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Morphological Diversity in *Laminaria digitata*

-different Species or different Phenotypes?

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Marine Coastal Development
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Abstract

Laminaria digitata-like specimens from the Trondheimsfjord, Hitra and Svalbard were studied using traditional morphological characteristics together with DNA barcoding of the mtDNA COI-gene. The phylogenetic analyses based on the mtDNA COI sequence data identified two species in the material from the mainland of Norway; *Laminaria digitata* and *Laminaria hyperborea*, while the material from Svalbard was identified as *Saccharina groenlandica*, which has likely been misidentified as *L. digitata* in the past.

Both *L. digitata* and *L. hyperborea* from Brænnebukta, the Trondheimsfjord, showed large seasonal variability in the morphology of the lamina, from a narrow lamina and a great angle between stipe and the lamina in February to a wide lamina and small angle between stipe and the lamina in September. Based on the large seasonality in lamina morphology identified in this study, the best way to distinguish between *L. digitata* and *L. hyperborea* is to look at the shape and flexibility of the stipe. *Laminaria hyperborea* has a stiff and close to circular cross sections of stipe, while *L. digitata* has a more flattened cross sections and bendable stipe.

Several different phenotypes of *L. digitata* were found in the same site in Brænnebukta, indicating that there are several factors affecting the morphology of the specimens; these comprise environmental factors, genetic differences, mechanical influences and shading between specimens.

Saccharina groenlandica collected around Svalbard seemed to be affected by local environmental conditions; showing large variability in morphology both of the stipe and the lamina depending on where they were collected. Since *S. groenlandica* is very plastic it is difficult to describe any distinct morphological characters that distinguish it from *L. digitata*, but results indicates that *S. groenlandica* has a shorter stipe than *L. digitata*. The results of the study suggest that *S. groenlandica* is more abundant than *L. digitata* in Svalbard waters.

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Introduction

Kelp forests are more diverse and productive than terrestrial forests (Steneck et al. 2002). The mainland of Norway and its islands have, with over 100 000 km, the second longest coastline in the world (Thuesen & Røvik 2014), and approximately 10 000 square kilometers of the Norwegian coast is covered with kelp forests (Steen 2009). Since the kelp in the genus *Laminaria* are some of the most important macroalgae in temperate and polar rocky shore lines (Bartsch et al. 2008), it is important to have a broad knowledge about this genus. Within *Laminaria* as well as within the family Laminariaceae there is great phenotypic plasticity (Kain 1979; McDevit & Saunders 2010). The morphologic plasticity identified in many macroalgae is a response to environmental factors and can make species identification difficult (Saunders 2005). Some of the confusion in the classification of *Laminaria* has been clarified and the more questionable species have been merged into established taxa (Kain 1979; Bartsch et al. 2008).

Species in the genera *Laminaria* Lamouroux (1813) and *Saccharina* Stackhouse (1809) were earlier included in the same genus; *Laminaria*. After phylogenetic analyses showed that *Laminaria* consisted of two clades (Yoon et al. 2001), it was suggested to divide the genus into *Saccharina* and *Laminaria* (Lane et al. 2006). The name *Saccharina* Stackhouse was available to use for one of the clades and also predated *Laminaria* Lamouroux, but *Laminaria* had been kept because of its common use (Lane et al. 2006). Both clades include both digitated and simple-bladed species (Bartsch et al. 2008).

Along the Norwegian coast, four species from *Laminaria* and *Saccharina* are known; *Laminaria digitata*, *Laminaria hyperborea* and *Saccharina latissima* (Rueness 1998) are most common, and a small species of *Laminaria* called *Laminaria gunneri* has been found a few times in Finnmark (Rueness 1977). In Svalbard waters three species have been found in previous studies; *Laminaria solidungula , L. digitata* and *S. latissima* (Svendsen 1959; Fredriksen & Kile 2012).

Saccharina groenlandica has been reported from the eastern Arctic and Atlantic Canada after DNA-barcoding specimens of kelp (McDevit & Saunders 2010). Formerly only three species of Laminariaceae (*Saccharina latissima*, *Laminaria digitata* and *Laminaria solidungula*) were known from that area. In the Pacific Ocean, *S. groenlandica* has been recorded as either having a branching or a non-branching lamina, and by that either resembling *L. digitata* or *S. latissima* (McDevit & Saunders 2010; Longtin & Saunders 2013). This is probably the reason why *S. groenlandica* was not detected in previous studies. Of all specimens sampled in the Maritime Provinces, 1/4th was digitated *S. groenlandica* and consequently being more abundant than *L. digitata* (Longtin & Saunders 2013). Similarly, earlier studies of macroalgae on Svalbard (Svendsen 1959; Fredriksen & Kile 2012) only registered three species from the genera *Saccharina* and *Laminaria*. An unpublished study using molecular systematics of kelp collected around Svalbard carried out by University Centre in Svalbard (UNIS) bachelor students (AB202-course report 2012), showed that *S. groenlandica* is also present around Svalbard. *Laminaria digitata* was found in one of three locations sampled, namely in the Kongsfjord. There are no earlier publications regarding *S. groenlandica* around Svalbard, and it is likely that specimens of *S. groenlandica* were mistakenly identified as *L. digitata*.

The last decades have seen few studies investigating the morphology of kelp in Norway and Svalbard. The new possibilities currently available utilizing DNA-barcoding in combination with thorough morphological studies allows for new perspectives in taxonomic research, allowing comparison of morphological and genetic variability (McDevit & Saunders 2009).

At the east coast of Svalbard there are two locations with kelp forest that are mentioned in the management plan from Sysselmannen (The Governor of Svalbard which is responsible for both environmental conservations and the police unit); Tommeløyane in Hinlopen and Rossøya, north of Hinlopen, these areas are considered to have a very high species richness (Sysselmannen 2013).

Morphology

All the three species examined consist of a holdfast or hapter, used to attach the kelp to a hard substrate (Fig. 1). The holdfast is not a root as known from land plants that take up nutrients and water, the algae takes up nutrients and water through the whole thallus (Rueness 1998). The stipe can be of variable length and the lamina is digitated and grows from the meristem, which is the transition zone between the stipe and the lamina and new cells are formed (growth zone) (Kain 1979). In the meristem the growth occurs by cell division (Rueness 1998). An experiment on growth rhythm in *Laminaria digitata* showed that in the southern parts of Norway the highest growth rate was from February to April (Sundene 1964), while in *Laminaria hyperborea*, the fast growth period was from January to June (Lüning 1969).

In a study of the morphology of *Laminaria hyperborea*, Sjøtun and Fredriksen (1995) concluded that the morphology of the kelp is more affected by wave exposure than tidal currents.

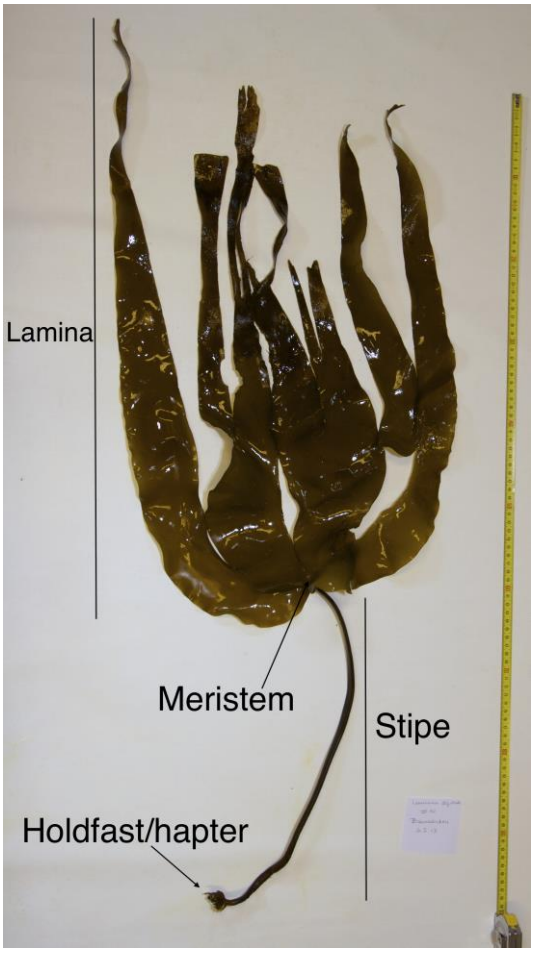

Figure 1: Morphology of *Laminaria digitata*.

Terms

Genotype: the genetic composition of an organism (Lawrence 1996).
Phenotype: visible or measurable biochemical and physical characteristics of an organism. Phenotype is the result of interaction between the environment and the genotype (Lawrence 1996).
Ecotype: a form within a species resulting from selection within a special habitat and being adapted genetically to that particular habitat. But different ecotypes can still breed with other individuals of the species (Lawrence 1996).

Laminaria digitata (Hudson) J.V.Lamouroux

The stipe of *Laminaria digitata* is by Printz (1953) described as slim, smooth and flexible. From the holdfast and up to around the middle of the stipe it is slightly thicker. From the middle and upwards towards the lamina, the stipe gets more flattened. The stipe will not secrete mucus if you cut it. The lamina is narrower in exposed sites, and wider in calm waters. The lamina has a digitated shape, with long segments that can either be broad or narrow. The color of the lamina is olive brown and specimens can be several meters long. *Laminaria digitata* is by Rueness (1977) described as quite variable in shape. On highly exposed areas along the west coast of Norway – a form with narrow lamina, an even transition to the stipe and deep and few rifts in the lamina occur, this morph is called f. *stenophylla*. On sheltered areas and on calm deep waters a form with a wider and thinner lamina occur, this morph is called f. *cucullata*. *Laminaria digitata* f.. *cucullata* has a heart shaped basis by the transition to the stipe and the lamina is less teared. In Norwegian this phenotype is called “skinnbroktare” (Jensen et al. 2000). The stipe of *L. digitata* can be up to 2-3 m and under some conditions the stipe is flatten, but not always (Rueness 1977).

A phenotype of *Laminaria digitata* from the Trondheimsfjord was described by Sundene (1958) which varied from the specimens he had collected on the Norwegian west coast and in the Oslofjord. This unidentified form was found in April and had a bullate and digitate lamina, the basal part of the lamina was either divided or undivided. The stipe was stiff and mostly smooth. The phenotype resembled *Laminaria hyperborea* but the lamina was not smooth (Sundene 1958).

An experiment was conducted where specimens of *Laminaria digitata* were moved from their normal habitat, to locations with other environmental conditions (Sundene 1964). That study demonstrated that if a *L. digitata* specimen with a narrow lamina was moved from an exposed area to calm waters, it obtained a wide, undivided lamina. In sites which are more exposed - specimens with wide lamina occur, but these will be digitated, f. *typica* or f. *ensifolia*. And in locations that are very exposed, the lamina will be very narrow and digitated, f. stenophylla (Sundene 1964).

There are two different phenotypes of *L. digitata* from Spitsbergen described by Kjellman (1877) from the specimens that he collected; f. *vera* which had a long, vigorous stipe. Some specimens had a thicker stipe close to the lamina, this part was also more flattened. It had a distinct transition between lamina and stipe. The stipe and lower part of lamina had a darker color. The specimens could be up to 300 cm long. The other phenotype was f. *latifolia*, which had a wide, egg shaped or elliptical lamina, which could be non-digitated or be parted in only a few pieces. The length of the stipe was variable, but versus the length of the lamina, it was fairly short. From Smeerenbergbay (now the Smeerenburgfjord) he collected an individual in July that he thought was needed to be mentioned, it had a very short, black stipe. The lamina was kidney-shaped and non-digitated, and from the top brim many dark, narrow pieces was attached, which was remains from the previous years lamina.

Laminaria digitata described from the Isfjord, Spitsbergen by Svendsen (1959), was very variable in shape, especially the lamina. The form varied from short stipe and a broad lamina, to long stipe

with a wide, non-digitated lamina or narrow lamina with deep rifts. The largest specimen found was 220 cm long.

Laminaria hyperborea (Gunnerus) M.Foslie

The stipe of *Laminaria hyperborea* is described by Printz (1953) as stiff and thick, being wider by the base and gradually getting narrower towards the lamina. The older the specimen the more woody and rough the stipe. A cross section of the stipe will make it secrete clear mucus. Specimens of *L. hyperborea* have a clear separation of lamina and stipe. The lamina of *L. hyperborea* is wider and shorter than the lamina of *Laminaria digitata*, and often have a heart shaped base. The color is chestnut and it can get several meters long (Printz 1953). The stipe of *L. hyperborea* can be 2-3 m long (Rueness 1998). *Laminaria hyperborea* is an indisputable distinct species according to Kain (1979), with its rugose stipe which has a slightly conical shape. In the fast growth season (January - June) there is almost a separation between the new and old lamina (Svendsen & Kain 1971). The lamina of *L. hyperborea* is according to Rueness (1977) normally shorter than stipe and rarely more than 1 meter long. In sheltered locations there can be found the cucullate shape, which has an undivided lamina (Svendsen & Kain 1971). The shape of the base of the lamina will be affected by the habitat, and can either be heart shaped or truncate. When there is little exposure there are fewer fingers in the lamina and the old part of the lamina is attached longer. In areas with greater wave action, the holdfast will be more developed, the stipe stiff and fairly straight and the lamina fan shaped, with a truncate base and divided into many segments. In locations with little wave action but strong current the stipe will probably be more flexible, the lamina will be divided and elongated, and be heart shaped at the base. This phenotype will resemble *L. digitata* in similar conditions (Kain 1971). The way to distinguish between a young *L. hyperborea* and a *L. digitata* is by looking at the transition zone, *L. hyperborea* will have a narrow transition zone (Kain 1971).

Saccharina groenlandica (Rosenvinge) C.E.Lane, C.Mayes, L.D.Druehl & G.W.Saunders

Saccharina groenlandica was described under two names; *Laminaria groenlandica* and *Laminaria cuneifolia* in Taylor (1957), which is both now regarded as *Saccharina groenlandica* (Guiry & Guiry 2014). *Laminaria cuneifolia* is described as being rather small. The stipe has mucilage ducts and is cylindrical and short (2-6 cm). The lamina is wedge shaped at the base and can often be constricted and showing its mucilage ducts. The lamina is 6-14 cm broad and 30-70 cm long. *Laminaria groenlandica* is also described as small. The holdfast is branched and narrow. The stipe is 30-75 cm long and solid, and being cylindrical at the base and more compressed above with mucilage ducts in the lower part. The shape of the lamina can range from an oblong shape to an obovate shape. The edges are undulate, and there are two rows of bullae (def: appearing blistered (Lawrence 1996)) near the center of the lamina. The shape of the base can be rounded or a broad wedge shape or heart-shaped. The lamina can be up to 1 m long and 25-80 cm in width and mucilage canals are always present (Taylor 1957). Anatomical examinations of *S. groenlandica* from Canada conducted in a study by McDevit and Saunders (2010) showed that mucilage ducts were present both in the lamina and the stipe. *Saccharina groenlandica* is described by Lindeberg and Lindstrom (2014) as being medium to dark brown in color. The stipe is cylindrical close to the holdfast and more flatten near the lamina. The stipe has microscopic mucilage ducts and can be up to 60 cm long with a branched holdfast. The lamina can be bullate when it is young but when it becomes older it will be more even and thicker. The lamina can split into several fragments and can be up to 2 m long.

Distribution

Laminaria digitata

Laminaria digitata is common along the whole coast of Norway (Printz 1953) and is found in the upper part of the sublittoral zone (Rueness 1977). Specimens grow both on exposed areas and in sheltered areas along the coast, and it can also be found in fjords where there is a current and there are little fresh water input (Printz 1953). Globally it is found in the North Atlantic (Bartsch et al. 2008); on the eastern side, from Brittany in south to Svalbard, Iceland and Russia in north (Kain 1979). On the western side of the Atlantic it is found from Cape Cod and to the northeastern coast of Greenland (Kain 1979).

Laminaria hyperborea

Laminaria hyperborea is found along the whole west coast of Norway, mostly in exposed areas and does not go far in to the fjords. The specimen gets larger the further north they are found. Along the coast of Skagerrak it grows deeper than further north (Printz 1953). According to Printz (1926) *L. hyperborea* mainly occur in the outer parts of the Trondheimsfjord, but it can also be found inside the fjord, as far in as Ytterøya. Globally it is restricted to the northeast Atlantic (Bartsch et al 2008), from Portugal in south to the North Cape of Norway. It is found at Iceland, but not in the Baltic Sea (Kain 1971).

Saccharina groenlandica

Saccharina groenlandica is found in the North Pacific; from California to British Colombia, and in the north by Alaska and Commander Island (Bartsch et al. 2008). On the Atlantic side, there has been some confusion about the taxonomy and thereby the distribution of *S. groenlandica* is unclear. But it is said to be found in Northern Labrador and Ellesmere Island (Taylor 1957), and on the west coast of Greenland, where it first was described by Rosenvinge (1893). The presence of *S. groenlandica* in Labrador and Newfoundland was confirmed in a paper by McDevit and Saunders (2010). Later it has also been discovered in the Maritime Provinces of Canada (Longtin & Saunders 2013).

Mucilage ducts

The use of mucilage ducts as a trait to distinguish between species has been debated among biologists, including Chapman (1975) and Kain (1979), which both concluded that this characteristic was too variable. Mucilage ducts are found in the stipe and/or lamina of some Laminariales species and are intercellular spaces in the cortex lined by secretory cells that are producing and secreting fucoidin (UW Friday Harbor Laboratories 2014). In the specimens of *Saccharina groenlandica*, *Laminaria digitata* and *Saccharina latissima* sequenced for the COI-gene by McDevit and Saunders (2010), they also examined whether mucilage ducts were the present or absent in specimens of *L. digitata* and *S. latissima* collected on both the west coast of the Atlantic Ocean and from Faroe Island and Ireland at the eastern side. They found that *S. groenlandica* had mucilage ducts both in its stipe and lamina, *L. digitata* had only mucilage ducts in the lamina, while mucilage ducts were absent in *S. latissima*. An experiment in cultivating *S. latissima* specimens from Canada and Great Britain in different temperatures performed by Burrows (1964) showed that *S. latissima* from Canada did not have mucilage ducts at any temperature, while the *S. latissima* specimens from Great Britain cultivated at warmer temperatures had produced mucilage ducts.

