

DNA BARCODING AND MUCILAGE DUCTS IN THE STIPE REVEAL THE PRESENCE OF *HEDOPHYLLUM NIGRIPES* (LAMINARIALES, PHAEOPHYCEAE) IN KONGSFJORDEN (SPITSBERGEN)¹

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The Arctic Ocean is a unique ecosystem hosting a biodiversity that has not yet been elucidated in full detail. There is increasing evidence that there are more kelp species constricted to Arctic/sub-Arctic habitats hitherto not well investigated, such as *Hedophyllum nigripes*, which is morphologically very similar to cold-temperate *Laminaria digitata*. *Hedophyllum nigripes* was originally described as *L. nigripes* by Agardh from Spitsbergen but has often been misidentified as *L. digitata* in the European Arctic. We initiated a systematic algal survey along a depth gradient (0–7.5 m) in Kongsfjorden (Spitsbergen) in June and July 2015 and thereby confirmed a predominant presence of *H. nigripes* (73%). *Hedophyllum nigripes* is occurring between 0 and 7.5 m while *L. digitata* was most abundant at 2.5 m depth. *Hedophyllum nigripes* individuals were generally younger (2.3 vs. 3.6 years) and stipe and blade length shorter (31 vs. 54 cm and 76 vs. 96 cm, respectively) compared to *L. digitata*. A combination of molecular (COI-5P) and morpho-anatomical tools (presence of sori and mucilage ducts in the stipe) was used to differentiate specimens of *H. nigripes* and *L. digitata*. Both kelp species were indistinguishable in most cases by external blade and stipe morphology. The different blade shapes represented different ontogenetic stages rather than phenotypic plasticity. The presence of mucilage ducts in the stipe was correlated with *H. nigripes* and changed with depth from 17%, 36%, and 85% at

2.5, 5, and 7.5 m, respectively. In addition, all summer fertile specimens were *L. digitata*.

Key index words: cytochrome *c* oxidase subunit I region; *Hedophyllum nigripes*; *Laminaria digitata*; mucilage ducts; phenotypic plasticity; Spitsbergen

Abbreviations: COI-5P, cytochrome *c* oxidase subunit I region; GI, genetic identification; MUC, Mucilage duct channel

Kelp are marine brown algae (class Phaeophyceae) and belong to the order Laminariales, which is based on the genus *Laminaria* (Lamouroux 1813). The genus *Laminaria* sensu lato (Bartsch et al. 2008) has gone through many taxonomic revisions. In 2006, a thorough multi-gene approach revealed *Laminaria* s.l. was polyphyletic, necessitating a split into two genera: *Laminaria*, containing all species affiliated to the type species *L. digitata*, and the resurrected genus *Saccharina* (Lane et al. 2006). Recently, Starko et al. (2019a,b) provided a multi-gene phylogenomic study of the Laminariales, which they interpreted as supportive of resurrecting *Hedophyllum* from *Saccharina*, necessitating the new combination *H. nigripes*.

The species *Laminaria digitata*, which is distributed in the temperate to polar North Atlantic, has been involved in taxonomic confusion (Kain 1979, Bartsch et al. 2008, Longtin and Saunders 2016, Guiry and Guiry 2019) owing to phenotypic plasticity. Originally, there have been two unresolved entities that were considered to be closely related to cold-temperate *L. digitata* due to their

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similar external morphology, both from Arctic to Sub-Arctic habitats: *L. nigripes* Agardh (1868; synonym of *Hedophyllum nigripes*; Starko et al. 2019a,b) and *L. gunneri* Foslie (1883). *Laminaria nigripes* was originally described from Spitsbergen (Agardh 1868), but was later also recorded in Russian waters further east, in Northern Norway and Greenland (Kjellman 1883, Ruess 1977, Pedersen 2011). This species was mainly distinguished from *L. digitata* by the presence of mucilage ducts in the stipe and blade. Further criteria were its broader blades, and rather short and blackish stipes (Agardh 1868), and the location of the basal sorus (Pedersen 2011). The presence of mucilage ducts as a taxonomic criterion has been widely used to differentiate species of *Laminaria* s.l. However, its usefulness has been questioned due to its variation along environmental gradients (Burrows 1964, Wilce 1965, McDevit and Saunders 2010). Longtin and Saunders (2015) demonstrated that mucilage ducts in the stipe of *H. nigripes* (as *Saccharina nigripes*) in the Canadian Atlantic can be taxonomically informative.

