A diploid in the Arctic – genetic and morphological variation of *Cochlearia groenlandica* L.

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MSc Thesis

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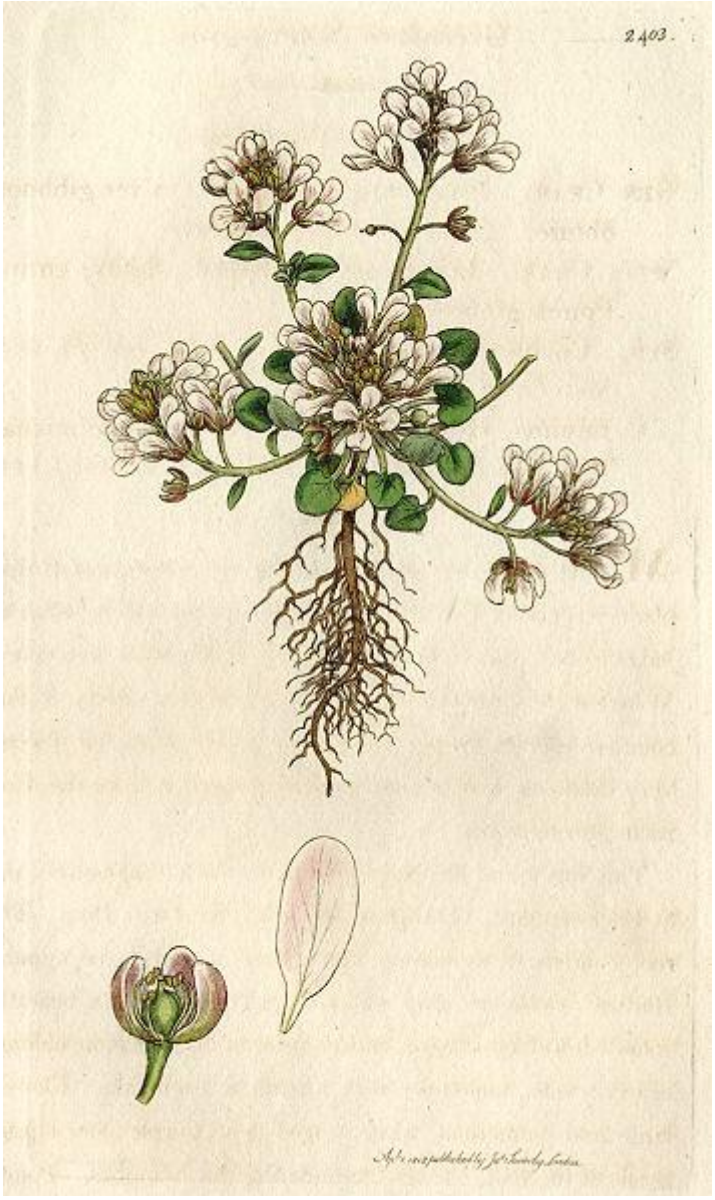

Greenland Scurvygrass (*Cochlearia groenlandica*), Sowerby, 1812.

2019

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Eirin Bruholt

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Abstract

The genus *Cochlearia,* together with its sister genus *Ionopsidium* Rchb., forms the tribe Cochlearieae, a rather small tribe which comprises about 30 taxa, which either x=6 or x=7 basic chromosome number. The x=6 series forms a polyploid complex, which is distributed throughout Europe, while the x=7 basic chromosome number is only found only in diploid plants (2n=14), which are widespread in the arctic. Plants with both basic chromosome number occur as diploids only in Iceland (2n=12, 14). As the Icelandic plants show morphologically similarity to the arctic plants, it has been debated whether they both should be included in the widespread arctic species *C. groenlandica*. The aim of this thesis was to obtain more knowledge about the relationships and taxonomic status of arctic *C. groenlandica*.

For this purpose, populations of *C. groenlandica* have been sampled from Alaska, Canada, West Greenland, East Greenland and Svalbard in addition to both coastal and alpine *Cochlearia* populations from Iceland. The collected populations represented various habitats such as beach cliffs, tundra, and urban areas. Single nucleotide polymorphisms (SNPs), derived from RAD-sequencing (RADseq), were applied to study the genetic variation of these populations, morphological variation were analyzed in plants cultivated under controlled conditions, and finally ploidy level and chromosome number were assessed by flow cytometry and chromosome counting.

The RADseq data showed a clear geographical structure among the *C. groenlandica* populations, which is in line with previous studies using RADseq to analyze *Cochlearia* populations. The populations from West Greenland constituted one genetic group, which was separated from a group consisting of populations from East Greenland, Svalbard and Alaska. The Canadian population had an intermediate position between these two groups. When the Icelandic populations were included, the Icelandic alpine population clustered closer to East Greenland and Svalbard than to the Icelandic coastal populations, in line with previous reports suggesting a genetic link between the Icelandic alpine populations and *C. groenlandica* populations in Svalbard.

For the morphological analyses, the same leaf and flower traits as have been used to study other *Cochlearia* populations were used to evaluate leaf shape, leaf base shape and leaf size, as well as petal length. There was a large variation in leaf shape and leaf base shape within and between the different populations, but no clear pattern which could be related to the geographically structure genetic variation. However, morphological differences between populations were found also after the plants had been grown in controlled conditions, which indicates that genetic factors or epigenetics underlie the observed phenotypic variation. Even though not consistently, there seemed to be a correspondence between leaf size and leaf shape and the habitat that the plants grew in, as all the tundra populations had the small lanceolate leaves with cuneate/trullate base, while costal and urban populations had significantly larger reniform leaves with subcordate/cordate base.

The flow cytometry analysis and chromosome counting confirmed that all the *C. groenlandica* populations were diploid with x=7, that there was no overlap with plants with x=6, and that *C. groenlandica* indeed seems to be a diploid in the Arctic.

The results in this thesis show that there are both morphological and genetic differences between the analyzed populations of *C. groenlandica*, but there are no indications that these constitute more

than one species. Previous suggestions to refer the Icelandic alpine populations to *C. groenlandica* are supported in this thesis, but it is still uncertain whether they might be referred to as ecotypes or subspecies. However, the Icelandic coastal populations do not show similar close associations to the Arctic.

1 Introduction

1.1 *Cochlearia*

The plant genus *Cochlearia* L. belongs to the family Brassicaceae in the order Brassicales. Together with its sister genus *Ionopsidium* Rchb., *Cochlearia* forms the tribe Cochlearieae, a rather small tribe which comprises about 30 taxa (Kiefer et al. 2013) and constitutes an isolated evolutionary lineage within the family. Koch et al. (1996) and Koch (2012) suggested, based on molecular evidence, that the genus is rather young, possibly with a post or late glacial origin.

Most of the *Cochlearia* species are biennial or perennial, but also annual species exist. In the vegetative phase, the leaves grow in rosettes and have a cordate to reniform shape, a wax-like surface and are succulent. The leaves on the flowering stems are more oblong-spathulate compared to the leaves of the sterile rosettes. The flower is a characteristic Brassicaceae flower with four white petals, two short and two long stamens. Most species produce flowers with honey scented nectar, but in some species nectaries are not present; such flowers are often associated with predominant inbreeding or selfing (Nordal and Laane 1990). The fruits in *Cochlearia* are globose small siliques.

Some of the morphological characters that commonly have been used to identify *Cochlearia* species are leaf shape and flower size (Lid and Lid 2005). These characters have, however, been reported to show phenotypic plasticity related to environmental conditions (Elkington 1984). This has further been documented by cultivation studies (Eriksen and Nordal 1989). Phenotypic plasticity was for instance shown to contribute to the morphological differentiation of ecotypes of *C. officinalis* in northern Norway (Eriksen and Nordal 1989).

Linné recognized the following five *Cochlearia* species: the boreal *C. danica* L. (1753), *C. officinalis* L. (1753), *C. anglica* L. (1759), *C. glastifolia* L. (1753) and the arctic *C. groenlandica* L. (1753). Of these, only *C. danica* has kept its taxonomical position unchallenged. Since then several taxa have been described; 24 accepted species and five subspecies according to Brassibase (Kiefer et al. 2013). Some of these are widespread species, others are local endemics, and still others need a critical re-evaluation (Koch 2012). *Cochlearia* is commonly found around most of Europe and abundant in arctic areas, particularly along the coast.

Taxonomically, the genus has always been considered complicated (Hultén 1970), most likely as a result of recent and ongoing speciation and recent chromosome evolution, which has taken place without corresponding morphological differentiation (Koch et al. 1996).

In the genus, two basic diploid series exist, based on the haploid numbers x=6 and x=7. The x=6 series, which has been suggested as more basal, is found from southern to northern Europe, while x=7 is more widespread in arctic regions (Gill 1971a, 1973, Nordal and Laane 1990, Koch et al. 1996). The x=6 series forms a polyploid complex, ranging from diploid species in Central and Southern Europe (*C. pyreneica* DC (1821), *C. aestuaria* (J.Lloyd) Heywood (1965)), through the autotetraploid *Cochlearia officinalis*, the hexaploid *C. danica* (2n=42) to the octoploid *C. anglica* (2n=48). The x=7 haploid chromosome number is so far only known from diploids in arctic and subarctic areas, e.g. *C. groenlandica* (2n=14). The 2n=14 taxa have been suggested to have originated by primary tetrasomy

(aneuploidy) from a 2n=12 species, e.g. *C. pyreneica* (Gill 1971), and considered cytologically and genetically different from the other *Cochlearia*-species (Saunte 1955).

Several studies have shown that the frequency of polyploidy increases with latitude in the northern hemisphere (Brochmann et al. 2004). It has been suggested that polyploids are better adapted to the extreme climates than diploids (Hagerup 1931). Others have suggested that polyploids have a selective advantage because of higher genetic variation resulting from recent hybridization (Manton 1950, Löve and Löve 1957, Johnson and Packer 1965, Johnson et al. 1965), or that polyploids seem better suited to colonize newly deglaciated areas than diploid, because they have a greater ecological adaptability (Stebbins 1950). For the 'arctic specialist' taxa, Brochmann et al. (2004) found a much higher frequency of polyploidy in the previous glaciated areas compared to areas that remained unglaciated during the last ice age, suggesting that polyploids are certainly more successful than diploids in colonization after deglaciation. Yet, for the genus *Cochlearia*, only diploids (2n=14) seem to be widespread in the Arctic, while the polyploid species of *Cochlearia* is found further south. This thesis will focus on the arctic diploid species, *C. groenlandica*.

1.2 Cochlearia groenlandica

Cochlearia groenlandica is, as the name indicates, an arctic species. The lectotype was designated by Reidar Elven and Inger Nordal (Elven and Nordal in Jonsell and Jarvis 2002: 69), and the type locality is Mudderbugten on Disko Island (West Greenland). It is the only registered *Cochlearia* species on Greenland, and is commonly found around the entire coast of Greenland (Rune 2011). All the inspected *Cochlearia* plants from Svalbard and Greenland are *C. groenlandica*, and the northern arctic *Cochlearia* plants from North America and Russia-Siberia conform morphologically to *C. groenlandica* (Alsos et al. 2019).

Cochlearia groenlandica is described to as self-compatible (Bjorå and Nordal in prep.). Svalbard populations have, thus, been reported to be autogamous, with high seed set in cultivated plants both when left to themselves or actively self-pollinated (Nordal and Laane 1990). As opposed to this, other *Cochlearia* species are known to be self-incompatible and thus obligate outcrossers, e.g. the polyploid *C. officinalis* from northern Scandinavian populations (Nordal and Laane 1990), and the diploid *C. pyreneica* and *C. aestuaria* from Central Europe (Koch 2002, Olsen et al., unpublished data).

Due to the rough environment, where *C. groenlandica* normally grows, its size is usually rather modest compared to other species of *Cochlearia.* It grows in a wide variety of habitats like open, not too dry or too wet ground on gravelly and sandy plains, sediments plains, open patches in moss tundra, frost-patterned ground, along seashores and other shores, as well as bird-cliff meadows (Rønning 1996). There are, of course, exceptions where the species can grow much larger when enough nutrition and space are given (Eriksen and Nordal 1989, Zmudczynska-Skarbek et al. 2013, Wojciechowska et al. 2015). Studies show that seabirds have fundamental importance for vegetation growth in poor arctic environments, also for *C. groenlandica* (Eriksen and Nordal 1989, Zmudczyńska-Skarbek et al. 2013, Wojciechowska et al. 2015). On Svalbard there has been reported occurrence of two morphological forms; tall plants were recorded in the vicinity of bird colonies, and the small and delicate plants were recorded in the open tundra and on substrates poor in nutrients (Eriksen and Nordal 1989, Odasz 1994, Rønning 1996).

The taxon currently known as *C. groenlandica* has several synonyms (Warwick et al. 2006, Kiefer et al. 2013); *C. arctica* Schltdl. ex DC, *C. fenestrata* R. Br. Ex DC., *C. oblongifolia* DC., *C. officinalis* var. *groenlandica* (L.) Gelert, *C. officinalis* subsp. *groenlandica* (L.) A. E. Porsild and *C. polaris* Pobed. Saunte (1955) suggested that probably all of the 2n=14 *Cochlearia* (including all *Cochlearia* in Greenland) should be included into one species; *C. groenlandica* of which *C. oblongifolia* should be given subspecific or varietal rank. Alternatively, she suggested that the Greenland plants might with equal right be grouped as *C. groenlandica, C. oblongifolia* and intermediate forms. Gill (1971) suggested to consider *C. arctica, C. groenlandica* and *C. oblongifolia* as varieties of *C. groenlandica* as their ranges overlap and they are morphologically similar. Nordal and Laane (1990) suggested to place all arctic diploids (2n=14), including the 2n=12 diploids from Iceland, into *C. groenlandica*. The Flora of North America reduced *C. arctica, C. oblongifolia* and *C. fenestrata* to one species; *C. groenlandica* (Al-Shehbaz and Koch 1993). Elven (2011), in the Panarctic Flora, noted that there are some morphological differences between *C. arctica* and *C. groenlandica,* which could be argued to be ecotypical, but still recognized *C. arctica* as a separate species, described as a predominant species in both arctic and non-arctic western Alaska, possibly present as rare also in northern Alaska. Löve and Löve (1976a) described the genus *Cochleariopsis* Á. Löve & D. Löve to include the *Cochlearia* taxa with x=7.