Deoxyribonucleic acid (DNA) barcoding

Cytochrome oxidase I (COI)

The 5' end of the cytochrome oxidase I (COI-5P) gene located in the mitochondrial genome has been proposed to be the sufficient barcode marker for species differentiation in the animal kingdom, with a few exceptions (Hebert et al. 2003). McDevit and Saunders (2009) showed that the mtDNA COI gene could also be used to differentiate among species of the brown macroalgae (Phaeophyceae) order Laminariales. Their study showed a divergence greater than 3% between species, between *Laminaria digitata* and *Laminaria hyperborea* the divergence was 3.04% and a intraspecific divergence of 0.00% - 0.46%, with the exception of one genus (McDevit & Saunders 2010).

Aim of the study

This study aims to:

- Describe the morphological variability in *Laminaria digitata* from chosen locations in mainland Norway and Svalbard.
  - To study if the variability in morphology is caused by genotypes, phenotypes or ecotypes.
- To identify if the morphological variability in *L. digitata* is of taxonomic relevance or if it represents within or between locations.

Hypothesis 1
Sheltered locations will give a stiff and long stipe and well-developed lamina of *Laminaria digitata*, while exposed areas will result in specimens with short stipes and lamina to avoid tearing.

Hypothesis 2
Seasonal variations will cause differences in the morphology of *Laminaria digitata*.

Hypothesis 3
Material commonly identified as "*Laminaria digitata*" found at Svalbard is a different species than the *L. digitata* found in the Trondheimsfjord.

Material and methods

Study area

Collection of *Laminaria digitata*-like individuals was carried out both in the Trondheimsfjord and on Svalbard during 2012-2013 (Table 1).

Table 1: Location, date and number of specimen collected at each sampling site.

Location	Date	Degree N	Degree E	Number of specimens
Brænnebukta	28.02.2013	63.45°	10.33°	23
Brænnebukta	06.05.2013	63.45°	10.33°	23
Brænnebukta	02.09.2013	63.45°	10.33°	21
Sauøya, Hitra	04.10.2012	63.67°	08.87°	4
Kapp Mitra	27.09.2013	79.12º	11.18º	8
Smeerenburgfjord	28.09.2013	79.69º	11.08º	5
Gyldénøyane, Hinlopen	02.10.2013	79.67º	19.69º	5

The collections in the Trondheimsfjord were mainly done in Brænnebukta, close to Trondheim (Fig. 2). The sampling was carried out, February 28th, May 6th and the last sampling September 2nd, 2013. Four additional *L.digitata* individuals were collected on Sauøya, Hitra on October 4th 2012 (Fig. 2).

Figure 2: Map of sampling locations in the Trondheimsfjord; Sauøya, Hitra and Brænnebukta, Trondheim. Source: Kartverket (2014).

On Svalbard, three sampling locations were visited during a UNIS-cruise autumn 2013 with RV Helmer Hanssen; Kapp Mitra (outlet of the Kongsfjord) on September 27th, the Smeerenburgfjord on September 28th and Gyldénøyane, Hinlopen strait on the 2nd of October (Fig. 3).

Figure 3: Map of Svalbard where the sampling locations, Kapp Mitra, the Smeerenburgfjord and Gyldénøyane are marked with red dots. Source: Norwegian Polar Institute (2014).

Collecting and handling of samples

The samples from Brænnebukta were collected by snorkling and utilizing an extended iron rake. The algae were kept in buckets with sea water in the field and at Trondhjem Biological Station (TBS) they were kept in a big tank with running sea water or in a net from the dock. The samples from Sauøya, Hitra was collected by SCUBA divers and frozen. At the cruise on Svalbard the kelp were collected by SCUBA divers and kept in tanks with running sea water on deck at *in situ* temperature.

All individuals were measured and morphological features described (Fig. 4, Table 2). The angle between lamina and stipe, was divided into 3 categories (Fig. 5). The cross sections of the stipes were categorized from 1-3 depending on their circularity (Table 2), this was calculated by dividing the height on the width of the cross section. Every specimen was photographed, both the whole thallus and special details such as the stipe-lamina angle, cross sections and coloration differences. The individuals collected in Brænnebukta were also photographed on an illumination box to document the different morphologies that can be found in one area. Tissue samples (1x1 cm) for DNA sequencing were taken from the meristem (Fig. 4). A total of 4 pieces of tissue samples were cut out from each individual. On the first sampling date, all of the samples were put directly in the freezer, and two pieces from each specimen were later dried on silica gel. On the other sampling dates, two pieces of tissue was dried directly on silica gel to avoid degradation of the DNA, and two pieces were put in a -20ºC freezer as backup. Cross sections were taken at the base of the holdfast, at the middle part and close to the lamina of each stipe (Fig. 4). The individuals collected at Sauøya, Hitra were frozen whole, and later taken up and measured as well as taken picture of and cut out tissue sample from.

Figure 4: Left: Measurements done to describe the morphology; width of lamina, total length, length of stipe and angle between lamina and stipe. Middle: Samples for DNA-extraction were cut out of the meristem. Right: Cross sections from the stipe were taken from the part close to the holdfast, approximately at the middle of the stipe and in the transition between stipe and lamina.

Table 2: Measurements performed on each sampled specimen.

		Measurements	**Categories**
Whole specimen	Total length	cm	
	Epigrowth	type	
	Angle between stipe and lamina	> 90° ± 90° < 90°	1 2 3
Stipe	Length	cm	
	Shape of middle part (height/width)	(0.25-0.5) (0.51-0.75) (0.76-1)	1 2 3
Lamina	Width of lamina	cm	

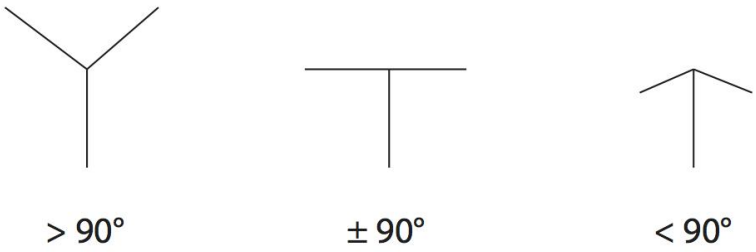

Figure 5: The three types of angle between lamina and stipe that were used to categories the specimens. > 90° = 1, ± 90° = 2, < 90° = 3.

DNA extraction

Cetyl trimethyl ammonium bromide (CTAB) extraction was used to extract DNA from the dried tissue samples based on a protocol modified from Doyle and Doyle (1987). DNA extraction kits often fail to extract high quality DNA for use in PCR, probably caused by high levels of polysaccharides, tannins and phenolics in kelp tissue (Lane et al. 2006). From each sample, 2-4 mg of tissue was cut off and put in 2 mL Eppendorf tube. The DNA-extractions were done in batches of 12 or 24 tubes, where the 12^{th} or the 24^{th} tube was the control and no tissue were added to these tubes. Qiagen stainless steel beads (3 mm) were added to each of the tubes before grinding with a Retsch Mixer Mill MM 400 (Haan, Germany). The samples were shaken for 160 seconds with a frequency of 25 Hz.

Six hundred µL of 2 % CTAB with 1% mercaptoethanol were added to the samples. The samples were then vortexed (VWR Analog Vortex Mixer, VM-3000, USA) and left on the bench for 1 hour in room temperature for incubation. The CTAB-buffer was used to dissolve the cell membranes to enhance DNA extraction. Every 15 minutes the samples were vortexed, except after the last 15 minutes. Chloroform-mix, 96:4 chloroform to isomylalchohol (500 µL) was added to separate the proteins and polysaccharides from the nucleic acids. The samples were shaken for ten minutes by hand, and vortexed twice. The samples were then centrifuged (Eppendorf Centrifuge 5424, Hamburg, Germany) for 5 minutes at 12,000 rpm (~13,500 g) to divide the samples into a water phase on top and an organic phase at the bottom. The DNA was found in the water phase and this supernatant was transferred to a new Eppendorf tube (1.5 mL). Cold isopropanol (2/3rds of the sample volume), which was stored in a -20ºC freezer, was added to precipitate the DNA. The samples were gently mixed and put in the freezer at -20ºC for 0.5-24hr. The samples were then centrifuged for 10 minutes with 13,000 rpm (~15.800 g) to collect the DNA into a pellet. The supernatant was poured out, while the DNA-pellet was left in the bottom the tube, and the tube were placed upside-down on lab tissue to dry. Six hundred µL 70% ethanol was added and the tubes were gently mixed and then centrifuged for 2 minutes at 12,000 rpm (~13,500 g). The ethanol was added to rinse the supernatant out of the DNA. The ethanol was poured out and the tubes were placed on lab tissue to dry. The latter procedure was repeated one time. The tubes with the DNA-pellets were placed upside-down on lab tissue and were left there until the ethanol was evaporated. 0.1 x Tris-EDTA-buffer (TE-buffer, 100 µL) was added to each tube and the samples were left in room temperature over night, and stored in a -20°C freezer until use.

The first DNA-samples were checked by using agarose gel electrophoresis, to see if the extraction had been successful and to make sure the samples had not been contaminated. This was done on an 0.7% agarose gel. Three µL GelRed (Biotium, USA) which visualizes the DNA under UV-light (Biotium 2014), was added as staining to the agarose gel. One µL loading dye (Thermo Scientific, Germany), which makes the template lie in the bottom of the well and visualizes the DNA-migration (Thermo Scientific 2013a), was mixed with 4 µL DNA-template and 3µL of the DNA-Loading dye mix were transferred to the wells in the gel for electrophoresis. GeneRuler 1 kb (Thermo Scientific, Germany), a mix of chromatography-purified individual DNA fragments, 250-10,000 bp (Thermo Scientific 2013b) and FastRuler Low Range (Thermo Scientific, Germany), a mix of five blunt-ended chromatography-purified individual DNA fragments, 50-1500 bp, was used as DNA ladder, which shows the size of double stranded DNA (Thermo Scientific 2013c). Three µL of one of them were added to first well and and the same amount of the other in the last well. The gel was run on 210 V for 7 minutes. The gel were checked under UV-light in GeneFlash (Syngene Bio Imaging).

Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) is a method to synthesize millions of copies of specific DNA sequences from a small amount of DNA (Erlich 1989). The gene targeted is cytochrome c oxidase I (COI), this marker has earlier shown to be able to differentiate between species of Phaeophyceae (McDevit & Saunders 2009) and is a standardized marker for identification of species (Hebert 2003). The forward primer used was GazF2 (biomers.net, Ulm, Germany) (5' CCAACCAYAAAGATATWGGTAC 3') and the reverse primer were GazR2 (biomers.net, Ulm, Germany) (5' GGATGACCAAARAACCAAAA 3') (Lane et al. 2007). The primers binds to the 3'ends of the separated DNA-strands, and the DNA-polymerase can start to synthesize a new strand from the 3'end of the primer (Erlich 1989). All of the DNA-samples were diluted 10x before PCR. The PCRs were run in 25 µL volumes containing 1x 12.5µL DreamTaq Green Master Mix (Thermo Scientific, Germany), 0.4 µM of each primer and 8.5µL water. Two µL of the diluted template was added. The last tube was the PCR negative (to check for contamination) and only DreamTaq Green Master Mix, primers and nuclease free water were added. The thermal profile for the PCR was: an initial denaturation at 94°C for 3 min followed by 35 cycles of 94°C for 30 s, 49°C for 45 s and 72°C for 1 min, with a final extension at 72°C for 10 min and a lagging phase at 4°C. For the PCR both Eppendorf Mastercycler ep gradient S (Hamburg, Germany) and GeneAmp PCR Systems 9700 (Foster City, USA) machines was used.

To determine if the DNA-extraction and PCR was successful, the PCR-product was checked on 1.5% agarose gel, this would also reveal if there was any contamination in the control or in the PCR-negative. Since DreamTaq Green Master Mix was used in the preparation of the PCR, loading dye was not needed when running the PCR-product on the gel. 3 µL of PCR-product was added to each well, Gene ruler 1 kb and Fast ruler LR was used as DNA ladder. The gel was run on 210 V for 8 minutes, and then checked under UV-light to see which individuals that gave bands on the gel. Bands on the gel means that there are DNA in the PCR-product.

Preparing for sequencing

Before sending the PCR-product for sequencing they had to be cleansed, this was done by using the E.Z.N.A. Cycle-Pure kit (Omega Bio-Tek Inc., Georgia, USA) and following the E.Z.N.A. Cycle-Pure Spin Protocol (Omega Bio-Tek Inc. 2009) to clean the DNA from primers, salts and nucleotides enzymes after PCR-reactions (Omega Bio-Tek Inc. 2009). The PCR-product was transferred to a 1.5 mL microcentrifuge tube and 100 µL CP-buffer was added to the microcentrifuge tube. They were vortexed (VWR Analog Vortex Mixer, VM-3000, USA) to mix and spun down to collect all the sample in the bottom of the tube. The samples were transferred to a HiBind DNA Mini Column placed in a 2 mL collection tube and centrifuged at 13,000 g for 1 minute (Eppendorf Centrifuge 5417R, Hamburg, Germany). The liquid in the collection-tube was discarded, and 700 µL DNA Wash Buffer was added. The samples were centrifuged at 13,000 g for 1 minute and the liquid in collection-tube was discarded. Then 500 µL of Wash Buffer was added and centrifuged at 13,000 g for 1 minute. The liquid was discarded and the empty HiBind Mini column was centrifuged at 13,000 g for 2 minutes to dry the column matrix. The HiBind DNA Mini column was placed in a 1.5 mL microcentrifuge tube and 20 µL Elution Buffer was added to the column and was left at the bench for 2 minutes in room temperature before they were centrifuged to elute the DNA from the filter in the column. 5 µL of the cleansed PCR-product was added to a 1.5 mL tube together with 5 µL of the forward primer (5µM GazF2), and sent to GATC Biotech for Sanger sequencing.

After checking the sequences, three individuals representing each species, plus one sequence of low quality where sent for sequencing using the reverse primer to ensure high quality sequences of the whole barcoding region. The same procedure as when sending the forward sequence was used,

except that the forward primer was switched with reverse primer (5µM GazF2).

Data analyses

The COI sequence data were analyzed in Geneious R7 (Biomatters, New Zealand). The sequences, both forward and reverse, was uploaded to Geneious R7 in chromatograph-format (.ab). The sequences were manually trimmed, and low quality bases in the start and the end of the sequences were removed, together with the primer sequences. The sequences were blasted with Megablast (Altschul et al. 1990) through Geneious R7 to identify similar sequences. For the specimens that had both forward and reverse sequences, these were assembled using «DeNovo Assembly» in Geneious. A selection of reference sequences of *Laminaria digitata*, *Laminaria hyperborea*, *Saccharina groenlandica* and related species submitted to GenBank (National Centre for Biotechnology Information 2014) by Dr. Saunders were imported into Geneious (Appendix I). The assembled sequences and the reference sequences were aligned using «Multiple Sequence Comparison by Log-Expectation (MUSCLE)» in Geneious R7, by using 100 iterations.

Selection of evolutionary model for phylogenetic analyses

In order to find the most suitable evolutionary model for the COI dataset, two different tests was used; jModelTest (Guindon & Gascuel 2003) and through MEGA 5.2.2 (Tamura et al. 2011). In jModelTest, the most fitting model was found to be TPM1uf+G when using the Bayesian Information Criterion (BIC). This model was, however not available in MrBayes 3.2.1 (Huelsenbeck & Ronquist 2001) ran in Geneious R7, and thus the second best model was chosen instead, the Hasegawa, Kishino and Yano (HKY) + gamma (G). In MEGA the most suitable model was found through «Find best DNA/Protein Models (ML)», with the settings «Automatic (Neighbor-joining tree)» for tree to use and «Complete deletion» for gaps/missing data treatment. The most suitable evolutionary model identified using MEGA was HKY+G.

Bayesian analysis

The inferences of phylogeny, in Bayesian analysis, are based on posterior probabilities, which are calculated using Bayes theorem, of phylogenetic trees (Huelsenbeck & Ronquist 2001). Maximization of the posterior probability is the optimal hypothesis (Mauro & Agorreta 2010). Bayesian analysis was done using MrBayes 3.2.1 (Huelsenbeck & Ronquist 2001) in Geneious R7 (Biomatters, New Zealand). MrBayes uses a variant of Markov chain Monte Carlo to perform Bayesian inference of phylogeny (Huelsenbeck & Ronquist 2001). The alignment of the selected specimens together with reference sequences downloaded from GenBank (Appendix x) was chosen, with the *Fucus serratus* sequence as an out-group. The evolutionary model used was HKY85 + G. Chain length was set to 1 000 000, and 100 000 as burn-in length. Molecular Clock with uniform branch lengths: exp: (1), was chosen. This created a file with raw trees. To create a consensus tree the support threshold was chosen to be greedy and set to 85%, and burn-in to 25%.

Maximum likelihood analysis

In Maximum likelihood it is mostly assumed that that there have happened an independent evolution of sites in the sequence, the probabilities of the different sites produces the likelihood. An average of the unobserved character states at the ancestral nodes gives the probability at any site (Yang & Rannala 2012). The Maximum Likelihood-analyses was done in Geneious R7, through PhyML (Guindon & Gascuel 2003). HKY85+G was chosen as the evolutionary model, and the phylogeny was tested using 1000 bootstrap replications.

Neighbor-joining analysis

Neighbor-joining is a pairwise distance method which is based on the presumption that the phylogenetic relationship between two sequences is related to the difference between them. These differences results from changes that have happened along the branches (evolutionary distance) (Mauro & Agorreta 2010). Neighbor-joining analysis were done in Geneious R7, where HKY was selected as the genetic distance model. The alignment of the selected specimens with reference sequences were used and *Fucus serratus* was chosen as out-group. The tree was resampled using 1000 bootstrap replicates. The consensus tree was set to have 85% support threshold.

Results

Three *Laminaria* species that did not have distinct species-specific morphological features were identified by DNA-sequencing of the COI-gene. The DNA-sequencing showed that there are two different species of *Laminaria* in Brænnebukta; *Laminaria digitata* and *Laminaria hyperborea* (Table 3). The specimens collected at Hitra were only *L. digitata*. The specimens collected on Svalbard, previously identified as *L. digitata*, were all the same species, namely *Saccharina groenlandica*. The sequenced *S. groenlandica* specimens had a difference in base composition from the reference sequences downloaded from GenBank, where base 148 was cytosine (C) instead of a thymine (T). After identifying which specimen belonged to which species using DNA sequence data, the variability of the morphological characteristics could be furthered examined.

Table 3: The number of specimens of each species that were identified in mid-Norway and Svalbard.