Through recent investigations at different sample sites, it became evident that kelps from the Eastern Canadian Arctic (Longtin and Saunders 2015) and Svalbard (Lund 2014, Moy 2015) did not mainly belong to *Laminaria digitata*, but to *Hedophyllum nigripes*. Thus, both species co-occur side by side in Arctic to sub-Arctic habitats and clear differentiation in the field cannot be easily completed by external morphology alone. Previous publications regarding the biogeography, ecology, and physiology of the European Arctic *L. digitata* populations may have partially dealt with *H. nigripes* (e.g., Wiencke 2004, 2011). Long-term monitoring at Hansneset in Spitsbergen (Svalbard) revealed an overall increase in kelp biomass since 1996–1998 and has been interpreted as a consequence of climate change. The biomass increase was mostly attributed to a 2.4 fold increase in *L. digitata* at 2.5 m depth (Bartsch et al. 2016). A systematic DNA barcoding (COI-5P) survey along a depth gradient between 0 and 7.5 m was conducted to reveal whether the species identity of *L. digitata* still holds or if both digitate kelp species co-occur at the investigated site in Kongsfjorden in a random or a zonal way. As well, the reliability of mucilage ducts as a clear distinctive character for species separation of *L. digitata* and *H. nigripes* in the field was of interest.

MATERIALS AND METHODS

Sample collection. Divers collected adult kelp at 2.5, 5, and 7.5 m depth from Hansneset, Kongsfjorden (78°99' N, 11°57' E) in June–July 2015 along the same transect as described in Bartsch et al. (2016). All depths were determined *in situ* by a dive computer (Uwatec, Scubapro Bottom Timer Digital 330) and corrected to chart datum (=lowest astronomical tide is 28 cm below mean low water spring tide) with the aid of the tide gauge in Ny-Ålesund (<http://vannstand.no/index.php/>

[nb/english-section/sea-level-data](#)). At each depth, divers randomly collected 40 digitate adult kelp specimens along a 30 m transect parallel to the coastline. In addition, we sampled all kelps in three random quadrats (0.5 × 0.5 m) at 0 m at a nearby site during low tide (Bartsch et al. 2016) where kelps grew in discontinuous patches. Specimens were considered “adult” when they were > 1-year-old. Adult *Hedophyllum nigripes* specimens were characterized by an age of 2.3 ± 0.8 y, a stipe length of 31 ± 16 cm, and a blade length of 76 ± 20 cm, while adult specimens of *Laminaria digitata* had a mean age of 3.6 ± 1.4 y, a stipe length of 54 ± 22 cm, and a blade length of 96 ± 23 cm. In contrast, at 0 m depth samples mostly contained juvenile kelp specimens not older than 1 y with small stipes (<5 cm) and blade lengths of 25 ± 15 cm (Table S1 in the Supporting Information). In addition, 10 small specimens were hand-picked at Kapp Guiszez just below the waterline during low water (Table S1). Immersion time for these specimens is unknown. Kapp Guiszez is an exposed site (Hop et al. 2016).

The algal samples were subdivided for morpho-anatomical examination, DNA extraction, and herbarium vouchers (Table S1). Tissue from the meristematic zone for DNA extraction was cut with a cork borer (\varnothing 2 cm), cleaned with a moist paper tissue and immediately frozen in liquid nitrogen, and stored at -80°C until freeze-drying. Before transportation to Germany, the samples were freeze-dried for 12 h at 1.030 mbar (Christ Alpha 1–4 LSG, Martin Christ, Osterode am Harz, Germany).

DNA extraction and amplification. Freeze-dried samples were ground with a Micro-Dismembrator (U Braun Biotech International, Melsungen, Germany). DNA was extracted with the Nucleospin[®] Plant II Kit and a prewash step with acetone was added (McDevit and Saunders 2009). The genomic DNA was cleaned with peqGold Cycle-Pure Kit following the manufacturer's protocol (VWR[®], Erlangen, Germany). The mitochondrial barcode marker COI-5P (650bp) was amplified using PCR primers GazF2 and GazR2 (Lane et al. 2007). The PCR mastermix contained 40.8 μL DNase-free H_2O , 5 μL Taq Puffer (10 \times), 1 μL dNTPs, and 1 μL each of GazF2 and GazR2 primers (Lane et al. 2007). PCR parameters followed those described in McDevit and Saunders (2009). Successful PCR products were purified with MinElute[®] PCR Purification Kit 50 (Qiagen, Hilden, Germany) following the manufacturers instruction.