All previous chromosome counts (Rice et al. 2014) indicate that *C. groenlandica* is indeed a diploid in the Arctic. However, considering the large distribution range of *C. groenlandica*, it cannot be ruled out without further investigations that 2n=12 diploid plants or higher ploidy levels are represented in arctic areas. The study by Olsen (2015) is a good example of how new information on chromosome numbers can change our common perception. Previously to that study, *Cochlearia* plants with 2n=14 were considered common in Iceland, whereas plants with 2n=12 were thought to be restricted to a limited area along the southern coast (Nordal and Laane 1990). Results by Olsen (2015) showed that plants with 2n=12 are actually found around the whole coast of Iceland.

1.3 *Cochlearia groenlandica* in Iceland?

Cochlearia groenlandica is distributed in arctic and subarctic areas, but there is some uncertainty as to whether *Cochlearia* on Iceland should be included in *C. groenlandica*. Iceland is the only known area where *Cochlearia* plants with different diploid chromosome number co-occur (2n=12, 14) (Gill 1971a, Nordal and Laane 1990, Koch et al. 1996, Koch et al. 1998). The *Cochlearia* plants in Iceland first of all grow in coastal areas with beach cliffs where they grow relatively larger in size and have either 2n=12 or 2n=14 (Olsen 2015). In addition, much smaller plants with 2n=14 are found in alpine areas where they grow in late snow beds. The Icelandic alpine *Cochlearia* plants are genetically, ecologically and morphologically separated from the geographically close coastal plants (Olsen 2015). Interestingly, the alpine plants seem to genetically group with *C. groenlandica* (2n=14) from Svalbard (Olsen 2015). Olsen (2015) only included material of *C. groenlandica* from Svalbard in her study and thus could not discuss whether there could be more separate links between the Icelandic *Cochlearia* plants (alpine or coastal) and different arctic areas.

The Icelandic alpine plants are not only genetically close to the *C. groenlandica,* but also share similar ecology as the late snow bed habitat on Iceland has much in common with the arctic tundra, where *C. groenlandica* plants is typically found in Svalbard (Olsen 2015). Previous analyses have also shown

that the *C. groenlandica* tundra plants in Svalbard are morphologically very similar to the Icelandic alpine plants (Nordal and Laane 1990). Due to this, Nordal and Laane (1990) referred the Icelandic plants to *C. groenlandica,* but further suggested that the ecological and morphological differentiated coastal and alpine plants might deserve subspecific recognition.

1.4 Aims

The aim of this thesis is to obtain more knowledge about the relationships and taxonomic status of the arctic *C. groenlandica*. Several recent studies have used molecular markers to investigate geographical structure and relationships within and among different *Cochlearia* taxa (Cires et al. 2011, Koch 2012, Brandrud 2014, Olsen 2015, Brandrud et al. 2017, Wolf 2017), but these have only to a little degree included material of *C. groenlandica* in their analyses. The study by Olsen (2015), which focused on *Cochlearia* in Iceland, however, established a clear genetic link to *C. groenlandica*. By collecting and analyzing more material of *C. groenlandica,* this thesis aims to answer questions left open by previous studies.

Restriction site Associated DNA sequencing (RADseq) is used to obtain genome-wide single nucleotide polymorphisms (SNPs) of multiple individuals from several populations. Results from flow cytometry and chromosome counting are obtained in order to determine ploidy levels and chromosome numbers. Morphological studies are performed on leaf and flower material and compared to the molecular and chromosome results. The material have either been collected during own field work or collected by other researchers.

The specific questions that will be addressed in this thesis are:

- Is genetic variation within *C. groenlandica* geographically structured?
- Is genetic variation within *C. groenlandica* related to variation in morphology?
- Is genetic variation within *C. groenlandica* related to variation in chromosome number or ploidy level?
- How are the Icelandic *Cochlearia* populations genetically related to *C. groenlandica*?

2 Material and method

2.1 Plant material

The plant material of *Cochlearia* was mainly sampled in western Greenland in August 2017 (fig. 1). Prior to the field work, the Herbarium of Greenland Vascular Plants, at the Natural History Museum of Denmark in Copenhagen, was visited to locate localities. In addition to locality, we noted what kind of habitat the plants grew in. The habitats from which we sampled plants included beach cliffs, road sides, gravelly plains, bare rocks, sediment plains, moss and sandy beaches, and alpine meadow/mossy tundra at around 500 m a.s.l. (fig. 2). The map of the localities was made in ARCMAP v. 10.4.1 (ESRI 2011), using GPS values with coordinates in decimal degrees.

At each locality, leaf samples were collected from ten individuals and instantly dried on silica gel for later DNA isolation. We selected only healthy, green leaves. The sampled individuals differed in size depending on the localities they grew in (fig. 3). Seeds were collected from the same or other individuals when possible. When seeds were not mature or present, living individuals were collected to be replanted in a growth chamber at the University of Oslo in order to collect seeds at a later time. Through contact with other researchers, we were able to retrieve silica dried material and seeds of *C. groenlandica* from Nuuk (western Greenland), silica dried material from Canada, and seeds from Alaska, eastern Greenland and Svalbard. Living plants and seeds from Iceland were already available from a previous project at the University of Oslo (Olsen 2015).

Plants from altogether 19 different populations were included in the analyses (Appendix Table A1) (in some of the genetic analyses like, the ALA1 population was split into two populations, ALA1_1 and ALA1_2, as they were sampled as two different populations, but geographically close to each other, and we only have information about one locality. These two were not separated in the morphological analyses). It would have been ideal to have included Russian and Siberian populations as well, but we were unable to retrieve any seeds or leaf material.

Figure 1: Map over the North Atlantic and Arctic areas with the localities of the sampled *Cochlearia* (see Appendix Table A1 for locality information and abbreviation of locality names.)

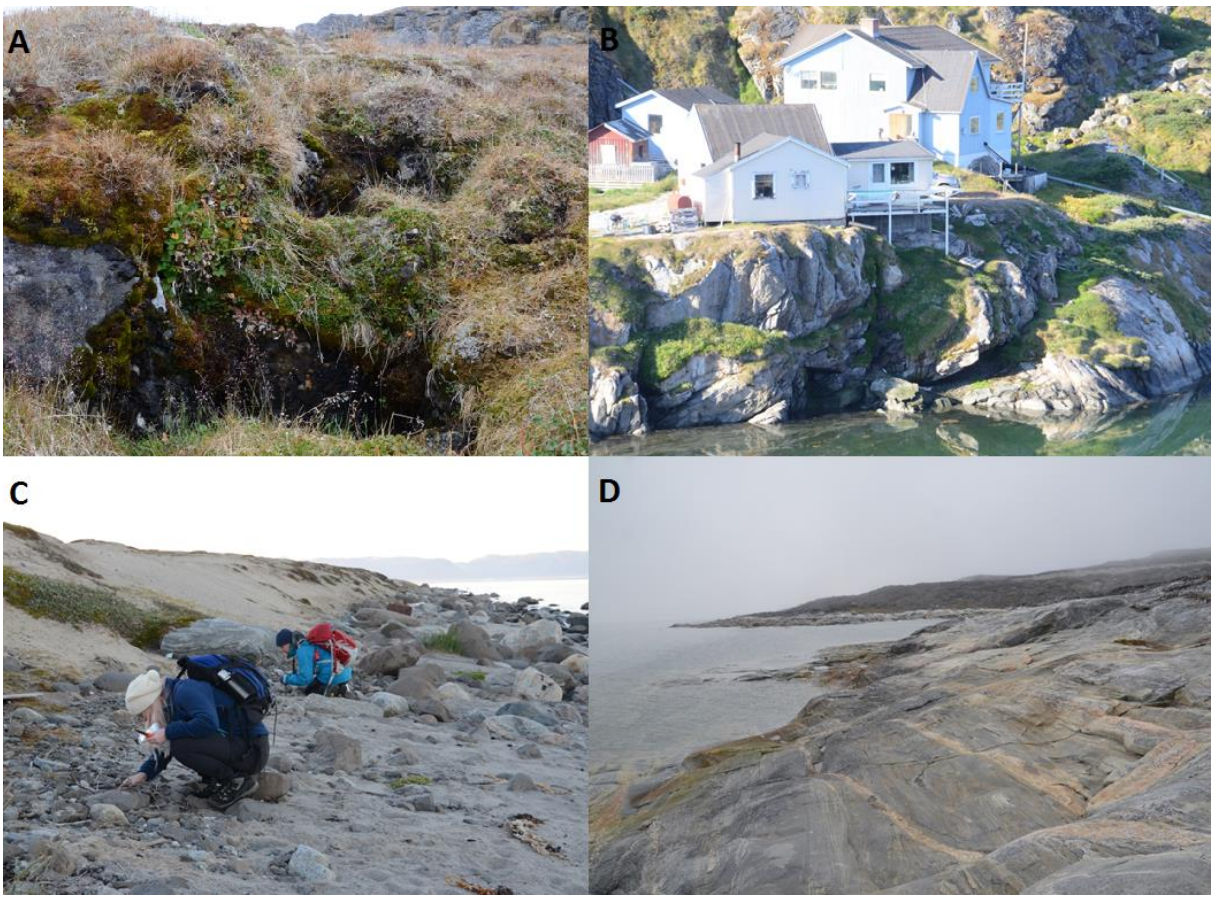

Figure 2: Different types of habitats from where *Cochlearia groenlandica* was sampled in West Greenland. A: Mossy tundra/alpine meadow (WGR1), B: Grassy cliff near beach in urban district (WGR2), C: Sandy beach (Mudderbugten, WGR6), D: Bare rocks (WGR5). Photos: Charlotte Sletten Bjorå.

Figure 3: Field photos of *Cochlearia groenlandica* depicting the size range of the samples. A: A plant from a grassy cliff in an urban district (WGR2). The plants were growing below a house providing high nutrition access. B: A plant from a moss patch at a sandy beach (WGR6). Photo: Charlotte Sletten Bjorå.

The seeds were stored dry and cold at 3°C until germination. In order to increase the germination rate, the seeds were subjected to stratification before sowing. Five ml distilled water was added on top of five layers of paper tissue, covered by filter paper in a petri dish. Ten healthy seeds were dispersed on top of the filter paper before covered with another filter paper, which was sprinkled with 2 ml water. Parafilm was used to seal the petri dishes before they were stored in a cold place (approximately 3°C) for about three weeks (Brandrud 2014).

The germinated plants were grown under controlled conditions in a growth chamber at the University of Oslo. As *C. groenlandica* is a biennial plant, and does not flower until the second season, the plants were allowed to develop leaf rosettes in summer conditions (18 hours of 18°C in light and 6 hours of 10°C in darkness) before they were moved to simulated winter conditions (10 hours of 9°C in light and 14 hours of 9°C in darkness) for approximately two months, and then moved to summer conditions once again to induce flowering.

Leaves were harvested from the leaf rosettes before they started flowering and either pressed to be used for leaf morphometry, or put on silica to be used for DNA extraction. When the plants produced

flowers, the flowers were collected for morphometry and flow cytometry, and flower buds for chromosome counting.

All plants, which were used for morphometry, flow cytometry and chromosome counting, were germinated from seeds in a growth chamber, and had at least one flowering season. None of the individuals brought back from field work were used for the analyses, only their seeds were harvested and sown for use.

2.2 RADseq analysis

DNA extraction

DNA was extracted from approximately 30 mg dried leaf material from approximately ten individuals from each of the 16 populations that were included in the RADseq analyses (Appendix Table A1). The E.Z.N.A. SP plant DNA kit (Omega Bio-tek, Norcross, USA) and the protocol issued by the manufacturer were used, with minor modifications as in Brandrud et al. (2017).
Carbide beads were used to crush the dried material in a 2 ml Eppendorf tube, disrupting them for 1 min at 20 Hz in a TissueLyser II, Retsch MMo1 (Retsch, Castleford, UK). The remaining protocol from the manufacturer was followed without any modifications except that the samples were eluted in 100 µl elution buffer. The extracted DNA was stored in DNA LoBind tubes (Eppendorf, Hamburg, Germany). Quantification and quality check of the extracted DNA were performed using both NanoDrop ND-1000 v3.10 Spectrophotometer (Thermo scientific, USA) and Qubit dsDNA BR assay kit (Life Technologies, Carlsbad, California, USA) with a Qubit fluorometer (Life Technologies).

To secure high-quality DNA for further procedures, the extracted DNA was cleaned with Nucleospin gDNA Clean-up (Macherey-Nagel, Düren, Germany). The manufacturer's protocol was followed without any modifications.

RADseq-library preparation

Next-generation sequencing technologies are making a substantial impact on many areas in biology, and so also on analysis of genetic diversity in and between populations. Restriction-site associated DNA sequencing (RADseq) is a method which delivers high resolution population genomic data from target genomes of any organism at reasonable costs. RADseq combines the use of restriction enzymes to cut DNA into fragments, and the use of molecular identifiers to associate sequence reads to particular individuals. A chosen restriction enzyme cuts the DNA from an individual, which produces a set of sticky-ended fragments. The fragments must be ligated to adapters that will bind to an Illumina flow cell before being sequenced on an Illumina machine (Davey and Blaxter 2010). The adapters contain a matching sticky-end and a barcode. The barcodes are used to recognize individuals in the subsequent analyses (Davey et al. 2011).

A single-digest RADseq library was prepared according to a protocol adapted from Baird et al. (2008) with modifications as in Paun et al. (2016) and Brandrud et al. (2017), except that the sub-libraries were kept separate throughout the procedure.

The library comprised of 84 samples (between five and seven individuals, with the highest DNA concentration, were selected from each of the 16 populations). The individuals were separated into seven sub-libraries where each individual was marked by a unique barcode combination. On each read there were three barcodes; one index barcode on the P1 adapter, one index barcode on the P2 adapter and one inline barcode on the P1 adapter (Appendix Table A2). Each of the seven P2_index adapters corresponded to a sub-library, and the 12 P1_inline adapters (combined with three P1_index adapters) discriminated individuals within the sub-library. The samples were diluted based on the quantification values to ensure the same amount of DNA (300 ng) was included from each individual: x µl DNA and 44-x µl Milli-Q water.