Location	Date	*Laminaria digitata*	*Laminaria hyperborea*	*Saccharina groenlandica*
Brænnebukta, the Trondheimsfjord	28.02.2013	20	3	
	06.05.2013	22	1	
	02.09.2013	15	6	
Sauøya, Hitra	04.10.2012	4		
Kapp Mitra	27.09.2013			8
The Smeerenburgfjord	28.09.2013			5
Gyldénøyane, Hinlopen	02.10.2013			5

Phylogenetic analyses

Bayesian analysis

The Bayesian analysis (Fig. 6) showed that the sequenced *Laminaria digitata*, *Laminaria hyperborea* and *Saccharina groenlandica* specimens (black font) could be identified to the reference sequence(s) of the same species derived from GenBank (Appendix I) (red font). The analysis support the monophyly of both the *Laminaria* and the *Saccharina* with a posterior probability of 1. *Laminaria digitata* and *L. hyperborea* represent sister species with a high posterior probability. *Nereocystis luetkeana* is a sister species to both the *Laminaria* and the *Saccharina* clades. *Fucus serratus* and *Saccorhiza dermatodea* were used as out-groups.

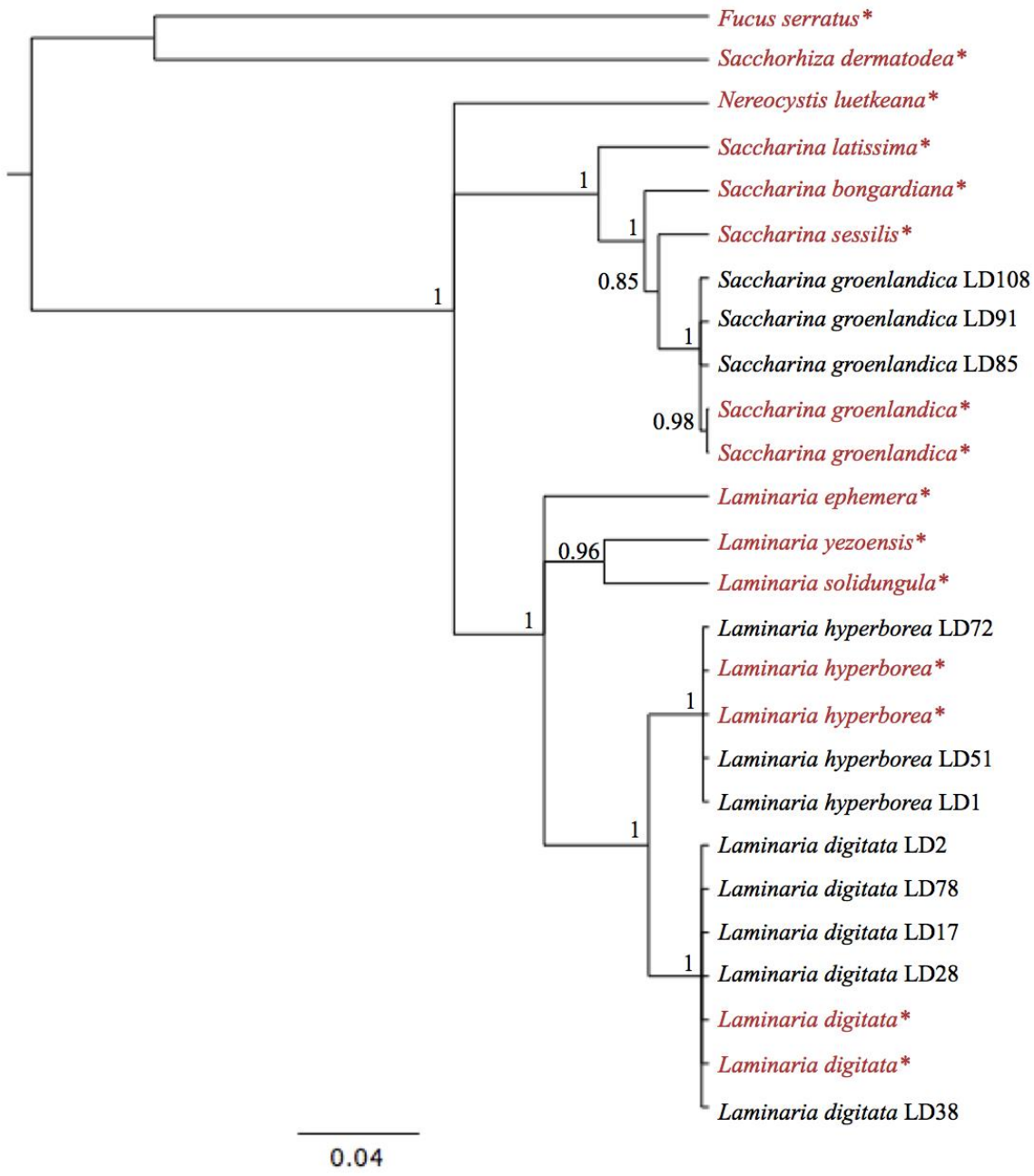

Figure 6: Bayesian consensus tree with posterior probabilities for the COI-gene. The specimens in black font are the sequenced specimens from the different sites in this study (Appendix I). The specimens labeled in red and marked with stars are the sequences which were downloaded from GenBank. Only posterior probability values above 0.8 were plotted in the tree.

Maximum likelihood analysis

In the Maximum likelihood consensus tree (Fig. 7) the sequenced *Laminaria digitata*, *Laminaria hyperborea* and *Saccharina groenlandica* specimens (black font) also formed clades with their respective reference sequence(s) derived from GenBank (Appendix I) (red font) as they did in the Bayesian analysis. According to the Maximum likelihood analysis *Laminaria* was polyphyletic, excluding *Laminaria solidungula*, *Laminaria yezoensis* and *Laminaria ephemera* from the *L. digitata*/*L. hyperborea* clade. The bootstrap support for the *L. digitata* clade was 99.7%, for *L. hyperborea* the bootstrap support was 98.9% and the joint clade of the two species have 97.5% support. The *S. groenlandica* clade had 91.1% support. *Nereocystis luetkeana*, *Saccharina latissima*, *Saccharina bongardiana*, *Saccharina sessilis* and *S. groenlandica* represent sister species, with varied support (65.6% to 92.8%). *Fucus serratus* and *Saccorhiza dermatodea* were used as out-groups.

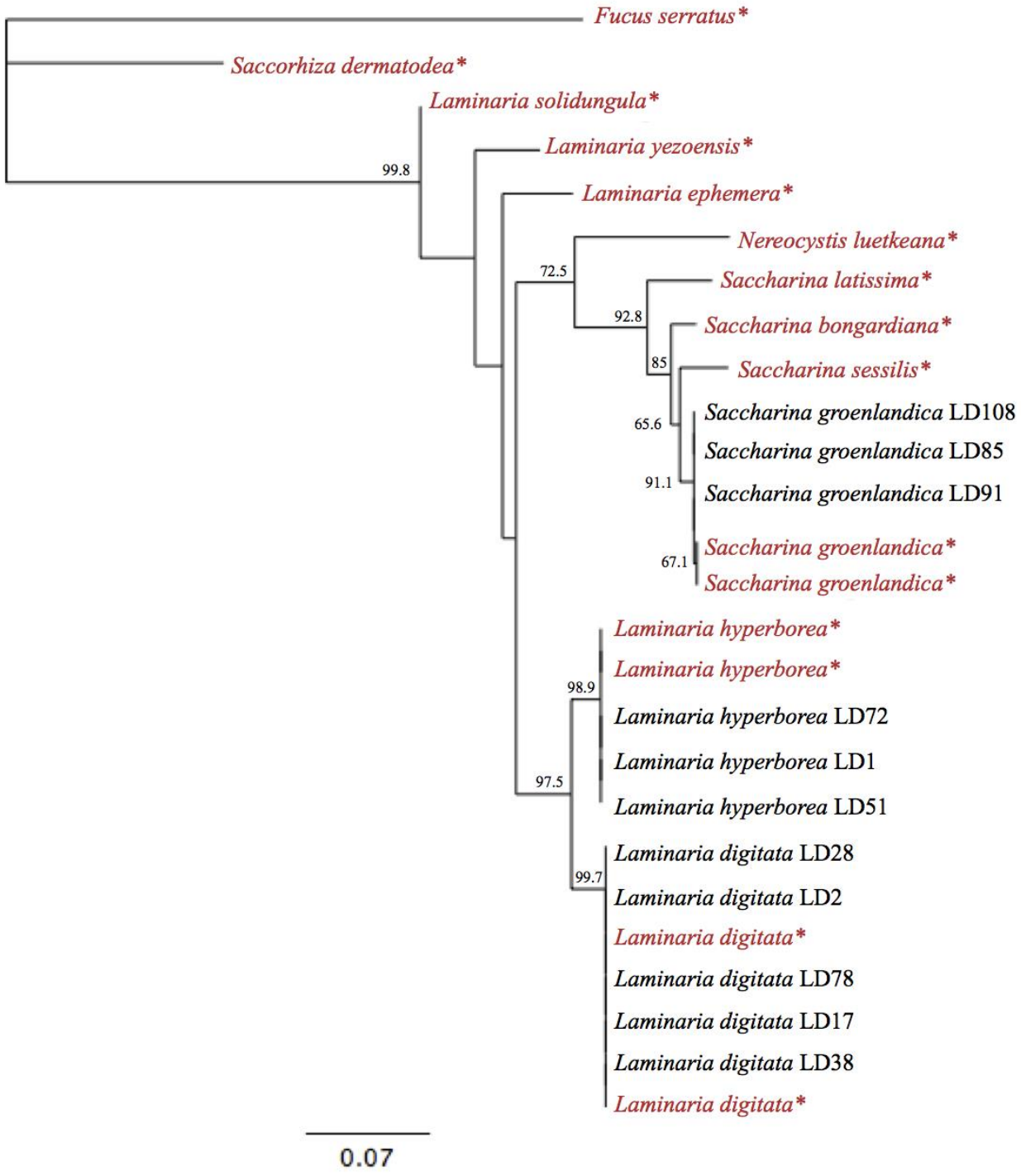

Figure 7: Maximum likelihood consensus tree with 1000 bootstrap replications for the COI-gene. The specimens in black font are the sequenced specimens from the different sites in this study (Appendix I). The specimens labeled with red and marked with stars are the sequences which were downloaded from GenBank. Only bootstrap values above 60% were plotted in the tree.

Neighbor-joining analysis

The Neighbor-joining consensus tree (Fig. 8) displayed that the sequenced *Laminaria digitata*, *Laminaria hyperborea* and *Saccharina groenlandica* specimens (black font) also can be identified to the reference sequence(s) of the same species derived from GenBank (Appendix I) (red font) . As in the Maximum likelihood analysis, *Laminaria* was polyphyletic and *Laminaria solidungula*, *Laminaria yezoensis* and *Laminaria ephemera* were not in the same clade as *L. digitata* and *L. hyperborea*. The bootstrap support for the *L. digitata* and *L. hyperborea*-clades was 100%, and the joint clade of the two species had 100% support as well. *Saccharina* is monophyletic with 99.1% bootstrap support. The clade of *S. groenlandica* had 99.9% support. *Nereocystis luetkeana* was not grouped with *Saccharina* in the Neighbor-joining tree. *Fucus serratus* and *Saccorhiza dermatodea* was used as out-groups.

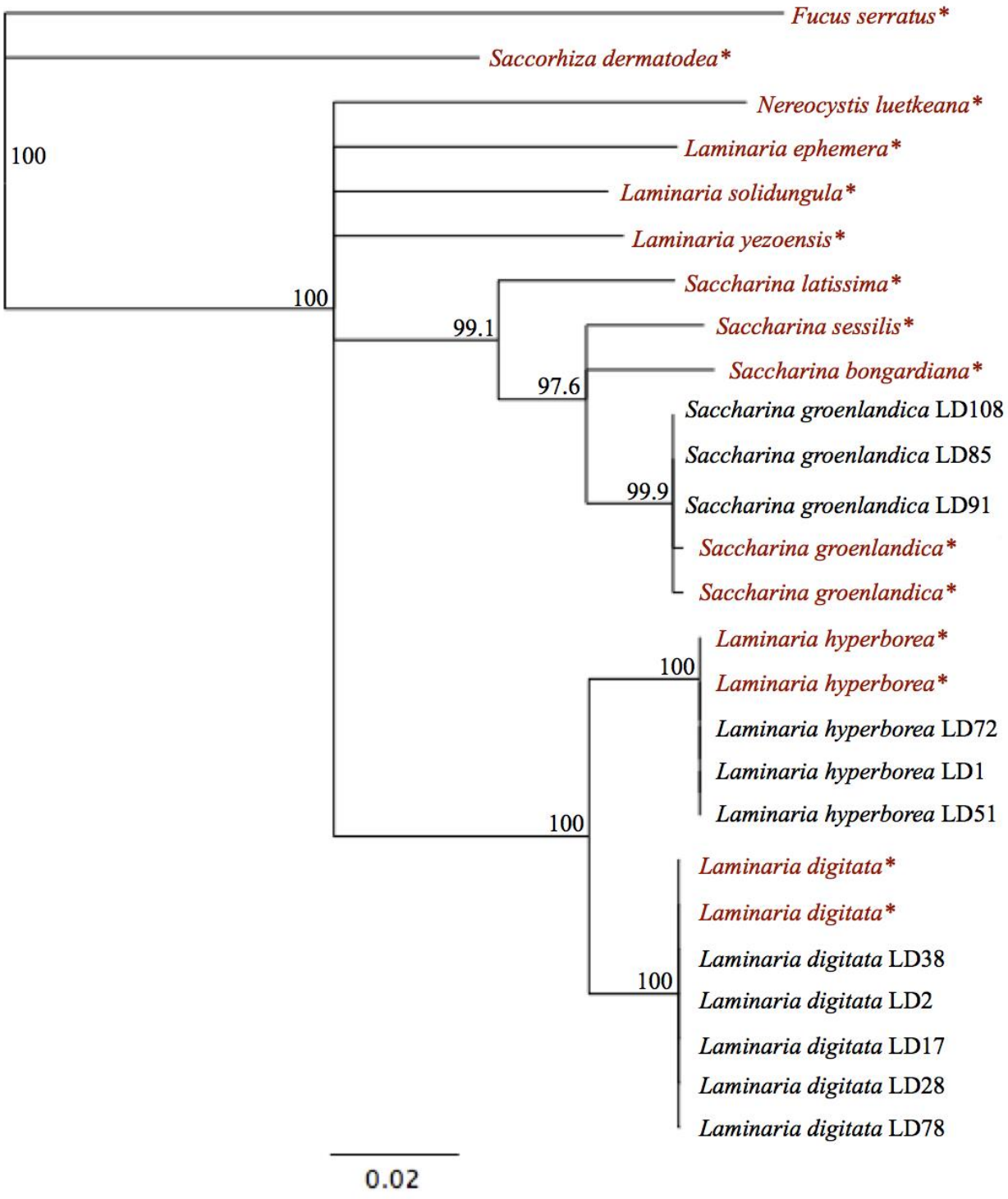

Figure 8: Neighbor-joining consensus tree with 1000 bootstrap replicates for the COI-gene. The specimens in black font are the sequenced specimens from the different sites (Appendix I). The specimens labeled with red and marked with stars are the sequences which were downloaded from GenBank. Only bootstrap values above 60% have been plotted.

Morphology

Brænnebukta, the Trondheimsfjord

In Brænnebukta, material was collected three times during 2013 (February, May and September), these collections show the great variation in the algal morphology during the seasons. Species-specific morphological characteristics were used to elucidate if there were both *Laminaria digitata* and *Laminaria hyperborea* in Brænnebukta. The DNA-sequencing showed that there were two species from the genus *Laminaria* in Brænnebukta, both *L. digitata* and *L. hyperborea*. In total there were found 57 *L. digitata* and 10 *L. hyperborea* in Brænnebukta (Table 3).

Laminaria digitata

February

Pictures show the characteristic of the lamina of the kelp (Fig. 9), and the angle between the stipe and lamina. The pictures taken of the *Laminaria digitata* specimen collected in February shows a great variation in the shape. From a large angle between stipe and lamina, seen in e.g. LD4 to a 90° seen in e.g. LD2 to a small angle seen in e.g. LD10. There were 7 specimen which were in category 1, 6 specimens in category 2 and 7 in category 3 (Table 4). When the specimen are lighted up from underneath it enhances the different coloration of the old (dark brown) and new tissue (bright brown). Some specimen have a curve in the area of the color change, e.g. LD18.

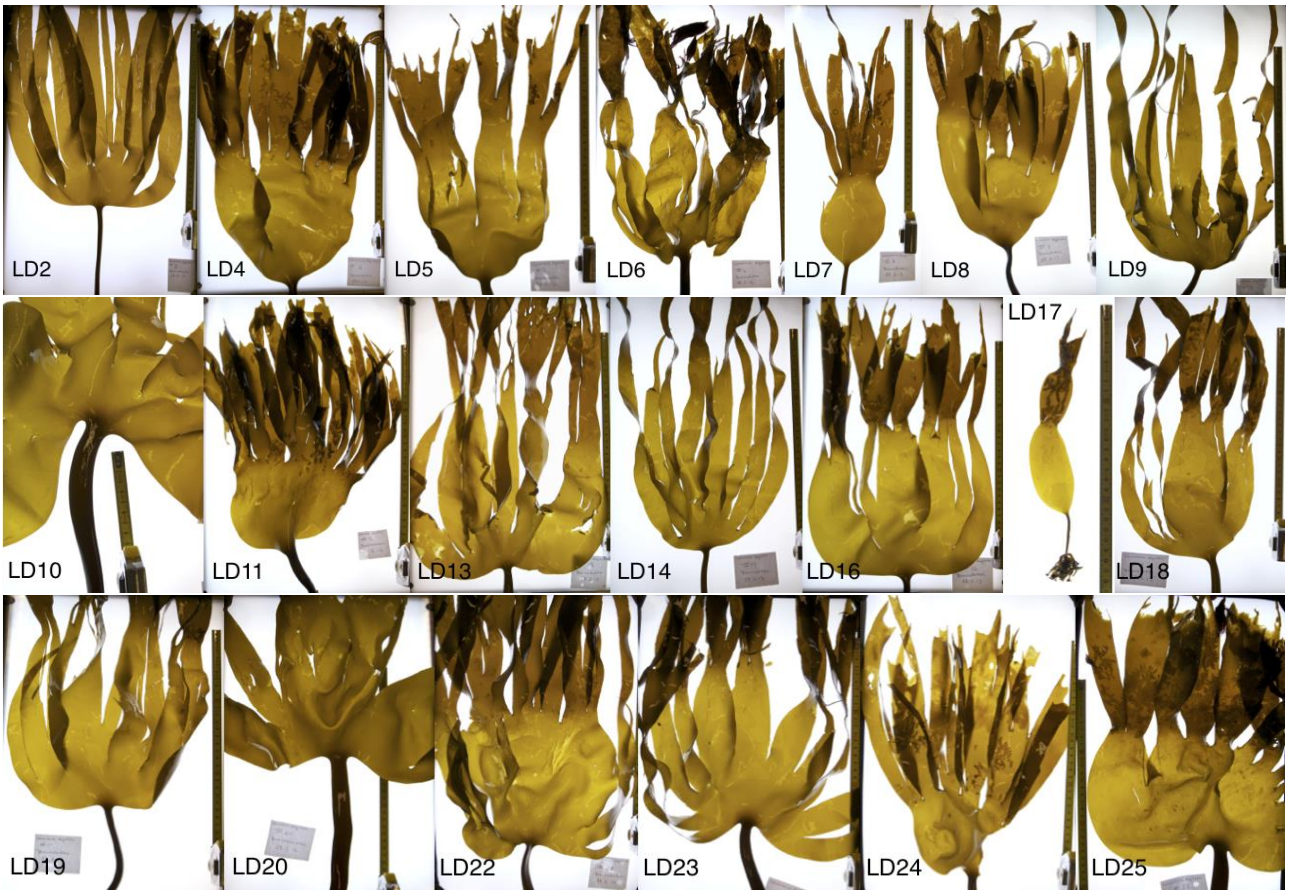

Figure 9: Morphology of *Laminaria digitata* sampled in February 2013 from Brænnebukta, the Trondheimsfjord. Detail pictures on a illumination box showing the angle between stipe and lamina. From top left: LD2, LD4, LD5, LD6, LD7, LD8, LD9. Middle row from left: LD10, LD11, LD13, LD14, LD16, LD17, LD18. Bottom left: LD19, LD20, LD22, LD23, LD24, LD25.