Species identification. The purified PCR products were sequenced at the Alfred-Wegener-Institute, Bremerhaven, Germany following the manufacturer's protocol for the BigDye[®] Terminator v3.1 Cycle sequencing kit (Applied Biosystems, Darmstadt, Germany) and using the PCR amplification primers. The sequence PCR parameters were as follows: initial denaturing at 96°C for 1 min; 25 cycles of 96°C for 10 s, 55°C annealing for 5 s and 60°C elongation for 4 min; and final elongation at 60°C for 4 min. The sequenced PCR products were purified with Agentcourt[®] CleanSeq[®] Kit (Agencourt Bioscience Corporation, Beverly, MA, USA) following the manufacturer's protocol. PCR products were transferred on the MicroAmp[®] Optical 96-well reaction plates (Applied Biosystems, Darmstadt, Germany) and analyzed in the sequencer (3130xl Genetic Analyzer, ABI, Prism, Applied Biosystems, Darmstadt, Germany). The raw data were edited and assembled using CLC Main Workbench 6.9.1 (Muehlthal, Germany). Sequences generated in Longtin and Saunders (2015) were added to each alignment prior to analysis to species level.

Morphological analyses. Morphological and anatomical analyses of all samples were performed as follows: digital photographs were taken, the lengths of the stipes and blades were measured to their nearest cm, and their ages were

determined by counting the growth rings from cross sections at the base of the stipe (Bartsch et al. 2016). Additionally, specimens were investigated for sori. The presence of mucilage ducts was verified in fresh blade and stipe material in a systematic manner. Small tissue fragments from the blade and stipe of each sample were cut and thin cross sections were obtained by hand with a razor blade. Stipe samples ($n = 20$) were taken 2 cm above the holdfast, in the middle of the stipe, and just below the meristematic zone.

Sample classification and selection for molecular identification. Following the concept of Longtin and Saunders (2015), *Hedophyllum nigripes* and *Laminaria digitata* were distinguished on anatomical grounds, and two different phenotypes were assigned to either species. Besides the verification of mucilage ducts via cross sectioning, additionally, the presence of a visible slime production after cutting of the stipe (Fig. S1 in the Supporting Information) was used as an indication for the presence of mucilage ducts in stipes. Furthermore, it was assumed that specimens which were fertile during the summertime belong to *L. digitata* (sensu Agardh 1868, Olischläger and Wiencke 2013). After a phenotypic classification of 172 specimens, a sample selection for genetic identification was conducted (Table 1). At least 30% of these collections were randomly selected for DNA barcoding, in addition to all fertile individuals and any individual that could not be identified by phenotype (Table 1). Unsuccessful sequencing of DNA reduced the final number of genetically identified individuals per depth (Table 1, Table S1).

RESULTS

Depth distribution of genetically verified specimens. From a total number of 172 individuals collected at Hansneset, 67 out of 77 processed individuals were successfully genetically identified (Table S1). Between 0 and 7.5 m depth, 19 specimens were genetically identified as *Laminaria digitata* and 48 as *Hedophyllum nigripes* (Table S1). The relative depth distribution of both species showed a gradient, with *H. nigripes* being present at all depths while *L. digitata* was not verified at 0 m and had a low relative contribution at 5 and 7.5 m and a maximum at 2.5 m (Fig. 1).

Gross morphology of genetically verified Hedophyllum nigripes and Laminaria digitata. There was an overall variation in the gross morphology of both species at Hansneset, probably representing different developmental stages. They varied from simple blade forms without any sign of digitation, to simple blades with emerging internal digits to campanulate

blades with a deep constriction between simple first year blades and digitate second year blades (thereby resembling the outer shape of *Ananas* fruits; in the following we will only use the term “campanulate”) and truly digitate forms (Fig. 2).

Most specimens at 0 m depth were digitate, and some individuals had a simple or campanulate blade morphology. All individuals (<80 cm total lengths) were 1-year old and had mucilage ducts in the blade but not in the stipe. All genetically verified specimens belonged to *Hedophyllum nigripes* and none to *Laminaria digitata* (Table S1).

All *Hedophyllum nigripes* specimens at 2.5 m depth had a digitate blade morphology with 4–6 fingers (Fig. 2), a horizontal to wide cuneate blade base and stipe lengths ranged between 13 cm and 42 cm (Table S1). Presence of mucilage ducts in the stipe was low (1 out of 6 individuals; Table S1). All *Laminaria digitata* specimens had a digitate blade morphology with 5–14 fingers (Fig. 2), and a heart shaped to horizontal to cuneate blade base. Stipe lengths ranged between 24 cm and 95 cm, and the maximum stipe length was considerably longer than for *H. nigripes* from the same depth (Table S1). Most *L. digitata* specimens at 2.5 m were fertile (81%) and bearing small sori at distal blade fingers. There were no mucilage ducts in the stipes.