Restriction enzyme PstI-HF (NEB, New England Biolabs, UK) was used for the digestion of genomic DNA at 37°C for about two hours. As Pstl-HF cannot be heat activated, the samples were cleaned with SPRI (Beckman Coulter, Indiana, USA) with no selection (1.8x), to remove the enzyme after restriction digestion. The 84 samples were normalized to a volume of 30 µl (90 ng DNA). Ligation of P1 adapters was done by adding 1.25 µl 100 nM P1 adapter, 1 µl 100 mM rATP (Promega, Fitchburg, USA), 1 µl NEB Buffer 2, 3.25 µl Milli-Q water, 3 µl 10x SmartCutBuffer and 0.5 µl 200 000 U T4 ligase (NEB) to each sample before incubating at 16°C overnight in a PCR machine without heated lid. To inactivate the enzyme, the samples were heat treated for 10 min at 60°C, before they were pooled into seven sub-libraries and randomly sonicated (stochastic shearing) using three cycles (45 sec on and 60 sec off, 4°C) on a Bioruptor Pico (Diagenode, Denville, US) to obtain an optimal size of 300-600 basepairs. Brandrud (2014) found that this setting is optimal for *Cochlearia*, but other settings were also tested in order to be sure which setting would result in the optimal size. MinElute Reaction Cleanup Kit (Qiagen, Venlo, Netherlands) was used to clean the sheared samples, eluting in 15 µl elution buffer, and subsequently SPRI size selection was performed on both sides (left 0.7x, right 0.55x). In order to remove the primers at the ends of the fragments, the Quick Blunting Kit (NEB) was used by adding 2.5 µl Buffer, 2.5 µl 100 mM dNTP and 1.0 µl enzyme to 19 µl DNA per sub-library and incubated for 30 min at room temperature. The samples were once again cleaned with MinElute Reaction Cleanup Kit, and dATP/adenine (Fermentas, Waltham, Massachusetts, USA) overhangs were then attached to the 3' end of the fragments by adding 2 µl (15U) Klenow Exo (NEB), 1 µl 100 mM dATP and 2 µl NEB Buffer to 15 µl DNA per sub-library before incubation at 37°C for 30 min. The samples were then again cleaned with MinElute Reaction Cleanup Kit. The P2 adapters were ligated to the DNA fragments by adding 5 µl 2 µM P2 adapter, 1 µl 199 mM rATP, 3 µl NEB Buffer 2, 0.5 µl T4 ligase to 20 µl DNA solution with subsequent incubation at room temperature for 30 min. The MinElute Reaction Cleanup Kit was then used for cleaning once more followed by left side size selection (0.65x) with SPRI. The sub-libraries were amplified using PCR as seven reactions each containing 12.5 µl Phusion Polymerase remix (NEB), 1 µl Solexa primer (10 µM), 7.5 µl water and 4 µl sub-library template (DNA), with the following cycling conditions; 30 sec at 90°C, followed by 18 cycles [10 sec 90°C, 30 sec 65°C, 30 sec 72°C], 5 min at 72°C, and incubation at 4°C. The resulting products were run on a 2% TBE gel for 30 min to verify successful amplification. The sub-libraries were cleaned with MinElute Reaction Cleanup Kit and left side SPRI size selection (0.65x).
The sub-libraries were quantified using Qubit, and submitted to the Norwegian Sequencing Centre where they were run on an Agilent 2100 Bioanalyzer (Agilent technologies, Santa Clara, USA) with a High sensitivity DNA Assay Kit (Agilent) to verify that the overall size range and quantity of the sub-libraries were optimal and whether the adapters had been removed. To measure the concentration of each sub-library, qPCR assay (KAPA Library Quantification Kits cat no KK4824, KAPA Biosystems,

Massachusetts, USA) was used, using a qPCR cycler (Lightcycler 96, Roche, Basel, Switzerland) ensuring that equal amounts of each sub-library were included in the final RAD-seq library before it was sequenced using pair-end sequencing (150 bp) in one Illumina HiSeq4000 lane. The sequencing service was provided by the Norwegian Sequencing Centre (www.sequencing.uio.no), a national technology platform hosted by the University of Oslo and supported by the "Functional Genomics" and "Infrastructure" programs of the Research Council of Norway and the Southeastern Regional Health Authorities.

Processing of RADseq reads

Approximately 380 million paired-end reads were returned from the Norwegian Sequencing Centre as a BCL file. This file was converted to a BAM file using ILLUMINA2BAM (part of the ILLUMINA2BAM Picard command-line tools; http://broadinstitute.github.io/picard/) implementing also the SECOND BARCODE option. The first part of the demultiplexing and quality filtering of the reads were done in BAMINDEXDECODER (part of the ILLUMINA2BAM Picard command-line tools). Demultiplexing was run twice in BAMINDEXDECODER, in the first run using the P1 index barcodes and in the second run the P2 index barcodes (Appendix Table A2). As the HiSeq4000 reads the second index (corresponding to our P1 index) as a reverse-complement relative to HiSeq2500 (which the adapters were designed for), we had to reverse-complement the barcodes in the barcode file used for demultiplexing. The output files from the last BAMINDEXDECODER run were converted to FASTQ files using SAMTOFASTQ (Picard-tools). As they contained pair-end reads, this resulted in two FASTQ files per BAM file. The FASTQ files were used as input files for the last part of the demultiplexing (based on the P1 inline barcodes), which was done in STACKS v. 1.47 (Catchen et al. 2011, 2013) using the program *process_radtags.pl*. This resulted in ca. 250 million (249 647 147) retained forward reads. The number of retained forward reads per sample varied between 1.4 to 6.5 million reads.

A reference catalog was made by Ovidiu Paun (University of Vienna) based on the demultiplexed reads. A catalog was build running the STACKS program *denovo_map.pl* with the settings m=5 (minimum number of identical, raw reads required to create a stack), M=1 (number of mismatches allowed between loci when processing a single individual) and n=1 (number of mismatches allowed between loci when building the catalog). The resulting average coverage was 58.9x. Then, RADtags that were polymorphic with up to 10 SNPs (single nucleotide polymorphism) per locus and covered in at least 50% of individuals were selected. In total 13,113 loci were included in the final reference.

To map the reads against this reference catalog, I first created an index and then mapped the references using BWA (Li and Durbin 2009). After this, the STACKS program *ref_map.pl* was run with the mapped files in order to execute the STACKS pipeline, executing each of the stacks components, and using the reference alignment to form stacks, and identify alleles.

The STACKS program *populations* was run with settings retaining only loci which were present in 80% of the individuals in a population, and only one SNP per locus (i.e. the first SNP on each locus) to minimize as much as possible linkage of markers. Using a population map, individuals were linked to their respective populations and output files of the following formats were produced to be used as input files for the downstream analyses: STRUCTURE, VCF and PHYLIP. As the four Icelandic populations turned out to be most divergent ones, *ref_map.pl* and *populations* were also run without the Icelandic populations. All analyses (except for those done by Ovidiu Paun) were run on the Abel

cluster, owned by the University of Oslo and the Norwegian metacenter for High Performance Computing (NOTUR).

In connection to the demultiplexing, it was discovered that some of the P1 adapters had been switched in the lab. This was edited in the final output files but three individuals from one of the Icelandic populations, one from Svalbard, one from East Greenland and one from West Greenland, which continued to misplace, were excluded from the analyses. By the end, 78 samples were left for the downstream analyses when the Iceland populations were included, and 58 samples when the Iceland populations were excluded.

Downstream analyses of SNPs

In order to investigate population structure, the programs AMOVA, STRUCTURE, R and SPLITSTREE were used.

Analysis of Molecular Variance (AMOVA) was used to estimate genetic divergence among and within populations, using the program ARLEQUIN v. 3.5.2.2 (Excoffier and Lischer 2010) with default parameters. PGDSpider v. 2.1.1.5 (Lischer and Excoffier 2012) was used to convert the PHYLIP input files (containing 12,304 SNPs with Iceland and 12,090 SNPs without Iceland) to ARLEQUIN input files. The individuals were sorted into populations, and higher level groups based on larger geographical regions.

STRUCTURE v 2.3.3 (Pritchard et al. 2000) was run through the Lifeportal at the University of Oslo (https://lifeportal.uio.no/). STRUCTURE assumes that within populations, the loci are at Hardy-Weinberg equilibrium, and uses Bayesian clustering to find the optimal number of clusters (K) that the dataset can be divided into, and then assigns individuals to these groups. The STRUCTURE input files contained 8255 SNPS with Iceland and 9220 SNPs without Iceland. The analyses were run with K=1-12 without Iceland and K=1-6 with Iceland included, with 10 replicates for each K. The admixture model and correlated frequencies were used, as admixture is a common feature of real data and the frequencies in the different populations are likely to be similar. The iterations were set to 1 000 000 and burn-in to 100 000. STRUCTURE HARVESTER (Earl and von Holdt 2012) and CLUMPAK (Kopelman et al. 2015) were used to summarize the results, using delta K (Evanno et al. 2005) to evaluate the number of clusters that best fit the data.

Principal component analysis (PCA) was run using R (R Core Team 2013), with the package ADEGENET v 2.1.1 (Jombart 2008, Jombart and Ahmed 2011). The STRUCTURE input files, which contained 8255 SNPs (with Iceland) and 9220 SNPs (without Iceland), were run with default settings.

NeighborNet was run in SPLITSTREE v 4.14.8 (Huson and Bryant 2006), using Jukes-Cantor as distance measure. The PHYLIP input files (containing 12,304 SNPs with Iceland and 12,090 SNPs without Iceland) were converted to NEXUS files using PGDSpider. The network was performed with each end node representing an individual.

2.3 Ploidy and chromosome analyses

Flow cytometry

In order to confirm that *C. groenlandica* individuals from the sampled localities were diploid, flow cytometry analysis was used to estimate relative DNA amount by flow cytometry (FCM). As previous analyses of *Cochlearia* leaves (Brandrud 2014) showed a high amount of endopolyploidy (often a result of endoreduplication) where chromosomes replicate without subsequent nucleus and cell division (Barrow 2006), petals were preferred for the FCM analysis, but when flowers were not available, leaves were used instead. The flowers and/or leaves of 55 individuals from 13 populations (Appendix Table A1) were packed in plastic bags with humid paper tissues, and sent to the Laboratory of Flow Cytometry, Institute of Botany, Academy of Sciences of the Czech Republic where the analysis was performed using the following protocol:

Carex acutiformis Ehrh., 2C = 0.41 pg (Lipnerová et al. 2013), was used as internal standard. Usually two petals or 0.5 cm^2 of leaf tissue of a *Cochlearia* sample and an appropriate amount of internal reference standard were chopped with razor blade in 0.5 ml of ice-cold Otto I buffer (0.1 M citric acid, 0.5% Tween 20). A crude suspension of nuclei was filtered through a nylon mesh (loop size 0.42 μm), incubated in room temperature for at least 5 min and stained with 1 ml of Otto II buffer (0.4 M $Na_2HPO_4 \cdot 12\ H_2O$) supplemented by AT-selective fluorescent dye DAPI (4',6-diamino-2-phenylindol) and 2-mercaptoethanol in final concentrations of 4 μg/ml and 2μl/ml, respectively. After short incubation (5 min) in room temperature, relative fluorescence intensity of at least 3000 nuclei was recorded using a Partec Space flow cytometer (Partec GmbH, Münster, Germany) equipped with the UV-LED chip (365 nm). The FCM results were expressed as fluorescence intensities relative to unit fluorescence intensity of the internal reference standard – i.e. *C. acutiformis*.

Two individuals with known chromosome number were included in the flow cytometry analysis as reference; one Icelandic sample (ICE4-1) reported to be 2n=14 (Olsen 2015), and one *Cochlearia pyreneica* (PYR_WEI-1) sample, from southwestern Europe, reported to be 2n=12 (Brandrud 2014).

Chromosome counting

As the relative fluorescence (RF) (based on the flow cytometry results) varied somewhat between individuals and populations, three plants with high, three plants with intermediate and three plants with low RF values were chosen for chromosome counting to confirm the actual chromosome number. Only plants that were flowering at the time were used.

I, with the help of assistants, collected the top of the inflorescence, which contained buds of different sizes and ages, and removed any siliques and flowers. The inflorescences were placed in 5 ml Eppendorf tubes with Carnoy's fixative I (ethanol: acetic acid 3:1), which was freshly prepared before use. The tubes were kept overnight at 4°C, and then transferred to a new 5 ml Eppendorf tube containing 70% ethanol before being shipped to Department of Botany, University of South Bohemia, Czech Republic where the chromosome counting was done by Magdalena Lučanová. The protocol used is as follows (Mandáková and Lysak 2016):

For chromosome preparation, a flower bud with white anthers was washed in distilled water twice for 5 min, then in 1 × citrate buffer twice for 5 min and then the buffer was changed for enzymatic

mix (0.3% pectolyase, 0.3% cellulase, 0.3% cytohelicase in citrate buffer). After 3 h digestion in 37°C, the enzymatic mix was changed for 1 × citrate buffer and the material was placed at 4°C for 15 min. Afterwards, the flower bud was placed onto a slide and tapped with a preparation needle. Then 20 µl of 60% acetic acid was added to the suspension and the slide was placed on a heating block at 50°C and the suspension was spread with a preparation needle for 30 sec. Then 100 µl of freshly prepared Carnoy's fixative was added for fixing of chromosomes, the liquid was drained and the dried slide was placed in a box at 4°C. Finally, 15 µl of Vectashield with 4',6–diamidino-2–phenylindole (DAPI) was added as a staining solution and the preparation was covered with a cover slip and fixed with nail polish. The chromosomes were observed under a Nikon Eclipse E600 microscope equipped with Nikon DS-Qi1Mc camera and the pictures were taken with NIS-Elements AR software. For every chromosome count at least 20 chromosome sets in mitotic metaphases were observed.

2.4 Morphometry analyses

For the morphometric analysis, flowers from 28 individuals from nine different populations (Appendix Table A1) were placed in 70% ethanol. These were the only populations which were flowering at the time. Rosette leaves from 47 individuals, from 13 different populations, were pressed using a plant press.

The morphological measurements of leaves and the flowers were done by hand, using a ruler and a protractor. Five leaves and five flowers were measured from each individual when possible. The maximum leaf length was measured as a vertical line from the leaf tip to the attachment of the petiole. The maximum leaf width was measured as the furthest distance between the leftmost and the rightmost points along a horizontal line drawn perpendicular to the leaf length line (fig. 4). Leaf base angle was measured as the angle degrees between the lines drawn from the point of attachment along the lower margins of the leaf (fig. 4). The petal length was measured as a vertical line from the tip to the base of the petal. The petal width was measured at the widest part of the petal. The sepal length was measured from the tip to the base of the sepal. The measurements were done by different people but the same method was used by all of them. These characters were used in previous morphometric studies of *Cochlearia* (Nordal and Laane 1990, Olsen 2015).

"Leaf ratio" was calculated by dividing the width with the length of the leaves, and "leaf size" was calculated by multiplying the length and the width of the leaves. The leaf angle values were used without any form for calculations. Summary statistics including mean, median, standard deviation and lower and higher quartiles (Q1, Q3) were calculated using EXCEL. Boxplots were made in R, using these values. A Kruskal-Wallis test was run in order to check whether there were any significant differences in means of the different characters between the populations.