Table 4: Categorization of the angle between stipe and lamina, and the shape of the middle cross section of the stipe of *Laminaria digitata* from Brænnebukta and Hitra. Angle categories 1-3: > 90° = 1; ± 90° = 2; < 90° = 3. Categories for the shape of the cross section of the stipe 1-3 (Height/width): 0.25-0.5 = 1; 0.51-0.75 = 2; 0.76-1 = 3.

Brænnebukta									Hitra		
28.02.2013			06.05.2013			02.09.2013			04.10.2012		
Specimen	Angle category	Shape of stipe	Specimen	Angle category	Shape of stipe	Specimen	Angle category	Shape of stipe	Specimen	Angle category	Shape of stipe
LD2	2	-	LD27	1	1	LD54	3	1	LD77	1	-
LD4	1	-	LD28	3	2	LD56	3	2	LD78	1	-
LD5	1	-	LD29	3	3	LD57	2	3	LD79	1	-
LD6	3	-	LD30	3	2	LD60	3	2	LD80	1	-
LD7	1	-	LD31	3	2	LD62	3	2			
LD8	1	-	LD32	3	2	LD63	3	2			
LD9	2	2	LD33	3	1	LD64	3	2			
LD10	3	1	LD34	3	2	LD65	3	2			
LD11	2	2	LD35	3	2	LD66	3	-			
LD13	3	3	LD36	3	2	LD69	3	-			
LD14	2	3	LD37	3	3	LD70	3	3			
LD16	2	3	LD38	3	2	LD71	2	2			
LD17	1	-	LD40	3	2	LD73	2	-			
LD18	1	2	LD41	3	2	LD74	3	-			
LD19	2	2	LD42	2	3	LD75	3	-			
LD20	3	2	LD43	3	2						
LD22	3	1	LD44	3	2						
LD23	3	2	LD45	1	3						
LD24	1	2	LD47	3	3						
LD25	3	2	LD49	3	1						
			LD50	3	3						
			LD52	3	3						

Figure 10 illustrates the variation in morphology of *L. digitata* collected in February. LD25 and LD7 have a large meristem resembling a flat area by the base of the lamina. While LD14 have deep rifts down to the meristem in the lamina. The lamina was also of highly variable regarding width, with CV (coefficient of variation) of 44% (Appendix II, Table 1). The ratio between the width and the length of the lamina was calculated, it showed a mean value of 0.52 and had a CV of 42% (Table 5).

Figure 10: Illustrates the variation of the morphology of *L. digitata* collected in February in Brænnebukta. From the left: LD25, LD7, LD8, LD19, LD14, LD23. Pictures of all collected specimens are found in Appendix III, Figure 1.

Table 5: Ratio between the width and length of the lamina of *Laminaria digitata* specimens (LD2-LD80) collected in Brænnebukta and Hitra. Coefficient of variation (CV) denotes ±CV% of mean value. The width and length measurements are found in Appendix II, Tables 1, 2, 3, 4.

Brænnebukta						Hitra	
28.02.2013		06.05.2013		02.09.2013		04.10.2012	
Specimen	Ratio (width/length)	Specimen	Ratio (width/length)	Specimen	Ratio (width/length)	Specimen	Ratio (width/length)
LD2	0.35	LD27	0.43	LD54	1.31	LD77	0.22
LD4	0.61	LD28	0.68	LD56	0.46	LD78	0.24
LD5	0.42	LD29	0.76	LD57	0.32	LD79	0.38
LD6	0.64	LD30	0.44	LD60	0.92	LD80	0.30
LD7	0.26	LD31	0.65	LD62	1.34		
LD8	0.48	LD32	0.56	LD63	1.56		
LD9	0.38	LD33	0.67	LD64	0.95		
LD10	0.91	LD34	0.63	LD65	1.08		
LD11	0.47	LD35	0.57	LD66	0.79		
LD13	0.48	LD36	0.57	LD69	0.57		
LD14	0.33	LD37	0.50	LD70	0.57		
LD16	0.53	LD38	0.51	LD71	0.49		
LD17	0.19	LD40	0.61	LD73	0.67		
LD18	0.31	LD41	0.42	LD74	0.58		
LD19	0.65	LD42	0.51	LD75	0.92		
LD20	0.87	LD43	0.62				
LD22	0.74	LD44	0.75				
LD23	0.72	LD45	0.52				
LD24	0.24	LD47	0.48				
LD25	0.82	LD49	0.81				
		LD50	0.62				
		LD52	0.59				
Mean	**0.52**	**Mean**	**0.59**	**Mean**	**0.83**	**Mean**	**0.29**
Standard deviation	**0.22**	**Standard deviation**	**0.11**	**Standard deviation**	**0.36**	**Standard deviation**	**0.07**
Coefficient of variation	**42%**	**Coefficient of variation**	**18%**	**Coefficient of variation**	**44%**	**Coefficient of variation**	**25%**

The cross sections of the stipe (Fig. 11) showed variation in the shape of the stipe. In most of the specimens the stipe was more flattened at the meristem of the lamina and gets more circular towards the holdfast. In most individuals the stipe also became more narrow just above the holdfast. The stipes were bendy and did not break by bending. There were not taken cross sections from the first 6 sampled individuals from February. The stipe of LD17 was so small that is was not taken cross section of it. The shape of the cross section from the middle part of the stipe was divided into categories where; 2 were in category 1, 8 were in category 2 and 3 were in category 3 (Table 4).

Figure 11: Morphological variations in cross section taken from three locations of stipes of the *Laminaria digitata* specimen collected in February from Brænnebukta, the Trondheimsfjord. Images shows discs from the stipes where the uppermost is taken close to the meristem, middle at middle part of stipes and the lowest close to the hapter. From top left: LD9, LD10, LD11, LD13, LD14, LD16, LD18. Bottom left: LD19, LD20, LD22, LD23, LD24, LD25.

May

The lamina of the specimens collected in May was on average larger, mostly in width, than the individuals from February (Appendix II, Table 1). While the mean width in February was 26 cm and a CV of 21%, while in May it was 48 cm, with a CV of 21% (Appendix II, Table 2). That meant that the specimens were too big to fit the whole lamina on the illumination box, and only part of the lamina is seen in the pictures. Figure 12 shows the base of the lamina and the majority of individuals had more or less a heart-shaped base of lamina, with a few exceptions (LD42, LD45). There were 2 specimens in category 1, 1 in category 2 and 19 in category 3 (Table 4). The calculated mean ratio between the width and length of the lamina was found to be 0.59 and had a CV of 18% (Table 5).

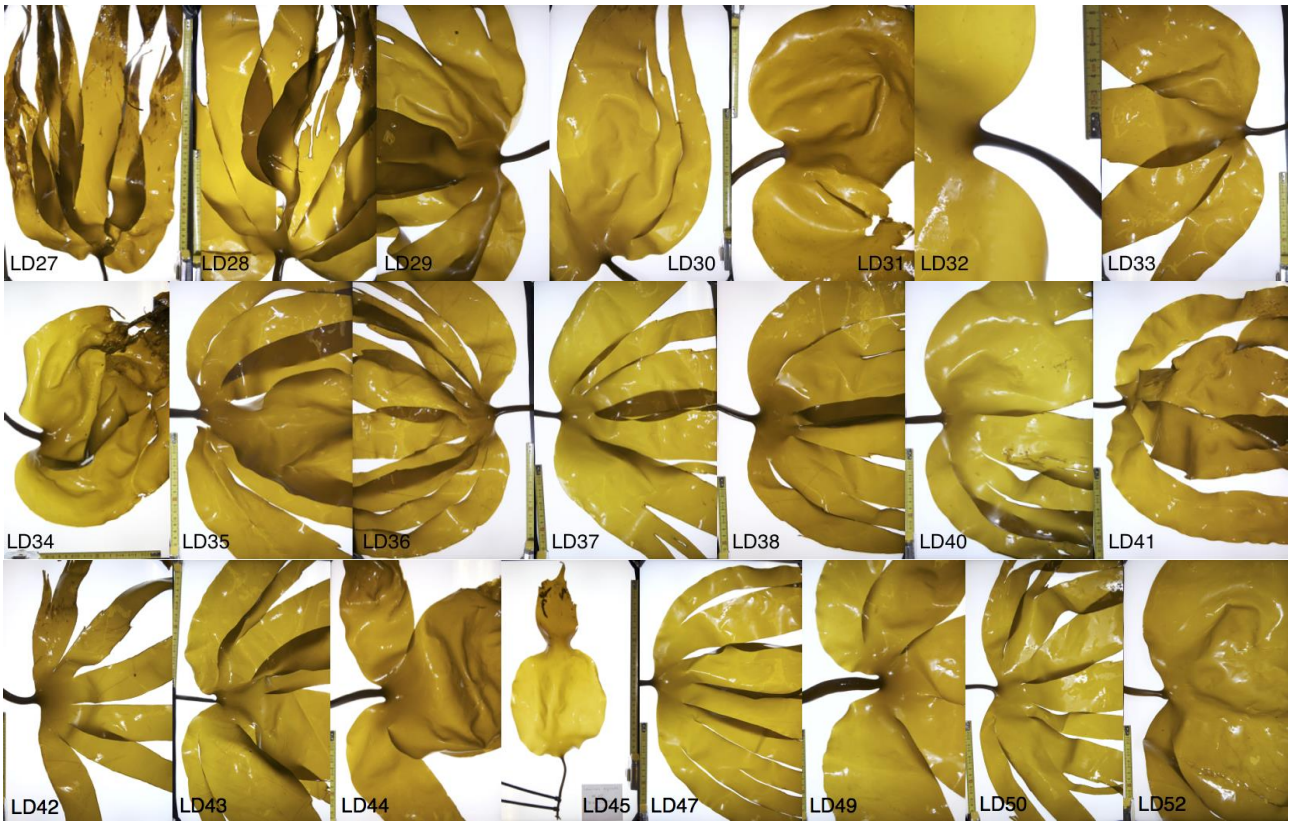

Figure 12: Morphology of *Laminaria digitata* sampled in May 2013 from Brænnebukta, Trondheimsfjord. Detail images showing the angle between stipe and lamina. Top left: LD27, LD28, LD29, LD30, LD31, LD32, LD33. Middle row from left: LD34, LD35, LD36, LD37, LD38, LD40, LD41. Bottom row from left: LD42, LD43, LD44, LD45, LD47, LD49, LD50, LD52.

In May there was a great variation in morphology of the *L. digitata* specimens. Figure 13 sum up the variation. The variation expands from LD34 which had a whole lamina with no deep rifts in it, to LD47 which had deep rifts in its lamina. There were also differences in the length of the stipe (Appendix II, Table 2).

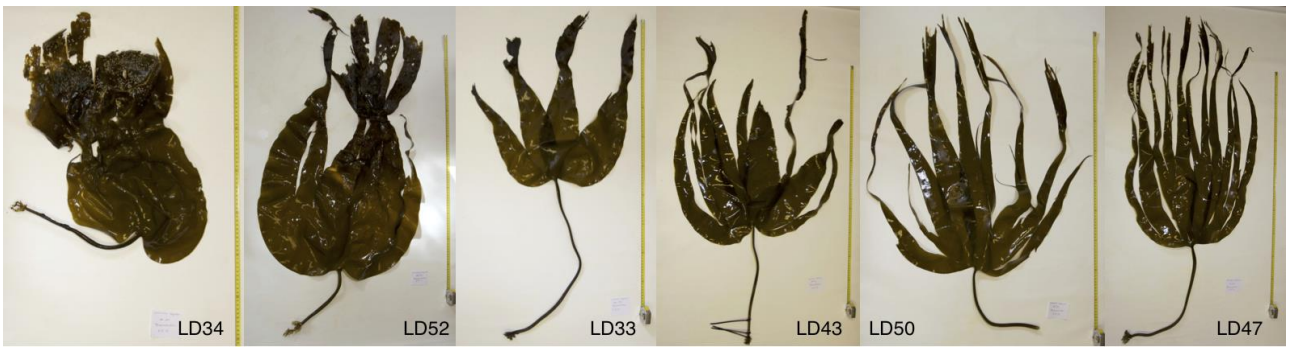

Figure 13: Differences in morphology of *Laminaria digitata* sampled in Brænnebukta, Trondheimsfjord in May 2013. From left: LD34, LD52, LD33, LD43, LD50 , LD47. Pictures of all collected specimens are found in Appendix III, Figure 2.

The stipes were to different degrees flattened except for LD46 (Fig. 14), which was a small individual that had a round stipe. Specimen LD34 was only slightly flattened, while the rest were clearly flattened. There were 3 specimens that were in category 1, 12 in category 2 and 7 in category 3 (Table 4). LD44 and LD49 were hollow in the middle. All of the stipes were bendy.

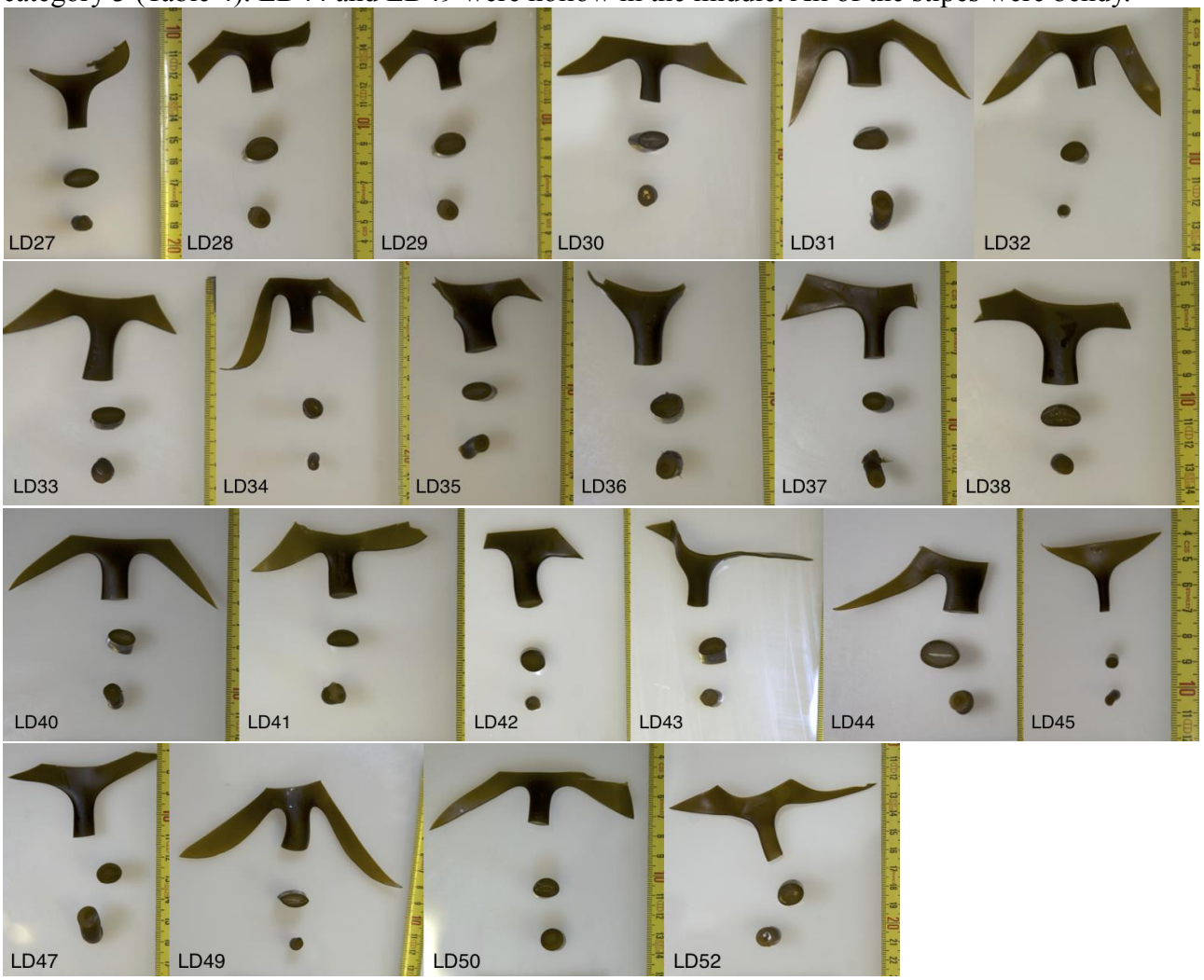

Figure 14: Morphological differences in cross section taken from three locations of the stipes of *Laminaria digitata* specimens collected in May 2013 in Brænnebukta, Trondheimsfjord. Images shows discs from the stipes where the uppermost is taken close to the meristem, middle at middle part of stipes and the lowest close to the hapter. From top left: LD27, LD28, LD29, LD30, LD31, LD32. Second row from left: LD33, LD34, LD35, LD36, LD37, LD38. Third row from left: LD40, LD41, LD42, LD43, LD44, LD45. Bottom row from left: LD47, LD49, LD50, LD52.

