or genetic group.

When this same PCA is annotated by site and genetic species it becomes clear that both species in site 1 (Sandycove) and 2 (Port na Happle) occupy a similar morphospace, but that site 3 (Galley Cove) and 4 (Trá Dhireáin Huston) show some stratification (Figure 4.4). Despite this stratification in site, there does not appear to be a noticeable differentiation between species at the same site. This indicates that site is a more important determining factor for *Laminaria* morphology than genetic species.

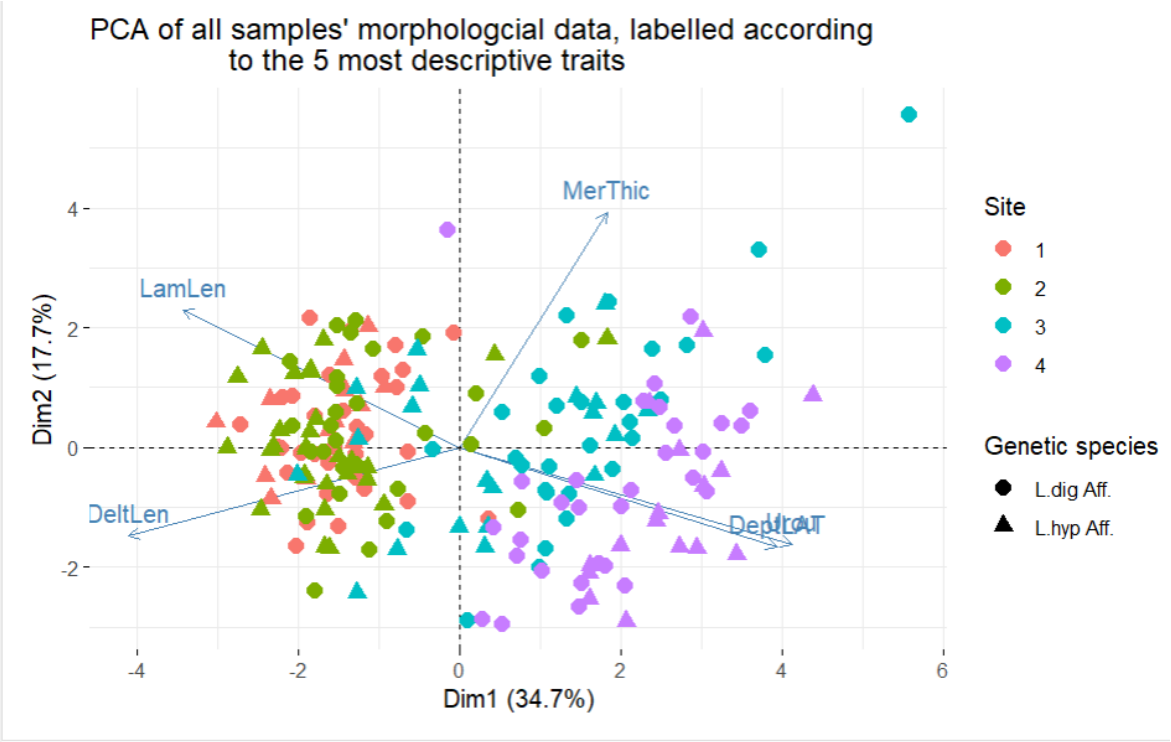

Figure 4.4. PCA of all quantitative morphological, including age and depth, with the top 5 contributing eigenvectors labelled. Primary site marked by colour and genetic group identified by K-means cluster analysis marked by shape. (N = 194). LamLen = Lamina length, MerThic = Meristem thickness, DeltLen = Stipe flexibility, Urou = Upper stipe cross-sectional roundness, DeptLAT = Depth of sample at Lowest Astronomical Tide. Site 1 = Sandycove; Site 2 = Port na Happle; Site 3 = Galley cove; Site 4 = Trá Dhireáin Huston. Dim1 explains 34.7% of the variation, while Dim 2 explains 17.7%. Note the separation of site 3 and particularly site 4 along the first axis (Dim1).

When each OTU is analysed using a correlogram, age and depth have a significant correlation with many morphological traits (Figure 4.5,a,b), indicating age and depth effects influence morphological traits, which is consistent with previous findings (Kain & Jones, 1963, 1977). As these samples were taken from across multiple sites there is a minimisation of site specific variables, allowing for better identification of species specific correlations. The difference in sampling number however will have influenced the significance level of many of the trait correlations between graphs in Figure 4.5a,b, as more samples allow for better testing of underlying signals. Notably, the morphological traits typically used for species identification (stipe flexibility, upper stipe roundness, holdfast volume and epiphyte coverage) all seem to be correlated with age and/or depth, with many of these correlations being significant. This raises concerns about use of these traits to accurately predict genetic species.

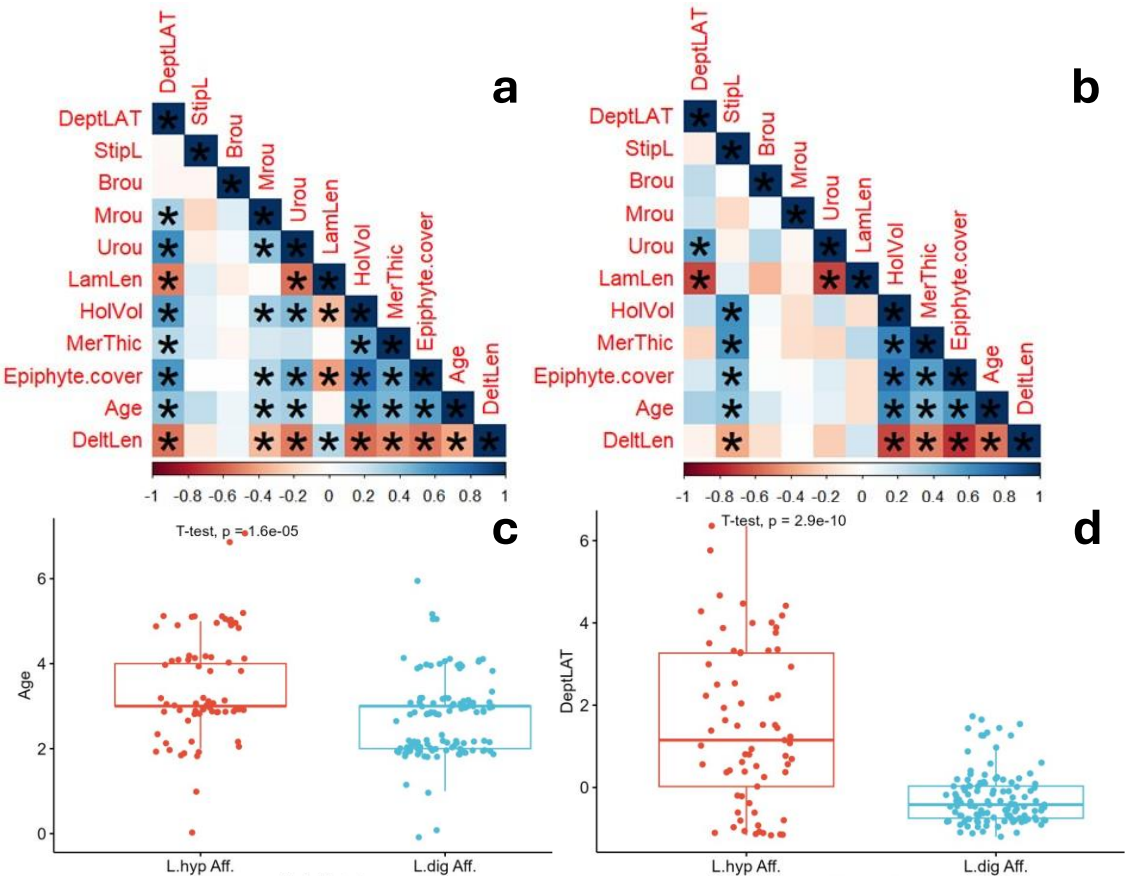

*Figure 4.5. a-d) a = L. digitata aff. (N = 101), and b = L. hyperborea aff. (N = 57) correlogram respectively, * indicates significance of p < 0.01. Scale bar below represents strength of correlation, from -1 to 1. Boxplots of c = Approximate age in years, and d = depth of samples adjusted to Lowest Astronomical Tide (m) for both L. digitata aff. and L. hyperborea aff. collected. Welch's T-test p-value included.*

The OTUs identified in this study show a significant difference in sampling depth and age, with *L. digitata aff.* having a much narrower range of depths at which it was sampled and being significantly younger than *L. hyperborea aff.* (Figure 4.5 c,d). It is important to note that the age and depth of samples is not a useful discriminating factor for individual specimen identification due to the high degree of variance and overlap between OTUs. Interestingly however, this study suggests that *L. digitata aff.* is not found at depths below 2m LAT, while *L. hyperborea aff.* was distributed across the sampled depth range. This may partially explain the differences in significant depth-related trait correlations seen between OTUs in Figure 4.5 (a,b), as the largest change in environmental variables (such as light and wave energy) that impact *Laminaria* morphology occur closer to the intertidal range, with deeper subtidal habitats showing greater stability (Kain & Jones, 1977). While age was also found to be significantly different, the mean age for both OTUs was approximately 3 years old, with the significance being attributable to *L. hyperborea aff.* having a skewed distribution towards older specimens and *L. digitata aff.* having a skew towards younger specimens

The traits that were identified as being most descriptive in the PCA (Figure 4.3) also have strong correlations with Age and Depth in the correlograms across both OTUs, and were tested for significance using Welch's t-test and visualised using boxplots (Figure 4.6). These are also typically the traits used to distinguish *Laminaria hyperborea* from *Laminaria digitata* in the field, and include stipe epiphyte coverage, stipe flexibility, and upper stipe roundness. Meristem thickness was also included as it was one of the most explanatory eigenvectors identified in the PCA, but interestingly did not show any significant difference between OTUs ($p = 0.74$). Epiphyte coverage showed a somewhat significant difference ($p = 0.0021$), but both species exhibited a wide range of coverage and so it is unreliable as a discriminating trait. Interestingly, upper stipe roundness and stipe flexibility showed highly significant differences between OTUs that were consistent with traditional species definition, with *L. hyperborea aff.* having more rigid and rounder upper stipes than *L. digitata aff.* This however, combined with the previously described correlation of these traits with age and depth, make them unreliable as discriminating traits. The significant positive correlation between age and depth in *L. digitata aff.* and insignificant positive correlation in *L. hyperborea aff.* also suggest that deeper individuals tend to live longer, further complicating any traits associated with depth or age to be deemed a reliable discriminating trait for any single specimen.

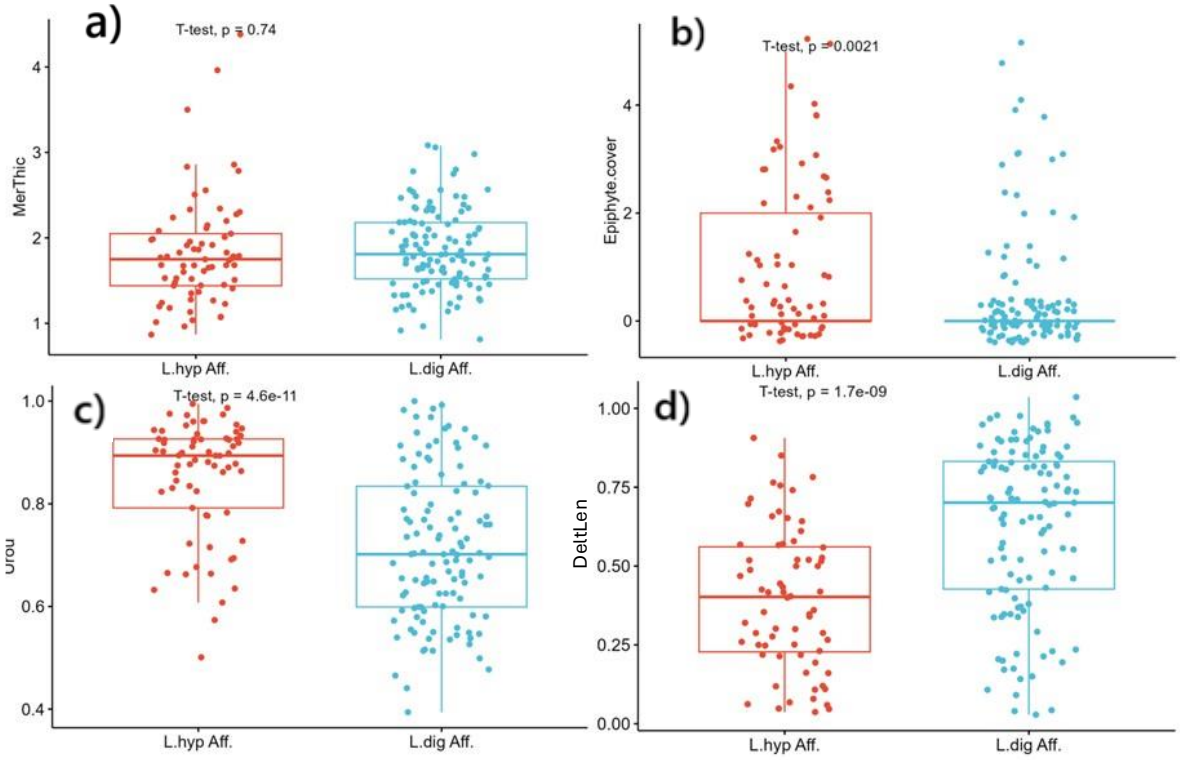

Figure 4.6. Boxplots of: a) Meristem thickness, b) Epiphyte coverage on stipe, c) Upper stipe roundness (Urou, 1= perfect roundness), and d) stipe flexibility (DeltLen, 0 = absolute rigidity,

1 = flexibility to 90° angle downwards from fulcrum) for both L. digitata aff. and L. hyperborea aff. collected. Welch's T-test p-value included.

4.4.3 Can evidence of hybridisation be identified in Laminaria*?*

To test for hybridisation, a DAPC was performed on all *Laminaria* using optimal k-means clusters (k = 2) as *a-priori* groups, and a kernel density plot with two discriminant components was created (Figure 4.8). The bimodal distribution of densities with little overlap indicates the distinct nature of these breeding groups and is evidence against the widespread hybridisation of *Laminaria* in Ireland. Interestingly, there seems to be one small peak of group 1 (*L. digitata aff.*) that shows similarity to group 2 (*L. hyperborea aff.*). This single specimen was found to have ~80% affinity to group 1 and ~20% affinity to group 2, indicating it may be of hybrid origin. This sample was observed in the north at Port na Happle and the uneven assignment between OTUs suggests it may be an F2 or F3 sporophyte, indicating that hybrids may be capable of introgressing into the parent populations. Group 1 showed several small peaks that may represent more historic introgression but was nevertheless quite distinct, as seen by its high and relatively narrow peak. Group 2 was however much more diverse as represented by its comparatively shallow and broad peak, which may also suggest directional introgression into *L. hyperborea*.