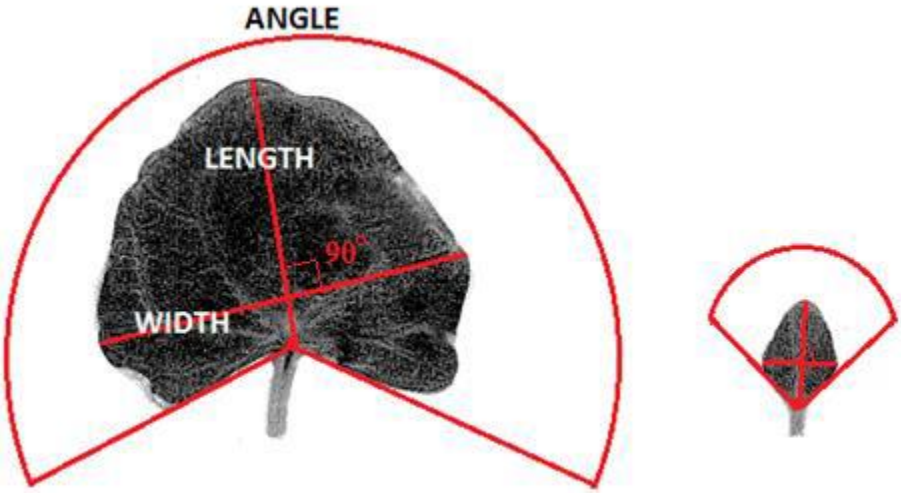

Figure 4: Illustration depicting how the measurements of the leaves were done. Examples of two leaves with very different size and shape are shown (the figure is borrowed from Olsen 2015).

3 Results

3.1 RADseq analysis

Downstream analysis of SNPs

STRUCTURE HARVESTER, which was used to select the optimal number of genetic clusters (K) from the STRUCTURE analysis, found the highest delta K value for K=2 and second highest for K=3 (Fig. 5, Appendix Figure A1a) when the Icelandic populations were included in the analysis.

With K=2 (not shown), the three Icelandic coastal populations (ICE1, ICE3, ICE5) were assigned to one cluster, while most other populations were assigned to the other cluster. The Icelandic alpine populations (ICE4) showed admixture between the two clusters, assigning only ca. 45% to the Icelandic cluster. One of the West Greenland populations (WGR7) and the Svalbard population (SVA) showed minor (<10%) assignment to the Icelandic cluster.

With K=3, the samples were divided into three clusters (blue, orange and purple in fig. 5). Also in this analysis, the Icelandic coastal populations (ICE1, ICE3, ICE5) were assigned to one cluster (orange) One cluster (blue) contained mainly the West Greenland populations (WGR), though WGR7 also here showed minor (<10%) assignment to the Icelandic (orange) cluster. The third cluster (purple) mainly consisted of the Alaskan (ALA), East Greenland (EGR) and Svalbard (SVA) populations. The Svalbard population, however, showed some (for most individuals <10%) to the West Greenland (blue) cluster. The Icelandic alpine populations (ICE4) assigned ca. 30% to the Icelandic (orange) cluster, but had its major (ca. 70%) assignment to the Alaska-East Greenland-Svalbard (purple) cluster. The Baffin population (BAF) from Canada assigned ca. 51% to the West Greenland (blue) cluster and ca. 49% to the Alaska-East Greenland-Svalbard (purple) cluster.

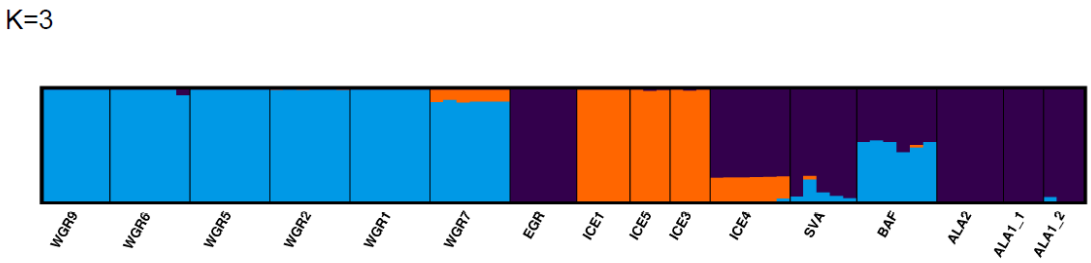

Figure 5: STRUCTURE analysis of 78 *Cochlearia* samples based on 8255 SNPs from RADseq data. The colors represent the number of genetic clusters (K=3). Each individual is represented by a vertical bar and populations are separated by vertical black lines. Names abbreviated according to Appendix Table A1.

In the analysis without the Icelandic populations, the highest delta K value was found for K=2 (fig. 5, Appendix Figure A1b), and the samples were divided into two clusters (blue and orange in fig 6). The West Greenland (WGR) populations assigned to one cluster (blue), while the Alaskan (ALA), East Greenland (EGR) and Svalbard (SVA) populations assigned to the other cluster (orange). One individual in the Svalbard (SVA) population showed some (ca. 17%) assignment also to the West Greenland (blue) cluster. Also in this analysis, the Baffin population (BAF) was admixed approximately 50-50 between the West Greenland (blue) and the Alaska-East Greenland-Svalbard

(orange) cluster. With K=3 (not shown), a third cluster was added, which subdivided the West Greenland samples. The most southern population (WGR7) assigned fully to this third cluster, whereas the two West Greenland populations (WGR1 and WGR2), which are close to WGR7, showed the 65% and 45% assignment to this cluster. With K=4 (not shown), the Alaskan populations formed a fourth cluster, and the Baffin population assigned ca. 50% to this cluster and ca. 50% to one of the West Greenland clusters. One of the Alaskan individuals assigned ca. 50% to the East Greenland-Svalbard cluster.

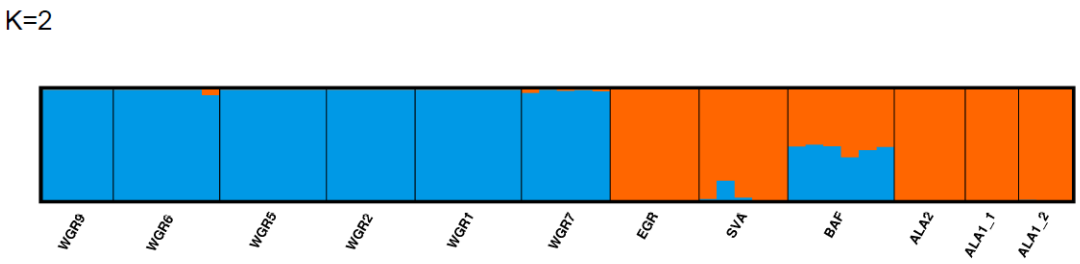

Figure 6: STRUCTURE analysis of 58 *Cochlearia groenlandica* samples when excluding the Icelandic populations, based on 9220 SNPs from RADseq data. The colors represent the number of genetic clusters (K=2). Each individual is represented by a vertical bar and populations are separated by vertical black lines. Names abbreviated according to Appendix Table A1.

In the PCA analysis, without Iceland, the first principle axis (PC1), which explained 14.39% of the total variation, showed a separation between the West Greenland (WGR) populations and the Alaskan (ALA), East Greenland (EGR) and Svalbard (SVA) populations (fig. 7). The Baffin population (BAF) was placed in between the two groups, corresponding to the admixture seen in the STRUCTURE analysis. The second principal axis (PC2), which explained 10.33% of the total variation, showed a separation of the East Greenland (EGR) and Svalbard (SVA) populations from the Alaskan populations. Further the West Greenland populations were separated according to a south-north gradient, with the most southern population (WGR7) found at low values along the axis.

One individual from Alaska (ALA1_1) was found closer to the Svalbard and East Greenland populations than to the remaining Alaskan individuals, corresponding to the result from STRUCTURE analysis with K=4 (not shown).

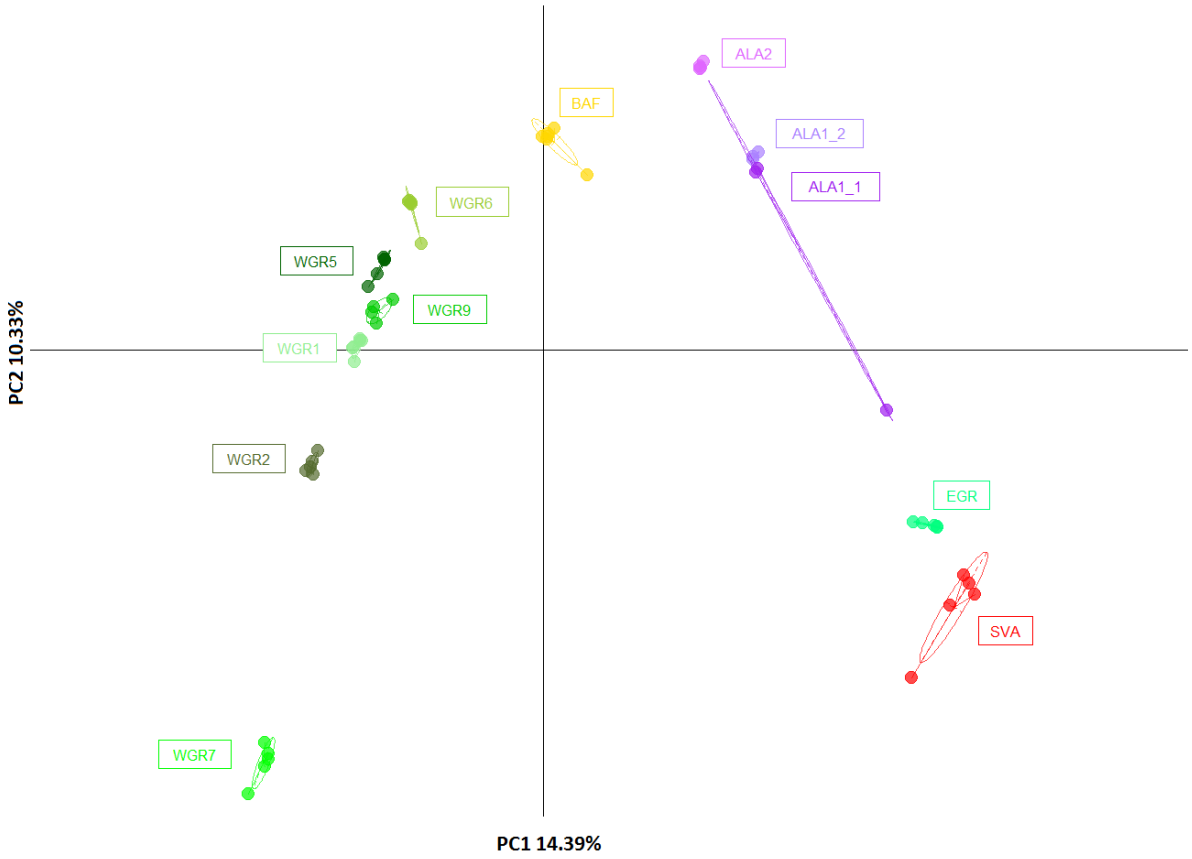

Figure 7: PCA analysis of 58 *Cochlearia groenlandica* samples, excluding the Iceland populations. The x axis represents PC1 and the y axis represents PC2. The variation explained by PC1 is 14.39% and for PC2 it is 10.33% of the total variation. The 95% inertia ellipses indicate the dispersion of each population around its center of gravity. Names abbreviated according to Appendix Table A1. Colors indicate the different countries; The United States (purple), Greenland (green), Iceland (blue), Canada (yellow) and Svalbard (red).

The NeighborNet performed by SPLITSTREE (fig. 8) grouped all individuals according to populations, except for one individual from Alaska, which like in the PCA analysis (fig. 7) showed to be closer to the Svalbard and East Greenland populations. Overall, the NeighborNet showed a similar geographical structure as was found in the STRUCTURE analysis and PCA.

When the Icelandic populations were included, the following pattern was seen: The largest split separated the three Icelandic coastal populations (ICE1, ICE3, ICE5) from the remaining populations. The Icelandic alpine population (ICE4) was closer to the East Greenland (EGR) and Svalbard (SVA) populations than to the Icelandic coastal populations. Otherwise, the same division was seen with the East Greenland (EGR), Svalbard (SVA) and Alaskan (ALA) populations grouping together on one side, the West Greenland (WGR) populations on the other side, and the Baffin population (BAF) in an intermediate position.

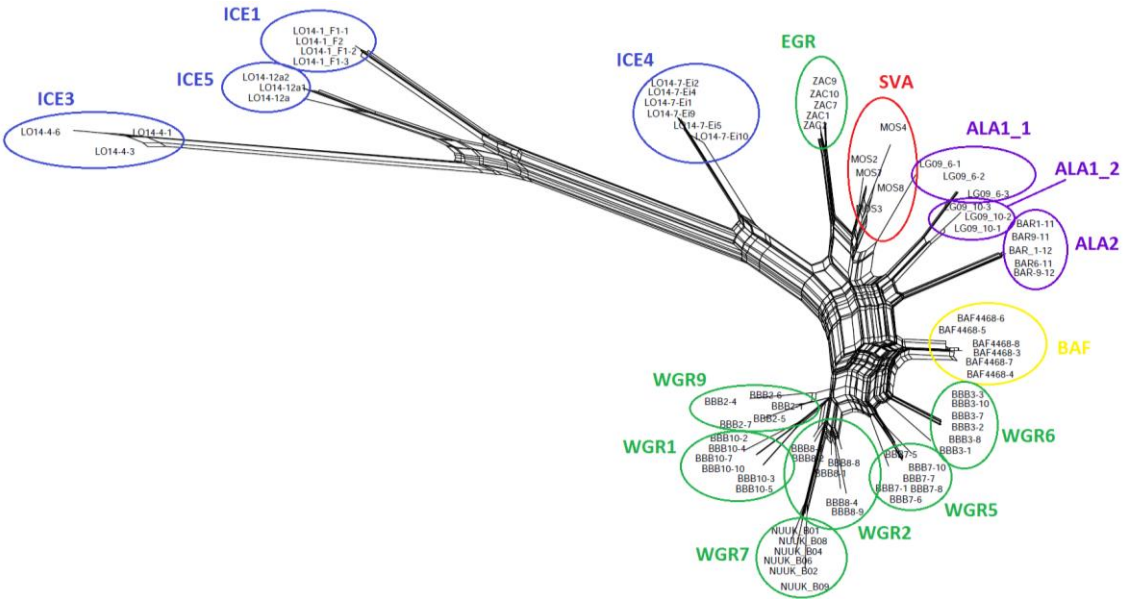

Figure 8: NeighborNet representing 16 *Cochlearia* populations based on 8255 SNPs obtained from RADseq data. Each node is representing one of the 78 individuals which are included in the analysis, and population names are abbreviated according to Appendix Table A1. Colors indicate the different countries; The United States (purple), Greenland (green), Iceland (blue), Canada (yellow) and Svalbard (red).