September

In September there were fewer *Laminaria digitata* specimens with a strong heart-shaped base of lamina (Fig. 15). Most had only a light heart-shape. LD57, LD71, LD73 had a perpendicular angle between stipe and blade. There were no specimens that ended up in category 1, 3 specimens were in category 2 and 12 were in category 3 (Table 4).

Figure 15: Morphology of *Laminaria digitata* sampled in September 2013 from Brænnebukta, Trondheimsfjord. Detail pictures showing the angle between stipe and lamina of specimen collected. LD75 is lacking from the figure. From top left: LD54, LD56, LD57, LD60, LD62, LD63. Middle row from left: LD64, LD65, LD66, LD69, LD70. Bottom row from left: LD71, LD73, LD74.

Figure 16 shows a selection of the variation of *L. digitata* sampled in September 2013 in Brænnebukta. Several individuals had torn tips and short lamina e.g. LD63 and LD70. The mean width of the lamina was 49 cm and the CV was 38% (Appendix II, Table 3). As in May there were individuals which had only a few rifts in their lamina e.g. LD57, and others that had deep rifts e.g. LD74. The mean ratio between the width and length of the lamina was calculated to be 0.85 and the CV was 44% (Table 5).

Figure 16: Variation in morphology of *Laminaria digitata*, showed by some of the most extreme forms found in Brænnebukta in September 2013. From left: LD57, LD56, LD71 , LD74, LD62, LD63. Pictures of all collected specimens are found in Appendix III, Figure 3.

Cross sections of stipes from *L. digitata* sampled in September showed a variation in shape (Fig. 17). Almost all of the collected specimens had flattened stipes, except LD75 that had a rounded stipe, but the length of the stipe was only 16 cm. LD54 had a particular flatten and broad stipe, especially near lamina. In category 1 there was 1 specimen, in category 2 there were 7 specimens and in category 3 there were 2 specimens (Table 4). The stipes was flexible in all of the specimens.

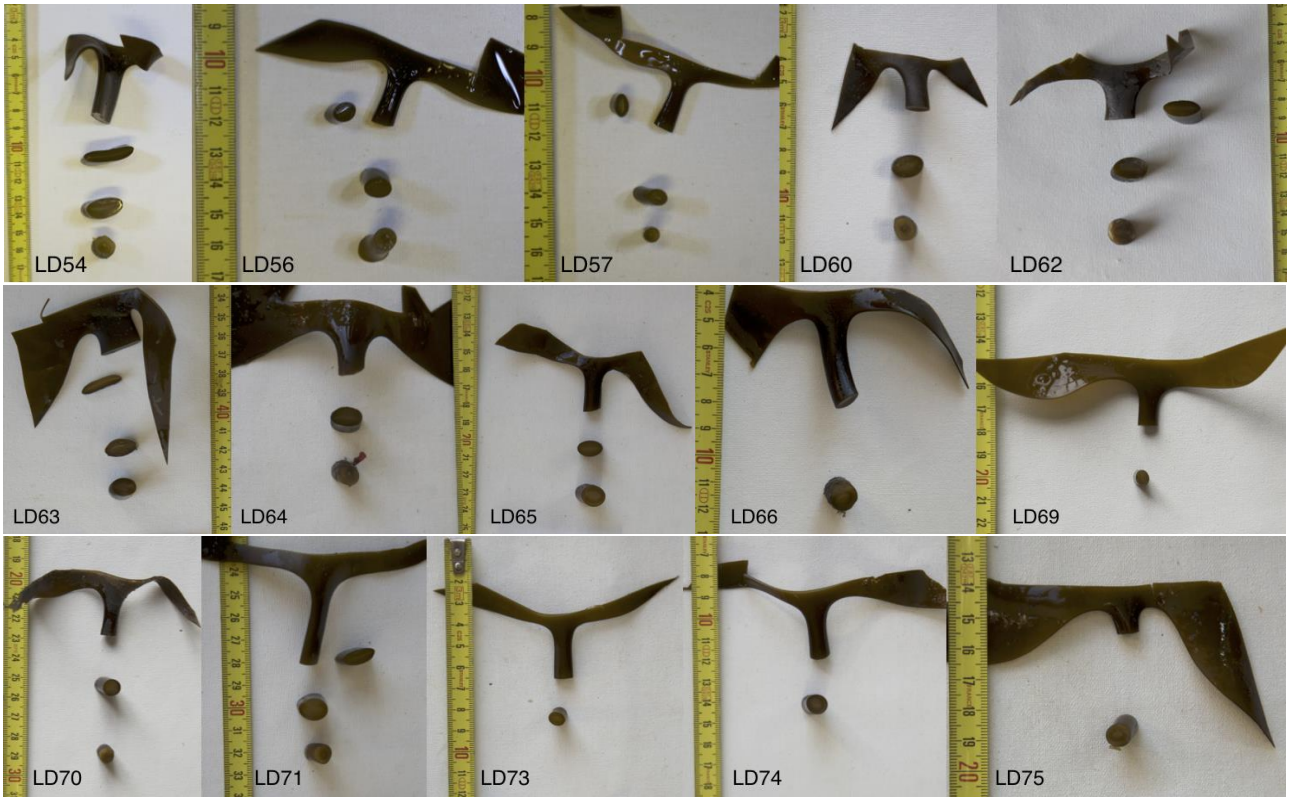

Figure 17: Morphological differences in cross section taken from three locations of the stipes of *Laminaria digitata* stipes collected in September 2013 in Brænnebukta, Trondheimsfjord. Images shows discs from the stipes where the uppermost is taken close to the meristem, middle at middle part of stipes and the lowest close to the hapter. On the pictures were there are three cross sections, the 3th cross section is the part next to the transition part. When there is only one cross section this is from close to the holdfast. From top left: LD54, LD56, LD57, LD60, LD62. Middle row from left: LD63, LD64, LD65, LD66, LD69. Bottom left: LD70, LD71, LD73, LD74, LD75.

Sauøya, Hitra

The *Laminaria digitata* specimens from Sauøya, Hitra had all a narrow and long lamina. LD79 and LD80 had deep rifts in their lamina, while both LD77 and LD78 had a rounded area by the meristem. The mean width of the lamina was 23 cm and the CV was 33% (Appendix II, Table 4). The mean calculated ratio between the width and length of the lamina was 0.29 and the CV was 25% (Table 5). They all had a 90° or wider angle between stipe and lamina so all the specimens were in category 1 (Table 4). Even though the stipes where mis-shaped after being frozen it seemed like they all had been flatten before they were frozen, which were also confirmed be the collector.

The specimens from Sauøya had been frozen before they were taken pictures and DNA-samples of. That caused that they had some mis-coloration, and the stipes were mis-shaped, so it was only taken picture on a white background (Fig. 18) and not on illumination box.

Figure 18: Morphology of *Laminaria digitata* specimens collected in October 2012 at Sauøya, Hitra. Specimen LD77 is the specimen to the right in the left picture. From left: LD77, LD78, LD79, LD80.

Laminaria hyperborea

February

The three collected *Laminaria hyperborea* specimens from Brænnebukta in February 2013 (LD1, LD12, LD21) had a rounded disc in the base of the lamina (Fig. 19). There were a narrowing of the lamina before the separation of the lamina fronds. The tissue had a difference in coloration above and underneath this narrowing, the tissue closer to the meristem is lighter than the tissue further out from the meristem. The angle between lamina and stipe is larger than 90˚ in the three collected specimens and is thereby in category 1 (Table 6).

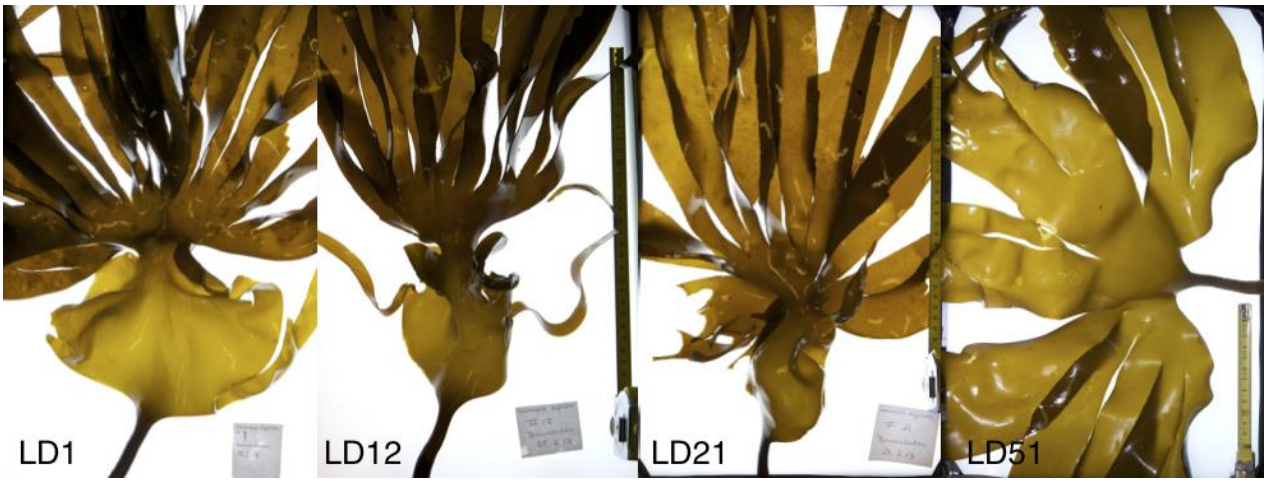

Figure 19: Morphology of the lamina of *Laminaria hyperborea* specimen sampled in Brænnebukta, Trondheimsfjord in February and May 2013, showing the angle between the blade and stipes. From left: LD1, LD12, LD21 (all from February), LD51 (May).

Table 6: Categorization of the angle between stipe and lamina, and the shape of the middle cross section of the stipe of *Laminaria hyperborea* from Brænnebukta. Categories for the angle: > 90° = 1; ± 90° = 2; < 90° = 3. Categories for the shape of the stipe (Height/width): 0.25-0.5 = 1; 0.51-0.75 = 2; 0.76-1 = 3.

Brænnebukta								
28.02.2013			06.05.2013			02.09.2013		
Specimen	Angle category	Shape of stipe	Specimen	Angle category	Shape of stipe	Specimen	Angle category	Shape of stipe
LD1	1	-	LD51	3	-	LD53	3	3
LD12	1	3				LD55	3	3
LD21	1	3				LD59	3	3
						LD67	3	3
						LD68	3	3
						LD72	2	3

May

In May there were only found one individual of *L. hyperborea* (LD51, Fig. 19), which had a wide and short lamina (Appendix II, Table 5). The shape of the base of the lamina was heart shaped, and found in category 3 (Table 6).

September

In September there were found 6 *Laminaria hyperborea* specimens in a kelp forest dominated by *L. digitata* (Fig. 20). LD72 had a 90° angle between stipe and lamina, while the remaining was heart shaped, which means that there were 1 in category 2 and 5 in category 3 (Table 6). The ratio (Table 7) between width and length of the lamina is greater in September than in February. When cutting in the meristem to collect DNA-sample, several of the specimens secreted mucus. The meristem was thicker than in the collected specimen of *Laminaria digitata*.

Figure 20: Morphology of *Laminaria hyperborea* sampled in September 2013 in Brænnebukta, Trondheimsfjord. Detail pictures shows the variation in the angle between stipe and lamina. From left: LD53, LD55, LD59, LD67, LD68, LD72.

Table 7: Ratio between the width and length of the lamina of *Laminaria hyperborea* specimens sampled in Brænnebukta in February, May and September 2013. The width and length measurements are found in Appendix II, Tables 5, 6, 7.

Brænnebukta					
28.02.2013		06.05.2013		02.09.2013	
Specimen	**Ratio (width/length)**	**Specimen**	**Ratio (width/length)**	**Specimen**	**Ratio (width/length)**
LD1	0.31	LD51	-	LD53	1.05
LD12	0.22			LD55	1.23
LD21	0.31			LD59	0.98
				LD67	0.91
				LD68	0.65
				LD72	0.75
Mean	**0.28**	**Mean**	**-**	**Mean**	**0.93**
Standard deviation	**0.05**	**Standard deviation**	**-**	**Standard deviation**	**0.21**
Coefficient of variation	**19%**	**Coefficient of variation**	**-**	**Coefficient of variation**	**23%**

Figure 21 shows the variation in *L. hyperborea* from February to September. Some of the *L. hyperborea* specimen left mucus on the canvas after they had been photographed. The mean width of the lamina in February was 18.2 cm and the CV was 20%, while in September the mean width was 48.1 cm and the CV was 25% (Appendix II, Table 7). The average ratio of the width and length of the lamina was calculated, this was 0.28 in February with a CV of 19%, in May the width measurement is lacking, and in September it was 0.93 and a CV of 23% (Table 7).

Figure 21: Seasonal differences in morphology of *Laminaria hyperborea* sampled in February, May and September in Brænnebukta, Trondheimsfjord. From left: LD12-LD21 (February), LD51 (May), LD53-LD72 (September). Pictures of all collected specimens are found in Appendix III, Figures 4 and 5.

The stipes of *L. hyperborea* were all rounded, close to circular and all were found in category 3 (Table 6), and they were either wider at the base by the hapter or the same size (Fig. 22). They were also less flexible than the stipes from the collected *Laminaria digitata*, and had a more "woody" character with rough surface in contrast to the smooth surface of *L. digitata*. Especially the thicker stipes of *L. hyperborea* specimens was hard to bend. It was not taken picture of the cross section of the specimen collected in May.

Figure 22: Morphological differences in cross sections taken from three locations of the stipes of *Laminaria hyperborea* sampled in February, May and September 2013 in Brænnebukta, Trondheimsfjord. Images shows discs from the stipes where the uppermost is taken close to the meristem, middle at middle part of stipes and the lowest close to the hapter. On the pictures were there are three cross sections, the 3rd cross section is the part next to the transition part. Top left: LD12 , LD21 (both collected in February), LD51 (May), LD53, LD55 (both collected in September). Bottom left: LD59, LD67, LD68, LD72 (collected in September).

Svalbard

Saccharina groenlandica

The DNA-sequencing showed that there was only collected *Saccharina groenlandica* in the material that resembled *Laminaria digitata* on Svalbard (Table 3). The morphology of *S. groenlandica* was found to vary from the different sampling sites.

Kapp Mitra (outlet of Kongsfjorden, Spitsbergen)

The specimens collected by Kapp Mitra had long and narrow laminae (Fig. 23). The mean width of the lamina was 24 cm and the CV was 31% (Appendix II, Table 8). The mean ratio between the width and length of the laminae was 0.39 and had a CV of 21% (Table 8). LD84, LD86, LD90 had dark areas by the edge of the laminae, on either one side or both sides. LD85 as a darker area across the lamina about 5 cm from the meristem.

Figure 23: Differences in morphology of *Saccharina groenlandica* specimens sampled at Kapp Mitra, outlet of the Kongsfjord in September 2013. From top left: LD81, LD82, LD83, LD84. From bottom left: LD85, LD86, LD89, LD90.

Table 8: Ratio of lamina (width/length) of the specimen sampled at Kapp Mitra, the Smeerenburgfjord and Hinlopen Strait in September and October 2013. The width and length measurements are found in Appendix II, Tables 8, 9, 10.

Kapp Mitra		Smeerenburgfjorden		Hinlopen	
Specimen	**Ratio (width/length)**	**Specimen**	**Ratio (width/length)**	**Specimen**	**Ratio (width/length)**
LD81	0.43	LD91	0.72	LD101	0.88
LD82	0.22	LD93	0.73	LD102	1.19
LD83	0.39	LD94	1.46	LD103	0.86
LD84	0.39	LD96	1.53	LD107	0.42
LD85	0.49	LD98	0.66	LD108	0.64
LD86	0.44				
LD89	0.34				
LD90	0.40				
Mean	**0.39**	**Mean**	**1.02**	**Mean**	**0.80**
Standard deviation	**0.08**	**Standard deviation**	**0.43**	**Standard deviation**	**0.29**
Coefficient of variation	**21%**	**Coefficient of variation**	**43%**	**Coefficient of variation**	**36%**

The angle between the lamina and the stipe was large in all the specimens collected at Kapp Mitra (Fig. 23) and they were all found in category 1 (Table 9). The stipes were all more flat near the blade and rounded towards the holdfast, 4 specimens were in category 2 and 3 specimens in category 3 (Table 9).

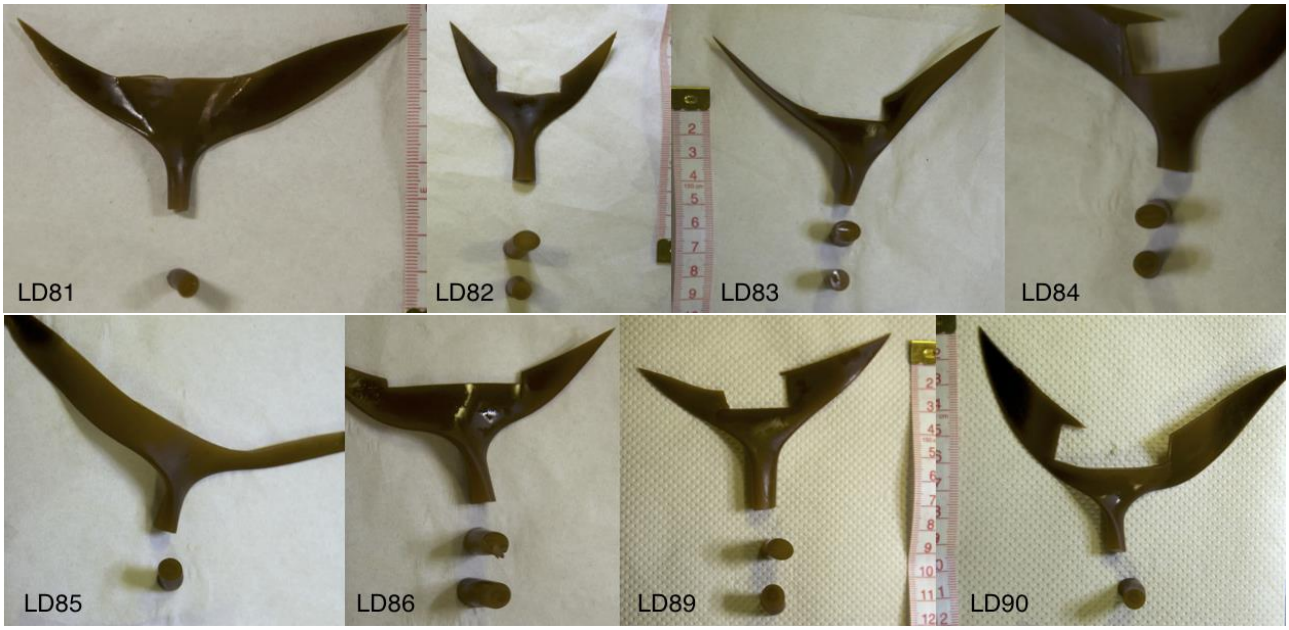

Figure 24: Morphological differences in cross section taken from three locations of the stipes of *Saccharina groenlandica* specimens collected at Kapp Mitra in September 2013. Images shows discs from the stipes where the uppermost is taken close to the meristem, middle at middle part of stipes and the lowest close to the hapter. From top left: LD81, LD82, LD83, LD84. From bottom left: LD85, LD86, LD89, LD90.