Hedophyllum nigripes individuals at 5 m depth had 3–4 blade fingers; some individuals had a campanulate blade morphology, with or without internal digits in the basal area of the new year's blade (Fig. 2). Only 4 out of 11 individuals had mucilage ducts in their stipes. The stipe lengths varied between 24 cm and 90 cm and the maximum stipe length was longer compared with individuals growing at 2.5 m depth (Table S1). Only one verified *Laminaria digitata* specimen was present at 5 m depth and had no mucilage ducts in blade or stipe (Fig. 2).

Only one out of 19 investigated specimens at 7.5 m depth was *Laminaria digitata* without mucilage ducts in the stipe (Table S1). The blade morphology of *Hedophyllum nigripes* was most variable at this depth ranging from simple blades with distal digits, to campanulate blades, and to blades with several fingers. Most specimens (24 out of 28) had mucilage ducts in the stipes, thus corresponding to the typical *H. nigripes* phenotype sensu Longtin and

TABLE 1. Overview of sample selection for genetic identification (GI) of *Laminaria digitata* and *Hedophyllum nigripes* collected at Hansneset after phenotypic pre-classification *sensu* Longtin and Saunders (2015; presence of mucilage ducts in blade and/or stipe) and presence of fertility and successful sequence results (last two columns).

Depth	<i>Laminaria digitata</i> phenotype	Samples for GI	% out of phenotype	<i>Hedophyllum nigripes</i> phenotype	Samples for GI	% out of phenotype	Unknown phenotype	Samples for GI	% out of phenotype	Total samples	Successful sequences	% GI
0 m	55 + 3 fertile	18 + 2 fertile	34%	0	0	0%	0	0	0%	58	13	22%
2.5 m	20 + 19 fertile	9 + 18 fertile	69%	2	2	100%	2	2	100%	43	23	53%
5 m	11	3	27%	26	6	23%	4	4	100%	41	12	29%
7.5 m	6 + 1 fertile	3 + 1 fertile	57%	23	17	74%	0	0	0%	30	19	63%

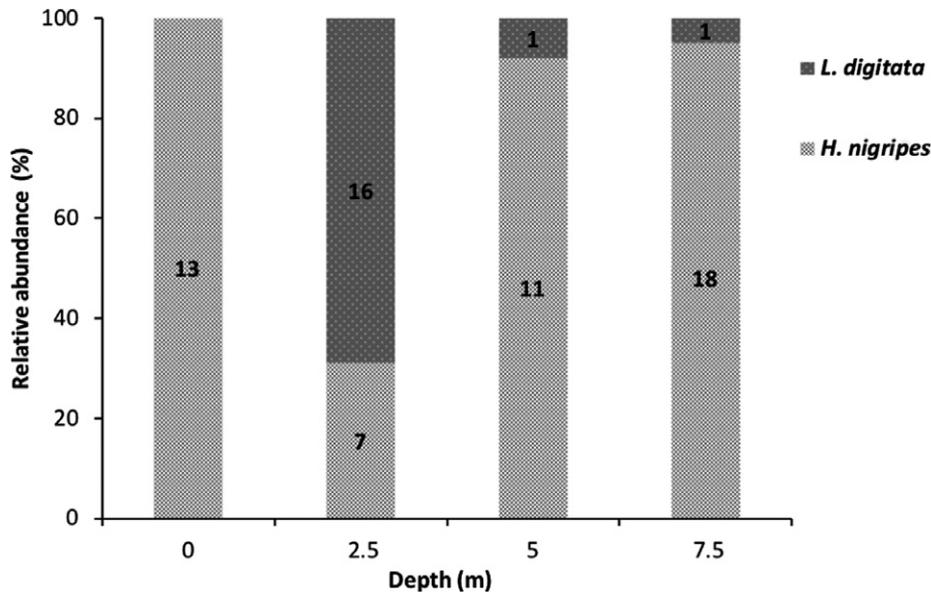


FIG. 1. Relative abundance of *Hedophyllum nigripes* and *Laminaria digitata* at 0 m (n = 13), 2.5 m (n = 23), 5.0 m (n = 12), and 7.5 m (n = 19) depth at Hansneset, Kongsfjorden (Spitsbergen).

Saunders (2015). The stipe lengths varied between 9 cm and 52 cm and were similar to individuals growing at 2.5 m depths (Table S1).

Presence of mucilage ducts in blade and stipe along the depth gradient. *Hedophyllum nigripes* plants showed mucilage ducts in their blades (70–100%) in all depths. The occurrence of mucilage ducts in stipes however varied with depth but not with age (Figs. 3 and 4). There was a significant increase in mucilage ducts in stipes by about 85% with depth (Fig. 3; ANOVA: $F_{1,2} = 28.09$, $P < 0.05$). *Laminaria digitata* plants had no mucilage ducts in their stipe. Specimens from 2.5 m (n = 16) and 7 m (n = 1) had mucilage ducts in their blade but only one *L. digitata* at 5 m had no mucilage ducts in the blade (Fig. 3).