The AMOVA analysis including the Icelandic populations (Appendix Table A3) showed that the main part of the variation was found among populations (86.62%) compared to within populations (13.38%) when no higher-level groupings were considered. When introducing higher-level groups as major regions (Alaska: ALA1, ALA2; West North Atlantic: BAF, WGR1, WGR2, WGR5, WGR6, WGR7, WGR9; East North Atlantic: EGR, SVA; Iceland: ICE1, ICE3, ICE4, ICE5), approximately the same amount of variation was explained among these higher groups (41.30%) as among populations (46.61%), and only 12.9% of the variation was explained within populations.

When Iceland was excluded from the AMOVA analysis (Appendix Table A3), generally the variation among populations within groups was higher than among groups (not shown). When comparing regions based on geographical distribution, among group variation was 20.27%, among-population variation within groups was 45.96% and within-population variation was 33.77%.

3.2 Ploidy and chromosome analyses

Flow cytometry

Out of the 55 individuals, which were analyzed with flow cytometry, two individuals were included as reference. One Icelandic sample (ICE4-1) has been reported to be 2n=14 (Olsen 2015), and a *Cochlearia pyrenaica* (PYR_WEI-1) sample, from southwestern Europe, has been reported to be 2n=12 (Brandrud 2014). The relative fluorescence intensity values obtained for the two reference samples were 0.666 (ICE4-1) and 0.720 (PYR_WEI-1). The other samples included in the analysis had values ranging from around 0.640 to around 0.740 (fig. 9). Because of this rather large range in values, we decided to have the chromosomes counted in order to confirm the ploidy level.

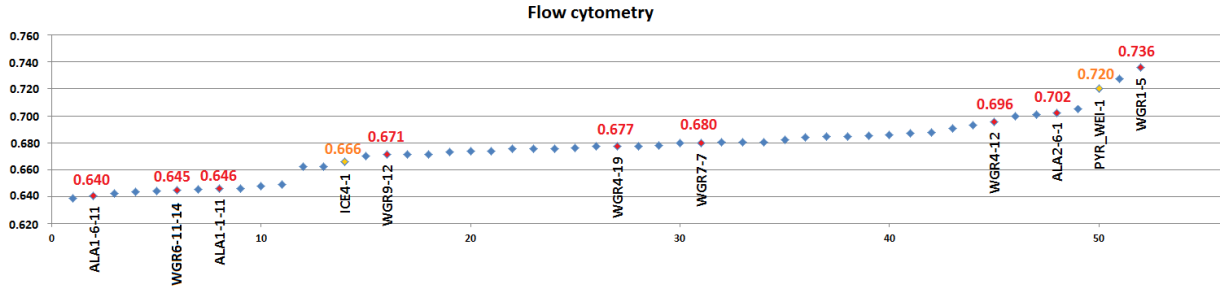

Figure 9: Relative fluorescence intensity values from the flow cytometry analysis of 55 *Cochlearia* samples, ranging between 0.640 and almost 0.740. Chromosome numbers were counted from three samples with values at the lower end of the range, three samples with values at the higher end of the range and three samples with intermediate values; these are highlighted with names, values and red color. Two reference samples, which have been chromosome counted previously (ICE4: 2n=14, PUR_WEI-1: 2n=12), are highlighted with names, values and orange color. Names abbreviated according to Appendix Table A1.

Chromosome counting

The nine individuals sent for chromosome counting represented the whole range of flow cytometry values (fig. 9); three with high values (fig. 10 a, b, c), three with intermediate values (fig. 10 d, e, f) and three with low values (fig. 10 g, h, i). The chromosome counts showed that all samples had a chromosome number of 2n=14 (fig. 10).

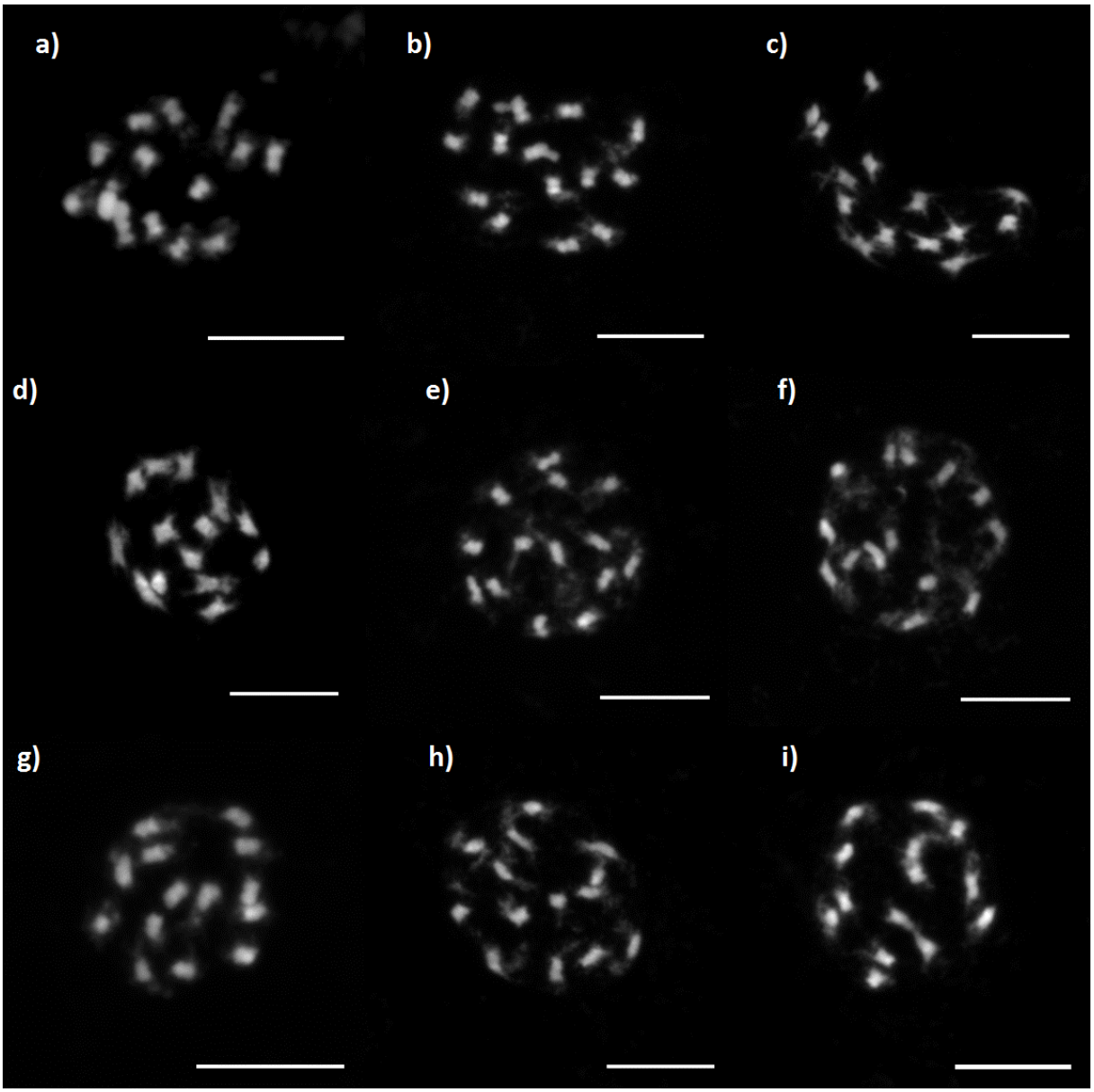

Figure 10: Microscope pictures of the chromosome sets of nine *Cochlearia groenlandica* samples, taken by Magdalena Lucanova at The Czech Academy of Sciences. Sorted from highest to lowest flow cytometry value; a) WGR1-5, b) ALA2-6.1, c) WGR4-12, d) WGR7-7, e) WGR4-19, f) WGR9-12, g) ALA1-1.11, h) WGR6-11.14, i) ALA1-6.11. Names abbreviated according to Appendix Table A1. The scale bar is 10 µm.

3.3 Morphometry analyses

The Kruskal-Wallis test showed that there was a significant ($p<0.05$) difference in leaf traits among the 13 populations included in the analysis (leaf surface area; $p = 2.042e-06$, leaf ratio; $p = 0.02587$, leaf angle; $p = 0.00294$). However, most of the populations showed high variation in all the measured leaf traits.

Higher values for leaf base angle (fig. 11A) indicate a more subcordate/cordate leaf base shape, while lower values indicate a more cuneate/trullate leaf base shape. The highest mean values for leaf base angle (Appendix Table A4) were found for several of the West Greenland populations (WGR3: 214.3 degrees, WGR5: 193.3 degrees, WGR7: 187.5 degrees, WGR1: 175.3 degrees, WGR6: 168.7 degrees), while the population with the smallest mean values was the Icelandic alpine population (ICE4: 102.0 degrees). Large within-population variation in leaf base angle (fig. 11A) was especially found for the Svalbard population (SVA) and some of the West Greenland populations (WGR4, WGR1). The Icelandic alpine population (ICE4) and two of the West Greenland populations (WGR5, WGR3) showed little within-population variation.

Higher values for leaf ratio (fig. 11B) indicate reniform leaf shape, while lower values indicate a lanceolate or ovate/orbicular leaf shape. The populations with the highest mean values for leaf ratio (Appendix Table A4) were several of the West Greenland populations (WGR2: 1.25, WGR7: 1.05, WGR8: 1.02, WGR4: 1.01, WGR6: 1.00), while the population with the smallest mean value was the Icelandic alpine population (ICE4: 0.61). Large variation in leaf ratio (fig. 11B) was found in many populations, especially the same populations as showed high variation in leaf base angle (SVA, WGR4, WGR1), but also in ALA2 and WGR3. Less variation in leaf ratio was found in one of the West Greenland populations (WGR5), the Icelandic populations (ICE4) and the other Alaskan population (ALA1).

Higher values for leaf size (fig. 11C) indicate large leaves, while lower values indicate smaller leaves. Most populations had overall small leaf size (around 1 to 3 cm^2; fig. 11C, Appendix Table A4) The East Greenland (EGR: 0.59 cm^2) and Svalbard (SVA: 0.68 cm^2) and one of the West Greenland populations (WGR1: 0.77 cm^2) had the smallest leaves. Two of the West Greenland populations were distinguished by having somewhat larger leaves (WGR8: 7.76 cm^2; WGR7: 5.03 cm^2). The same patterns were found also when analyzing leaf length and leaf width separately (Appendix Table A4).

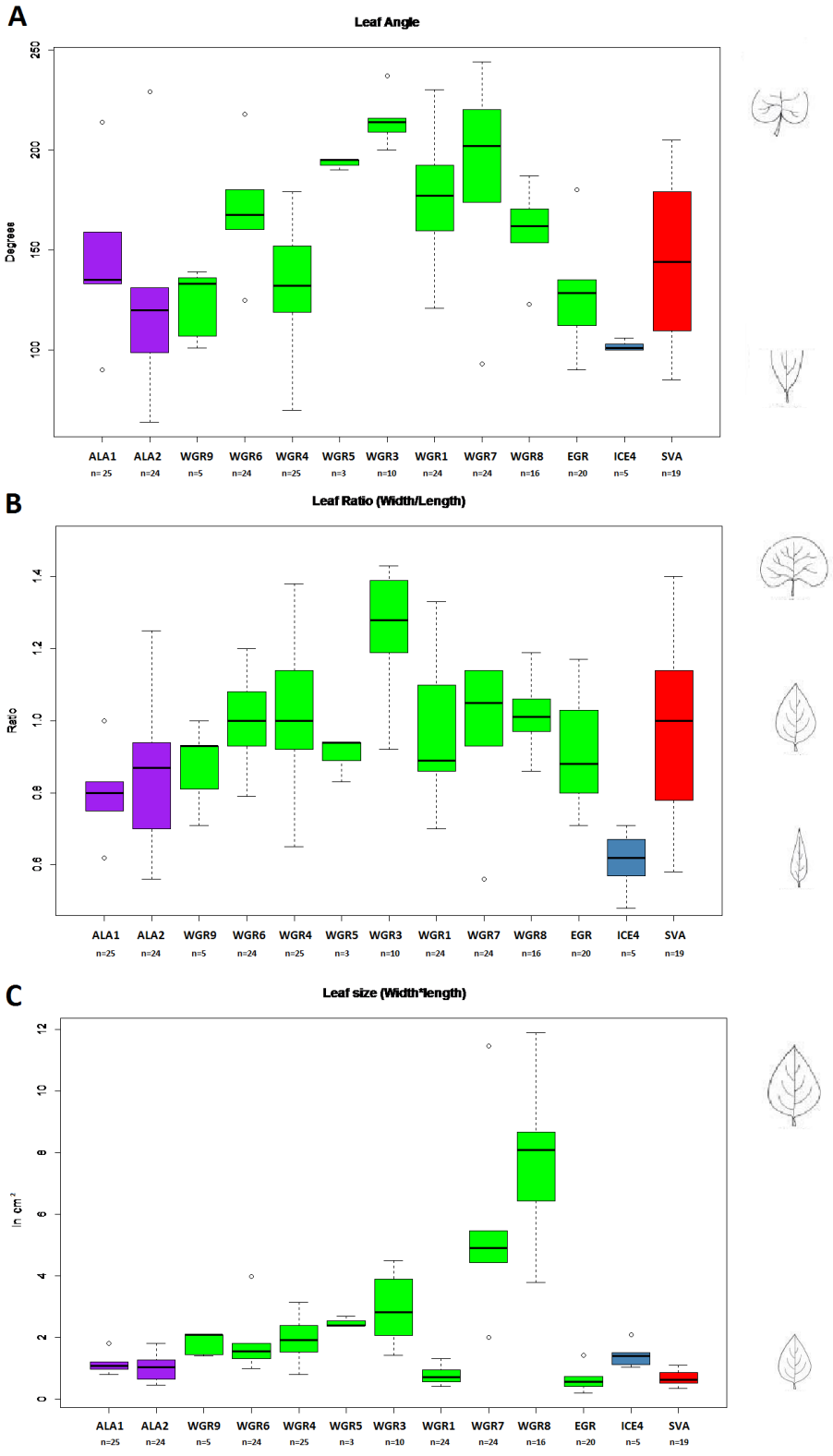

Figure 11: Boxplot from the morphometric analysis of 13 *Cochlearia groenlandica* populations, including the Icelandic alpine population (names abbreviated according to Appendix Table A1). The average of five leaves from an individual was calculated, and the total number of leaves measured is indicated below the population names. The colors indicate the different countries; The United States (purple), Greenland (green), Iceland (blue) and Svalbard (red). Drawn leaf figures in the right margin are from the University of Florida (http://plants.ifas.ufl.edu/education).
A) Leaf base angle. Higher values indicate a subcordate /cordate leaf base shape, while lower values indicate a cuneate/trullate leaf base shape.
B) Leaf ratio (leaf width divided by length). High values indicate reniform leaf shape, while lower values indicate lanceolate or ovate/orbicular leaf shape.
C) Leaf size (leaf width multiplied with length). Higher values indicate larger leaves, while lower values indicate smaller leaves.