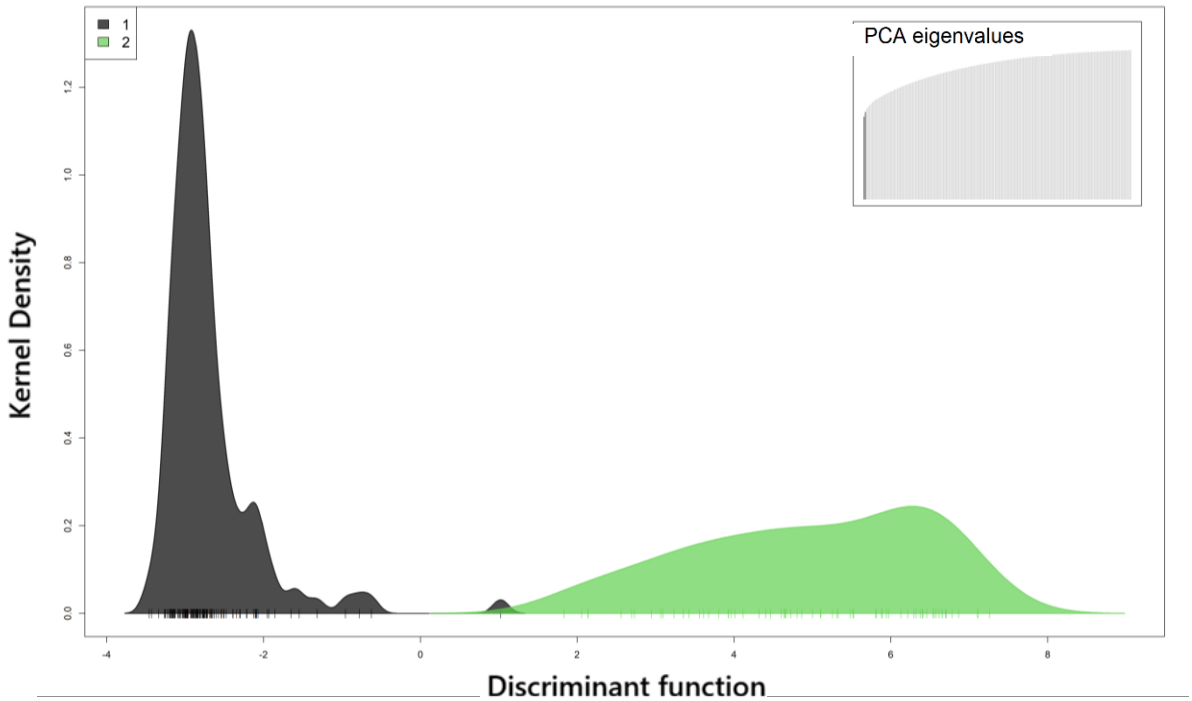

Figure 4.7. DAPC of all Laminaria *SNPs (N = 193), 719 biallelic SNPs, 1000 cross validation, X PC retained. 1* = L. digitata aff*, 2* = L. hyperborea. aff*, according to k = 2 assignment. PCA eigenvalues as a proportion of total variance in inset.*

4.4.4 Species diversity

When analysed across populations, higher observed heterozygosity (*Ho*) and gene diversity (*Hs*, *Ht*) was found for *L. hyperborea aff.* compared to *L. digitata aff.* despite the lower total number and less geographically distributed samples (Table 4.1). *Dest* indicates that there is low population differentiation in both *L. digitata aff* (Dest = 0.01) and *L. hyperborea aff.* (Dest = 0.02*)*. Fixation indices (Fst) also differed between OTUs, with *L. digitata aff.* (Fst = 0.05) having a slightly higher value than *L. hyperborea aff.* (Fst = 0.04). The greater sampling range and number of *L. digitata aff.* may have increased Fst values, while alignment of both species to an outgroup (*L. ephemera*) would reduce total Fst values due to selection of more conserved SNPs across the *Laminaria* genome. Global Fis for both breeding groups was negative, indicating an overall outbreeding tendency for Irish *Laminaria*, however the Fis for *L. hyperborea aff.* was substantially more negative (-0.41) than for *L. digitata aff.* (-0.19).

Table 4.1. Diversity statistics across populations of each Laminaria *OTU. 430 biallelic SNPs used for L. digitata aff., 305 biallelic SNPs for L. hyperborea aff.*

	Definition	***L. digitata aff*** **. (*n* = 124)**	***L. hyperborea aff.*** **(*n* = 69)**
Ho	Observed heterozygosity	0.18	0.37
Hs	Within population gene diversity	0.15	0.26
Ht	Overall gene diversity	0.16	0.27
Dst	Gene diversity among samples	0.01	0.01
Fst	Fixation index	0.05	0.04
Fis	Inbreeding coefficient	-0.19	-0.41
Dest*	Population differentiation	0.01	0.02

*(Jost, 2008)

4.4.5 Refugial signals

Populations with increased Private alleles (Pa) were found in both OTUs but with differing distribution (Table 4.2). While *L. digitata aff.* had Pa = 166 on the east coast (Sandycove) and Pa = 128 on the west coast (Trá Dhireáin Huston), *L. hyperborea aff.* showed refugial signals primarily in the north with Pa = 50 (Port na Happle) with a slightly elevated Pa = 21 on the West coast (Trá Dhireáin Huston). This indicates that the west coast may contain refugial sites for both species, but that *L. hyperborea aff.* shows a more northernly refuge compared to *L. digitata aff.* which shows an eastern refugial signal.

Table 4.2. Population genetic diversity statistics for L. digitata aff. *and* L. hyperborea aff. *nInd = Number of individuals (corrected for sample size), Ho = Observed Heterozygosity, He = Expected Heterozygosity, FIS = Inbreeding coefficient, Pa = Private allele*

Species (n)	Site (n)	Lat	Lon	nInd	Ho	He	Fis	Ar	Pa
***L. digitata* (124)**	**Bagginbun (3)**	52.17668	-6.82917	1.681	0.172	0.094	-0.257	1.112	0
	Cushendall (2)	55.08373	-6.05504	1.740	0.143	0.090	-0.111	1.109	0
	Killary (2)	53.59706	-9.77032	1.745	0.176	0.106	-0.164	1.118	0
	Galley cove (28)	51.46144	-9.73986	20.177	0.172	0.142	-0.090	1.128	26
	Port na Happle (29)	55.1806	-6.72414	22.563	0.179	0.148	-0.081	1.143	26
	Sandycove (31)	53.28821	-6.12029	23.874	0.196	0.154	-0.117	1.147	166
	Trá Dhireáin Huston (29)	53.23125	-9.73373	22.174	0.184	0.146	-0.088	1.139	128
***L. hyperborea* (69)**	**Galley cove (16)**	51.46144	-9.73986	8.704	0.361	0.241	-0.291	1.245	14
	Port na Happle (27)	55.1806	-6.72414	17.341	0.377	0.251	-0.309	1.251	50
	Sandycove (13)	53.28821	-6.12029	7.751	0.356	0.232	-0.322	1.242	16
	Trá Dhireáin Huston (13)	53.23125	-9.73373	7.233	0.386	0.245	-0.360	1.262	21

4.4.6 L. digitata aff. *diversity*

Population level statistics were calculated for *L. digitata aff.* in Table 4.2, with negative Fis values being calculated for every population, ranging from -0.088 in Trá Dhireáin Huston to -0.257 in Bagginbun. Sandycove notably has a lower Fis (-0.117) than the other primary populations, indicating heterozygote excess. Both the observed heterozygosity (0.196) and Allelic richness (1.1472) was highest in the Sandycove population, indicating a region of high diversity and evolutionary potential. Similarly, uHe can be seen to allow be highest in Sandycove (Figure 4.8a).

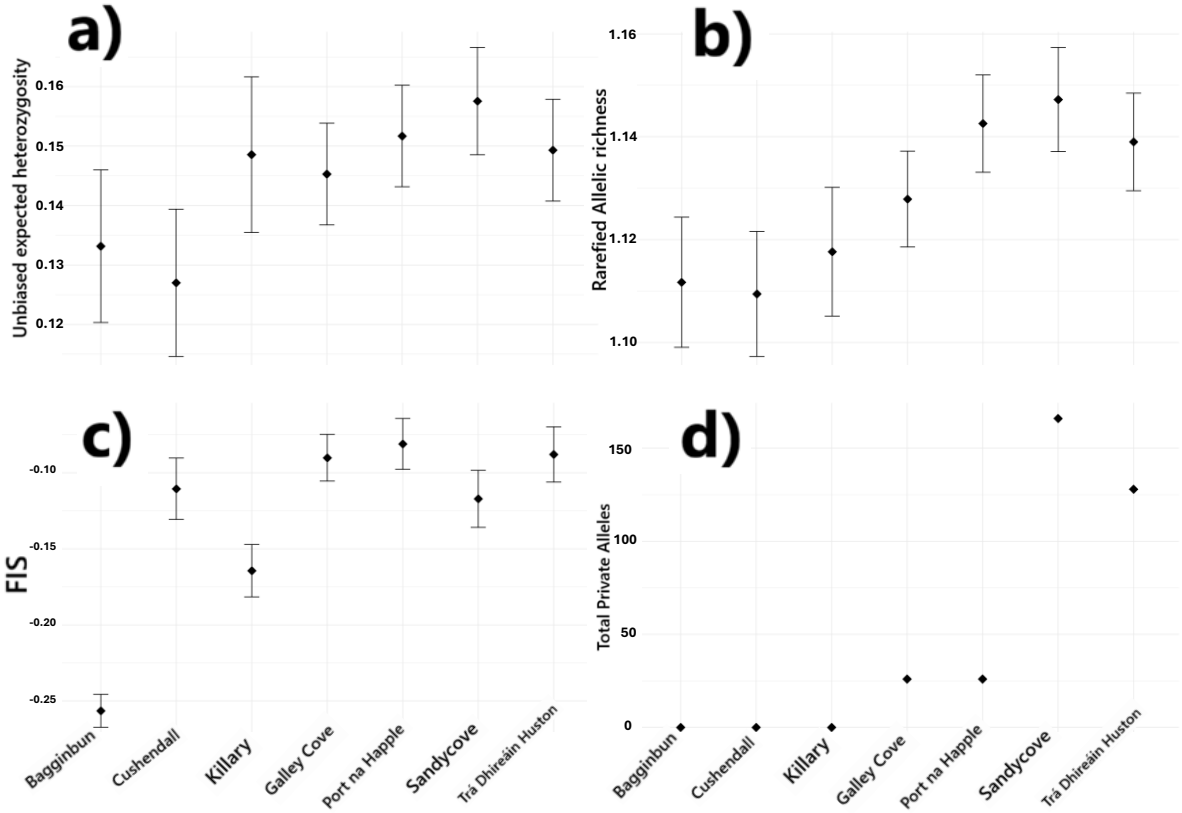

Figure 4.8. a) Unbiased Expected heterozygosity (uHE), b) Rarified Allelic richness, and c) Fis for each population of L. digitata aff. including standard error bars. Total private alleles also included in d. All populations in alphabetical order, as labelled in c and d. Note the exceptionally high amount of private alleles in Sandycove and Trá Dhireáin Huston.

4.4.7 L. hyperborea aff. diversity

All populations of *L. hyperborea aff.* showed comparatively high levels of *Ho*, from 0.356 (Sandycove in the east) to 0.386 (Trá Dhireáin Huston in the west). Similar patterns existed for Allelic Richness, which ranged from 1.242 to 1.262 in these same populations. Notably *L. hyperborea aff.* shows extremely negative values for Fis, ranging from -0.291 in Galley cove to -0.360 in Trá Dhireáin Huston. Additionally, the Ar and Ho of *L. hyperborea aff.* across all populations was much higher than in *L. digitata aff.*, indicating it is substantially more diverse and has greater adaptive diversity. Patterns of uHe closely follow those of Ho and Ar (Figure 4.9).

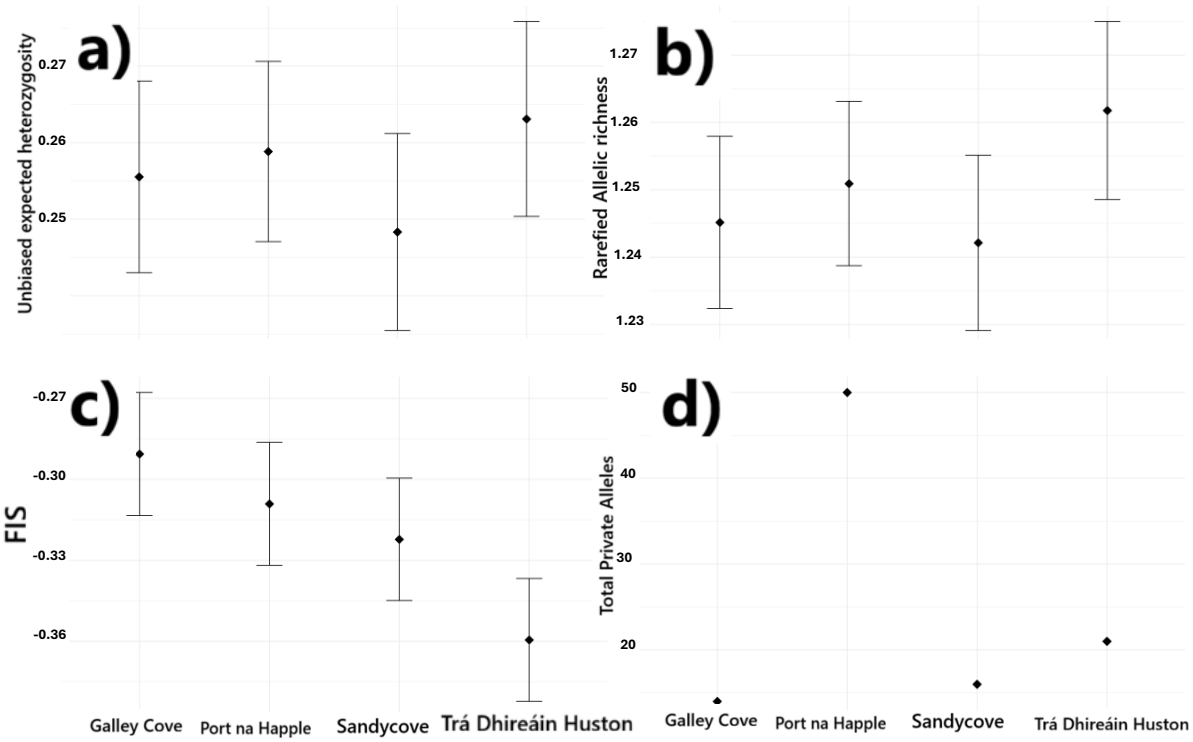

Figure 4.9. a) Unbiased Expected heterozygosity (uHE), b) Rarified Allelic richness, and c) Fis for each population of L. hyperborea aff. including standard error bars. Total private alleles also included in d). All populations in alphabetical order, as labelled in c and d. Note the higher amount of private alleles in Port na Happle

4.4.8 L. digitata aff. population structure

Optimal clustering was found to be at k = 4 in k-means clustering analysis of *Laminaria digitata aff*. Clustering from k = 2-7 was conducted and DAPC was performed on each of these clusters. These were divided into populations when visualised on a posterior membership compoplot to identify structure across k-means clusters and populations (Figure 4.10). This indicates that Sandycove is a very distinct genetic cluster as it separates out consistently across k assignments, followed by Port na Happle (k = 3) and then Trá Dhireáin Huston (k = 4). Interestingly for optimal k = 4, Galley cove forms a genetic cluster with Killary and Bagginbun, while Cushendall shows affinity towards Port na Happle, with a slight Galley cove assignment. K = 5 divides three Sandycove samples and one Cushendall sample into a new group, while the remaining Cushendall sample clusters with Port na Happle. K = 6 further divides Sandycove samples into another cluster, indicating there is quite a lot of structure and diversity at this site compared to other populations. K = 7 subdivides Port na Happle into two distinct groups and one Cushendall associated group, indicating the possible existence of admixture. It is of note that Trá Dhireáin Huston has consistent and strong reassignment despite similar Ho and Ar as in Port na Happle and Sandycove, suggesting it is quite a distinct population.