Fertility. Most *Laminaria digitata* specimens (93%) were fertile, but no specimen of *Hedophyllum nigripes* were fertile (Table S1). At 0 m, only 5% fertile specimens were collected. At 2.5 m, 44% of all sampled individuals were fertile, thereof 14 genetically verified *L. digitata* and no *H. nigripes*. At 5 m depth no collected specimen was fertile and at 7.5 m depth only one out of 30 specimens without genetic identification.

Gross morphology of *Hedophyllum nigripes* from Kapp Guisnez. All genetically verified records (9 out of 11 samples) from Kapp Guisnez were *Hedophyllum nigripes* (Table S1). The blade and stipe phenology were extremely different compared to specimens from Hansneset (Fig. 5), therefore, it is considered to represent a different phenotype. All stipes were very short (2.5–22 cm), flattened and with a blackish colour and a shiny surface. The extraordinary blade shape of some specimens was characterized by being wider than long (Fig. 5) compared to being longer than wide which is typical for *Laminaria digitata*. In total, all (but one) of the specimens were

short and blade length varied between 18 and 65 cm, although they had an age of 2–4 y. In addition, there was one other specimen of *H. nigripes* with a very long stipe (120 cm) and blade length (133 cm). Surprisingly, 8 out of 9 *H. nigripes* specimens from Kapp Guisnez lacked mucilage ducts in stipes and 7 out of 9 lacked mucilage ducts in blades (Table S1). They had no visible slime production at all and thus were similar to the 0 m *H. nigripes* individuals collected at Hansneset. The overall gross morphology was very different.

DISCUSSION

Presence of *Hedophyllum nigripes* in Kongsfjorden and the Arctic. The present study, in addition to Lund (2014) and Moy (2015), supports Agardh's separation of Arctic digitate kelp species into *Hedophyllum nigripes* (as *Laminaria nigripes*; Agardh 1868) and *Laminaria digitata* and verify the presence of *H. nigripes* in its type habitat at Spitsbergen, European Arctic. In all, 67 specimens collected at Hansneset, Kongsfjorden (Spitsbergen) between 0 and 7.5 m depth were genetically identified via COI-5P. In all, 18 specimens were *L. digitata* and 49 specimens *H. nigripes* (73%; Table S1). Sampling along a depth gradient at Hansneset, Kongsfjorden (Spitsbergen) indicates that *H. nigripes* is the dominant species. We thus conclude that *H. nigripes* was overlooked or misidentified as *L. digitata* in prior studies in Svalbard (e.g., Wiencke 2004, 2011, Wiencke et al. 2007, Hop et al. 2012, Bartsch et al. 2016). Recent evidence suggests that *H. nigripes* is a widespread species in northern hemispheric cold temperate to Arctic locations. Molecularly-verified specimens of *H. nigripes* are found from British Columbia over Alaska in the northeastern Pacific to the Canadian Arctic, Atlantic Canada, and Svalbard in the Atlantic



FIG. 2. Gross morphology of *Laminaria digitata* (A and B) and *Hedophyllum nigripes* (C–E) collected at Hansneset, Spitsbergen. Scale bars = 10 cm. Individual numbers refer to Table S1. (A) Specimen with a campanulate shape blade morphology, dark brown (ID 103, age: 3 years). (B) Specimen with sorus, long stipe and slender digitate blade shape (ID 160, age: 1 year). (C) Specimen with an unsplit blade and a lighter brown-greenish color at the edges of the blade (ID 125, age: 1 year). (D) Specimen with a broad blade morphology and a long, round stipe (ID 155, 3 years). (E) Specimen with a campanulate shape blade morphology, internal digits in new blade just formed (ID 140, 3 years). [Color figure can be viewed at wileyonlinelibrary.com]

(Lund 2014, Moy 2015, Longtin and Saunders 2016, Starko et al. 2019, this study) while other Atlantic records from Greenland, Iceland, and Northern Norway (Guiry and Guiry 2019) need molecular confirmation.