The Kruskal-Wallis test showed that there was no significant ($p<0.05$) difference in petal length among the eight populations included in the analysis ($p=0.1105$). Most populations had petals length means around 0.4 cm (fig. 12, Appendix Table A4). Though not significant, two of the West Greenland populations had somewhat longer petals than the remaining populations (WGR7: 0.52 cm; WGR9: 0.48 cm). These two populations had also slightly wider petals. The Svalbard population had the lowest mean value for petal length (SVA: 0.36 cm).

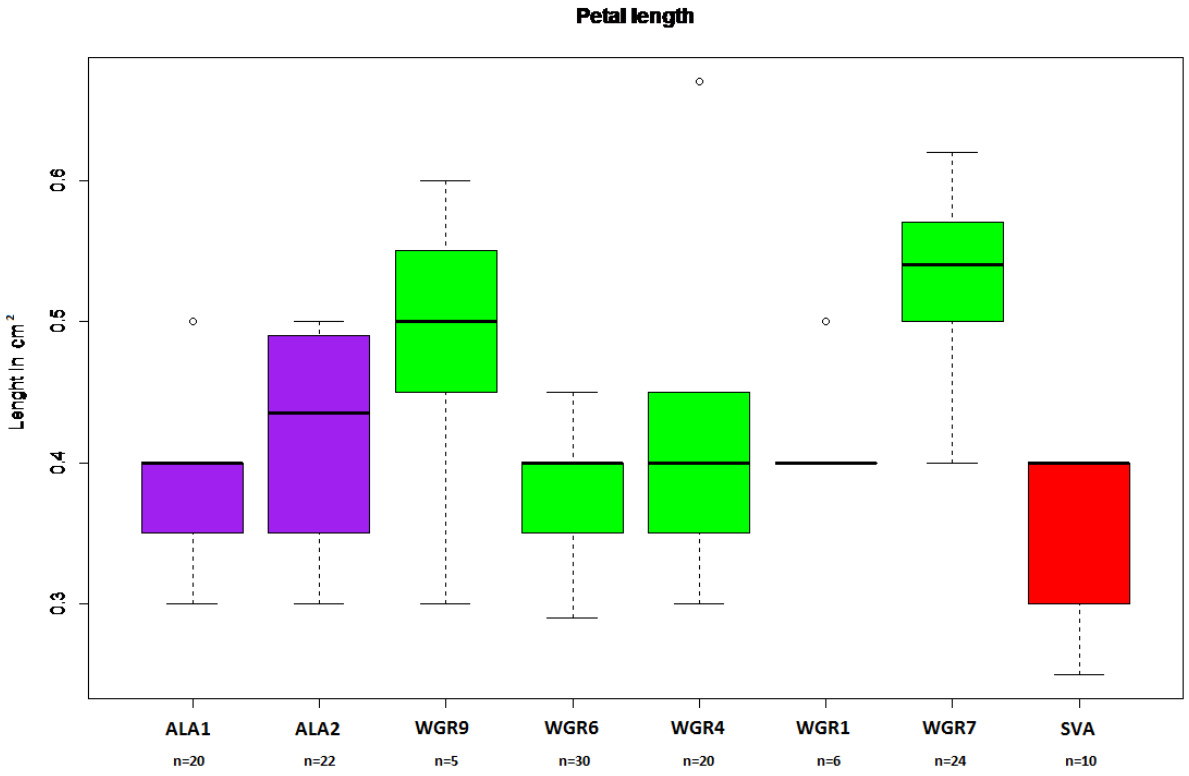

Figure 12: Boxplots depicting the variation in petal length of *Cochlearia groenlandica* from eight populations (names abbreviated according to Appendix Table A1). The average of five petals from an individual was calculated, and the total number of petals measured is indicated below the population names. The colors indicate the different countries; The United States (purple), Greenland (green) and Svalbard (red).

4 Discussion

4.1 Is genetic variation within *C. groenlandica* geographically structured?

The RADseq data in this study revealed a clear geographical structure among the different *C. groenlandica* populations. This is in line with previous studies, which have used RADseq to analyze the genetic variation of *Cochlearia* populations in Norway (Brandrud 2014, Brandrud et al. 2017) and Iceland (Olsen 2015). Brandrud (2014) and Brandrud et al. (2017) found partly a geographical structure among *C. officinalis* populations from Northern Norway, and partly an ecotypic differentiation corresponding to three previously recognized ecotypes (Nordal and Stabbetorp 1990). Olsen (2015) found clear geographical structure among Icelandic *Cochlearia* populations, separating alpine populations from coastal populations, but she also recognized genetic groups among the coastal populations corresponding to a southern, an eastern and a western group.

Based on RADseq data all individuals in this thesis were assigned to distinct populations, even within the focus area West Greenland from where most populations were included. Even geographically close populations were genetically distinguishable. The AMOVA analyses showed that variation within populations was relatively low compared to variation between populations. Such a pattern is expected for a mainly selfing species (Hamrick and Godt 1996) as *C. groenlandica*, and has been described from other small, isolated populations of *Cochlearia* (Nordal and Laane 1990). Only one individual (from an Alaskan population) did not assign unambiguously to a population, and was genetically closer to the East Greenland and Svalbard populations. This could potentially be a result of long-distance dispersal, but as this was found for only one individual it might more likely be a result of contamination of samples in the lab.

All analyses in this thesis used to describe the geographical structure of the *C. groenlandica* populations (figs. 5, 6, 7, 8) show a clear structure corresponding to larger geographical regions. The West Greenland populations are grouped together, and clearly separated from the group including the East Greenland and Svalbard populations. Separation between the western and eastern part of the North Atlantic region is a common pattern found in several other studies of the genetic structure in arctic plant species (e.g. *Cassiope tetragona* D. Don, Eidesen et al. 2007a; *Salix herbaceae* L., Alsos et al. 2009). Eidesen et al. (2013) compared genetic patterns of 17 widespread arctic-alpine plant species to identify general patterns of genetic discontinuity and connectivity. The major genetic borders that they identified correspond to physical barriers to gene flow. The North Atlantic Ocean and the Greenland ice cap constitute one such barrier that corresponds well with the genetic separation found in this thesis.

The Alaskan populations turned out to be genetically closer to the East Greenland and Svalbard populations than they are to the West Greenland populations. This pattern is somehow surprising as these two regions are geographically quite separated on either side of the previous mentioned strong barrier established by the Atlantic Ocean and Greenland ice cap. Further, Eidesen et al. (2013) also suggested the Arctic Ocean as a strong barrier. In previous studies of arctic plant species, Alaskan populations have more often been grouped with East Siberian populations in a genetically distinct Beringian cluster (e.g. *Arctous alpinus* L. Nied., Eidesen et al. 2013; *Draba nivalis* Liljebl.,

Skrede et al. 2009), corresponding to the non-glaciated area during the Pleistocene glaciations, which served both as a refugium and a source for interglacial recolonization of the adjacent areas (Abbott and Brochmann 2003, Westergaard et al. 2010). However, genetic patterns from some studies also suggest a possible connectivity from Alaska through northern parts of arctic Canada to northeastern Greenland (e.g. *Dryas octopetala* L., Skrede et al. 2006; *Cassiope tetragona*, Eidesen et al. 2007a).

In some studies, a clear genetic connection between West Greenland and the eastern part of Arctic Canada has been found (e.g. *Cassiope tetragona*, Eidesen et al. 2007a; *Vaccinium uliginosum* L., Eidesen et al. 2007b; *Salix herbaceae*, Alsos et al. 2009), whereas in other studies populations from the eastern part of Arctic Canada have a closer link towards the Beringian area, but also with some affinities to West Greenland (e.g. *Oxyria digyna* L. Hill., Wang et al. 2016). In this thesis, the *C. groenlandica* population from Baffin (Arctic Canada) has an intermediate position between West Greenland on one side and the Alaskan populations on the other side, which makes sense if one expects an eastward colonization from the Beringian area through North America to West Greenland (Eidesen et al. 2013, Wang et al. 2016).

Despite the existence of large-scale general patterns of genetic and phylogeographic structure within the Arctic as suggested by Eidesen et al. (2013), for other parts of the world it has often been stated that phylogeographic patterns are to a large degree species-specific (Taberlet et al. 1998, Soltis et al. 2006). This is because of stochasticity related to dispersal, establishment and reproduction of single species. Although, migration routes for arctic plants, in order to achieve their present-day distribution, remain for the most-part unknown, long-distance dispersal in arctic plants has taken place at much higher rates than previously thought, which has been demonstrated for several species (Alsos et al. 2007, 2015). Alsos et al. (2015) showed that several species colonized Svalbard postglacially via multiple dispersal event from several source regions situated 280 to >3000 km away. This is probably facilitated by wind over frozen sea ice, driftwood or birds (Alsos et al. 2007). Further, although the Arctic and Atlantic Oceans are strong barriers relative to continuously inhabitable continental areas, these barriers are not impermeable (Dahl 1963). There are for instance several molecular studies, which have shown that gene flow has taken place across the Atlantic Ocean (Bennike 1999, Hagen et al. 2001, Skrede et al. 2009, Alsos et al. 2007, 2015). The genetic patterns found for *C. groenlandica* are likely to have been influenced both by general large-scale migration routes observed for arctic plans as well as stochastic long-distance dispersal events.

4.2 Is genetic variation within *C. groenlandica* related to variation in morphology?

When observing *C. groenlandica* in the field, it was evident that different populations vary with regard to leaf morphology; populations consisting of smaller plants often have lanceolate leaf shape, while populations consisting of larger plants have cordate/reniform leaf shape. This difference in leaf morphology was also to some degree seen in the plants cultivated under controlled conditions, which suggests that variation in leaf morphology is not only environmental affected, but also a result of underlying genetic factors (Nordal and Laane 1990). Leaf traits have previously been recognized as informative in *Cochlearia* when comparing coastal and alpine plants on Iceland (Nordal and Laane 1990, Olsen 2015), and also when comparing ecotypes of *C. officinalis* in northern Norway (Nordal

and Stabbetorp 1990). However, for the *C. groenlandica* populations included in this thesis, there seems not to be any clear correspondence between the genetic large-scale structuring observed based on the RADseq data and the observed variation in leaf morphology.

When analyzing cultivated plants, a significant difference in leaf traits, such as shape and size, was found between the populations (fig. 11). Three of the populations from West Greenland (WGR3, WGR7, WGR8) seemed to have considerably larger leaves (mean >3 cm^2) than the other populations. Two of these populations (WGR3 and WGR7) were growing in urban habitats, and therefore probably got more nutrients from humans, pets and other animals, which are attracted to inhabited areas, as well as having a habitat which is more sheltered compared to harsher environments. The population WGR8 was growing in salt marsh in a protected fjord. Previous studies have found *C. groenlandica* and *C. officinalis* to grow much bigger in size when growing near nutrient rich bird cliff colonies (Eriksen and Nordal 1989, Zmudczyńska-Skarbek et al. 2013, Wojciechowska et al. 2015). Eriksen and Nordal (1989) reported that *C. officinalis* bird cliff colony populations were not only morphologically but also genetically different.

The tundra populations from Alaska (ALA1, ALA2), West Greenland (WGR1), East Greenland (EGR) and Svalbard (SVA), on the other hand, turned out to have rather small leaves (mean ca. 1 cm) compared to the populations which grew in urban or coastal habitats. This might be due to genetic adaptation to the more severe tundra habitat and shorter growing season they are exposed to.

Nordal and Laane (1990) reported arctic or alpine *Cochlearia* plants from Iceland, Svalbard and Norway to have small lanceolate leaves. The leaf ratio mean of the Svalbard plants included in this thesis seems to correspond to measurements done on Svalbard plants by Nordal and Laane (1990), however, the overall variation in leaf ratio obtained for the Svalbard plants in this thesis was much larger than what was the case in Nordal and Laane 1990.

Although not fully corresponding, there seems to be some correlation between leaf size and shape and the habitat the plants grow in. All of the *C. groenlandica* samples in this study grew in habitats with harsh environments as a result of short summers and long winters, but some were additionally exposed to high salt concentrations from coastal waves, while others were exposed to drought due to high sun exposure in the cliff and tundra habitats and still others to high access to nutrients. As the differences in leaf morphology were observed not only in the field, but also when the plants were grown in controlled conditions, it is obvious that variation in leaf morphology is driven not only by changing environmental conditions but also as a result of adaptation to the prevailing habitat. From the results in the present study, it is not possible to conclude whether the differences can be explained by underlying genetic factors or whether epigenetics is a likely reason. Brandrud et al. (2017) suggested that the morphological differences between ecotypes of *C. officinalis* could be explained by partly phenotypic plasticity, genetics and epigenetics. Epigenetic signals such as DNA methylation and RNA or histone modifications can be inherited across generations (Zhang 2008, Jablonka and Raz 2009). Epigenetic variation is often considered as important for populations with low genetic diversity as a source of phenotypic variation (Kalisz and Purugganan 2004, Richards 2008, Yi et al. 2010, Medrano et al. 2014).

Although there are differences in leaf size and shape between populations in this thesis, some of the variation that was found might be due to the measuring method, which was done by several persons. It was particularly challenging to obtain consistent results for the measurements of the leaf base

angle, and outlier measurements were found for most populations (fig. 11A). As small differences in measuring of the leaf angle may results in large differences, it was hard to find the “golden way” of measuring. To mitigate the eventual measuring-errors, all outliers were measured over again. A better method for future studies of leaf shape could perhaps be to use a computer program developed for landmark-based morphometrics.