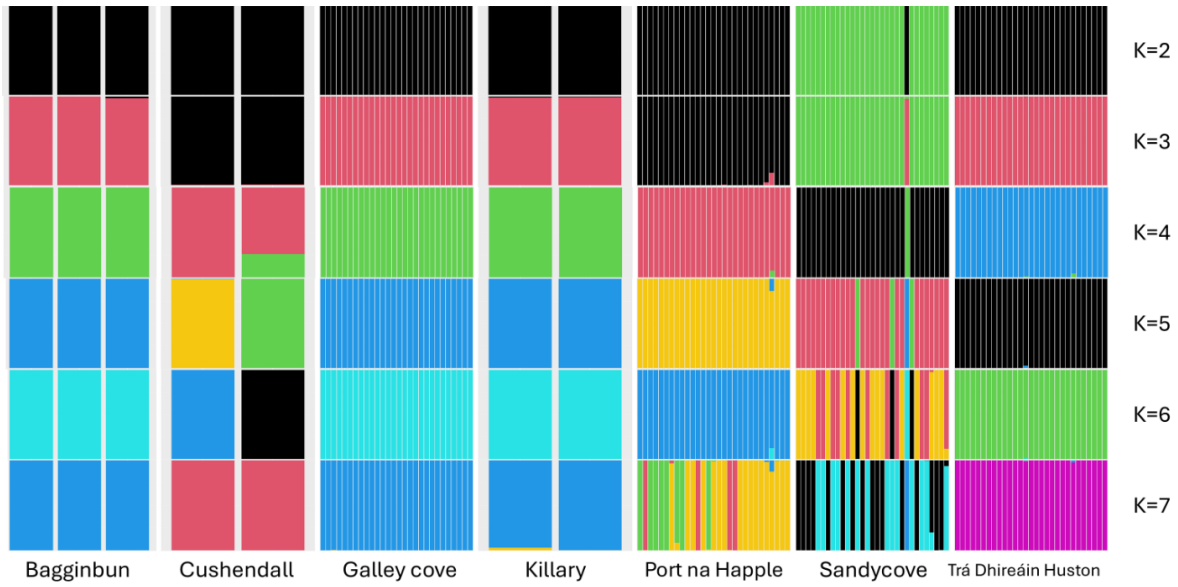

Figure 4.10. Posterior membership probability, according to DAPC, of all L. digitata aff. *(N = 124) using 719 biallelic SNPs according to k = 2-7. Retained PCs 1000x cross validated for each k cluster. Each column is an individual and the proportion of the column in each colour represents the affinity of that individual to said group.*

When analysed by cluster (Figure 4.11), the primary populations on the east and west both showed high Pa (128 and 164 respectively). The east however showed high levels of other diversity metrics (Ar, uHe) which were comparatively low in the west, potentially indicating lower effective population size in the west. Interestingly, the northern cluster showed low Pa but relatively high diversity in Ar and uHe, which may be a function of admixture, possibly via geneflow from more southernly populations.

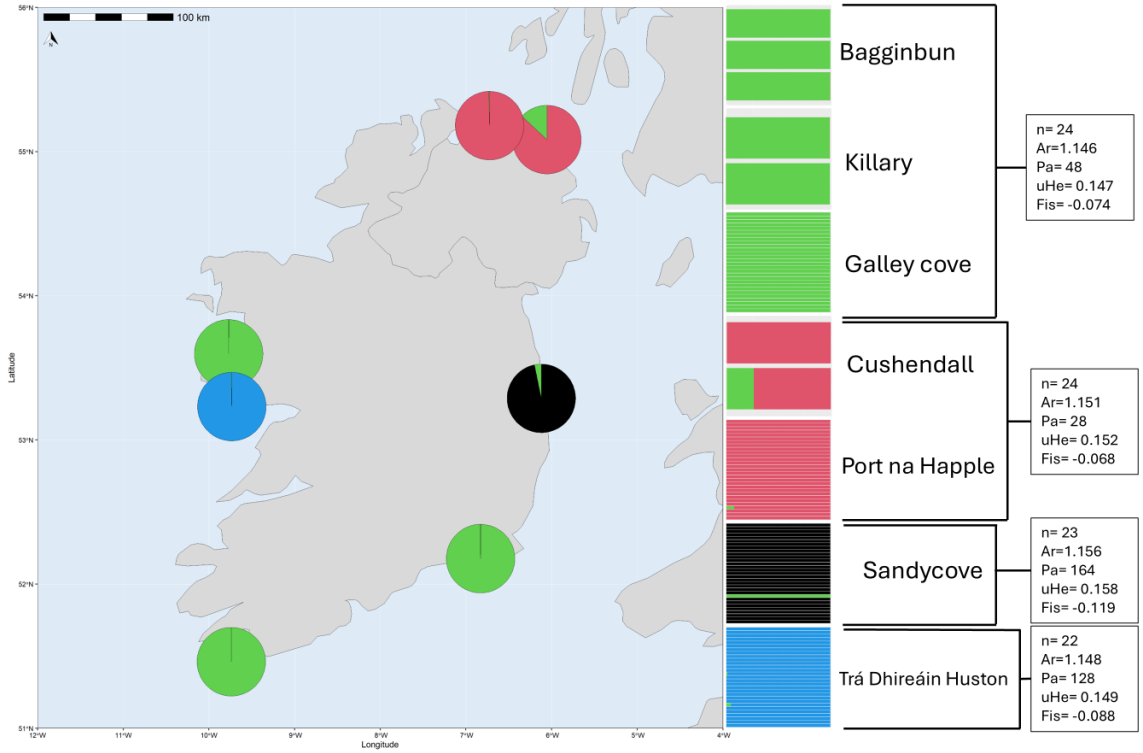

Figure 4.11. proportion of k = 4 k-means cluster assignments as proportion of grouped site genotypes for L. digitata aff. *Optimal k-cluster posterior membership assignments on right, with sample number (*n*) corrected for sample size according to Nei (1978), rarefied allelic richness (Ar), total private alleles (Pa), unbiased expected Heterozygosity (uHe), and fixation index (Fis) for each cluster.*

Results of DAPC of *L. digitata aff.* (Figure 4.12) indicate high divergence of the Sandycove population along the first principal component, while the second axes separates the remaining primary populations. Galley cove and Trá Dhireáin Huston showed some similarity, but were still relatively distinct. The minor populations at Bagginbun and Killary showed similarity to the southern population at Galley cove, while Cushendall showed some similarity to Port na Happle, but one sample showed attraction to the Sandycove cluster. The DAPC derived posterior membership compoplot indicate that Galley cove and Bagginbun show some affinity towards one another (Figure 4.12, right), as well as to Killary, and that these southern sites had affinity towards samples in both the east and north. The high affinity of some samples in Sandycove and Port na Happle to this Bagginbun/Galley cove genotype indicate geneflow might be preferentially along the east coast northwards, although lower sampling at Bagginbun may produce some statistical artifacts that could influence this observed pattern. This can be seen in Figure 4.12, where the southern cluster genotypes seem to be represented at the eastern and northern sites. Interestingly, the population at Cushendall does not show the same assignment pattern as Bagginbun despite similarly low sampling, with very distinct population assignment and low affinity to any other group. The lack

of assignment of Trá Dhireann Huston genotypes to other populations indicates high differentiation. Overall, the primary populations had good reassignment to their sampled populations, with very little assignment of samples between the primary populations, but many samples across populations showed affinity to Bagginbun genotypes. There is a general pattern of more northern populations having affinity to southern genotypes, but not vice versa, suggesting south to north geneflow.

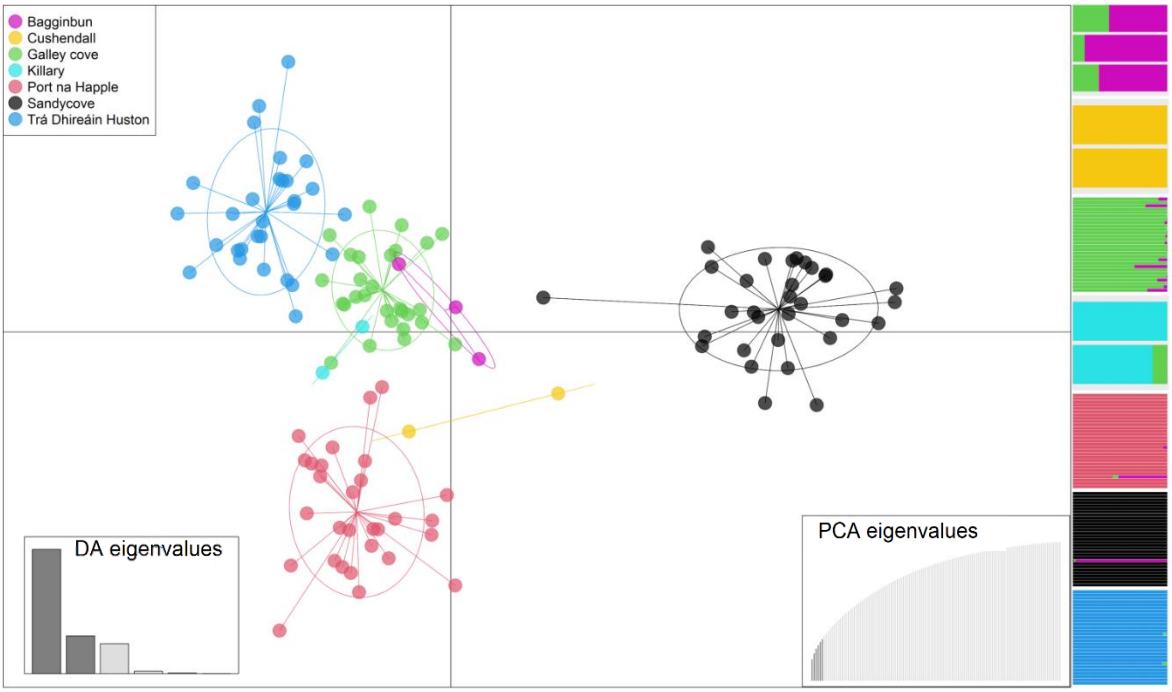

Figure 4.12. DAPC of all L. digitata aff. SNPs (N = 124), 719 biallelic SNPs, 1000 cross validation, 6 PCs retained, 29.7% variance retained. Posterior membership compoplot probabilities on right with each horizontal bar representing one sample. PCA eigenvalues as a proportion of total variance in inset on right. DA eigenvalues in inset on left. The eigenvalue plots show the amount of genetic information retained by the PCA (on the right) and the discriminant function (DA, on the left).

Pairwise Fst in Figure 4.13 indicates high divergence at Sandycove (mean Fst = 0.1998), with pairwise Fst being highest when compared to Trá Dhireann Huston (0.253), which also showed the next highest mean Fst (0.1255). Galley cove showed the lowest mean Fst for primary populations (mean Fst = 0.0963), and Port na Happle was similarly low (mean Fst = 0.1023). Killary and Cushendall both showed similarly low mean Fst (0.0947 and 0.099 respectively), but interestingly Bagginbun had a somewhat elevated mean Fst (0.117) when compared to the other minor populations. This furthers the hypothesis that geneflow is primarily from the south to the north, with Port na Happle possibly representing a region of admixture between northward geneflow on the east and west coast.

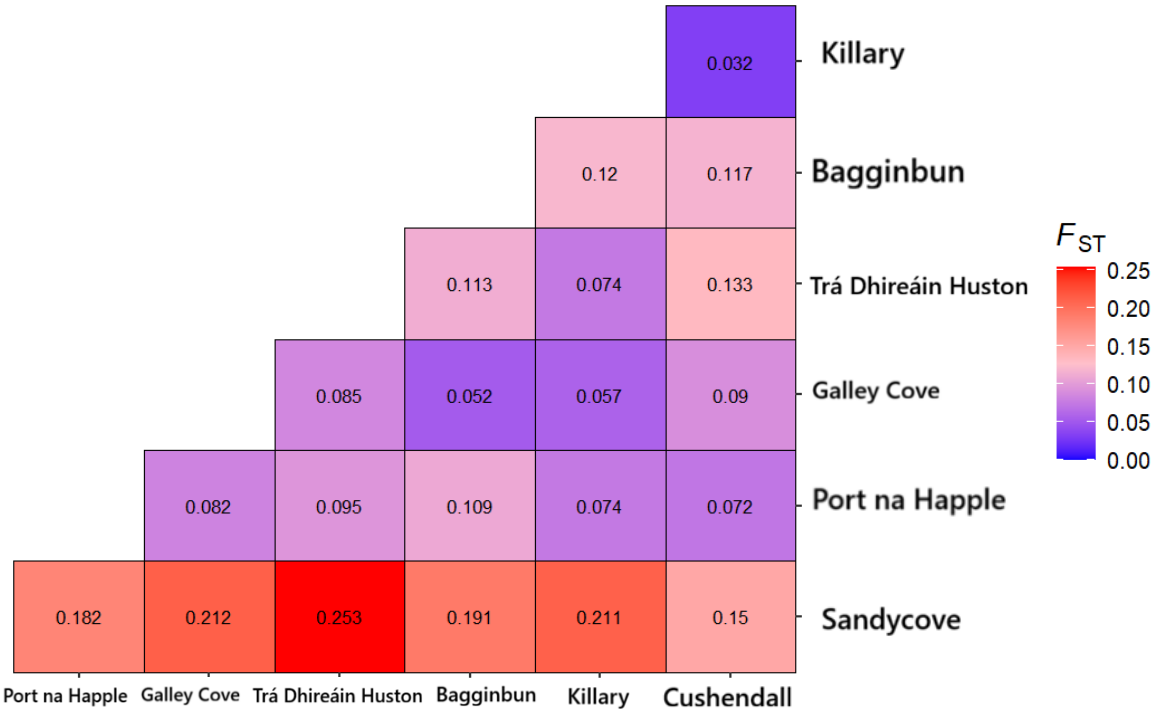

Figure 4.13. Pairwise Fst of L. digitata aff *(N = 124) biallelic SNPs = 430. The higher the number, the greater the pairwise differentiation.*

4.4.9 L. hyperborea aff. Population structure

For *L. hyperborea aff.* optimal clustering was found to be at k = 2 in k-means clustering analysis. Clustering from k = 2-4 was performed and DAPC was performed on each of these clusters. These were divided into populations when visualised on a posterior membership compoplot to identify structure across k-means clusters and across populations (Figure 4.14). Interestingly the optimal k = 2 clusters showed a clear distinction between populations, with a south/west and north/east group being very distinct. K = 3 showed some admixture within the north/east cluster, while k = 4 separated out the south/west cluster into distinct groups with little admixture. This indicates that the populations within the north/east group show more structure compared to the populations in the south/west group despite geneflow. Interestingly, this same kind of admixture does not seem to occur in *L. digitata aff.* populations (Figure 4.11), which are instead primarily assigned to separate genotype clusters.