Table 9: Categorization of the angle between stipe and lamina, and the shape of the middle cross section of the stipe of *Saccharina groenlandica* from Kapp Mitra, the Smeerenburgfjord and Hinlopen. Categories for the angle: > 90° = 1; ± 90° = 2; < 90° = 3. Categories for the shape of the stipe (Height/width): 0.25-0.5 = 1; 0.51-0.75 = 2; 0.76-1 = 3.

Kapp Mitra			Smeerenburgfjord			Hinlopen		
Specimen	**Angle category**	**Shape of stipe**	**Specimen**	**Angle category**	**Shape of stipe**	**Specimen**	**Angle category**	**Shape of stipe**
LD81	1	-	LD91	3	2	LD101	3	3
LD82	1	2	LD93	3	2	LD102	3	3
LD83	1	2	LD94	3	2	LD103	3	3
LD84	1	2	LD96	3	2	LD107	3	3
LD85	1	3	LD98	3	3	LD108	2	3
LD86	1	3						
LD89	1	2						
LD90	1	3						

The Smeerenburgfjord, Spitsbergen

In the Smeerenburgfjord, the *Saccharina groenlandica* specimens had a broad and shorter lamina (Fig. 25) than the specimens found at Kapp Mitra. The mean width of the lamina was 52 cm and had a CV of 23% (Appendix II, Table 9). The mean ratio between the width and length of the lamina was 1.02 and the CV was 43% (Table 8). All of the specimens had a heart-shaped lamina-stipe angle, and found in category 3 (Table 9). LD96 had an especially thick lamina. The specimens had a dark brown color, and LD98 had a darker area by the meristem.

Figure 25: Morphological variation of *Saccharina groenlandica* sampled from the Smeerenburgfjord in September 2013. From left: LD91, LD93, LD94, LD96, LD98.

All the collected specimens had flattened stipes from around ½ and up, and more rounded towards the holdfast (Fig. 26). LD94 had a particular broad stipe in the transition from the lamina to the stipe, it was 5 cm in width. There were 4 specimens found in category 2 and 1 in category 3 (Table 9).

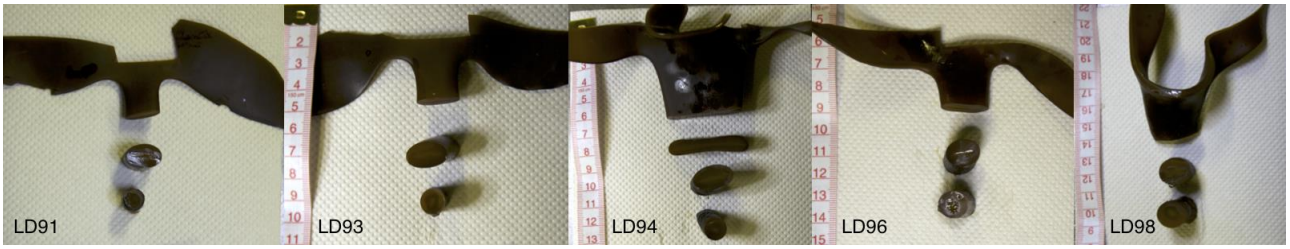

Figure 26: Morphological differences in cross sections taken from three locations of the stipe of *Saccharina groenlandica* collected in the Smeerenburgfjord. Images shows discs from the stipes where the uppermost is taken close to the meristem, middle at middle part of stipes and the lowest close to the hapter. On the pictures were there are three cross sections, the 3th cross section is the part next to the transition part. From left: LD 91, LD93, LD94, LD96, LD98.

Gyldénøyane, Hinlopen strait

By Gyldénøyane, the *Saccharina groenlandica* specimens also had a wide lamina (Fig. 27). The mean lamina length was 41 cm and the CV was 33% (Appendix II, Table 10). The mean ratio between width and length was 0.80 with a CV of 36% (Table 8). All of the specimens sampled at Gyldénøyane had a lighter color by the meristem. LD101 and LD103 had some darker areas on the lamina; these were more coarse than the surrounding tissue. The angle between the stipe and the blade were lightly heart-shape in all, and 1 was found in category 2 and 4 in category 3 (Table 9).

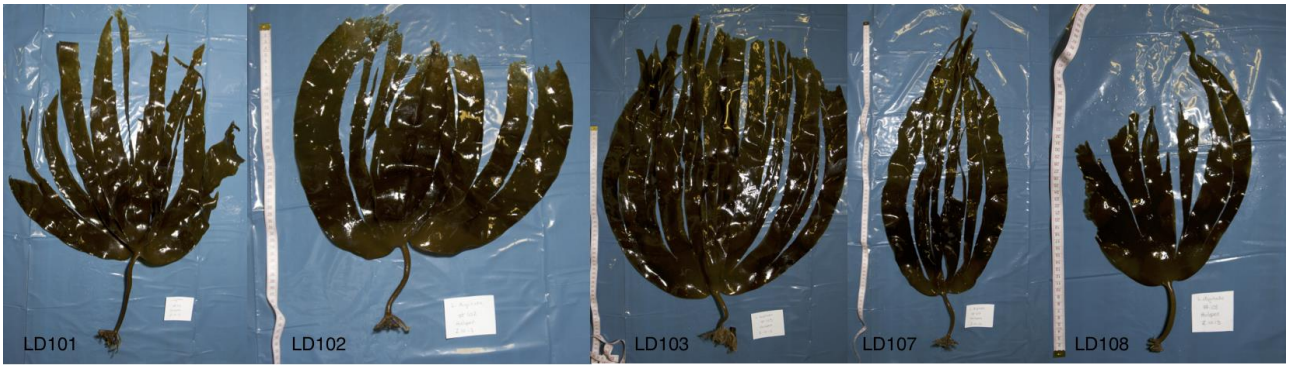

Figure 27: Morphological variation of *Saccharina groenlandica* sampled at Gyldénøyane, Hinlopen Strait in October 2013. From left: LD101, LD102, LD103, LD107, LD108.

LD101, LD102 and LD103 had stipes which were slightly flatten from the middle part and up to the blade, while LD107 and LD108 had rounded stipes the whole way (Fig. 28). All of them ended up in category 3 (Table 9).

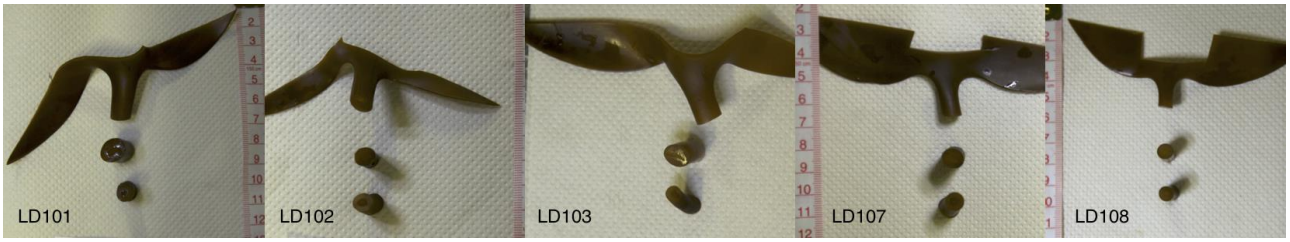

Figure 28: Morphological differences in cross sections taken from three locations of the stipe of the *Saccharina groenlandica* specimen sampled at Gyldénøyane, Hinlopen Strait in October 2013. Images shows discs from the stipes where the uppermost is taken close to the meristem, middle at middle part of stipes and the lowest close to the hapter. From left: LD101, LD102, LD103, LD107, LD108.

Length of stipe and lamina

Figure 29 illustrates the mean length of specimens of the collected species at different dates and sites. The blue part of the bar visualizes the average length of the stipes, while the orange part of the bar shows the average length of the lamina. The percentages indicates how much of the thallus that consists of lamina or stipe. The figure shows that the longest mean total length of *Laminaria digitata* were found in May in Brænnebukta, which was 129 cm and the coefficient of variation (CV) was 21% (Appendix II, Table 2). In February the mean length was 93 cm and the CV was 21% and the lamina was a smaller fraction of the thallus than in May and September, with 53%. In September the mean length was 96 cm with a CV of 17% and on Hitra it was 118 cm. The mean length of the stipes of *L. digitata* did not vary too much between the different sampling dates and sites (Appendix II, Tables 1, 2, 3, 4). The mean length of lamina was more varied, in February it was 49 cm (CV = 23%), 81 cm (CV = 21%) in May, in September the mean length was 61 cm (CV = 24%) and at Hitra it was 79 cm (CV = 23%) (Appendix II, Tables 1, 2, 3, 4). The lamina made up between 53% and 67% of the thallus.

The mean length of *Laminaria hyperborea* was 115 cm in February and 101 cm in September, in May it was only collected one *L. hyperborea* and that was 145 cm long (Appendix II, Tables 5, 6, 7). The mean length of the stipe of *L. hyperborea* is quite similar in February and September, 50 and 49 cm, with a CV of respectively 6% and 17%, making up respectively 56% and 52% of the thallus. The specimen from May individual had a stipe length of 80 cm. The mean lamina length was longer

in February than in September, respectively 65 cm (CV = 2%) and 52 cm (CV = 12%).

Saccharina groenlandica had a shorter mean length than found in *L.digitata* and *L.hyperborea*. At Kapp Mitra the mean length was 76 cm (CV = 13%), at the Smeerenburgfjord it was 90 cm (CV = 26%) and at Hinlopen Strait the mean length was 68 cm (CV = 31%) (Appendix II, Tables 8, 9, 10). At Kapp Mitra and Hinlopen Strait, the mean stipe length were a smaller fraction of the length, than in the Smeerenburgfjord. At Kapp Mitra the mean stipe length was 15 cm, with a CV of 53%, at the Smeerenburgfjord the mean length was 33 cm and the CV was 22%, in Hinlopen Strait the mean length of the stipes were 14 cm and the CV was 35% (Appendix II, Tables 8, 9, 10). The mean length of the lamina did not vary much between the locations, at Kapp Mitra it was 60 cm, it was 57 cm in the Smeerenburgfjord and 54 cm in Hinlopen Strait (Appendix II, Tables 8, 9, 10). But the fraction of the thallus that the lamina made up varied, making up 80% at Kapp Mitra and Hinlopen Strait, and 63% in the Smeerenburgfjord.

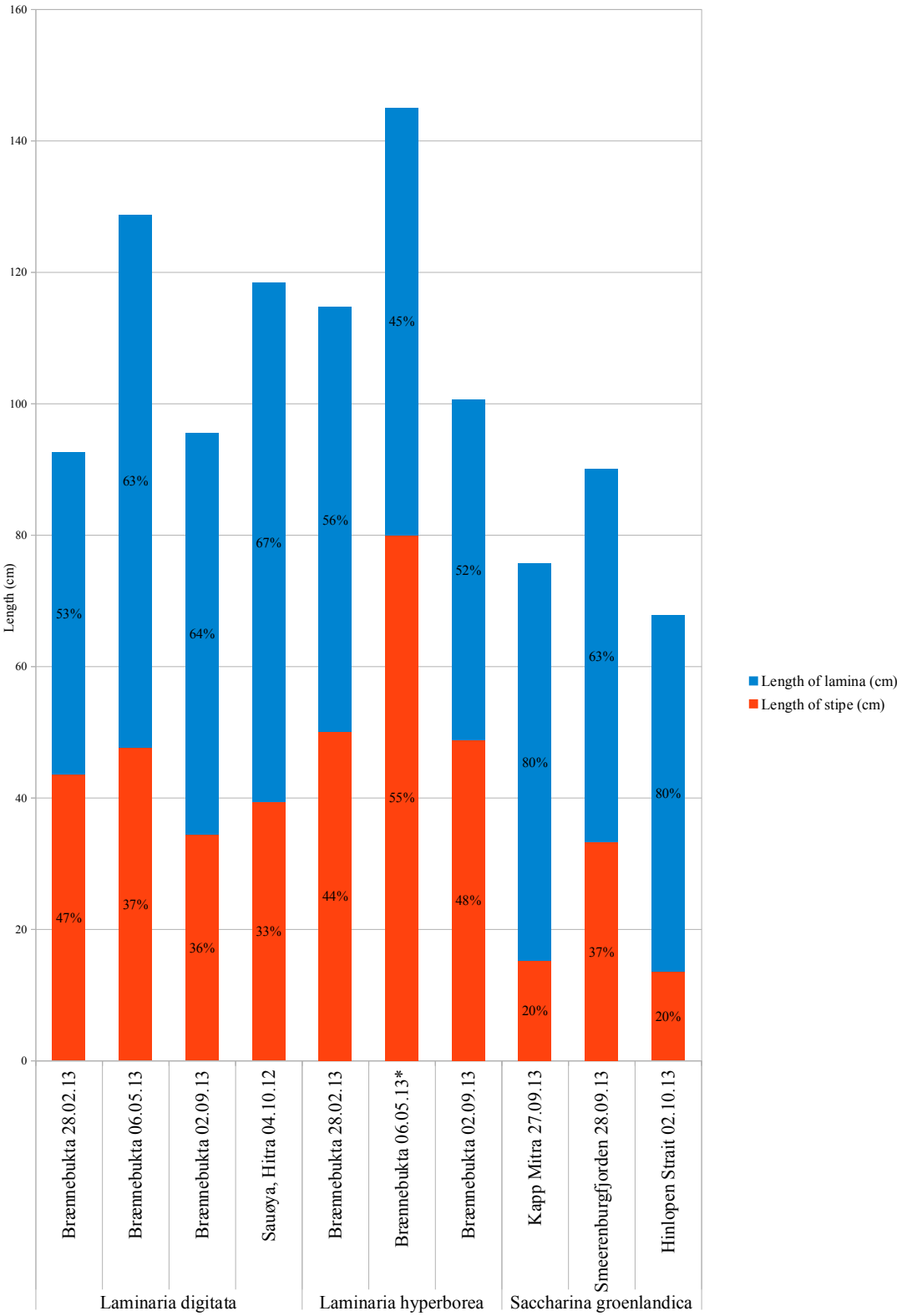

Figure 29: Mean length of lamina and stipes in specimens of *Laminaria digitata* and *Laminaria hyperborea* collected in Brænnebukta and at Hitra, and *Saccharina groenlandica* collected at Kapp Mitra, Smeerenburgfjord and Hinlopen Strait. Mean lamina length and length of stipe is indicated respectively in orange and blue, and the percentage of the total length for each part consists of is marked in each bar. The measurements and statistics are shown in Appendix II, Tables 1-10. *Only one *L.hyperborea* was collected in Brænnebukta in May 2013.

Epigrowth

In February there were most epigrowth on the old tissue of *Laminaria digitata* collected at Brænnebukta, on the new tissue there was little or no epigrowth. The epigrowth found on *L. digitata* was mainly bryozoans, mostly *Electra pilosa* (Fig. 30, left) and some *Membranipora membranacea*. There were bryozoans on the stipe of some of the specimens, and then primarily close to the holdfast. A couple of specimens had some small rhodophytes attached to their stipe. There were less epigrowth on *L. digitata* in May than in February; three specimens had no epiphytes, while many had just a small amount of bryozoans on the stipe. There were some individuals that had some growth of *Spirorbis spirorbis* on the lamina (Fig. 30, right). In September there appeared to be more epiphytes on the lamina than both in February and May. All of the specimens had some form of epiphytes on the lamina. There was an abundance of bryozoans on several individuals, the lamina of LD62 was almost entirely covered. Both *E. pilosa* and *M. membranacea* were present.

On the specimens of *Laminaria digitata* from Hitra there were no epiphytes on the lamina of LD77 and LD78, but LD78 had a little bryozoans on the stipe. LD79 and LD80 had some bryozoans on the lamina.

On *Laminaria hyperborea* sampled in February there was a little bryozoan growth on the older tissue of the lamina and epigrowth of rhodophytes on stipes. In May there were no epigrowth on the lamina, but there was plenty on the stipe; rhodophytes, bryozoans and *S. spirorbis*. All of the sampled individuals of *L. hyperborea* in September had epigrowth on their lamina, especially bryozoans and rhodophytes. There were more epigrowth on the stipes of the specimens collected in February and May than the specimens collected in September. In February the most common epigrowth were bryozoans and *Palmaria palmata*. In May there were also bryozoans and *P. palmata*, but also other rhodophytes as *Phycodrys rubens* and *Ptilota gunneri*.

The sampled *Saccharina groenlandica* specimens from Kapp Mitra had no visible epigrowth, neither on the lamina nor the stipe. On the specimens from the Smeerenburgfjord there were three individuals that had bryozoans both on the lamina and the stipe; LD91, LD93 and LD96. LD94 had only a few bryozoans on the upper part of the stipe, while specimen LD98 had no epiphytes. The specimens collected in Hinlopen had little epigrowth, only a few bryozoancolonies.