There were no significant differences in petal length between the populations. The West Greenland populations WGR9 and WGR7 had slightly longer petals, and the Svalbard population had slightly shorter petals. WGR9 grew in grassy cliffs by the coast and WGR7 grew in urban habitations. Plants from these populations were of overall large size, both when observed in the field and when cultivated under controlled conditions. The Svalbard population grew in a tundra habitat, and the plants were of overall small in size still when cultivated in controlled conditions. Nordal and Laane (1990) have previously used flower size (as well as seed size and mode of reproduction) to discuss whether *Cochlearia* plants on Iceland should be grouped with *C. officinalis*, and have determined this trait to be informative when comparing *Cochlearia* plants. However, this character seems not to be important for discrimination between populations or genetic groups within *C. groenlandica*.

4.3 Is genetic variation within *C. groenlandica* related in chromosome number or ploidy level?

The produced flow cytometry data and chromosome counts from several populations across different arctic regions have added considerable knowledge about *C. groenlandica* and confirm that as of today all known observations of *C. groenlandica* are 2n=14. The genetic variation found within *C. groenlandica*, thus, does not correspond to variation in ploidy levels or basic chromosome numbers. It was quite clear from the flow cytometry and chromosome counting that all the analyzed populations of *C. groenlandica* were diploid. The relative fluorescence (RF) values varied from 0.640 to almost 0.740, but chromosome counting of selected samples confirmed that the whole range represented diploidy.

Polyploidy is common in *Cochlearia,* and the different ploidy levels within the x=6 series are mainly related to distinct species (e.g. *C. pyreneica* 2n=12, *C. officinalis* 2n=24, *C. aestuaria* 2n=48). A similar ploidy series has so far not been found for the basic chromosome number x=7, which is widespread in the Arctic and North Atlantic areas (Gill 1971a, 1973, Nordal and Laane 1990, Koch et al. 1996), and all chromosome counts reported for *C. groenlandica*, as presently circumscribed (Elven 2011), are 2n=14 (Rice et al. 2014). A couple of 2n=12 counts have been linked to the *C. groenlandica* name, but these are from Iceland (Koch et al. 1996, Koch et al. 1998) and most likely not *C. groenlandica* (Olsen 2015). The 2n=12 Icelandic *Cochlearia* plants were previously only reported to be found in a restricted part of the southern coast of Iceland until Olsen (2015) counted and found it to be more widespread; the 2n=12 plants are found along the whole coast of Iceland, and are genetically quite distinct from *C. groenlandica* found in arctic areas (Olsen 2015; see discussion below).

Löve and Löve (1976a) described the genus *Cochleariopsis* to include taxa with x=7 previously described as *Cochlearia*. However, this new genus has generally not been accepted by other experts (Elven 2011), as the morphological differences between plants with x=6 and x=7 to a large degree are

minor, and the chromosome difference probably is a result of tetrasomy (Gill 1971, 1973, Nordal and Laane 1990). Olsen (2015) concluded that the Icelandic *Cochlearia* plants did not form genetic clusters according to chromosome number (2n=12 and 2n=14). This is in accordance with the RADseq results from this thesis, where the Icelandic coastal populations with 2n=14 (ICE1, ICE5) are genetically closer to the Icelandic coastal population with 2n=12 (ICE3) than they are to the arctic *C. groenlandica* populations with 2n=14 (see further discussion below).

4.4 How are the Icelandic *Cochlearia* populations genetically related to *C. groenlandica*?

When adding the Icelandic *Cochlearia* populations to the dataset of *C. groenlandica*, the coastal populations (ICE1, ICE3, ICE5) were genetically clearly separated from the *C. groenlandica* populations. However, the Icelandic alpine populations (ICE4) turned out to be genetically closer to the East Greenland and Svalbard populations of *C. groenlandica*. Based on the results from the STRUCTURE analysis, this population was admixed between the cluster consisting of the other Icelandic populations (orange cluster, fig. 6), and the cluster consisting of East Greenland, Svalbard and Alaska (purple cluster, fig. 5), but with largest assignment to last mentioned cluster. The same pattern was found in the network analysis (fig. 8), separating the Icelandic coastal populations from the remaining populations, and with the Icelandic alpine population close to the East Greenland and Svalbard populations. The same genetic link between Icelandic alpine populations and *C. groenlandica* was also found by Olsen (2015), who included two Icelandic alpine populations (of which one was the same as the one used in this thesis). Both populations were genetically closer to the Svalbard populations than they were to the Icelandic coastal populations.

The morphological analyses showed that the Icelandic alpine population (ICE4) had small leaf size, cuneate/trullate leaf base shape and lanceolate leaf shape, comparable to what was found by Nordal and Laane (1990) and Olsen (2015). Olsen (2015) did not include *C. groenlandica* from Svalbard in her morphological analysis, but relied on other studies (Nordal and Laane 1990) when she concluded that the Icelandic alpine plants and plants from Svalbard were morphologically similar. The results from this thesis confirm that Icelandic alpine plants are similar in leaf size to the *C. groenlandica* tundra populations from East Greenland, Svalbard and Alaska. The leaves of the Icelandic alpine population were even more extreme when it comes to the cuneate/trullate leaf base shape and lanceolate leaf shape compared to the Svalbard tundra populations, which overall seemed to have larger variation in leaf shape and leaf shape base.

Based on similarity in genetics, morphology and chromosome number, Olsen (2015) suggested that the Icelandic alpine plants and the Svalbard plants share a common evolutionary history, and that the Icelandic alpine plants should be referred to *C. groenlandica*. In this thesis, when analyzed together with an even larger sampling of *C. groenlandica* populations representing other arctic regions, this conclusion has been supported.

Olsen (2015) further suggested that the observed genetic patterns could be a result of long-distance dispersal between Iceland and Svalbard or other arctic areas. Dispersal of seeds to (or from) Iceland has been heavily discussed in the tabula rasa versus glacial survival debate (Nordal 1987). Supporters

of the tabula rasa theory have suggested dispersal of diaspores, with or without adaptations for long dispersal, to occur with strong winds carrying diaspores across sea-ice in winter (Brochmann and Steen 1999), as ice rafted debris (Buckland and Dugmore 1991) or with aerial transport, ocean currents and migrating birds (Nordal 1987, Johansen and Hytteborn 2001). The studies by Alsos et al. (2007, 2015) also suggested that for plant colonization of Svalbard, the limiting factor was establishment and not dispersal per se; the hardiest species have colonized the arctic archipelago more frequently than the less hardy ones, regardless of morphological adaptations. It is therefore not unreasonable to argue that *C. groenlandica* might have dispersed from Svalbard to Iceland, or the other way around. Olsen (2015) included only *C. groenlandica* populations from Svalbard in her genetic analysis and was unable to evaluate the Icelandic alpine plants in a larger arctic setting. With more arctic regions included in this thesis, it is clear that the link from the Icelandic alpine plants to the Arctic is indeed towards the eastern part of the North Atlantic Ocean, as they group closest with the East Greenland and Svalbard populations. This corresponds to the earlier discussed large-scale general patterns of genetic and phylogeographic structure within the Arctic as suggested by Eidesen et al. 2013.

The Icelandic coastal populations (ICE1, ICE3, ICE5) were isolated from each other by larger splits in the network analysis than the variation found among the *C. groenlandica* populations, which represent a much larger geographical distribution. This is especially true for the separation between the 2n=12 coastal population (ICE3) and the two 2n=14 coastal populations (ICE1, ICE5). However, in Olsen (2015), where a larger sampling of Icelandic populations were included, no clear association between genetic structure and chromosome number (2n=12, 14) was found. The separation observed in this thesis between the 2n=12 and 2n=14 coastal populations, thus, more likely corresponds to the geographical structure found by Olsen (2015), where the 2n=14 populations (ICE1, ICE5) belong to western group (which also included 2n=12 populations in the study by Olsen (2015)) and the 2n=12 population (ICE3) belongs to a southern group.

The Icelandic coastal populations were unfortunately not included in the morphological analyses in this thesis (due to lack of living plants). However, Olsen (2015) analyzed the morphology of the Icelandic coastal populations and found that they had overall larger leaves with more subcordate/cordate leaf base angle and ovate/orbicular leaf shape, separating them from the Icelandic alpine population, and much more similar to what was found in this thesis for the West Greenland populations (WGR7, WGR8) growing in urban habitats close to the coast.

Olsen (2015) concluded that the Icelandic coastal population with 2n=14 were genetic and morphological different from the alpine plants with 2n=14, but did not discuss the evolutionary relationships or origin of these plants. An obvious question would have been whether these plants could have a separate link to 2n=14 plants in the Arctic. Based on the results in this thesis, where populations from several arctic regions were included, such a scenario seems however not to be the case, and it is more likely that the Icelandic coastal 2n=14 plants have originated separately in Iceland.

4.5 Further Research

Even though clear geographical genetic structure was found for the analyzed *C. groenlandica* populations, the overall genetic variation seemed relatively limited compared to the observed variation between the Icelandic populations. Ideally populations of more *Cochlearia* species should have been included to evaluate the delimitation of *C. groenlandica*, but based on the present results neither variation in genetics, morphology or chromosome number indicate that the analyzed populations constitute more than one species. For future research it would, however, be important to include populations covering the whole circumarctic distribution of *C. groenlandica,* first of all Russian and Siberian populations, which were unfortunately not available for this thesis. Without a better coverage of the whole distribution area, conclusions about dispersal patterns are only preliminary, for instance the inferred relationship between the Alaskan and East Greenland/Svalbard populations. As several taxa have been described within the arctic area, it would also be especially important to compare material that in morphology and distribution correspond to what has been described as *C. groenlandica, C. arctica* and *C. oblongifolia* in order to consider whether they should be treated as species on their own right, or alternatively as subspecies or ecotypes. The specimens from the Alaskan populations in my analyses could potentially be either *C. groenlandica* or *C. arctica* as they are collected in an area where both taxa are reported to occur (Elven 2011). Based on my data, these populations did not differentiate neither genetically nor morphologically from the other *C. groenlandica* populations and gave no indications of a separate taxon. However, a much larger sampling specifically aimed at this question would be needed to make any firmer conclusions.

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Appendix

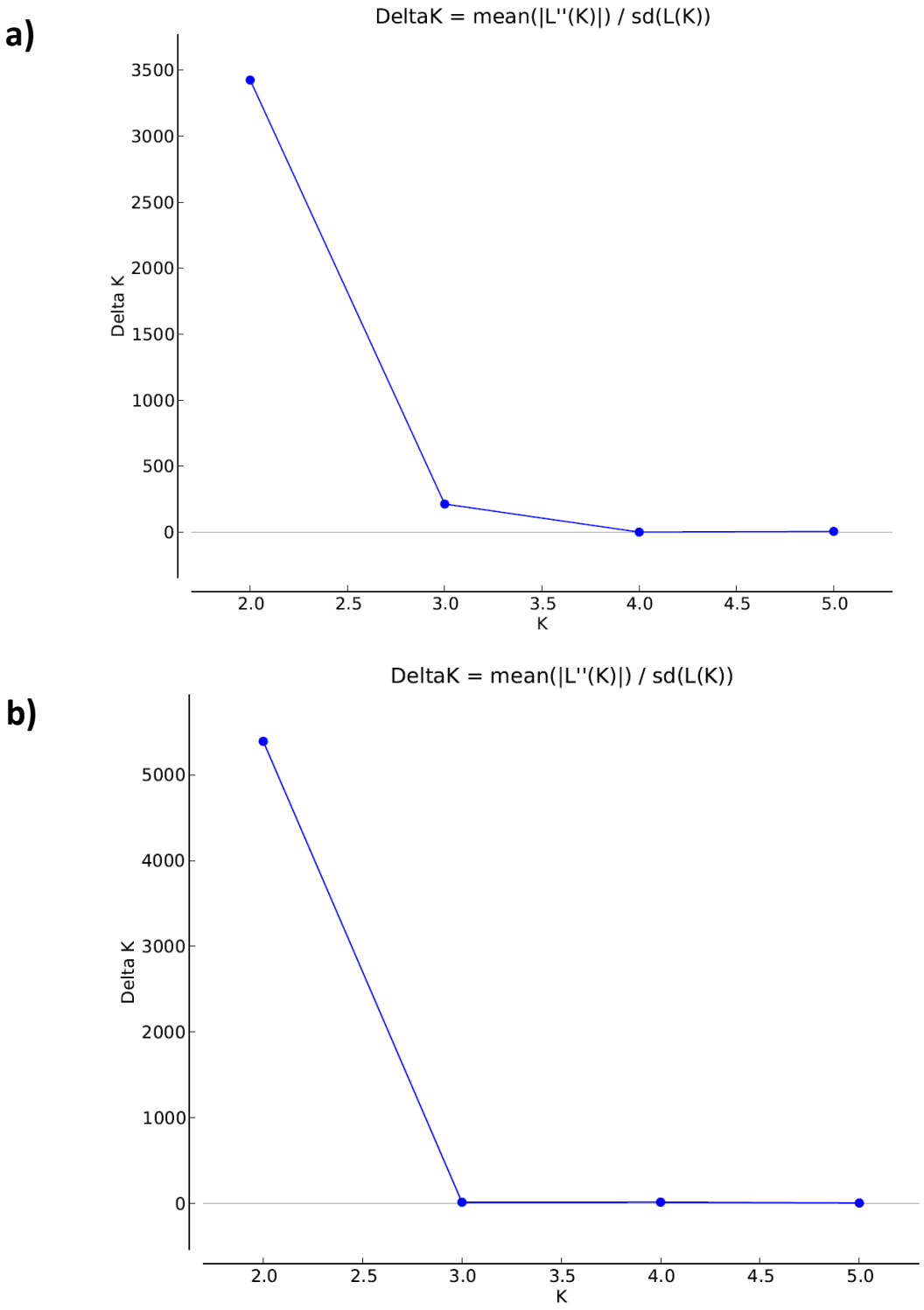

Figure A1: Delta K values used to evaluate the optimal number of K from the STRUCTURE analysis. a) With Iceland included, delta K indicates K=2 to be the most optimal number of groups, but also K=3 has a delta K value higher than 0. b) Without Iceland included, delta K indicates K=2 to be the optimal number of groups.

Table A1: Collection data of *Cochlearia* in the Arctic and North Atlantic. "Name" is name used in this thesis, "Collection number" is the number used by the collectors. "RADseq", "Flow cyt." and "Morph." indicate the number of individuals remaining from the RADseq library, for flow cytometry and morphology analyses (petals in parentheses). Collectors: AKB= Anne Krag Brysting, CSB= Charlotte, Sletten Bjorå, EB= Eirin Bruholt IN= Inger Nordal, LNO=Luka Natassja Olsen.