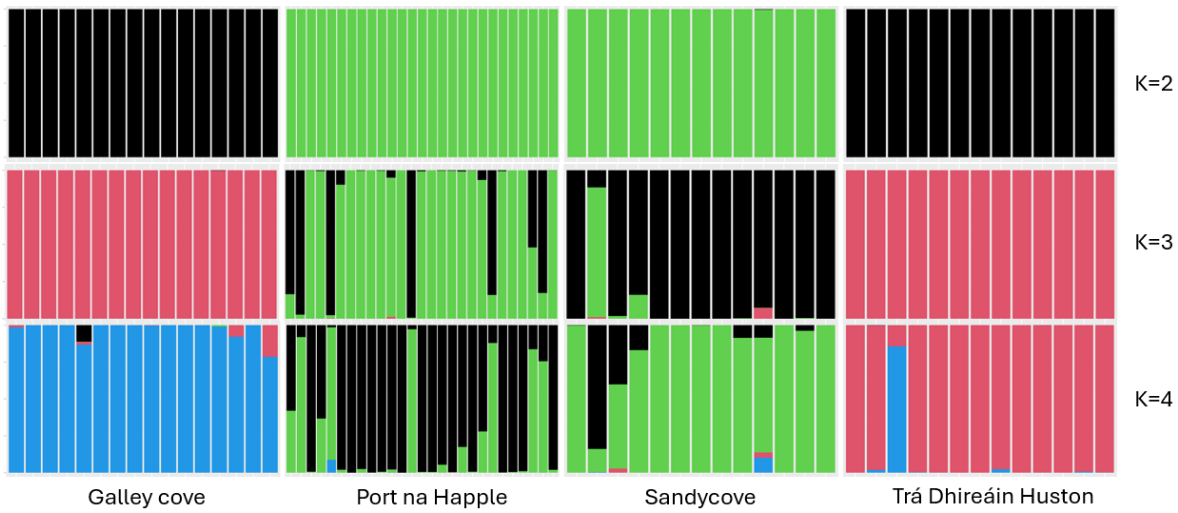

Figure 4.14. Posterior membership probability, according to DAPC, of all L. hyperborea aff. SNPs (N = 69), using 704 bi-allelic SNPs, 1000 cross validation, and 6 PCs retained. Each column is an individual and the proportion of the column in each colour represents the affinity of that individual to said group.

This south-west/north-east divide can be visualised in Figure 4.15, which pools genotype assignment for k = 2 of all samples at a site. While diversity metrics (Ar, uHe, Pa) were higher in the north-east cluster, this may in part be due to higher sampling. Interestingly, there was a greater excess of heterozygotes in the south-west cluster (Fis = -0.261) than the north-east cluster (Fis = -0.219), which might point to population sub-structuring in the north-east, producing Wahlund effects.

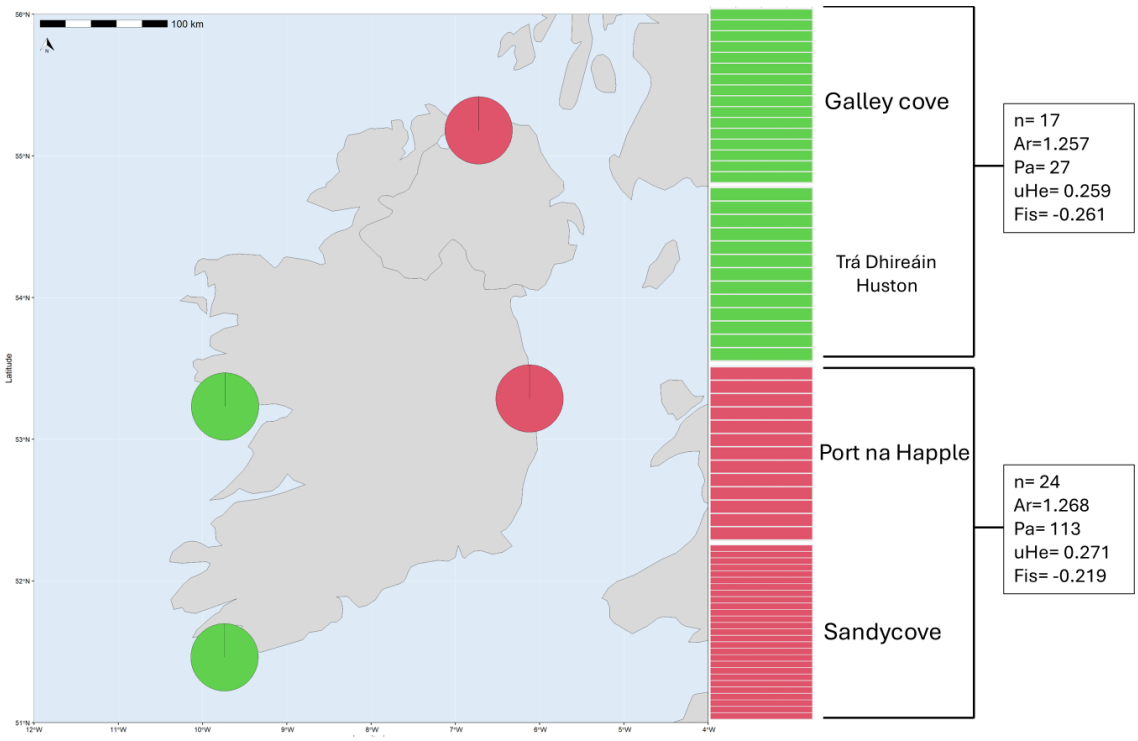

Figure 4.15. proportion of k = 2 k-means cluster assignments as proportion of grouped site genotypes for L. hyperborea aff. *Optimal k-cluster posterior membership assignments on right, with sample number (n) corrected for sample size according to Nei (1978), rarefied allelic richness (Ar), total private alleles (Pa), unbiased expected Heterozygosity (uHe), and fixation index (Fis) for each cluster.*

DAPC for *L. hyperborea aff.* showed clear distinction between the sampled populations (Figure 4.16). Interestingly, the first PC distinguishes between north/east and south/west populations while the second axes further separates out the north and east, and south and west populations respectively. A posterior membership compoplot shows clear reassignment of all specimens to their source population, with one single northern specimen showing a small degree of reassignment to the eastern genotype, which suggests some potential geneflow (Figure 4.16, right).

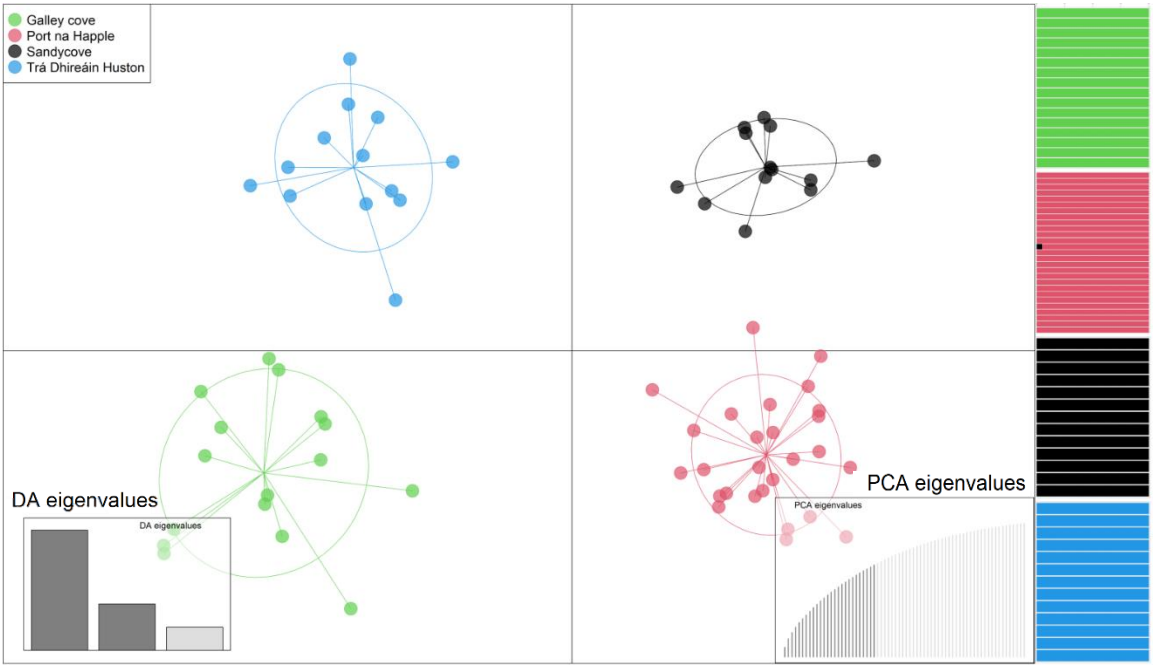

Figure 4.16. DAPC of all L. hyperborea aff. SNPs (N = 69), 704 biallelic SNPs, 1000 cross validation, 26 PCs retained. 68.8% variance conserved. Posterior membership probabilities on right with each horizontal bar representing one sample. PCA eigenvalues as a proportion of total variance in inset on right. DA eigenvalues in inset on left. The eigenvalue plots show the amount of genetic information retained by the PCA (on the right) and the discriminant function (DA, on the left).

The pairwise Fst in Figure 4.17 indicates high divergence of the east (Sandycove, mean Fst = 0.1001) and west (Trá Dhireann Huston, mean Fst = 0.1057) populations compared to the south (Galley cove, mean Fst = 0.0837) and north (Port na Happle, mean Fst = 0.0887) population. This indicates a similar patten observed in *L. digitata aff.* that there may be long

distance dispersal from southern to northern populations, but that this doesn't necessarily include the sampled east and west populations.

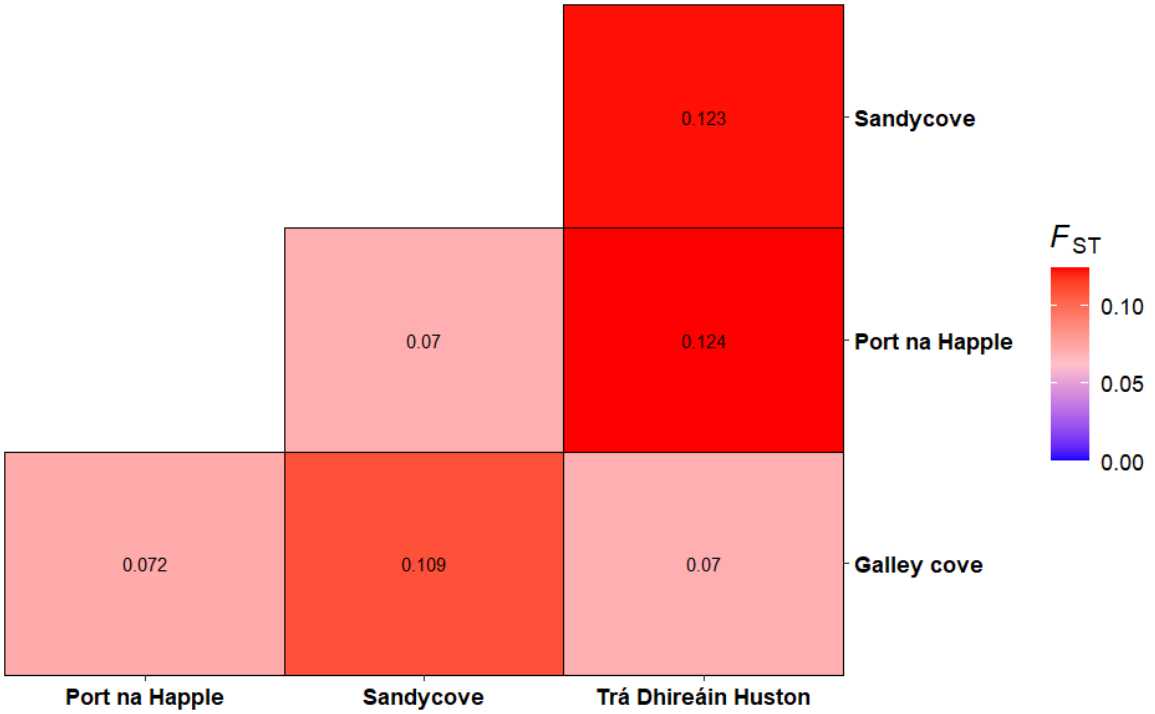

Figure 4.17. Pairwise Fst of L. hyperborea aff (N = 69). The higher the number, the greater the pairwise differentiation.

4.4.10 Isolation By Distance

A model of Isolation By Distance was constructed for both species (Figure 4.18*)* and was found to be highly significant ($p = 1e^{-04}$, $p = 0.001$) for both OTUs. *L. digitata aff.* has an $R^2 = 0.28$, and *L. hyperborea aff.* has an $R^2 = 0.217$. The Mantel r statistic for *L. digitata aff* was 0.5291 while *L. hyperborea aff.* had a Mantel r of 0.5071, and both were highly significant ($p = 9.999e^{-05}$). Interestingly, and IBD model for *L. hyperborea* using clockwise distance from the most southernly population both increased the strength and significance of the model ($p = 1e^{-04}$, $R^2 = 0.257$), suggesting geneflow along the west coast to be a more important explanatory factor (Appendix A).

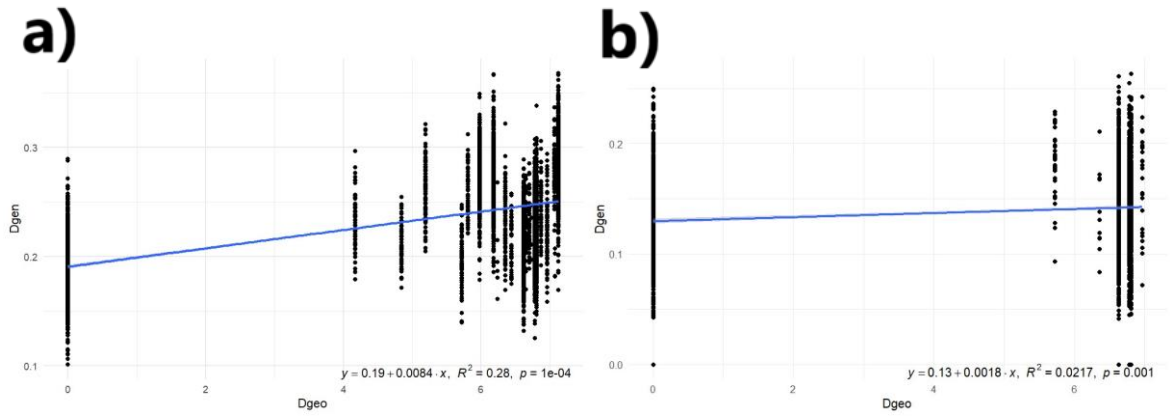

Figure 4.18. IBD of: a) L. digitata *aff. (N = 124) and; b)* L. hyperborea *aff. (N = 69) respectively. Genetic dist/1- Genetic dist against log(geographic distance) using minimum marine geographic distance as measured on Google Earth respectively. 10, 000 permutations. Equation of the line given at the bottom, along with R^2 (explanatory power) and p (significance).*

4.5 Discussion

4.5.1 Are Irish Laminaria species distinct?

The major divide seen in the species-naïve *Laminaria* phylogenetic tree in Figure 4.2 is evidence for two major groups existing, and corresponds precisely to the two groups identified by optimal k-means clustering. These however, do not correspond well to in-field identifications of *Laminaria hyperborea* and *Laminaria digitata* based on established morphological traits typically used for identification. While on a species level there appear to be broad trends, the individual effects (such as age and depth) appear to be more deterministic of many of these traits typically used for identification. This indicates poor species definition according to the morphological species concept, and highlights the need for a more comprehensive approach to macroalgal species definition (McCoy et al., 2020), but confirms the existence of two distinct *Laminaria* species in Ireland according to the phylogenetic species concept.