Zonated co-occurrence of both kelp species. During our study, it became evident that *Hedophyllum nigripes* and *Laminaria digitata* co-occur in the same habitat

with a clear depth zonation pattern: while *L. digitata* is absent from 0 m but the most abundant kelp at 2.5 m depth, *H. nigripes* is dominant at 0 m, 5 m, and 7.5 m depth at Hansneset (Fig. 1). Although sampling at 0 m was limited to a small sample area at Hansneset, the infralittoral fringe specimens from Kapp Guisseez were also genetically verified as *H. nigripes*. The observed zonation pattern is unusual,

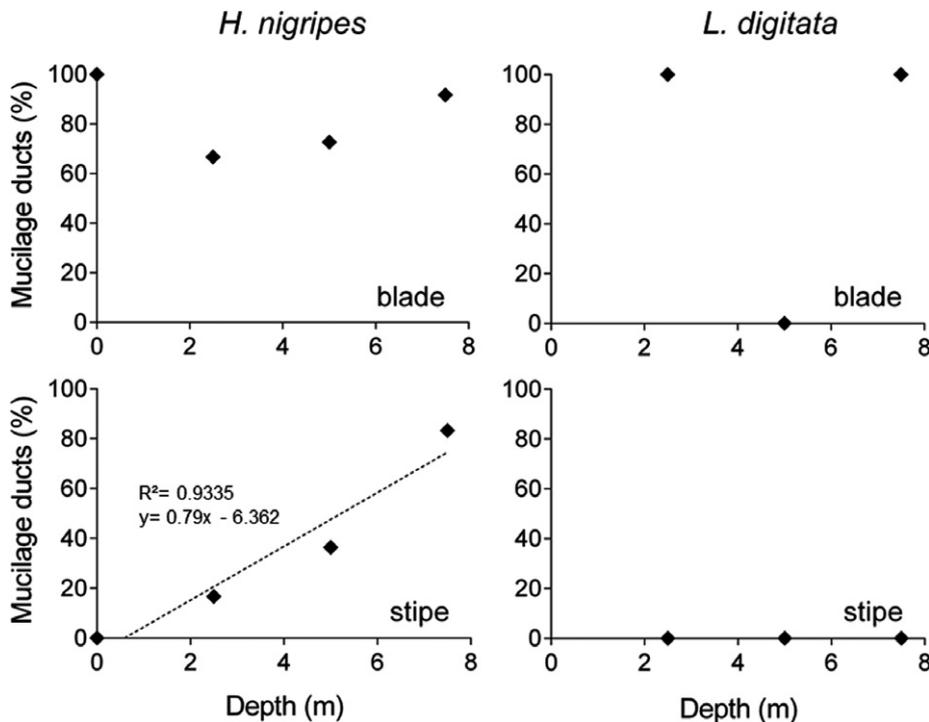


FIG. 3. Relative abundance of mucilage ducts in stipes and blades of *Laminaria digitata* and *Hedophyllum nigripes* from Hansneset, Spitsbergen. *L. digitata* sample size: 0, 2.5, 5, 7.5 m: $n = 0$, $n = 16$, $n = 1$ and $n = 1$, respectively. *H. nigripes* sample size: 0, 2.5, 5, 7.5 m: $n = 13$, $n = 7$, $n = 11$, $n = 18$, respectively. Correlation between relative mucilage duct abundance and depth. The regression line of best fit is indicated by dashed line ($R^2 = 0.93$, $P < 0.05$).

especially for *L. digitata*. In the temperate North Atlantic region, *L. digitata* is always growing in the infralittoral fringe zone (e.g., Bartsch and Kuhlenskamp 2000, Robuchon et al. 2014). *Laminaria digitata* is readily outcompeted in greater depths by other perennial kelp species, such as *L. hyperborea* (Kain 1979, Pehlke and Bartsch 2008), which might explain its preference in the shallow zone. While *L. hyperborea* is not present at our investigation site, there are two other potentially competing perennial kelp species in Kongsfjorden: *Alaria esculenta* and *Saccharina latissima*. Both co-occur with *L. digitata* at 2.5 m depth (Hop et al. 2012, Bartsch et al. 2016). Results from a laboratory study suggest that *A. esculenta* may outcompete *L. digitata* under certain conditions (Zacher et al. 2019), but the competitive strength of *H. nigripes* is completely unknown. It cannot be fully excluded that the unusual zonation pattern is a sampling artifact. The sampling protocol did not consider the potential patchiness of kelps and thereby does not reflect the true situation in the field. It is well known that kelps have a limited dispersal and often recruit in clumps (e.g., Dayton 1985).

All investigated *Laminaria digitata* specimens of the present study were fertile and had sori between June and July 2015. Olischläger and Wiencke (2013) suggested preferential summer fertility of *L. digitata* in Kongsfjorden. No fertile *Hedophyllum nigripes* individuals were recorded during the investigated summer period, but many were observed in autumn and spring (I. Bartsch, unpub. data), which confirms Agardh's assumption that the fertile period of both species does not overlap. This may favor reproductive

isolation even though dormant perennial gametophyte stages have been reported in diverse kelp species (Hoffmann and Santelices 1991).