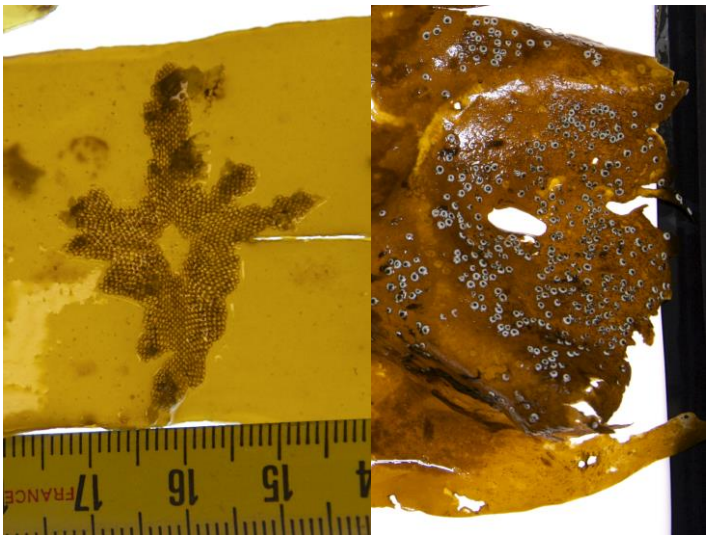

Figure 30: Left picture: *Electra pilosa* on specimen LD19. Right picture: *Spirorbis spirorbis* on specimen LD34.

Discussion

Phylogeny

All phylogenetic analyses run for the kelp COI sequences resulted in the same well-supported clades identifying the analyzed material from Norway as *Laminaria digitata* and *L. hyperborea*, and the analyzed material from Svalbard as *Saccharina groenlandica* (Figs. 6, 7, 8). *Laminaria solidungula*, *Laminaria yezoensis*, *Laminaria ephemera* and *Nereocystis luetkeana* had more uncertain placements in the phylogenetic trees. The *S. groenlandica* reference sequences differer by a single point mutation from the Svalbard material, possibly due to a mutation in the Svalbard population or in the population from British Columbia, Canada, where the reference specimens were collected (Ratnasingham & Hebert 2007). The Bayesian analysis produced a tree similar to the phylogeny shown by McDevit and Saunders (2010), where *Laminaria* and *Saccharina* were both monophyletic and *Nereocystis leutkeana* was in a clade of its own, though their phylogeny was based on Neighbor-joining. Both in the Maximum likelihood analysis and in the Neighbor-joining analysis, *Laminaria* was polyphyletic, possibly due to the fairly few species of *Laminaria* included in the analyses. In the Maximum likelihood tree, *N. luetkeana* was included in the *Saccharina*-clade (Fig. 7), as it was in the phylogenetic analyses of McDevit & Saunders (2009) utilizing COI sequences and in Lane et al. (2006) utilizing genes of both nuclear and plastidic origin, suggesting that there are some uncertainties regarding the relationship of *N. luetkeana* and *Saccharina*.

The reference sequences used were all from specimens submitted to GenBank by Dr. Saunders, the reason that I chose only sequences uploaded by him is because he has done a lot of work in this field. And a weakness in GenBank is that there is no quality control, that means that there may be wrongly identified species in the database. These uncertainties is of major concern and should be further elucidated.

Morphology

Laminaria digitata

The seasonal variability of lamina morphology in *Laminaria digitata* found in Brænnebukta was considerable changing from specimens of lamina category 1 and 2 dominating in February to mostly specimens of lamina category 3 in May and September (Table 4). The greatest variation in the shape of the lamina was found in February, which is the period were the growth rate of the lamina is the highest and that is probably the reason why there is a large variation in morphology. It seems like when the lamina is growing rapidly it takes some time before it splits into “fingers”, and when it does split in the spring the lamina probably gets wider and the angle between the stipe and lamina gets smaller. The measurements of the width of the lamina also supports that the lamina is wider in September than in especially February and to some extent in May. The average length (Appendix II, Tables 1, 2, 3) of the lamina is longest in May, this suggests that in September the wear from different environmental factors have made the outer parts of the lamina to shed, and in February the lamina is still in its new-growth phase.

Different phenotypes of *Laminaria digitata* were found in the same site (Brænnebukta); f. *cucullata*, f. *typica* and the form that was only found in the Trondheimsfjord (Printz 1953; Sundene 1964; Sundene 1958). This finding contradicts the suggestion that *L. digitata* morphology is affected by only environmental factors (Sundene 1964), alternatively the Brænnebukta site experience a mid-exposure level that favor several phenotypes. Another possibility is that genetic

differences can affect the morphology of individuals collected only centimeters apart. A third explanation can be that the neighboring kelp affects each other by shading or mechanical influence which may alter the growth. The unidentified form that Sundene (1958) found in the Trondheimsfjord can be a seasonal variation of f. *cucullata*.

From Hitra only a few individuals were collected; these were all of f. *typica*, with a narrow and long lamina. If *L. digitata* f. *typica* dominates in more exposed localities as been suggested (Sundenes 1964), this indicates that the exposed site at Hitra relative to the more sheltered site at Brænnebukta, do change the morphology of this species. Alternatively, lamina shape may depend more on season than environmental factors, but since the specimens from Hitra were sampled in October, they would more likely have a wider lamina compared to lamina of specimens collected in Brænnebukta.

The stipe morphology varied much less throughout the year, suggesting characteristics of the stipe may be a more useful diagnostic character to identify kelp species. Small specimens of *L. digitata* have rounded, narrow stipes that vary in size but not with season.

When studying the pictures taken on the illumination box (Figs. 9, 12, 15), less light seems to be going through the lamina in September than in February and May. This can be related to lamina thickness (self shading) and pigmentation (higher towards summer). In addition, epigrowth will make the lamina darker due to biofilm (microorganisms), larger macroalgae and epifauna.

Laminaria hyperborea

Some of the specimens that were collected in Brænnebukta had some of the most common characteristics of *Laminaria hyperborea*, such as the circular, rough stipe. Although *L. hyperborea* is not common in the Trondheimsfjord (Jensen et al. 2000), these specimens were identified to *L. hyperborea* also by their COI sequences. All of the individuals that were identified as *L. hyperborea* by DNA sequencing had a close to circular cross section of stipe and fell into category 3 of the shape of the stipe (Table 4). The stipes had rough surfaces (enhancing epigrowth) and were far more stiff in contrast to *Laminaria digitata*.

The specimens of *L. hyperborea* sampled in February did not have the typical *L. hyperborea* characteristics with the heart shaped base and angle category 3, they had a rather large angle between stipe and lamina, which fell in category 1. This is probably caused by the fast growth of the new lamina from the meristem in the winter season, when the lamina has still not divided into fragments. It seems like the angle between stipe and the lamina decrease and the lamina broadens as the lamina splits up. As in *L. digitata*, the lamina was wider in September than in February (Appendix II, Tables 5, 6, 7), and the shape of the lamina of all the collected specimens fell in to category 3, which suggested that when the lamina gets wider the shape of the base of the lamina gets more heart-shaped and the angle smaller. The average length of the lamina is shorter in September possibly due to by environmental factors like wave exposure and epigrowth that wear the lamina down. The lamina was longer than the stipe in 7 of 10 individuals; this contradicts the species description by Rueness (1977) where it says that the lamina is usually shorter than the stipe.

There was only one *L. hyperborea* sampled in May, which makes it hard to say anything about the morphological variation of the species in this season. One could still see the contours of the disc-shape seen in February, and the rifts in the lamina was still not very deep, this is probably due to that the *L. hyperborea* was still in the fast growth period.

The stipe will most likely not change its morphology through the seasons, while the lamina will.

This is why I believe that it is much safer to distinct the *L. hyperborea* by using its stipe, both its roundness (stipe category 3), roughness and the diameter, that it is equal or thicker close to the holdfast. In younger or smaller individuals this characteristic might not be as accurate as in the bigger specimens collected.

Another morphological character that seems to distinguish the two *Laminaria* spp is the meristem, which seems to be thicker in *L. hyperborea* compared to *L. digitata* (personal observation).

The reason why *L. hyperborea* was found this far into the Trondheimsfjord can be because Brænnebukta lies on the southern side of the fjord, the side where the current goes into the fjord (Bakken et al. 2000), which could bring the algae further in from outer coastal areas into the fjord systems. According to Printz (1953) *L. hyperborea* is mostly found in exposed areas and is not found far into fjords, so it would have been interesting to study the occurrence of *L. hyperborea* at several locations in the fjord, also on the northern side, where the outwards current is located and the travel from the coast is longer.

Saccharina groenlandica

The morphology of *Saccharina groenlandica* sampled on Svalbard differed from each location, which suggests that local conditions have a great impact on the morphology of *S. groenlandica*. At Kapp Mitra the collected specimens had a long, narrow lamina and the angle between the stipe and the lamina was greater than 90°, which means that all the collected specimens fell in category 1 of the shape of the lamina. If the phenotypes of *S. groenlandica* also are partly a product of the environmental exposures as seen in *L. digitata* (Sundene 1964), the specimens collected at Kapp Mitra may experience stronger currents than in the two other sites that were sampled on Svalbard. The specimens sampled from the Smeerenburgfjord had an average wider lamina than specimens from the other two sampling sites on Svalbard. The stipes in these specimens were also considerably longer, which make them resemble a typical *Laminaria digitata*. The lamina was broad and relatively short compared to the stipe and all of the sampled specimens had a heart shaped base of the lamina. In Hinlopen the collected specimens had relatively short stipes and a broad and long lamina, which suggests less exposure in the sampling site according to Sundene (1964).

The shape of the stipes of *Saccharina groenlandica* was never in category 1 (Table 9), at the Hinlopen site there were only stipe of category 3 and in the Smeerenburgfjord there were 4 in category 2 and 1 in 3, while at Kapp Mitra they were spread almost even. Which indicates that environmental factors also can have an effect on the shape of the stipe.

Since only one season was sampled at Svalbard it cannot be drawn any conclusions about the seasonal variation in *S. groenlandica*, but I believe that there is variation through the seasons in Svalbard compared to that found in the Trondheimsfjord. On Svalbard the sea ice can affect the wear and tear of the lamina, and thereby maybe the overall morphology. Ice cover parts of the year will also probably affect the growth rate of the lamina, and in areas where the ice stays long in the spring the specimens may experience less growth because of little light. Under the ice cover there will be no wave exposure either, which also will affect the morphology of the lamina.

Some of the specimens of *Laminaria digitata* that Kjellman (1877) described from Spitsbergen resemble the *Saccharina groenlandica* specimens that were sampled at Svalbard in 2013, especially the specimens that were collected in the Smeerenburgfjord. These had also a kidney shaped lamina and had dark areas near the meristem, like the specimens that Kjellman described. After reading his

descriptions of *L. digitata* from Svalbard, like that the length of the stipe was variable, but fairly short compared to the lamina and comparing with the specimens that have been sequenced as *Saccharina groenlandica*, it seems like the specimens that he described possibly was *S. groenlandica*. Kjellmann collected a couple of specimens in July that had old tissue still attached, causing a distinct shape with two morphological different structures and color. With several collections through the year, one could look at the variation of morphology through the year.

As in the Maritime Provinces of Canada (Longtin and Saunders 2013), the result of this study suggests that *Saccharina groenlandica* can be more abundant than *Laminaria digitata* in Svalbard. Both in this study and the AB202-report from UNIS (2012) showed that *S. groenlandica* was found in more sites at Svalbard than *L. digitata*. There is also the matter of fact that a phenotype of *S. groenlandica* resembles *Saccharina latissima* (Longtin & Saunders 2013), so there is the possibility that there can be *S. groenlandica* that also has been regarded as *S. latissima* at Svalbard. There is also the possiblity that *Laminaria gunneri* found in Finnmark (Rueness 1977), also represent *S. groenlandica*, but this requires further studies.

Since *Laminaria digitata* was not found during our sampling on Svalbard, it is difficult to say anything distinct about their characteristic compared to *Saccharina groenlandica*. It can be morphological differences between *L. digitata* in the Trondheimsfjord and at Svalbard.

This study shows that we still do not know the species composition in the kelp forests of Svalbard, and that there is still work that needs to be done regarding mapping of kelp forests. The management plan from Sysselmannen (2013) only mentioned two areas, but do we know enough about the species diversity in the other kelp forests around Svalbard?

Species comparison

The inner parts of the Trondheimsfjord are formerly believed to contain mostly *Laminaria digitata* (Jensen et al. 2000). This study indicates that there is also a considerable amount of *Laminaria hyperborea*, at least in Brænnebukta. *Laminaria digitata* and *L. hyperborea* are quite similar in form and both have variation in the morphology throughout the year, but the shape and texture of the stipe is different in the two species. According to my data the stipe is the best way to distinguish between *L. digitata* and *L. hyperborea*. *Laminaria hyperborea* usually has a stipe that will fall in the category 3 in stipe cross section, while stipes from *L. digitata* can fall in either category (1-3). The texture of the stipe is also different between the two species, at least in the older specimens of *L. hyperborea*, the stipe is rougher and not as smooth as in *L. digitata* and it is also stiffer, this agrees with the species descriptions by Rueness (1977), where he describes the stipe of *L. digitata* as flexible and *L. hyperborea*s stipe as non-flexible. Another trait that can be observed from the pictures is that the stipe of *L. hyperborea* is either cone shaped or has the same circumference from lamina to hapter, while the stipe of *L. digitata* will be thinner just before the hapter. The shape of the base of the lamina is too variable to use as a diagnostic characteristic, it appears that it is mainly a seasonal or age variation in the morphology of the lamina of *L. digitata* and *L. hyperborea*, since in February there are found more lamina-stipe angle of category 1 in *L. hyperborea* than in *L. digitata*.

Saccharina groenlandica seems to be more plastic than both *L. digitata* and *L. hyperborea*, concerning the morphology of both the stipe and the lamina which varied greatly between locations. Even though it is hard to say too much about the plasticity in these two latter species since they in this study is described from only one or two sites. It is difficult to describe any distinct morphological characters of *S. groenlandica* since it is quite variable from site to site, probably due to environmental conditions. But it seems like *S. groenlandica* at Svalbard on average has a shorter stipe than *L. digitata* (Appendix II, Tables 8, 9, 10) found in the Trondheimsfjord and that this can

be used as a trait to distinguish the two species. In literature the stipe of *S*. *groenlandica* is described up to 70 cm long, depending on the phenotype (Taylor 1957), which is longer than the average measurements of *L*. *digitata* from the Trondheimsfjord. As in *L*. *digitata*, there are specimens of *S*. *groenlandica* where the stipe gets thinner near the hapter.

Without doing any color measurements, it seems like the *S*. *groenlandica* specimens had a darker color on the lamina and stipe than *L*. *digitata* (with a few exceptions), indicating higher pigmentation of the major light havesting pigments Chl *a*, Chl *c* and fucoxanthin. In addition the thicker the lamina, the higher absorption of light in the visible range (400-700 nm) making lamina darker (less light transmitted or reflected) (Hilstad 2005).

Trouble shooting

The reference sequences used were all from specimens submitted to GenBank by Dr. Saunders, the reason that I chose only sequences uploaded by him is because he has done a lot of work in this field. And a weakness of GenBank is that there is no quality control, which means that there may be wrongly identified species in the database. Phylogenetic analyses can be uncertain if several taxa is missing, but if the reference sequences used are wrongly identified will it for sure give incorrect phylogeny.

The use of mucilage ducts as a taxonomic character has been debated (Kain 1979). Since anatomical examinations of stipe and lamina is time consuming, these examinations was not performed. In hindsight it would have been interesting to examine this character in the different species.

When looking back at the work with the morphological traits, I see that I should have included a cross section by the transition zone between stipes and meristem as well. That would have given a better view of the shape at different positions of the stipe. The stipe categories were also too wide, which makes lightly flattened cross section also end up in category 3. But for simplicity it was important not to have too many categories.

One weakness with not doing my own collections at Svalbard is that I do not know the morphological variability of the collected populations, since the SCUBA diver got asked to collect kelp that looked like *Laminaria digitata*, there can be other phenotypes of *Saccharina groenlandica* that could have been overlooked. So that has to be taken into consideration for possible studies in the future. It would also be interesting to look at how the morphology changes through the year, and also how the depth influences the morphology.

Future perspectives

There is still much work to be done regarding identification and mapping of kelp along the Norwegian coast and in the Arctic. For *Saccharina groenlandica* it would be interesting to study the seasonal variations of the morphology, and especially see how wave action, current speed and ice cover affects morphology of the lamina. Up-to-date literature about morphological characters and distribution of arctic kelp species is also scarce, which is a big task that should be done in the near future. In the Trondheimsfjord it would be interesting to know the fraction of *Laminaria hyperborea* in kelp forests dominated by *L*. *digitata* and how far into the fjord *L*. *hyperborea* is found.

In the phylogeny of kelp there are still uncertainties in some clades and there are several currently accepted species of *Laminaria* that are not sequenced, such as *Laminaria nigripes* and *Laminaria gunneri*, which can either be separate species or be a part of already established species.

Conclusions

Using DNA barcoding of the mtDNA COI-gene to differentiate between kelp species was successful, and could document the presence of *Saccharina groenlandica* at Svalbard.

Several factors seems to have an effect on the morphology on both *Laminaria digitata* and *Laminaria hyperborea*, these comprise environmental factors, genetic differences, mechanical influences and shading between specimens, together with seasonal variations.

The best way to distinguish between *L. hyperborea* and *L. digitata* is to look at:
A) The cross section of the stipe together with its flexibility.

A1) *Laminaria digitata* has oval shaped cross section of the stipe and the stipe is flexible.
A2) The cross section of the stipe of *L. hyperborea* are circular and the stipe is stiffer than in *L. digitata*.

Saccharina groenlandica is affected by local environmental conditions, showing a large morphological variability between the sampling sites, this variability makes *S. groenlandica* difficult to distinguish from *L. digitata* when looking only at the morphology.

All three of the studied species showed a large morphological plasticity. Figures 31, 32 and 33 illustrates some of the phenotypes found in the different species.

Figure 31: Three different phenotypes of *Laminaria digitata*. The left and right are more extreme in morphology, while the middle is the most regular form of specimens examined.

Figure 32: Three different phenotypes of *Laminaria hyperborea*. The left and right are more extreme morphology, while the middle is the more common shape found during sampling.

Figure 33: Three different phenotypes of *Saccharina groenlandica*. The left and right are more extreme morphology, while the middle is the common form.