4.5.2 Are Laminaria species morphologically distinguishable?

No clear structure was observed when morphological traits were analysed via PCA and annotated by in-field identification using commonly used distinguishing traits (Figure 4.3). This provides additional support to the results seen in Figure 4.2 that *L. hyperborea* and *L. digitata* are not well segregated by morphological traits. Evidence that environmental effects are more important for morphology than genotype is seen when the PCA was annotated by site (Figure 4.4), in particular for site 3 (Galley cove) and 4 (Trá Dhireáin Huston).

Both OTUs display similar correlations between morphological traits which indicates that many allometric growth patterns and ecophysiological influences on morphology are shared

between these closely related species. The positive correlation between age and depth in both OTUs point towards a relationship between depth and longevity in Irish *Laminaria*, something previously identified in *L. hyperborea* in Scotland (Kain & Jones, 1977). These findings suggest that *Laminaria* that grow at lower depths live longer, have shorter lamina, rounder upper stipes, thicker meristems, more rigid stipes, and more stipe epiphyte growth; all of which are characteristics typically attributed to *L. hyperborea*. The misattribution of species can in part be attributed to stipe upper roundness (Appendix D) and stipe flexibility (Appendix E). In particular, site 4 appeared to have the most misattribution of morphological species, and this can be seen by the most typical discriminating traits being inverse of what might be anticipated. Here *L. digitata* showed many typical signs of *L. hyperborea* morphology, including being sampled at deeper depths (Appendix F), having rounder upper stipes (Appendix G), greater epiphyte growth on the stipe (Appendix H) and more rigid stipes (Appendix H). These likely help explain why Figure 4.4 shows a greater segregation of morphological variance by site for site 4, but fails to fully account for the segregation of site 3 samples, indicating there are more site-associated variables underlying these shifts in morphology.

The highly significant difference in depth at which the breeding groups were found (Figure 4.5d) suggest ecological niche differentiation with *L. digitata aff.* having a distinctly intertidal to shallow subtidal preference and being completely absent at depths below 2 metres at Lowest Astronomical Tide. Conversely, *L. hyperborea aff.* was found distributed across sampled depths, including in the intertidal. Due to the positive correlation of age with depth, and many key morphological traits seemingly being a function of both depth and age, many of the intertidal *L. hyperborea aff.* individuals may be mistaken for *L. digitata* when sampling due to younger age and ecophysiological constraints that promote a typical *L. digitata* morphology. Similarly, *L. digitata aff.* specimens growing at lower depths and which are older may be mistaken for *L. hyperborea aff.* individuals. Previous studies have suggested that *Laminaria* age is often a function of both depth and exposure (Kain, 1971; Klinger & DeWreede, 1988), but these studies lacked confirmation of genotype. It is therefore plausible that upper stipe roundness is also a function of depth and wave exposure and that stipe flexibility is a function of age, as annual growth rings are added. The increase of holdfast volume with age also makes biological sense, as growth tends to include the annual addition of haptera, and is included as a consideration in estimations of approximate age (Kain & Jones, 1963). Additionally, stipe flexibility may be ecologically more beneficial at shallower depths by allowing for greater movement in high-energy environments, while more rigid stipe would allow for greater access of the lamina to light at lower depths.

Interestingly for *L. hyperborea aff.* stipe length had a significant correlation with many of the traits related to age, but notably not depth (Figure 4.5 b). This is in contrast to *L. digitata aff.* which showed no significant correlation of stipe length with any measured variables (Figure 4.5. a). This discrepancy between species may be due to lower sampling number of *L. hyperborea aff.,* meaning that fewer sites were sampled, making correlations more sensitive to the site-specific effects. This however may also be a real underlying discriminating difference between species, and further research to examine in detail is needed. Another notable difference is the lack of significance in many of the same depth correlations for *L. hyperborea aff.*, excluding a positive upper stipe roundness and negative lamina length correlation. Similar age related correlations exist however, with holdfast volume, meristem thickness, and epiphyte cover all positively correlating with age, and stipe flexibility negatively correlating with age. While there appear to be correlations with depth, many of these are insignificant which may be a function of lower sample size in this OTU. This indicates a similar pattern as seen in *L. digitata aff.*, where older specimens have larger holdfasts, thicker meristems, more epiphytes and less flexible stipes, and deeper specimens have rounder upper stipes. This further provides evidence that the traditionally used distinguishing traits between *L. hyperborea* and *L. digitata* are a function both age and depth of the individual (in addition to any environmental variables) rather than being strictly genetically determined. Therefore the assumption that *Laminaria hyperborea* and *Laminaria digitata* can be reliably identified by traditionally used morphological traits can be refuted as trait variability is more determined by ecophysiological constraints than phylogenetic species identity. While species may show greater segregation of discriminating traits at lower depths, it is recommended that molecular tools be used to confirm identification where species-specific research is being performed.

This indicates that molecular tools are required for reliable species discrimination between *L. digitata* and *L. hyperborea,* especially when there is a lack of sampling depth or approximate age to constrain ecophysiological effects on the morphology of samples, and species-specific reporting is necessary. This furthers recent suggestions by Mauger et al. (2021) that there is poor species discrimination by morphology alone, and who developed molecular tools for rapid identification of cryptic species including *L. digitata* and *L. hyperborea*. This rapid identification however is based on the mitochondrial cytochrome c oxidase subunit I gene (COI) which is assumed to be strictly maternally inherited in kelp due to their oogamous mode of reproduction. Hybridisation and introgression between sister species may result in erroneous identification using these tools alone, which should be considered when using them. Cross-validation of patterns resolved from genomically distributed SNP markers, such

as in this study, with these mitochondrial barcoding markers would further bolster the reliability of these barcodes as well as potentially identify deviations due to false assumptions in reproductive inheritance or hybridisation.

4.5.3 Can evidence of hybridisation be identified in Laminaria?

Evidence for hybridisation can be found in the DAPC kernel density plot in Figure 4.8, where an individual of *L. digitata aff.* sampled from the north at Port na Happle had an approximately 20% attribution likelihood to *L. hyperborea aff.* This further complicates species definition as it has long been debated whether *L. digitata* and *L. hyperborea* were interfertile (Bartsch et al., 2008), but these contradicting reports may also be due to poor species identification, as suggested by the data presented here**.** Hybridisation does not appear to be common enough to homogenise genomic variation between OTUs but introgression is plausible and further investigation comparing both nuclear and plastid markers should identify the extent of hybridisation and whether there is directionality to it.

4.5.4 Population diversity

L. digitata aff.

L. digitata had the highest levels of Ar and uHe in Sandycove (east), as well as having the lowest Fis of all primary populations, and the highest Pa (166). Galley cove had the lowest uHe and Ar as well as a relatively high Fis, suggesting that the southern populations may be less diverse and show more signals of inbreeding than more northernly ones. Interestingly, both Port na Happle in the north and Trá Dhireáin Huston in the west showed similarly high diversity (uHe, Ho, He, Ar), despite a high number of Pa found in the west (128) when compared to the north (26). While the northern site had the same number of Pa (26) as the southern site, the increased diversity found in the north and low Pa compared to the west suggests that this may be a region of admixture. This may be due to current flow being primarily in a south to west to north to east direction and a lack of potential source populations existing off the south-west coast of Ireland in the open Atlantic. Minor population diversity statistics can be heavily influenced by chance due to low sampling, however the low Fis in Bagginbun specifically may warrant further investigation on the south-east coast. This may indicate that there is a poorly differentiated and widespread southern genotype cluster and that this is a common source of propagules for more northernly populations. The high levels of private alleles on both the west and east coast suggests that these may be glacial refugia, however the lower Fis and structure seen in higher k assignments (Figure 4.11) in Sandycove may indicate admixture with unsampled populations in the Irish Sea and along the British coast. A comprehensive study sampling the Irish and British coastlines in the Irish sea as well as populations on the islands

(e.g. Isle of Man) would help resolve whether these are truly refugial signals or whether they are a function of geneflow from divergent populations, and could help build on previous research in the region (King, McKeown, et al., 2020; Schoenrock et al., 2020).

L. hyperborea aff.

Trá Dhireáin Huston in the west showed the highest Ar, Ho, and uHe of all populations for *L. hyperborea aff.,* while Port na Happle in the north showed the highest He, however it is important to note that He and Ar can inflated by the higher sample size of Port na Happle (Eugenia Barrandeguy & Victoria García, 2021). While Port na Happle did contain a large number of Pa (50), this may also be influenced by uneven sampling, which was a function of more putative *L. hyperborea* being identified as *L. digitata* than vice versa. It is notable however that Trá Dhireáin Huston showed a slightly elevated Pa compared to similarly sampled sites at Galley cove and Sandycove. This, in conjunction with the high levels of other metrics of diversity, indicates that the west coast may harbour refugia for *L. hyperborea*. The west coast also appears to have the lowest Fis, and as a similar pattern of higher diversity and refugial signals correlating with lower Fis was seen or *L. digitata* for Sandycove, it may point towards outbreeding tendencies being more pronounced in more diverse refugial populations in *Laminaria*. In contrast to *L. digitata* however, *L. hyperborea* showed reduced diversity at Sandycove on the east coast, highlighting the need to accurately identify these species when conducting diversity research or enacting conservation measures in order to obtain reliable and meaningful results. This may be due to historical biogeographic differences between the two species and/or ecophysiological differences, such as presence of suitable habitat at different depths or seasonal differences in spore release and current flow. Galley cove however showed similarly lower levels of Fis between the species, indicating that the forces that may be causing Galley Cove populations to outbreed less is similar between species, and may be related to ocean currents.

4.5.5 Refugial signals

Areas of long-term population persistence have been previously modelled to exist for both study species in the south of Ireland and English Channel region (Assis, Araujo, et al., 2018). This aligns with the other diversity statistics, indicating that *L. digitata aff.* seems to have more of its diversity distributed on the east and west coast of Ireland, while *L. hyperborea aff.* seems to be relatively more diverse in the north and potentially the west. This may be a function of niche differentiation between the species, and further research into species specific habitat modelling may help to explain this. Trá Dhireáin Huston in particular shows a level of private

alleles, diversity, as well as divergence from other populations for both species, making this region a good potential candidate for a glacial refuge for *Laminaria*. Increased standing genetic diversity on the east coast for *L. digitata aff.* and potentially for *L. hyperborea aff.* in the north may point towards further refugia, however these may be influenced by influx of unsampled variation from other coastal populations in the Irish sea such as from Britain or the Isle of Man (Mooney et al., 2018). This indicates that these two breeding groups do not exhibit the same signs of refugial population persistence in regard to their genetic signals in Ireland that is suggested by the long-term persistence models by (Assis, Araujo, et al., 2018). This species specific disparity is crucial to the understanding of the diversity and evolution of north-east Atlantic *Laminaria* and would benefit from more in-depth study that included more sampling from coastlines across Ireland and the Irish sea.

Interestingly it was previously found that *L. digitata* is likely to have a European refuge in Ireland based on a north-west site in Donegal (Neiva et al., 2020). *L. hyperborea* was previously shown to have refugial signals in the south of Ireland (Lough Hyne) with diversity decreasing northwards (Schoenrock et al., 2020), which contrasts with the findings of this study which suggest higher diversity exists in the north and west. This indicates that better sampling of both species paired with molecular confirmation of species identification is needed in order to resolve this tension.

4.5.6 Are Laminaria populations distinct?

Overall, both species' primary populations in the north, south, east, and west were highly distinct. Minor populations in *L. digitata aff.* showed affinity with nearby primary populations, with the notable exception of Killary in the west, which had a southern affinity. Interestingly, *L. hyperborea aff.* showed south-west/north-east clustering, with some genotype affinity being identified between the north and east populations.

The southern populations of *L. digitata aff.* at Galley cove appear to be more distributed across the posterior membership compoplot (Figure 4.11), indicating higher rates of gene flow from these populations, which is consistent with a broadly south-west to north-east current flow**.** Previous research has found a broad latitudinal correlation with diversity in the north-east European range of *L. digitata* (Neiva et al., 2020), which appears to hold true for this study too. *L. hyperborea aff.* appears to be very diverse and outbreeding but with distinct populations. The optimal $k = 2$ clusters segregated the north-east and south-west into groups, and $k = 3$ indicated that there was some geneflow within the north-east cluster, while $k = 4$ indicated that southern and western populations were more distinct from one another.

Both species showed signs of east and west divergence, with southern and northern populations showing signals that suggest a general south to north geneflow to be likely. For *L. digitata*, long distance dispersal events between south and north may be facilitated by current dynamics that exclude the west coast site, and further sampling to increase the resolution of population structure along the west coast is needed to resolve this observation. This may point towards complex hydrodynamics that create a barrier to propagule dispersal within this region, isolating portions of the west coast from long distance dispersal, in particular in the Galway Bay region. This would suggest that northern sites might be regions of admixture as propagules progressively travel from more southernly populations northwards in line with prevailing oceanic currents.

4.5.7 Isolation by Distance

Both species displayed a significant ($p = 1e^{-4}$, $p = 0.001$) pattern of Isolation By Distance, with similar R^2 values for both *L. digitata aff.* (0.28) and *L. hyperborea aff.* (0.217). Notably, an IBD model using minimum marine distance (MMD) was assumed to be most explanatory of variation, but for *L. hyperborea aff.* a clockwise movement from the most southernly population (Galley Cove) had a higher R^2 value despite being equally significant (Appendix A). This suggests that *L. hyperborea aff.* has preferential geneflow from the south along the west coast instead of the east coast, as this is the primary difference between clockwise distance and MMD for *L. hyperborea aff.*

4.5.8 Mating system and niche segregation

One notable result is the highly negative Fis for both species in this study, but particularly for *L. hyperborea aff.*, further highlighting the need for accurate discrimination of these species when conduction species-specific research.

This is in contrast to the literature for these species which often report positive *Fis (Fouqueau et al., 2024; Schoenrock et al., 2020)*, typically assumed to indicate an inbreeding tendency. Recent GBS analysis on a related kelp (*Saccharina latissima*) in the north Atlantic however has seen similar negative Fis (Bråtelund et al., 2024), potentially indicating an underlying biological signal of outbreeding. This signal may also be explained through stochastic hybridisation events, and in particular the introgression of these hybrids into *L. hyperborea aff.*, and further research comparing ecological niche, morphology, plastid genomes and nuclear genomes is needed to test this hypothesis. Another plausible explanation for the Fis disparity may be the tendency for longer lived species to have greater selection against inbred individuals (Duminil

et al., 2009), which may be a selection pressure at both generations. It has been shown that kelp that are inbred show lower sporophyte developmental success, but it is likely that undescribed pheromones or gamete kinship recognition mechanisms also play a role, which may promote outbreeding (Camus et al., 2021).