Name	Collection no.	Country	Locality	Habitat	Latitude	Longitude	Date	Collectors	RADseq	Flow cyt.	Morph.
ALA1	LG09-A-48-6, LG09-A-48-10	USA	Seward Peninsula, Nome, Alaska	-	64°29'44.8''	165°25'49.9''	2009	A. L. S. Gustaffson	6	5	25 (20)
ALA2	COGR	USA	Barrow, Alaska	Tundra	71°31'05''	156°54'8''	08.07.2015	J. Prevey	5	6	24 (22)
BAF1	4468	Canada	Cape Dorset, Qikiqtaaluk region, Nunavut	Beach	64°00'00''	76°00'00''	03.07.2015	J.M. Saarela, R.D. Bull	6	-	-
WGR1	BBB10	Greenland	Præstefjeldet (Palasip Qaqqaa), Sisimiut	Tundra	66°57'44.3''	53°43'17.3''	10.08.2017	EB, CSB, AKB	6	5	24 (6)
WGR2	BBB8	Greenland	Paaraarsuk, Sisimiut	Urban, beach cliff	66°56'14.5''	53°40'31.9''	08.08.2017	EB, CSB, AKB	6	-	-
WGR3	BBB9	Greenland	Christian 28-ip Aqq., Sisimiut	Urban, grassy cliff	66°56'21.1''	53°40'14.3''	08.08.2017	EB, CSB, AKB	-	2	10
WGR4	BBB4	Greenland	Røde elv, Qeqertarsuaq	Sandy beach	69°15'4.31"	53°29'19.1''	06.08.2017	EB, CSB, AKB	-	6	25 (20)
WGR5	BBB7	Greenland	Tipiooq Kangilleq, Asiaat	Bare coastal rock	68°43'09''	52°49'08''	07.08.2017	EB, CSB, AKB	6	1	3
WGR6	BBB3	Greenland	Mudderbugten, Disko Island	Gravelly beach	69°43'51.08"	51°55'51.8''	04.08.2017	EB, CSB, AKB	6	6	24 (30)
WGR7	COCARC	Greenland	Nuuk	Urban	64°11'26.81''	51°41'43.59''	03.09.2017	I. B. D. Jacobsen	6	6	24 (24)
WGR8	COCARC 04	Greenland	Kobberfjorden	Saltmarsh	64°81'08.6''	51°23'27.72''	22.08.2017	I. B. D. Jacobsen	-	2	16
WGR9	BBB2	Greenland	Close to Sermermiut, Illulissat	Grassy cliff	69°12'21.3''	51°08'56.6''	03.08.2017	EB, CSB, AKB	5	1	5 (5)
EGR	ZACK	Greenland	Zackenberg	Tundra	74°47'31.39''	20°52'08.48''	??.??.2015	L. Stewart	5	6	20
ICE1	LO.14-1	Iceland	Stokkseyri, Arnessýsla	Beach cliff	63°83'85.83''	21°07'51.94''	11.08.2014	LNO, AKB, IN	4	-	-
ICE3	LO.14-4	Iceland	Ingòlfshöfði, Austre-Skaftafellssýsla	Beach cliff	63°42'03.06''	16°63'77.78''	13.08.2014	LNO, AKB, IN	3	-	-
ICE4	LO.14-7	Iceland	Eirìkstaðir, Norður-Mùlasýsla	Alpine	65°14'34.44''	15°47'17.5''	14.08.2014	LNO, AKB, IN	6	1	5
ICE5	LO.14-12	Iceland	Hafnir, Gullbringusýsla	Beach cliff	63°93''59.2''	22°68''89.4'	17.08.2014	LNO, AKB, IN	3	-	-
SVA	MOS	Svalbard	Moskuslaguna, Adventfjorden, Nordeskjöld Land	Tundra	78°14'43.12''	15°43'51.17''	01.09.2017	P. B. Eidsen	5	5	19 (10)

Table A2: a) The P1 adapters used for the RADseq protocol, with the barcodes highlighted in bold red. b) The P2 adapters used for the RADseq protocol, with the barcodes highlighted in bold red.

a)

P1t_D506_25	AATGATACGGCGACCACCGAGATCTACAC**TAATCTTA**ACACTCTTTCCCTACACGACGCTCTTCCGATCT**ACTGAT**TGC*A
P1t_D506_4	AATGATACGGCGACCACCGAGATCTACAC**TAATCTTA**ACACTCTTTCCCTACACGACGCTCTTCCGATCT**TGACCA**TGC*A
P1t_D506_23	AATGATACGGCGACCACCGAGATCTACAC**TAATCTTA**ACACTCTTTCCCTACACGACGCTCTTCCGATCT**GAGTGG**TGC*A
P1t_D506_XY	AATGATACGGCGACCACCGAGATCTACAC**TAATCTTA**ACACTCTTTCCCTACACGACGCTCTTCCGATCT**CTCATC**TGC*A
P1t_D507_25	AATGATACGGCGACCACCGAGATCTACAC**CAGGACGT**ACACTCTTTCCCTACACGACGCTCTTCCGATCT**ACTGAT**TGC*A
P1t_D507_4n	AATGATACGGCGACCACCGAGATCTACAC**CAGGACGT**ACACTCTTTCCCTACACGACGCTCTTCCGATCT**TGACCA**TGC*A
P1t_D507/In23	AATGATACGGCGACCACCGAGATCTACAC**CAGGACGT**ACACTCTTTCCCTACACGACGCTCTTCCGATCT**GAGTGG**TGC*A
P1t_D507/InXY	AATGATACGGCGACCACCGAGATCTACAC**CAGGACGT**ACACTCTTTCCCTACACGACGCTCTTCCGATCT**CTCATC**TGC*A
P1t_D508/In25	AATGATACGGCGACCACCGAGATCTACAC**GTACTGAC**ACACTCTTTCCCTACACGACGCTCTTCCGATCT**ACTGAT**TGC*A
P1t_D508/In4	AATGATACGGCGACCACCGAGATCTACAC**GTACTGAC**ACACTCTTTCCCTACACGACGCTCTTCCGATCT**TGACCA**TGC*A
P1t_D508/In23	AATGATACGGCGACCACCGAGATCTACAC**GTACTGAC**ACACTCTTTCCCTACACGACGCTCTTCCGATCT**GAGTGG**TGC*A
P1t_D508/InXY	AATGATACGGCGACCACCGAGATCTACAC**GTACTGAC**ACACTCTTTCCCTACACGACGCTCTTCCGATCT**CTCATC**TGC*A

P1b_D506_25	[Phos]**ATCAGT**AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT**TAAGATTA**GTGTAGATCTCGGTGGTCGCCGTATCAT*T
P1b_D506_4	[Phos]**TGGTCA**AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT**TAAGATTA**GTGTAGATCTCGGTGGTCGCCGTATCAT*T
P1b_D506_23	[Phos]**CCACTC**AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT**TAAGATTA**GTGTAGATCTCGGTGGTCGCCGTATCAT*T
P1b_D506_XY	[Phos]**GATGAG**AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT**TAAGATTA**GTGTAGATCTCGGTGGTCGCCGTATCAT*T
P1b_D507_25	[Phos]**ATCAGT**AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT**ACGTCCTG**GTGTAGATCTCGGTGGTCGCCGTATCAT*T
P1b_D507_4	[Phos]**TGGTCA**AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT**ACGTCCTG**GTGTAGATCTCGGTGGTCGCCGTATCAT*T
P1b_D507/In23	[Phos]**CCACTC**AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT**ACGTCCTG**GTGTAGATCTCGGTGGTCGCCGTATCAT*T
P1b_D507/InXY	[Phos]**GATGAG**AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT**ACGTCCTG**GTGTAGATCTCGGTGGTCGCCGTATCAT*T
P1b_D508/In25	[Phos]**ATCAGT**AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT**GTCAGTAC**GTGTAGATCTCGGTGGTCGCCGTATCAT*T
P1b_D508/In4	[Phos]**TGGTCA**AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT**GTCAGTAC**GTGTAGATCTCGGTGGTCGCCGTATCAT*T
P1b_D508/In23	[Phos]**CCACTC**AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT**GTCAGTAC**GTGTAGATCTCGGTGGTCGCCGTATCAT*T
P1b_D508/InXY	[Phos]**GATGAG**AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT**GTCAGTAC**GTGTAGATCTCGGTGGTCGCCGTATCAT*T

b)

IndP2t_2s	[Phos]AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC**CGATGT**ATCAGAACAA
IndP2t_3s	[Phos]AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC**GATCAG**ATCAGAACAA
IndP2t_4s	[Phos]AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC**TGACCA**ATCAGAACAA
IndP2t_6s	[Phos]AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC**GCCAAT**ATCAGAACAA
IndP2t_9s	[Phos]AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC**ACTTGA**ATCAGAACAA
IndP2t_10s	[Phos]AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC**TAGCTT**ATCAGAACAA
IndP2t_12s	[Phos]AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC**CTTGTA**ATCAGAACAA

Table A3: Results from AMOVA analyses including a) 16 populations and 78 samples of *Cochlearia*, describing the distribution of genetic variation between and within populations, as well as between higher level groups, selected based on geographical distribution; 1) ALA1 and ALA2, 2) BAF and WGR1, WGR2, WGR5, WGR6, WGR7 and WGR9, 3) EGR and SVA, and 4) ICE1, ICE3, ICE4 and ICE5. Based on 12,304 SNPs obtained from RADseq data. b) 12 populations and 58 samples of *Cochlearia groenlandica*, describing the distribution of genetic variation between and within populations, as well as between higher level groups, selected based on geographical distribution; 1) ALA1 and ALA2, 2) WGR1, WGR2, WGR5, WGR6 and WGR9, 3) EGR and SVA, 4) WGR7 and BAF. Based on 12,090 SNPs obtained from RADseq data.

Source of variation	d.f.	Sum of squares	Variance components	Percentage variance
(a) All: 16 populations, 78 individuals				
Among populations	14	59131.38	791.03406	86.62
Within populations	63	7696.900	122.17302	13.38
Geographical area: 4 groups: (Alaska), (WGR), (EGR+SVA),(ICE)				
Among groups	4	33509.93	417.15964	41.30
Among populations within groups	10	25621.45	470.83347	46.61
Within populations	63	7696.900	122.17302	12.09
(b) Without Iceland: 12 populations, 58 individuals				
Among populations	10	24659.01	424.22900	64.49
Within populations	47	10978.18	233.57837	35.51
Geographical area: 4 groups: (Alaska), (WGR), (EGR+SVA), (WGR7+BAF)				
Among groups	3	11345.63	140.24218	20.27
Among populations within groups	7	13313.38	317.89860	45.96
Within populations	47	10978.18	233.57837	33.77

Table A4: Morphometry statistics including mean, standard error and standard deviant of a) leaf traits and b) petal traits. Names abbreviated according to Appendix Table A1. The average of five leaves from an individual was calculated, and the total number of leaves measured is indicated in the first column (n)

a)

n	Name	Leaf ratio (in cm^2)			Leaf size (in cm)			Leaf length (in cm)			Leaf width (in cm)			Leaf angle (in degrees)		
		Mean	Std. Error	Std. Dev	Mean	Std. Error	Std. Dev	Mean	Std. Error	Std. Dev	Mean	Std. Error	Std. Dev	Mean	Std. Error	Std. Dev
n=25	ALA1	0.79	0.04	0.09	1.1	0.05	0.23	1.18	0.02	0.11	0.93	0.02	0.12	143.36	6.06	30.29
n=24	ALA2	0.86	0.04	0.19	1.01	0.08	0.38	1.08	0.05	0.22	0.91	0.04	0.21	120.88	6.82	33.42
n=4	WGR9	0.88	0.05	0.1	1.82	0.15	0.33	1.44	0.06	0.14	1.33	0.04	0.08	123.2	7.11	15.9
n=24	WGR6	1.00	0.02	0.1	1.65	0.13	0.57	1.28	0.05	0.23	1.27	0.04	0.17	168.71	3.84	18.79
n=25	WGR4	1.01	0.04	0.19	1.96	0.13	0.64	1.4	0.06	0.32	1.37	0.05	0.23	134.04	5.17	28.85
n=3	WGR5	0.90	0.03	0.05	2.5	0.08	0.14	1.67	0.05	0.09	1.5	0	0	193.33	1.36	2.36
n=10	WGR3	1.25	0.06	0.18	3	0.35	1.1	1.52	0.07	0.23	1.91	0.15	0.46	214.3	2.91	9.21
n=24	WGR1	0.97	0.04	0.18	0.77	0.05	0.27	0.88	0.02	0.1	0.86	0.04	0.22	175.33	5.89	28.85
n=24	WGR7	1.05	0.05	0.26	5.03	0.24	1.75	2.21	0.09	0.43	2.25	0.09	0.43	187.46	8.82	43.22
n=16	WGR8	1.02	0.02	0.08	7.67	0.55	2.2	2.71	0.1	0.39	2.77	0.12	0.46	160.75	4.15	16.59
n=20	EGR	0.94	0.03	0.14	0.59	0.06	0.27	0.79	0.04	0.19	0.73	0.04	0.16	127.2	4.79	21.44
n=5	ICE4	0.61	0.04	0.08	1.43	0.17	0.37	1.54	0.13	0.29	0.92	0.04	0.1	102	1.02	2.28
n=19	SVA	0.97	0.05	0.21	0.68	0.05	0.22	0.85	0.05	0.21	0.79	0.03	0.12	141.63	8.25	35.98

b)

n	Name	Petal ratio (in cm^2)			Petal length (in cm)			Petal width (in cm)			Sepal length (in cm)		
		Mean	Std. Error	Std. Dev	Mean	Std. Error	Std. Dev	Mean	Std. Error	Std. Dev	Mean	Std. Error	Std. Dev
n=20	ALA1	0.42	0.02	0.07	0.39	0.01	0.07	0.16	0.01	0.03	0.27	0.01	0.04
n=22	ALA2	0.39	0.01	0.05	0.41	0.02	0.08	0.16	0.01	0.04	0.24	0.01	0.03
n=5	WGR9	0.41	0.03	0.06	0.48	0.05	0.1	0.19	0.01	0.02	0.25	0.02	0.04
n=30	WGR6	0.34	0.02	0.11	0.38	0.01	0.04	0.12	0.01	0.04	0.22	0.01	0.03
n=20	WGR4	0.51	0.03	0.12	0.41	0.02	0.09	0.21	0.01	0.04	0.27	0.01	0.04
n=6	WGR1	0.42	0.02	0.06	0.42	0.02	0.04	0.18	0.01	0.03	0.23	0.02	0.05
n=25	WGR7	0.46	0.01	0.07	0.52	0.01	0.07	0.24	0.01	0.03	0.31	0.01	0.05
n=10	SVA	0.41	0.05	0.14	0.36	0.02	0.06	0.14	0.01	0.04	0.21	0	0.02