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Appendix I

Reference sequences downloaded from GenBank February 2014:

Fucus_serratus gi|183397516|gb|EU646716.1|
Laminaria_digitata_gi|262179452|gb|GU097704.1|
Laminaria_digitata gi|262179454|gb|GU097705.1|
Laminaria_ephemera gi|262179456|gb|GU097706.1|
Laminaria_hyperborea gi|211906633|gb|FJ409155.1|
Laminaria_hyperborea gi|211906635|gb|FJ409156.1|
Laminaria_solidungula gi|211906655|gb|FJ409166.1|
Laminaria_yezoensis gi|211906657|gb|FJ409167.1|
Nereocystis_luetkeana gi|262179490|gb|GU097723.1|
Saccharina_bongardiana gi|262179504|gb|GU097730.1|
Saccharina_groenlandica gi|262179512|gb|GU097734.1|
Saccharina_groenlandica gi|262179514|gb|GU097735.1|
Saccharina_latissima gi|262179708|gb|GU097832.1|
Saccharina_sessilis gi|211906739|gb|FJ409208.1|
Saccorhiza_dermatodea gi|211906747|gb|FJ409212.1|

Appendix II

Table 1: *Laminaria digitata* in Brænnebukta 28.02.13

Specimen	Shape of lamina	Shape of stipe	Lamina width (cm)	Length of stipe (cm)	Length of lamina (cm)	Total length (cm)
LD2	2	-	24.0	35.0	69.0	104.0
LD4	1	-	25.0	41.0	41.0	82.0
LD5	1	-	20.0	40.0	48.0	88.0
LD6	3	-	29.0	49.0	45.5	94.5
LD7	1	-	10.5	38.0	41.0	79.0
LD8	1	-	20.0	34.0	41.5	75.5
LD9	2	2	24.0	45.0	63.0	108.0
LD10	3	1	52.0	48.0	57.0	105.0
LD11	2	2	18.0	44.0	38.0	82.0
LD13	3	3	30.0	57.0	62.0	119.0
LD14	2	3	21.0	43.0	63.0	106.0
LD16	2	3	31.0	36.0	59.0	95.0
LD17	1	-	4.0	6.5	21.0	27.5
LD18	1	2	17.5	37.0	56.0	93.0
LD19	2	2	32.0	51.0	49.0	100.0
LD20	3	2	40.0	55.0	46.0	101.0
LD22	3	1	34.0	66.0	46.0	112.0
LD23	3	2	37.0	48.5	51.5	100.0
LD24	1	2	10.0	45.5	42.5	88.0
LD25	3	2	33.5	51.5	41.0	92.5
Mean			**25.6**	**43.6**	**49.1**	**92.6**
Standard deviation			**11.28**	**11.93**	**11.26**	**19.12**
Coefficient of variation			**44%**	**27%**	**23%**	**21%**

Table 2: *Laminaria digitata* in Brænnebukta 06.05.13

Specimen	Shape of lamina	Shape of stipe	Lamina width (cm)	Length of stipe (cm)	Length of lamina (cm)	Total length (cm)
LD27	1	1	27.0	66.0	63.0	129.0
LD28	3	2	62.0	76.0	91.0	167.0
LD29	3	3	72.0	65.0	95.0	160.0
LD30	3	2	37.0	67.5	84.5	152.0
LD31	3	2	50.0	32.0	77.0	109.0
LD32	3	2	56.0	23.5	100.0	123.5
LD33	3	1	43.0	64.5	64.5	129.0
LD34	3	2	34.0	31.0	54.0	85.0
LD35	3	2	60.0	51.0	105.0	156.0
LD36	3	2	47.0	58.0	82.0	140.0
LD37	3	3	45.0	42.5	89.5	132.0
LD38	3	2	43.0	63.0	84.0	147.0
LD40	3	2	49.0	18.0	80.0	98.0
LD41	3	2	32.0	44.0	75.5	119.5
LD42	2	3	40.0	39.5	78.0	117.5
LD43	3	2	59.0	47.5	95.5	143.0
LD44	3	2	50.0	71.5	66.5	138.0
LD45	1	3	18.0	10.0	34.5	44.5
LD47	3	3	47.0	55.0	97.0	152.0
LD49	3	1	65.0	58.5	80.0	138.5
LD50	3	3	50.0	35.5	81.0	116.5
LD52	3	3	63.0	29.0	106.0	135.0
Mean			**47.7**	**47.7**	**81.1**	**128.7**
Standard deviation			**13.21**	**18.54**	**17.15**	**27.51**
Coefficient of variation			**28%**	**39%**	**21%**	**21%**

Table 3: *Laminaria digitata* in Brænnebukta 02.09.13

Specimen	Shape of lamina	Shape of stipe	Lamina width (cm)	Length of stipe (cm)	Length of lamina (cm)	Total length (cm)
LD54	3	1	80.0	46.0	61.0	107.0
LD56	2/3	2	29.0	28.5	63.5	92.0
LD57	2	3	12.0	26.5	38.0	64.5
LD60	3	2	57.0	37.5	62.0	99.5
LD62	3	2	81.0	43.5	60.5	104.0
LD63	3	2	56.0	55.5	36.0	91.5
LD64	3	2	53.0	45.5	56.0	101.5
LD65	3	2	60.0	29.0	55.3	84.3
LD66	3	-	51.0	30.2	64.3	94.5
LD69	3	-	42.0	17.0	74.0	91.0
LD70	3	3	25.0	41.0	44.0	85.0
LD71	2	2	37.6	53.0	76.0	129.0
LD73	2	-	47.0	24.0	70.0	94.0
LD74	3	-	55.0	23.0	94.5	117.5
LD75	3	-	56.0	16.0	61.0	77.0
Mean			**49.4**	**34.4**	**61.1**	**95.5**
Standard deviation			**18.61**	**12.56**	**14.92**	**15.71**
Coefficient of variation			**38%**	**36%**	**24%**	**16%**

Table 4: *Laminaria digitata* at Sauøya, Hitra 04.10.12

Specimen	Shape of lamina	Shape of stipe	Lamina width (cm)	Length of stipe (cm)	Length of lamina (cm)	Total length (cm)
LD77	1	-	19.0	15.0	85.0	100.0
LD78	1	-	14.5	29.5	60.0	89.5
LD79	1	-	31.5	43.0	83.0	126.0
LD80	1	-	26.0	70.0	88.0	158.0
Mean			**22.8**	**39.4**	**79.0**	**118.4**
Standard deviation			**7.51**	**23.40**	**12.83**	**30.55**
Coefficient of variation			**33%**	**59%**	**16%**	**26%**

Table 5: *Laminaria hyperborea* in Brænnebukta 28.02.13.

Specimen	Shape of lamina	Shape of stipe	Lamina width (cm)	Length of stipe (cm)	Length of lamina (cm)	Total length (cm)
LD1	1	-	20.5	51.0	66.0	117.0
LD12	1	3	14.0	43.0	64.0	107.0
LD21	1	3	20.0	56.0	64.0	120.0
Mean			**18.2**	**50.0**	**64.7**	**114.7**
Standard deviation			**3.62**	**6.56**	**1.15**	**6.81**
Coefficient of variation			**20%**	**13%**	**2%**	**6%**

Table 6: *Laminaria hyperborea* in Brænnebukta 06.05.13

Specimen	Shape of lamina	Shape of stipe	Lamina width (cm)	Length of stipe (cm)	Length of lamina (cm)	Total length (cm)
LD51	3	3	-	80.0	65.0	145.0

Table 7: *Laminaria hyperborea* in Brænnebukta 02.09.13

Specimen	Shape of lamina	Shape of stipe	Lamina width (cm)	Length of stipe (cm)	Length of lamina (cm)	Total length (cm)
LD53	3	3	58.0	68.0	55.0	123.0
LD55	3	3	59.0	58.0	48.0	106.0
LD59	3	3	59.0	52.5	60.5	113.0
LD67	3	3	43.0	39.0	47.0	86.0
LD68	3	3	36.0	44.0	55.7	99.7
LD72	2	3	33.5	31.0	44.5	75.5
Mean			**48.1**	**48.8**	**51.8**	**100.5**
Standard deviation			**12.01**	**13.44**	**6.20**	**17.49**
Coefficient of variation			**25%**	**28%**	**12%**	**17%**

Table 8: *Saccharina groenlandica* at Kapp Mitra 27.09.13

Specimen	Shape of lamina	Shape of stipe	Lamina width (cm)	Length of stipe (cm)	Length of lamina (cm)	Total length (cm)
LD81	1	-	24.5	6.5	57.5	64.0
LD82	1	2	13.0	27.0	60.0	87.0
LD83	1	2	25.2	25.0	64.0	89.0
LD84	1	2	26.0	9.0	67.0	76.0
LD85	1	3	37.6	7.0	77.0	84.0
LD86	1	3	23.8	16.0	54.0	70.0
LD89	1	2	16.0	20.0	47.0	67.0
LD90	1	3	23.0	11.0	57.0	68.0
Mean			**23.6**	**15.2**	**60.4**	**75.6**
Standard deviation			**7.33**	**8.09**	**9.05**	**9.84**
Coefficient of variation			**31%**	**53%**	**15%**	**13%**

Table 9: *Saccharina groenlandica* at Smeerenburgfjorden 28.09.13

Specimen	Shape of lamina	Shape of stipe	Lamina width (cm)	Length of stipe (cm)	Length of lamina (cm)	Total length (cm)
LD91	3	2	36.0	21.0	50.0	71.0
LD93	3	2	47.0	32.0	64.0	96.0
LD94	3	2	67.0	40.0	46.0	86.0
LD96	3	2	52.0	36.0	34.0	70.0
LD98	3	3	59.0	37.0	90.0	127.0
Mean			**52.2**	**33.2**	**56.8**	**90.0**
Standard deviation			**11.78**	**7.40**	**21.43**	**23.36**
Coefficient of variation			**23%**	**22%**	**38%**	**26%**

Table 10: *Saccharina groenlandica* at Gyldénøyane, Hinlopen 02.10.13

Specimen	Shape of lamina	Shape of stipe	Lamina width (cm)	Length of stipe (cm)	Length of lamina (cm)	Total length (cm)
LD101	3	3	53.0	22.0	60.0	82.0
LD102	3	3	40.4	12.0	34.0	46.0
LD103	3	3	54.0	11.0	63.0	74.0
LD107	3	3	33.0	13.0	78.0	91.0
LD108	2	3	23.0	10.0	36.0	46.0
Mean			**40.7**	**13.6**	**54.2**	**67.8**
Standard deviation			**13.24**	**4.83**	**18.82**	**20.79**
Coefficient of variation			**33%**	**35%**	**35%**	**31%**

Appendix III

Figure 1: *Laminaria digitata* specimens collected in Brænnebukta, Trondheimsfjord on 28.02.2013. Picture of LD22 is missing.

Figure 2: *Laminaria digitata* specimens collected in Brænnebukta, Trondheimsfjord on 06.05.2013.

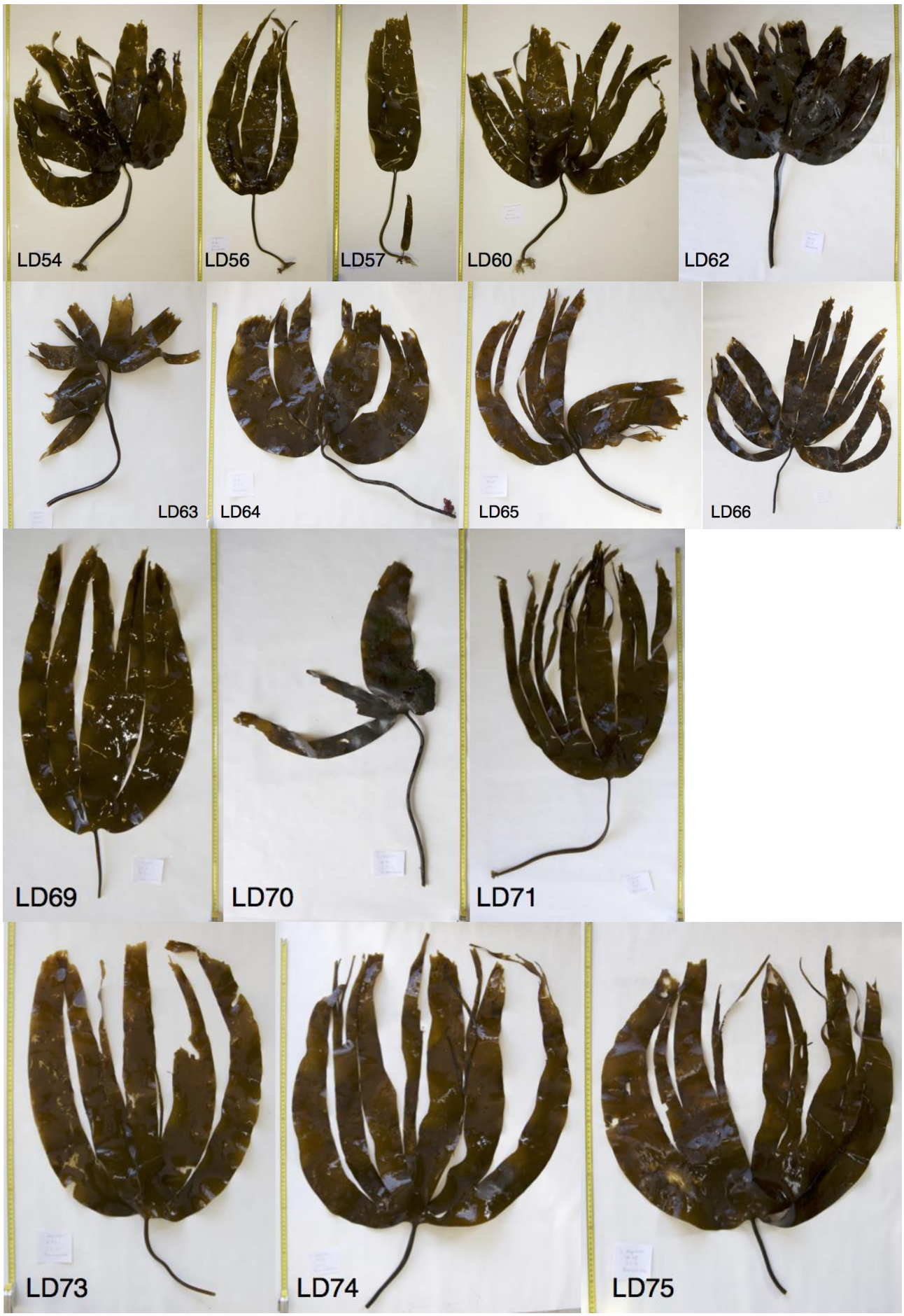

Figure 3: *Laminaria digitata* specimens collected in Brænnebukta, Trondheimsfjord on 02.09.2013.

Figure 4: *Laminaria hyperborea* specimens collected in Brænnebukta, Trondheimsfjord on 28.02.2013 (LD1, LD12 and LD21) and 06.05.2013 (LD51).

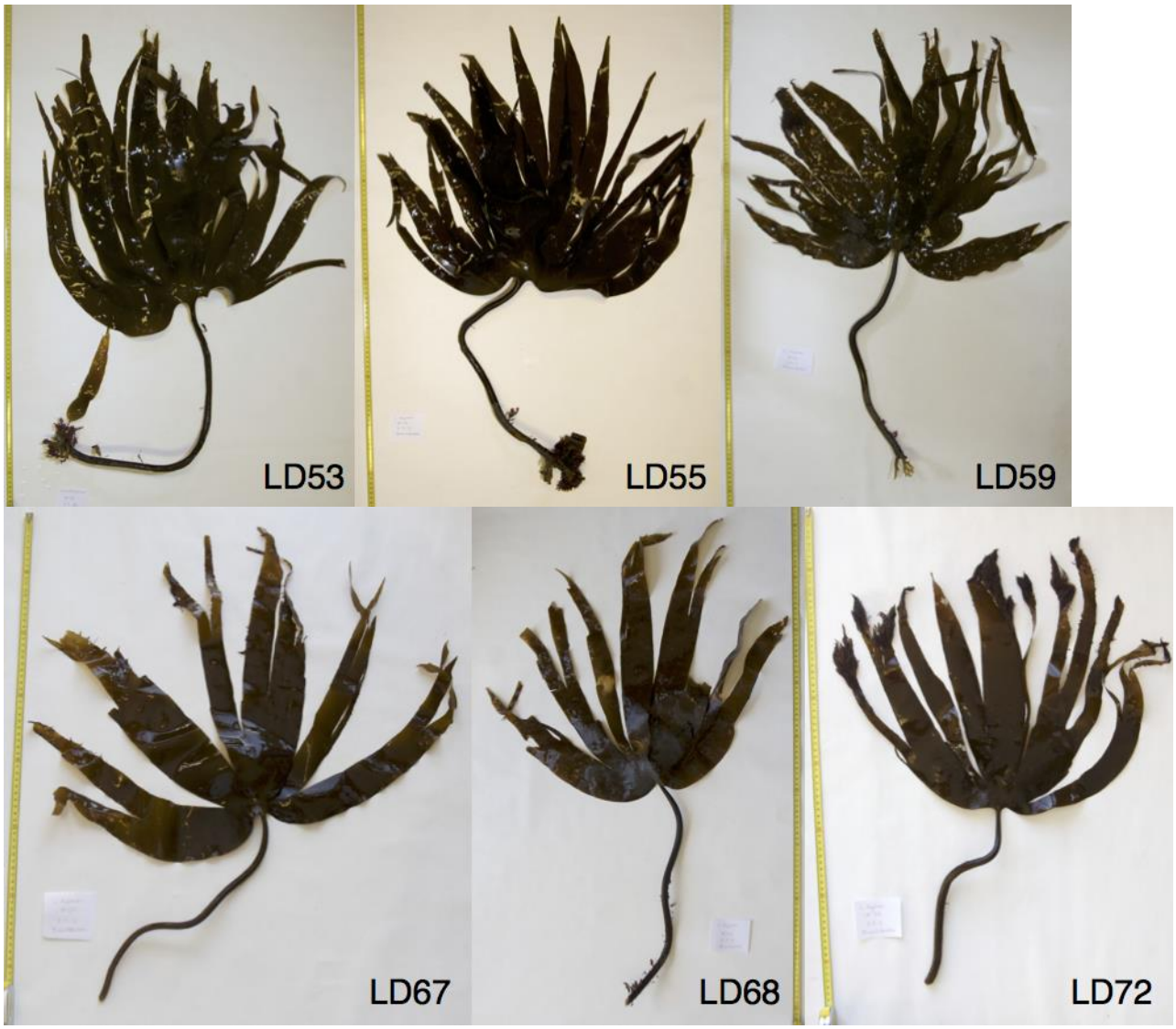

Figure 5: *Laminaria hyperborea* specimens from Brænnebukta, Trondheimsfjord collected 02.09.2013.