The complete absence of *L. digitata aff.* below 2m LAT at these sites also suggests that *L. digitata* recruitment may be limited by environmental factors such as light availability/quality. These results suggest that *L. digitata* tends be more competitive in highly disturbed intertidal environments with high light exposure, while *L. hyperborea* is a generalist that may be more competitive at more stable lower depths where *L. digitata* is at its niche edge, possibly due to limitations in photosynthetic potential and PAR (Photosynthetically Active Radiation) variation with depth (Kain & Jones, 1964, 1965, 1969). This realised niche difference has previously been reported in removal experiments (Kain & Jones, 1975), indicating inter-specific competition for light in the subtidal, and disturbance near the intertidal as a driving forces behind this segregation of niches by depth. Any observed ecological differentiation in sporophyte presence is necessarily a function of gametophyte habitat suitability for sporophyte production as well as the sporophyte ability to persist in those environments. Further research into the ecological differences between gametophytes of *L. digitata* and *L. hyperborea* may provide clarity on the importance of interspecific competition versus niche differentiation, as sporophyte presence is a direct result of both gametophyte persistence and syngamy success. Gametophyte niche differentiation may be a key driver of sympatric speciation in systems like this with significant overlap in sporophyte presence. Where *L. digitata* gametophyte presence is limited by depth, this would functionally act as a pre-zygotic barrier to gene flow, while failure of successful syngamy and/or sporophyte production would indicate a post-zygotic barrier.

4.5.9 Future research and knowledge gaps

Further research into the physiological tolerance ranges for gametophytes of *Laminaria* may uncover key differences between species that correlate to depth related constraints, such as behaviour in red and blue light, UV exposure, thermal stability, or desiccation tolerance (Kain & Jones, 1964, 1965, 1969, 1977). This would give a “theoretical niche” for the gametophyte that would necessarily include but not be limited to the theoretical niche of the sporophyte, and may help explain the depth-related niche segregation seen in the sporophytes of each species in this study.

The historical conflation of morphotype with phylogenetic species has led both to the overclassification of *Laminaria* species as well as poor resolution of species identity when molecular testing was conducted alongside morphometric assessment (Henry, 2018; Lund, 2014). This raises serious concerns about the existing literature on *L. digitata* and *L. hyperborea* that assumes species are reliably identifiable by morphology alone, and in the absence of such confirmation, strongly divergent outliers in terms of diversity may be due to inclusion of misidentified individuals. This is of particular concern when sampling in shallow waters where the niche of both species overlaps. For example, previous research that identified extremely high diversity and divergence in *L. hyperborea* in Irelands Lough Hyne also noted that this was the only population that wasn't sampled below 2 m depth using SCUBA (Schoenrock et al., 2020), suggesting the findings of this paper may be partially confounded at this site.

Research into differences in genetic diversity between gametophyte populations and sporophyte populations at specific locations would also help identify the extent to which gametophyte banking influences the observed population-level diversity of kelp. While methodologically difficult, such patterns of inheritance may be inferred via use of plastids that are assumed to be maternally inherited, and contrasting these to nuclear signals and through use of linkage maps. Genomic nuclear linkage to haplo-types (as defined through use of plastid markers) at a higher rate than would be expected via random assortative mating may indicate differences in vegetative propagation of gametophytes sexes, with female vegetative reproduction resulting in a higher linkage. Conversely, if linkage is identified but this is not correlated to plastid haplotypes then this would indicate that male gametophyte vegetative propagation is dominant.

Future diversity studies on these species should record normalised depth of sampling as well as use molecular tools, such as those by Mauger et al. (2021). Sampling for specific species should aim to select specimens from the intertidal for *L. digitata*, and <2 m LAT for *L. hyperborea*, paired with examination for rounder upper stipes, more rigid stipes, and higher epiphyte coverage. Where species identity is vital for the study however, molecular confirmation is essential in order to obtain good results. This would result in more reliable data as well as help uncover whether depth has any effect on intra-specific diversity, such as through the hypothesised MultiAnnual Delayed (MAD) gametophyte dynamics, and through linkage of nuclear and plastid markers. It may be prudent to reconceptualise kelp sporophytes as producers of recombinant gametophytes, rather than merely as the primary life stage and

source of spores, in order for us to better understand kelp genetic structure and niche and thus better conserve kelp forests.

Chapter 5 Discussion

Kelp are important habitat-forming species that provide the basis of structurally complex and phylogenetically diverse ecosystems (Steneck et al., 2003). Increased pressures on kelp, such as climate change, harvesting, and other human activity, pose direct threats to the survival of many kelp and the habitats they form (Krumhansl et al., 2016; Smale, 2020). With increasing economic demand for kelp and kelp-derived products the necessity for better baseline data on kelp populations and their diversity is a requirement for their successful conservation and sustainable exploitation (Hofmann et al., 2025).

In this thesis, the diversity, connectivity, and presence of refugia of the three most common Irish kelp; *Saccharina latissima*, *Laminaria digitata*, and *L. hyperborea* are examined in an effort to fill in a geographical knowledge gap in a kelp diversity hotspot of the north-east Atlantic. Additionally, this thesis is the first instance of extensive morphological and molecular comparisons of populations of north-east *Laminaria*, providing a holistic analysis of phenotypic plasticity and how this is related to phylogenetic species interpretation. These findings are placed in a broader context via the first systematic review of kelp population genetics for the north-east Atlantic, providing a framework for future research to more effectively target knowledge gaps and synthesise findings across taxa. Cumulatively, these findings highlight the importance of studying kelp with consideration for their biogeographical history as well as their unique phylogenetic position in order to accurately assess and conserve their diversity and inform their utilisation.

5.1 Integrated approach: How molecular tools can help form new natural history hypotheses

The combination of molecular tools and morphological data in Chapter 4 of this thesis recontextualises a number of previous findings regarding both *L. digitata* as well as *L. hyperborea*. Accurate identification of species when examining diversity is important, and the substantial overlap in phenotypic traits typically used to discriminate between species raises concerns over the accuracy of previous studies, particularly when some populations appear to have significant outliers (Schoenrock et al., 2020; Schübert, 2022). The segregation of sites by PCA in Figure 4.4 despite the poor resolution of species may result in some sites having species with more misleading morphologies, potentially leading to statistical artefacts, such as inflated allelic richness, private alleles, Fis, or Fst when comparing sites. This indicates that any future studies that aim to undertake species specific diversity or ecological testing of *Laminaria* in the north-east Atlantic should genetically confirm their sample identities in order

to produce reliable and meaningful results (Mauger et al., 2021). Similarly, the identification of potential hybrid specimens in this thesis suggest that organelle based markers (such as mitochondrial COI) for rapid identification may not be entirely reliable owing to uniparental inheritance, and that these should be paired with nuclear barcode markers where ambiguity arises about the genetic identity of a sample using these markers (Schübert, 2022).

Furthermore, the observed niche segregation of genetically confirmed *L. digitata* to depths above 2m LAT is the precise depth previously reported to be the limit for presence of perennial *L. digitata* (Lüning, 1979). This limit was previously described as being due to a persistent growth rate into the darker months of the year, possibly an adaptation to the sublittoral fringe, which precludes it from persisting long-term at greater depths with less light. The significant ($p < 0.01$) correlation of specimen age with depth however suggests that *L. digitata* persist longer at lower depths despite the apparent mismatch of growth and photosynthetic capacity. The similarly positive but non-significant correlation seen between depth and age in *L. hyperborea* despite the persistence of this species at lower depths suggest that there may be influences at shallower depths that prevent specimens of both species from reaching older ages. This points to the potential of balancing selection between strategies that seek to maximise resources for growth in the intertidal (e.g. light) with the increased pressures that seem to correlate with earlier death. This suggests contrasting evolutionary trajectories for these two species that can broadly be placed on a fast versus slow life history strategy continuum (Stott et al., 2024). Additionally, *L. digitata* specimens morphologically identified as *L. hyperborea* were typically sampled between 0-2m depth LAT, while *L. hyperborea* identified morphologically as *L. digitata* were typically above the LAT (Appendix C). This suggests a shared morphological plasticity and, despite apparent emerging niche segregation, that the morphotypes typically associated with either species may better be understood as a shared strategy that is dependent on the depth of the individual.

These findings have profound implications on the utilisation and conservation of both species. The greater misidentification of deeper *L. digitata* specimens as *L. hyperborea* in this study might suggest a systemic bias in surveying efforts that overestimate *L. hyperborea* presence. When combined with indications that *L. hyperborea* tends towards a slower life history strategy, with older individuals that apportion more diversity within individuals than among populations, this could provide a false confidence of population health and therefore lead to unidentified loss of important genetic diversity. Conversely, *L. digitata* may be more heavily impacted by rising sea-level changes which are estimated to have a global mean increase of ~20 cm by 2050 (Hamlington et al., 2024), as well as by an increase in sea surface temperatures (Garcia-Soto et al., 2021) due to its narrower range of suitable depth. *L. digitata*

has previously been shown to display inhibited sporogenesis due to increased temperature (Martins et al., 2019), which may cause selective pressures against more intertidally adapted genotypes.

5.2 The big picture: trends in kelp biogeography

The findings in this thesis suggest that the hypothesised refugia for *S. latissima*, *L. digitata*, and *L. hyperborea* have species-specific structure across the Irish coasts not present in previous models (Assis, Araujo, et al., 2018). Indeed, the results of all three species suggest an east/west component to connectivity, refugial signals, and overall diversity in addition to a previously identified north/south distinction. This north/south structure was identified on a European scale in the Chapter 2 Review for *L. digitata* and *S. latissima*, and was typically attributed to glaciation, but this east/west structure may better be explained by the opening of the Irish Sea and rapid shifting of suitable habitat (Becker et al., 2015; Scourse et al., 2018). This pattern may also be a function of asymmetric connectivity, as suggested by *L. digitata* in this study, which showed signs of northernly genotypes having similarity to southern populations but not the other way around. This broadly aligns with the prevailing direction of the North Atlantic current (Krauss, 2012), and correlates well to previous suggestions that Ireland may have been a source for recolonisation of north-eastern European coastlines after the LGM (Neiva et al., 2020). The relative isolation seen on the east coast populations by all three species however also points towards the existence of smaller scale structures such as the Irish Sea front potentially providing a barrier to geneflow and effectively isolating refugial populations. Considering these results, the minimal sampling identified in Scotland, the Faroe Islands, and Iceland in the review in Chapter 2 might suggest that there is additional refugial diversity to be found in these coastal European Atlantic fringes. These trends identified in all study species for which new data was collected suggest that a greater effort should be placed into sampling and understanding Irish kelp populations, and perhaps also other shallow sessile marine life, as these refugia are unlikely to have only sheltered kelp. In fact, the very nature of kelp as foundation species suggests that Ireland may have provided important coastal habitat for many species in Europe during the Last Glacial Maximum.

5.3 The small picture: are we neglecting gametophytes?

Assumptions made when analysing genetic signals of diversity may impact data from organisms with different mating systems differently. Kelp notably has an heteromorphic

haplo-diplontic lifestyle, with large diploid sporophytes that produce spores that develop into microscopic haploid gametophytes, which in turn undergo syngamy to produce the sporophyte.

The focus on the visibly obvious sporophyte and the assumptions carried over from the expertise honed in many animal and plant studies run the risk of overlooking the importance of the free-living gametophyte stage in population dynamics. While there has been some recognition of gametophyte importance in these dynamics, it has been traditionally difficult to study, and is only recently being revisited in light of greater access to suitable molecular tools (Schoenrock, McHugh, et al., 2021). Nonetheless, the ecological role of this "seed bank of microscopic forms" in population persistence and the underlying genetic patterns we observe has been largely been left undiscussed, and the evidence presented in Chapter 3 and 4 suggest that the gametophyte plays an under-recognised role in the life cycle of wild kelp.

Gametophytes in laboratory culture have shown long term survival, continuous growth and fragmentation, as well as sporophyte production induced by both specific light intensities and qualities that can vary with species (Ebbing et al., 2025; Edwards et al., 2016; Ratcliff et al., 2017). This light induced sporophyte production is of particular interest because sporophyte presence is the typical metric for kelp presence and thus sampling, and it suggests that there may be gametophyte persistence in regions unsuitable for sporophyte production. Ebbing et al. (2025) summarises studies using light to induce both vegetative and sexual reproduction in gametophytes, and notes that vegetative growth is often induced by red light, and sexual reproduction is often induced by blue light. This suggests that perhaps gametophytic vegetative reproduction occurs primarily in shallower water, as red light is filtered out, and that deeper gametophyte may be biased towards sexual reproduction, although the inter-study variability is high and there appear to be more complex dynamics impacting these processes that are not well understood yet (Ebbing et al., 2025). This as well as DNA barcoding approaches discussed by Schoenrock *et al*. (2021) suggest that there may be a significant unseen bank of gametophytes persisting in the environment, vegetatively reproducing, and only producing sporophytes when environmental conditions are suitable (Kain & Jones, 1977) and a complimentary gamete is present. Should this be the case, it would indicate that half of the independently living life-cycle components of the kelp are being overlooked in many genetic studies, and that this haploid generation is constrained by its own ecological niche. This is a crucial consideration for biogeographic processes and conservation genetics, as it highlights the existence of unsampled standing variation in the gametophyte generation that must be inferred through the proxy of existing sporophytes. This life cycle phenomenon may provide greater genetic resilience for populations in stable populations (as per Assis, Serrão,

et al. (2018)) due to the hedging of genetic diversity between generations as well as between vegetatively and sexually reproducing strategies. This lack of consideration for non-sporophyte life stages may also impact understanding of dispersal events, as gametophytes may become locally persistent via vegetative fragmentation. Differences between male and female gametophytes may also uncover the relative role of each sex in this dynamic, and whether vegetative propagation and/or contribution towards syngamy is more common in either sex.

Gametophyte niche segregation and persistence in the environment as well as vegetative reproduction may help explain some of the complex genetic patterns observed in kelp. The observed pattern of negative Fis across all species studied in this thesis could arise from individual heterozygous genotype combinations being differently represented in male and female gametophytes by chance, which upon continuous vegetative reproduction, could give rise to multiple sexual recombination events from the same genotype and thus give an apparent increased signal of outbreeding. This hypothesis is further bolstered by male gametophyte fragmentation possibly being part of kelp reproductive strategy (Destombe & Oppliger, 2011), and future studies should address this potential disparity in their molecular diversity research, potentially through contrasting maternally inherited plastid and bi-parental nuclear markers. The comparatively negative Fis values observed in *L. hyperborea* may be partly due to gametophytic persistence in this species being greater than in its sister species, possibly due to a more stable sub-tidal distribution. A more stable subtidal niche may also promote more intergenerational geneflow with gametophyte persistence giving rise to comparatively lower Fis values in *L. hyperborea* than *L. digitata*. It has recently been hypothesised that MultiAnnual Delayed (MAD) gametophytes may represent a distinct life stage for kelp, and that MAD gametophytes may have a narrower environmental range at which sporophyte production is successful, potentially leading to the emergence of fast and slow life-history strategies at the haploid stage Ebbing et al. (2025). This could plausibly explain the observed Fis disparity and apparent depth-related niche segregation observed in this study, and the comparison of MAD gametophyte behaviour between *Laminaria digitata* and *Laminaria hyperborea* could provide key evidence to explaining the apparent sympatric divergence of these sister species. There is immense difficulty in studying these systems in the wild, but a recent simulation study modelling the behaviour of partial clonality and differential population sizes between the diploid and haploid phases of haplodiplontic organisms has found that an increased population size in haploid life stages is associated with more negative FIS values, likely as a result of drift being twice as impactful in haploids when compared to diploids (Stoeckel et al., 2021). Interestingly, it also found negative effects on FIS at extremely

high rates of clonality, likely due to the divergence of haploid and diploid generations into their own species as seen neotenic ferns such as *Lomariopsis lineata* or *Vittaria appalachiana* (Pelosi et al., 2025). This however is unlikely to be a major factor in this context, as no sporophyte clones were identified in the dataset, and these members of *Laminaria* are largely deemed to be sexually reproducing. This suggests that the hypothesis of large populations of partially clonal gametophytes, particularly in regions of long-term persistence, may explain the high levels of outbreeding tendencies identified in regions identified as glacial refugia.