Morphological variation. The great phenotypic plasticity in kelps has contributed a lot to taxonomic confusion within the order Laminariales in the past (Kain 1979, Bartsch et al. 2008, Demes et al. 2009). The observed campanulate blades, beside digitate blades and undivided blades of both species, seem to be a late spring stage, where the new undivided blade is still connected to remnants of the previous year's digitate blade (Figs. 2, 5). The general morphological similarity of the two species (Lund 2014, Longtin and Saunders 2015, 2016, this study) is likely an expression of their occurrence in similar habitats which may induce similar morphotypes (Henry 2018). It is recognized that distinct morphological characters in kelps have evolved independently several times in diverse genera (Starko et al. 2019a).

A few 2- to 3-year-old specimens of *Hedophyllum nigripes* from the exposed location Kapp Guisnez compared to semi-exposed Hansneset (Gontar et al. 2001, Hop et al. 2002, Voronkov et al. 2013) exhibited a very unusual external morphology. *H. nigripes* is able to tolerate semi-exposed to exposed habitats, which is known from the Bay of Fundy (Canada) (Longtin and Saunders 2016). These specimens from Kapp Guisnez are present in its compressed form: an extremely wide and short digitate blade with a torn appearance and its short-compressed stipe. These specimens, however, resemble morphological descriptions of Agardh (1868) for *H. nigripes* (*Laminaria nigripes*; Fig. 5). Agardh's (1868) type