5.4 Shortcomings and future research

While this work has demonstrated both the importance of Irish kelp populations and identified substantial inconsistencies with *Laminaria* species identification, more sampling needs to be done in future studies. The limitations of GBS as a SNP based genotyping method in kelp include extraction of high quality and quantity of DNA, which is exceptionally difficult for kelp due to their abundance of polysaccharides and polyphenols (Bråtelund et al., 2024; Pearman et al., 2024; Ramakrishnan et al., 2017; Snirc et al., 2010; Wilson et al., 2016). These typically involve an intense grinding step to powder the sample, as well as a step to remove single-stranded nucleic acids, steps to chelate polyphenolics (typically using a type of PVP), as well as an ethanol washing step to reduce polysaccharide content. The precise methodologies however vary widely between studies, and often depend on resources such as time, cost, and labour availability. The cost of GBS also limits the sampling number, and a reasonable balance between site number and samples per site needs to be struck. Additionally, the identification of potential hybrids of *Laminaria* and possible directional introgression needs further confirmation, possibly through haplotype analysis of plastid markers in these samples and comparison to the SNP data in this study. Future research should utilise the findings in this thesis to design sampling regimes that compare these well characterised populations to other locations, for which cheaper genotyping options such as SSRs would be appropriate. This may even include samples from this work that act as typical representatives of their populations, for which dried tissue is archived and still available, for direct future comparison. It is with this in mind that the individual GBS data will be made available in NCBI SRA as part of a BioProject to allow other researchers to access sequences from either confirmed phylogenetic species for comparison.

Additional sampling of more sites would also help to disentangle the observed effect that site seemed to have on the morphology of *Laminaria*. Future morphological studies that control for depth according to LAT using transects parallel to the shoreline could help identify genotypic

differences in morphology within sites, and compare these among sites to establish the role of abiotic factors such as wave exposure. This study design could also identify species specific morphology changes with age, and determine whether there are interactive developmental effects with depth and wave exposure. While existing papers have studied how exposure and current flow impact relative growth rate (Kregting et al., 2013; Kregting et al., 2016; Millar et al., 2020), more general comparisons that control for depth (and the related eco-physiological gradients associated with it) are scarce (Bartsch et al., 2008). Fundamental to these future endeavours, however, is the avoidance of conflation of *Laminaria* species by morphology alone as identified in this thesis.

Underlying these distributional patterns of sporophytes are the distributional patterns of the gametophytes, but gametophytes are more difficult to study *in-situ* than sporophytes. While some research has been done on defining the ecological niche of some kelp gametophytes (Mohring et al., 2013), more often the focus has been on latitude as an explanatory factor for niche differentiation (Oppliger et al., 2012; Van Den Hoek et al., 1995). Recent attention to the role of the gametophyte stage in the biogeography of kelp (Edwards, 2022) is supported by the findings here, suggesting further research into the differentiation of the gametophytic niches of species to be crucial. Specifically, the differences between genetically confirmed *L. hyperborea* and *L. digitata* could produce important results that help explain the differential distribution of these species not along latitudinal gradients, but also along depth gradients. Future research that surveys presence of these species along depth gradients at different latitudes may provide clues to the underlying adaptation of *Laminaria* to differing environmental conditions, which may be of vital importance to conserving these species and the habitat they produce in a changing climate.

The use of population genetics for analysing kelp is well established, with early studies using allozyme data (Valero et al., 2001), it was recognised early that treating them similarly to seed plants in data analysis risks making assumptions that may distort interpretation (Schiel & Foster, 2006). The results of this thesis suggest high levels of outbreeding in Irish kelp, with species specific differences. While *S. latissima* had high levels of outbreeding, they were similar to recent findings that used a similar methodological approach (Bråtelund et al., 2026). More interestingly, the comparison of the *Laminaria* sister species revealed that *L. hyperborea* was substantially more outbreeding than *L. digitata*. This tentatively points towards support for the recently proposed MAD gametophyte hypothesis (Ebbing et al., 2025), in which gametophytes that find themselves in conditions unsuitable for sporophyte development seemingly delay sporogenesis and vegetatively reproduce via fragmentation indefinitely. The proposers of this hypothesis suggest a hedging of strategies between fast (k-selection) and

slow (r-selection) life history strategies of gametophytes. To test whether the difference in sporophyte depth seen between *L. digitata* and *L. hyperborea* is, in part, due to differential use of a MAD gametophyte life cycle a maternally inherited plastid DNA study across depths would be necessary. Additionally, comparison of physiological limitations of gametophytes from both species may further disentangle the role of gametophyte 'seedbanks' in the observed differences in outbreeding and niche depth for these species.

While directionality of geneflow was inferred via posterior-membership assignment in this study (Chapter 4), it was not directly modelled or measured. Future work using hydrodynamic modelling could implement this for comparison to genetic signals (Fouqueau et al., 2024). This may also be inferred in future studies at larger spatial and time scales by using haplo-type mapping, and may provide data regarding which refugia acted as source populations in post-glacial recolonisation.

Future research, as identified in Chapter 2, should prioritise greater collaboration between researchers in an effort to document and compare diversity of all kelp across the north-east Atlantic. The recent call for the establishment of the a European Board of Macroalgal Genetic Resources provides an ideal platform for collaboration and knowledge sharing, as well as more efficient use of research funding (Hofmann et al., 2025). Future sampling that includes specimens from all regions where structural clusters were identified in Chapter 2 could provide better data for broad biogeographic questions such as population dynamics during glacial cycles as well as providing a foundation for more effective conservation. This has been somewhat demonstrated by a recent large scale study of *S. latissima* (Jaugeon et al., 2025), to which this thesis adds important resolution in the most diverse region identified.

5.5 Concluding remarks

Overall, this thesis fills important genetic diversity knowledge gaps for some of Europe's most prominent kelps in what appears to be one of its most diverse regions. The findings of all population genetic studies of kelp in the north-east Atlantic are synthesised and provide a base for future research. Chapter 2 additionally highlights knowledge gaps in the existing research, and allows research to concretely and systematically address these gaps in the future. As such, the aims of chapter 2 can be considered fulfilled, and come at a point in time where European kelp researchers have previously highlighted the need for such work (Hofmann et al., 2025).

The results from Chapter 3 and 4 confirm the hypothesis that Irelands kelp have significant population genetic diversity and that this is likely, in part, a result of glacial refugia. Additionally, the findings suggest that different kelp species show differing patterns of refugial diversity, and

that the east and north-east coasts of Ireland may unexpectedly be a region of high diversity for some kelp such as *L. digitata*. The possible identification of a hybrid *Laminaria* and even directional introgression provides a fertile ground for future studies and raises further questions about the evolutionary history of this genus in the north-east Atlantic where it seemingly evolved, and partially addresses one of the core aims of chapter 4. Future efforts to sample more broadly, or re-analysing the putative hybrid using alternative methods would provide more evidence for potential hybridisation, however it can be stated that hybridisation appears not to be a widespread phenomenon in Ireland. Additionally, the findings in this thesis strengthen previous doubts about the use of morphology alone for identification of north-east Atlantic *Laminaria* species, and identify that the highly plastic phenotypes are more likely to be a function of abiotic factors than due to genotypic ones. This addresses one of the key aims of this thesis, and helps to close the disparity between species concepts that has been left unaddressed in the literature as research moved from primarily morphological to molecular methods. Future population genetic research must also necessarily include greater consideration for the gametophyte life stage and attempt to challenge the assumptions of genetic theories and ideological frameworks that have been developed for use in organisms that neither share a similar mating system nor recent evolutionary history.

It is hoped that the results from this thesis are utilised by the burgeoning aquaculture industry in Ireland such that a robust industry can grow to meet market demands for kelp, with minimal negative genetic impact on the native wild populations that form the foundation for Irelands kelp forest ecosystems. This includes greater collaboration and addressal of knowledge gaps (Chapter 2), use of local kelp genetic material and mitigation of gene pool pollution and genetic erosion (Chapter 3 & 4), and the greater use of molecular tools when collecting *Laminaria* sporophytes. This should be done in tandem with a revision of the practices of wild-harvesting as they pertain to *Laminaria*, as current practices neglect to consider poor species resolution, indicating that the current understanding of *Laminaria* ecology and population dynamics may be unsuitable for the sustainable exploitation of these natural populations (Chapter 4.)

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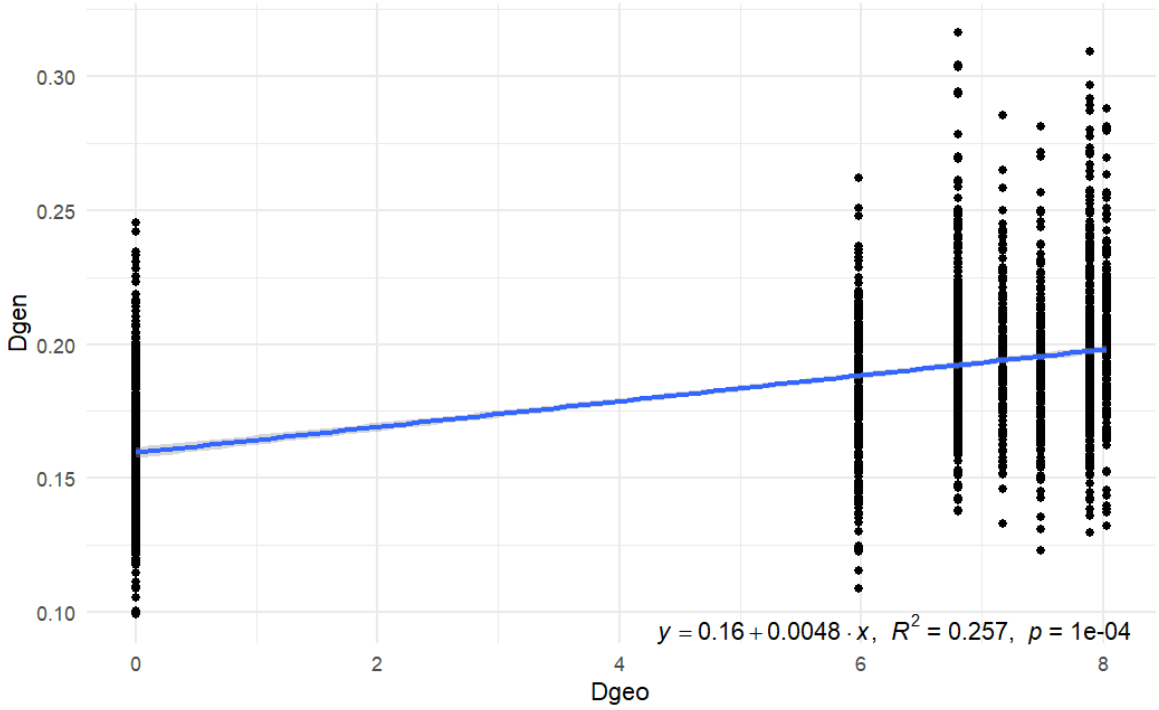
Appendix A



Appendix A.1. IBD of L. hyperborea *aff. (N = 69). Genetic dist/1- Genetic dist against log(geographic distance) using clockwise distance from the most southernly population (Galley cove) as measured on Google Earth respectively. 10, 000 permutations.*

Appendix B

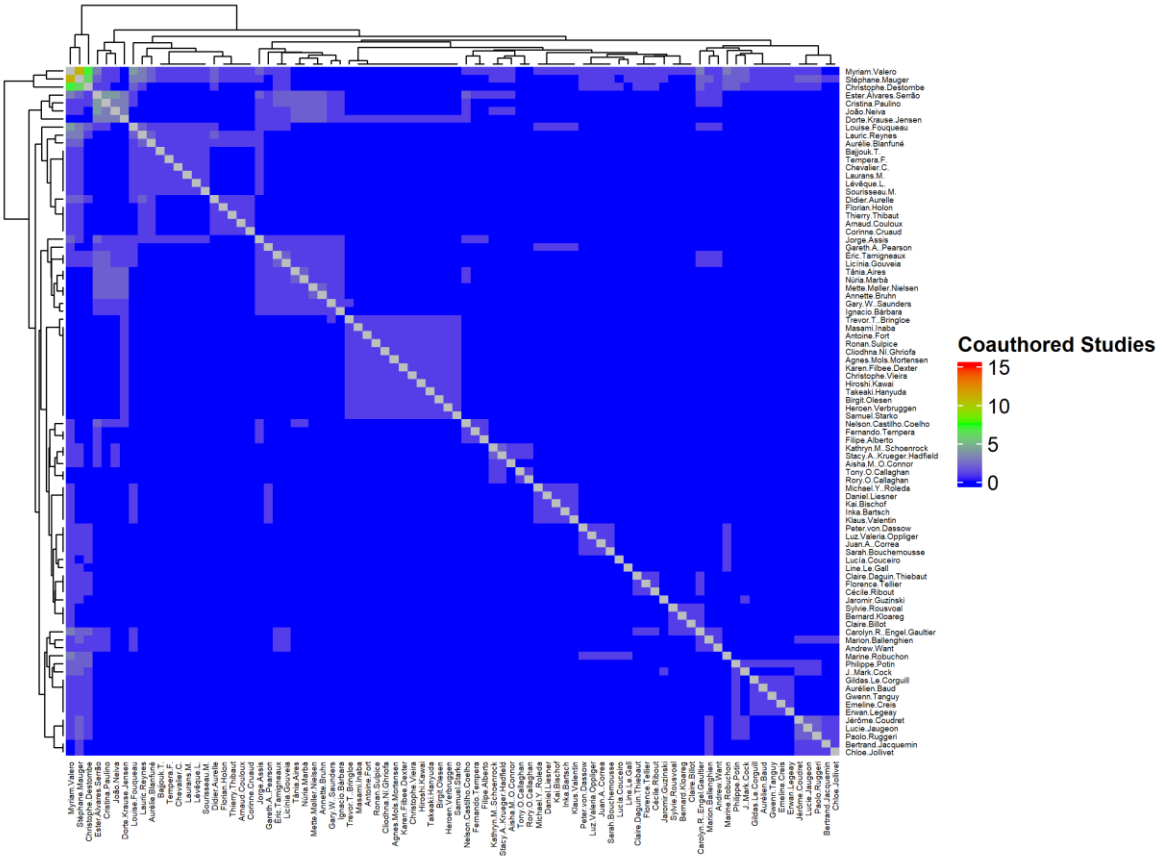


Figure B.1. Heatmap of co-authorship of the primary network identified in network analysis.

Appendix C

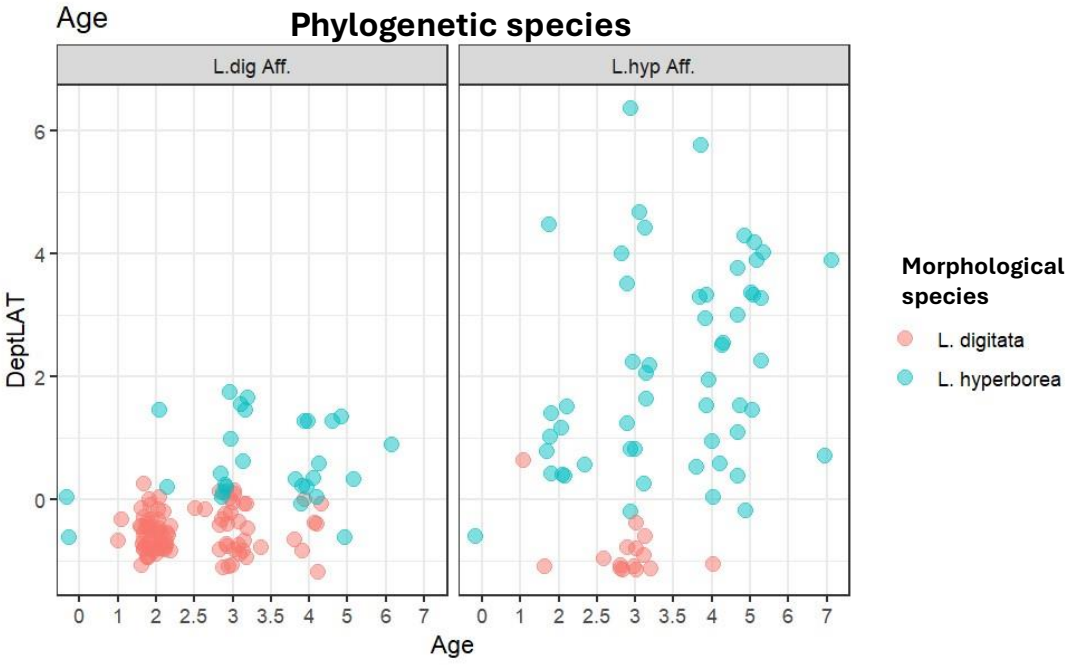


Figure C.1. Depth and age of samples according to morpho- and phylospecies

Appendix D

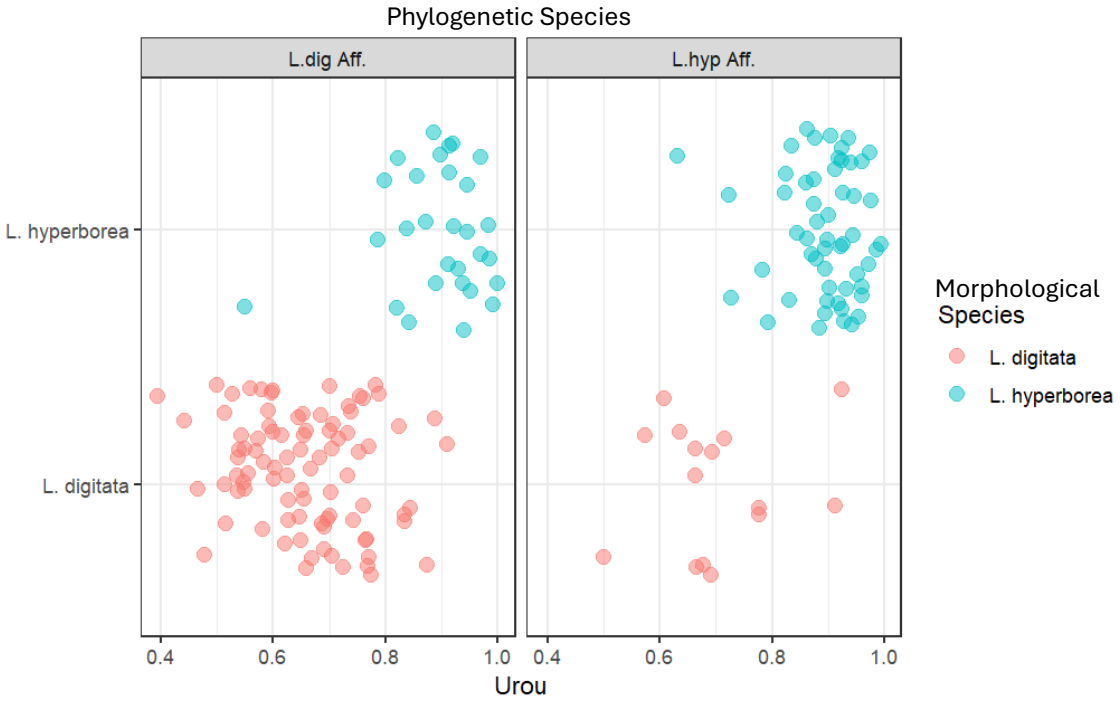


Figure D.1. Upper stipe roundness of samples according to morphospecies (colour) and phylospecies (box), 1 = perfectly round, while 0 = perfectly flat.

Appendix E

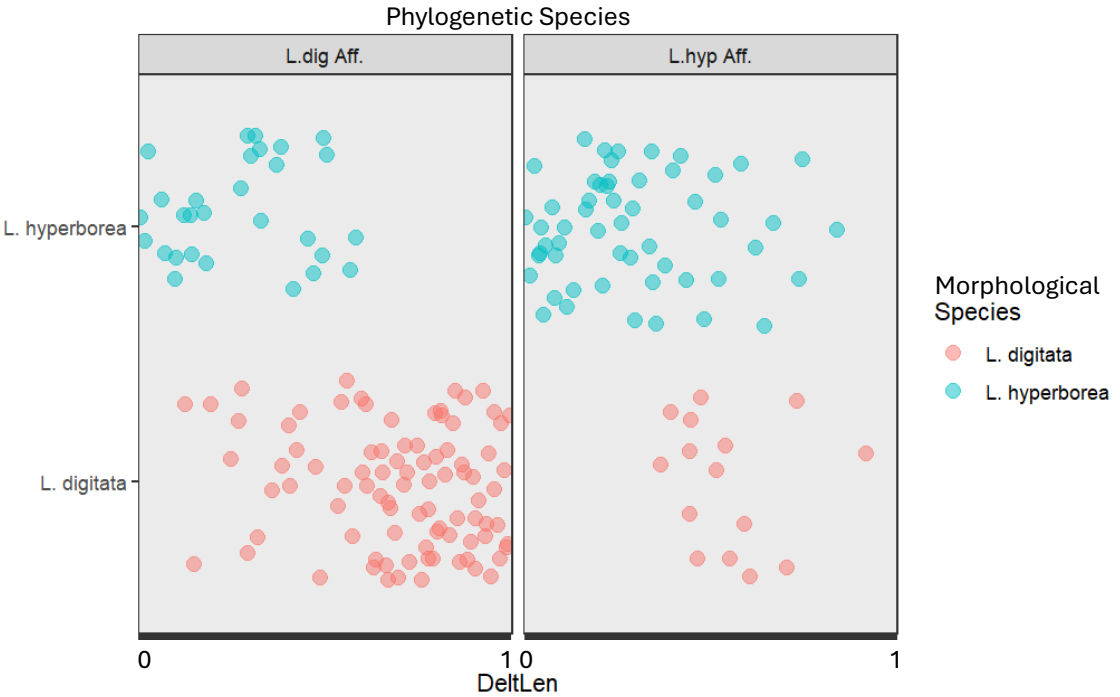


Figure D.1. Stipe flexibility of samples according to morphospecies (colour) and phylospecies (box), 1 = bends at 90° under standard weight, while 0 = doesn't bend at all under standard weight.

Appendix F

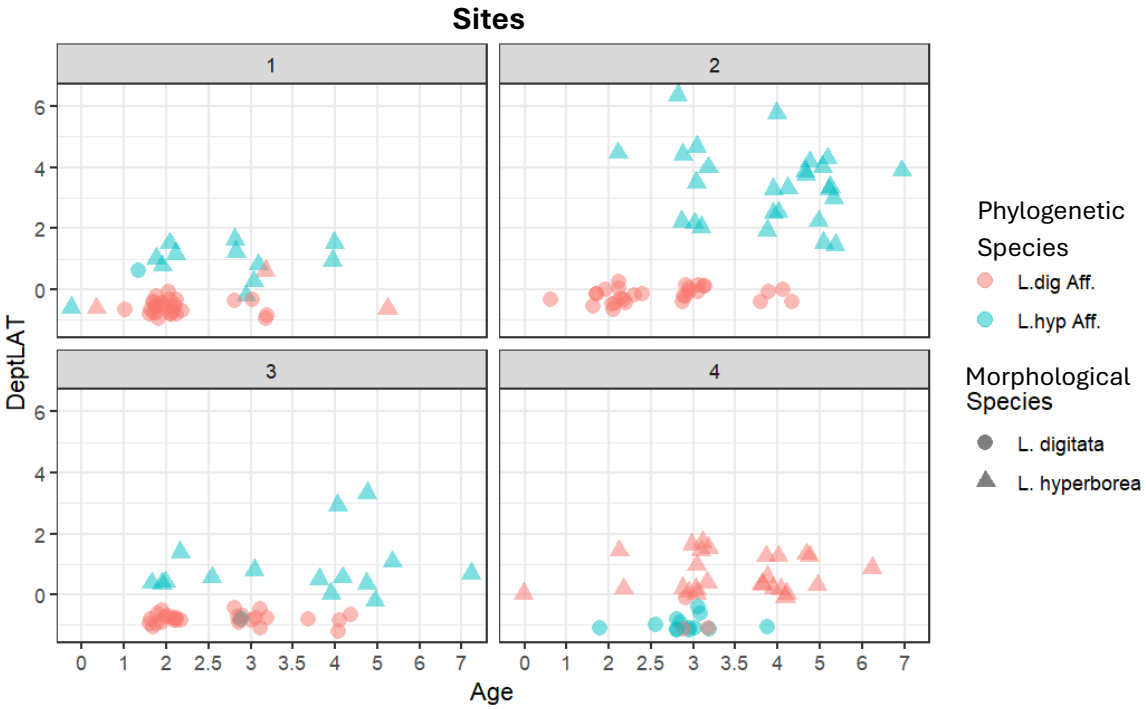


Figure F.1. Depth and age of samples according to morpho- and phylospecies for each site. Note the high rates of species misattribution at site 4 in particular, highlighting the need for molecular identification to account for such inter-site variance.

Appendix G

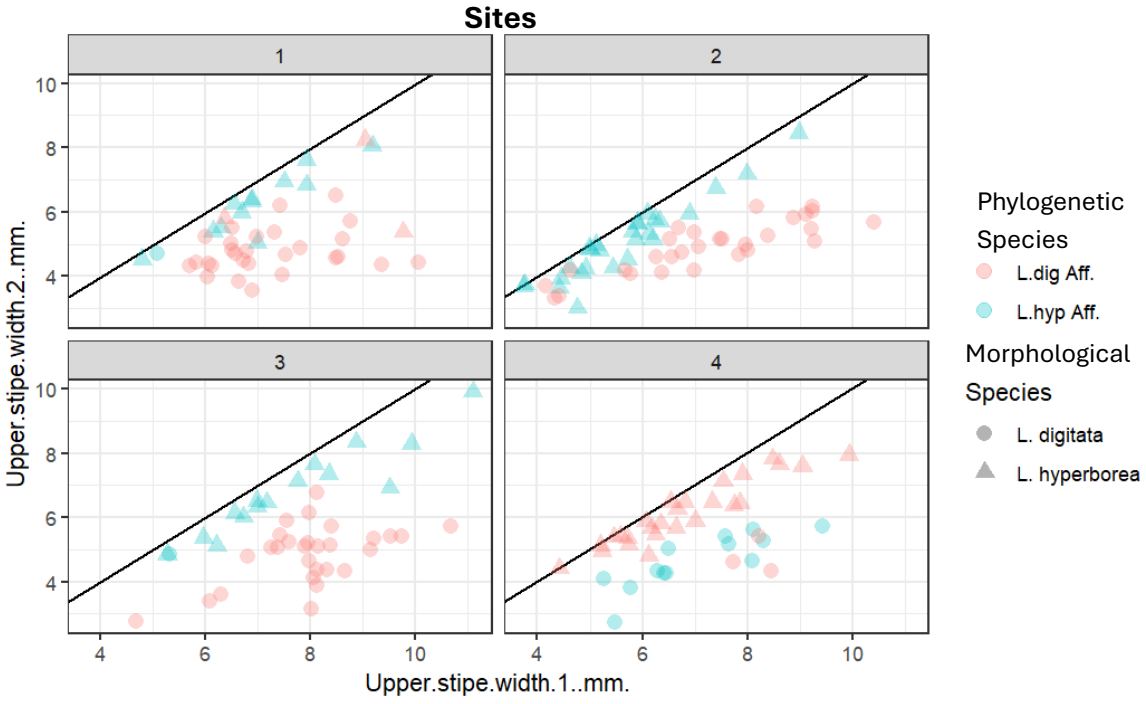


Figure G.1. Cross-sectional width of samples at the upper stipe according to morpho- and phylospecies for each site. Black line indicates perfect roundness. Note that both species have similar absolute sizes, but that L. dig Aff. *shows consistently flatter stipes at all sites except for site 4, which shows the inverse, and is associated with species misidentification.*

Appendix H

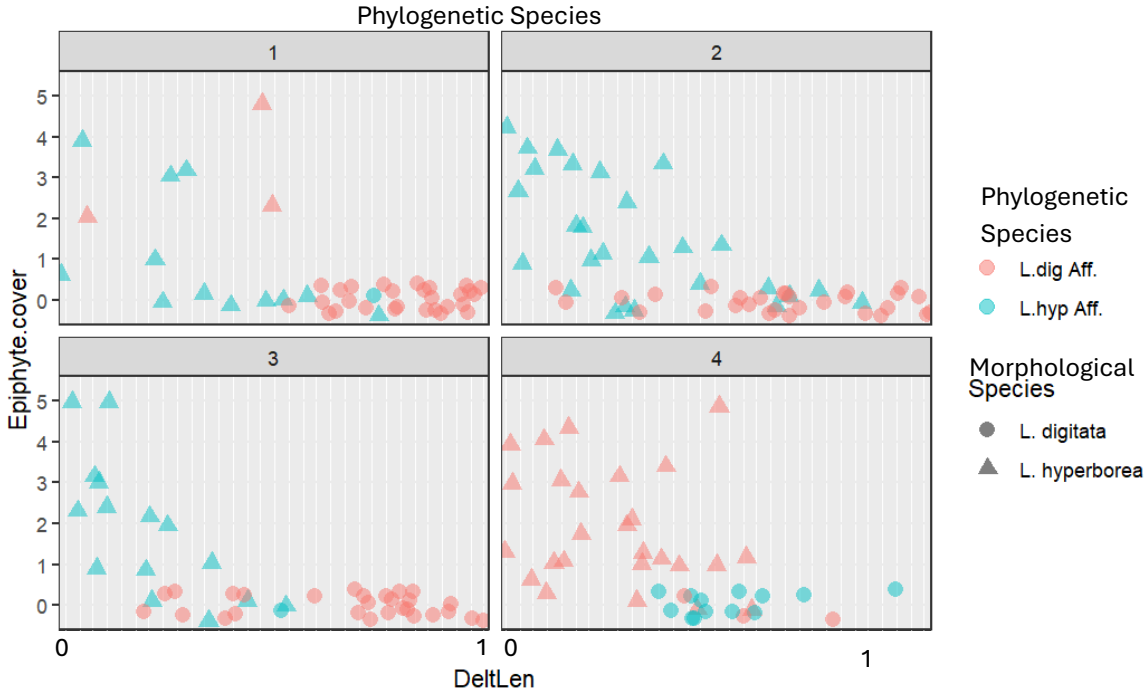


Figure H.1. Epiphyte coverage of stipe according to Braun-Blanquet scale and stipe flexibility (DeltLen) according to morpho- and phylospecies for each site. Note that site 4 suggests that genetic L. digitata *here have epiphyte coverage and stipe rigidity more similar to* L. hyperborea *at the other sites, and vice versa, leading to the most frequent misattribution at this site.*