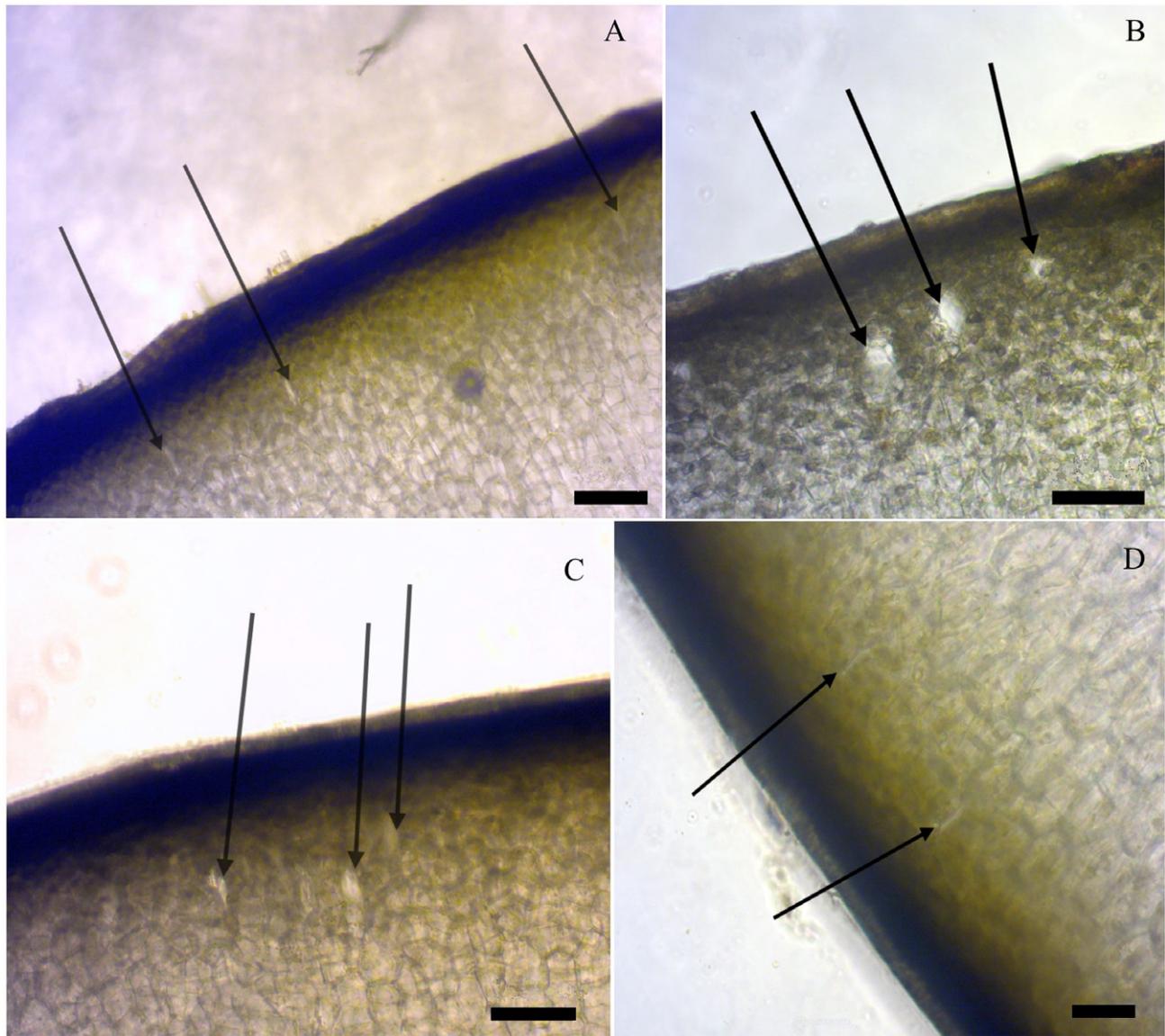


FIG. 4. Mucilage ducts in stipes of *Hedophyllum nigripes* (cross sections). All mucilage ducts are indicated by arrows. (A) Mucilage duct channel of individual #155 at 2.5 m depth, (B) mucilage duct channel of individual #104 collected at 5 m depth, (C) mucilage duct channel of individual #126 collected at 7.5 m depth, and (D) mucilage duct channel of individual #181 collected just below the waterline at Kapp Guisnez. A–C: from Hansneset. For individual numbers see Table S1. Scale bars = 50 μm . [Color figure can be viewed at wileyonlinelibrary.com]

description mentions “a rather short, blackish stipe flattened just above the holdfast, with a dark brown blade, often reniform, split into fingers and resulting in ensiform tails. Although blades of *L. digitata* and *L. nigripes* are similar in their form, *L. nigripes* tends to grow wider than long” (English translation of Latin text). Four specimens of *H. nigripes* from Kapp Guisnez had a blackish stipe color with a shiny surface, the blade color was slightly darker compared to *L. digitata* in the fresh state, the stipes were extremely short, and the blades were wider than long, and the tips were quite tattered (Fig. 5). This phenotype fits to the general characterization of the genus *Hedophyllum* described by Starko et al. (2019a).

Mucilage ducts. The presence or absence of mucilage ducts can vary with changing environmental conditions (Kjellman 1883, Burrows 1964, Wilce 1965). This character came into discredit but was recently resurrected as valid criterion for species discrimination between *Hedophyllum nigripes* and *Laminaria digitata* in Atlantic Canada (Longtin and Saunders 2015). The current study confirmed the presence of mucilage ducts in the stipe, but its absence did not unequivocally discern *H. nigripes* from *L. digitata*. Therefore, it supports Longtin and Saunders (2015) discovery’s partially. However, in fresh field material no “pseudo-mucilage ducts” were identified, which were described by Longtin and



FIG. 5. Gross morphology of *Hedophyllum nigripes* collected at Kapp Guisnez, Spitsbergen just below the low water mark. Scale bars = 5 cm. (A) Specimen with very a short stipe and with a blackish color. The digitate blade is wider than long and dark brown. (B) Specimen with a very short stipe and with a blackish color. The blade is digitate and has a blackish color at the meristem zone. (C) Specimen with a short stipe and an extraordinary blade shape. The blade has a digitate morphology. The meristem zone has a darker color than the blade tips. (D) Specimen with a very short stipe and blackish color. The meristem zone is darker compared to the blade tips. [Color figure can be viewed at wileyonlinelibrary.com]

Saunders (2015) in herbarium collections as putative infections of endophytes. Interestingly, the number of mucilage ducts in the stipes of *H. nigripes* increased with depth (Fig. 3). Although it is possible that very small mucilage ducts in juvenile specimens were undetected due to hand-made cross sections, this is unlikely due to a systematic and precise procedure for each specimen: every stipe was investigated 3×20 times at each stipe zone by cross sections.

In light of the current results, it is likely that *Hedophyllum nigripes* is a dominant member of the kelp community in European Arctic to Sub-Arctic habitats. This is supported by its lower temperature

affinities for growth and gametogenesis compared to Arctic *Laminaria digitata* (Franke 2019). It is likely that more hitherto neglected or unconfirmed species are present in Arctic environments as it has become evident for the Baffin Island (Küpper et al. 2016). The identity of *L. fissilis* (Agardh 1868), for example, is still unresolved and was regarded as valid entity by Guiry and Guiry (2019).

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Figure S1. Visible slime production in a stipe of *Hedophyllum nigripes*. Slime threads between finger and the cut stipe are visible.

Table S1. List of kelp samples used in this study, with phenotype sensu Longtin and Saunders (2015), species name after genetic identification, sample location, collection depth, age, presence of sorus, presence of mucilage ducts in blade and stipe, stipe and blade length and GenBank accession numbers for barcode (COI) sequences.