Received: 18 December 2018 | Accepted: 21 October 2021

DOI: 10.1002/edn3.262

Environmental DNA
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ORIGINAL ARTICLE

Fingerprinting Arctic and North Atlantic Macroalgae with eDNA – Application and perspectives

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Funding information
Independent Research Fund Denmark, Grant/Award Number: 8021-00222 B

Abstract

Macroalgae are key primary producers in North Atlantic and Arctic coastal ecosystems, and tracing their fate and distribution is vital to improve our understanding of their ecological role and provision of ecosystem services. Recent advances from environmental DNA (eDNA) have added a new capacity to fingerprint and trace macroalgae. However, further development of resources for amplifying and identifying macroalgal eDNA are much needed. Here, we examined the performance in terms of resolution and specificity of two 18S primers (18S-V7 and 18S-V9) recently applied in identifying macroalgae from eDNA. We also built a local barcode database for primer 18S-V7 with 31 widespread Arctic and North Atlantic macroalgal species to complement the existing DNA databases. Furthermore, we applied metabarcoding of eDNA to identify macroalgae in Arctic marine sediments (Disko Bay, W. Greenland) and evaluated the contributions from our local barcode database. We identified macroalgal DNA from 19 families across 11 orders in surface (0–1 cm, with both primers) and subsurface (5–10 cm, with 18S-V7 primer) sediments. The barcode database developed here with the 18S-V7 primer improved the identification of unique families, from 16 to 19 families, thereby strengthening the taxonomic assignment possible relative to pre-existing barcode reference sequences. Overall, this study demonstrates the feasibility of eDNA to resolve contributions of macroalgae in Arctic marine sediments, and enhances the fingerprinting resolution. We thereby document a novel pathway to answer key questions on the ecological role and fate of macroalgae in the Arctic.

KEYWORDS
Arctic, eDNA, fate of macroalgae, kelp, macroalgal tracer, marine biodiversity, marine vegetation, metabarcoding, mini-barcodes, sediment

1 | INTRODUCTION

Macroalgae are the dominant contributors to primary production in the coastal ocean (Duarte, 2017; Duarte & Cebrian, 1996; Gattuso et al., 2006), and contribute significantly to global marine carbon sequestration through export of organic carbon to sinks in coastal marine sediments and the deep ocean (Duarte & Cebrian, 1996; Krause-Jensen & Duarte, 2016; Krumhansl & Scheibling, 2012;

Ortega et al., 2019). However, as macroalgae typically support carbon sequestration beyond their habitat, there is a need for fingerprinting tools to trace macroalgal export and identify macroalgae contributions to organic matter in marine sediments (Geraldi et al., 2019). Macroalgal C-sequestration thereby contrasts to that of seagrass meadows, mangroves, and salt-marshes, which grow on soft seafloor and mainly accumulate carbon in the sediments below the habitats (Duarte & Cebrian, 1996; Duarte & Krause-Jensen, 2017; Duarte et al., 2005, 2013).

In the northern hemisphere and particularly in the Arctic, macroalgae are widely distributed along the vast shoreline with the prevalence of rocky shores, and their distribution tends to expand in response to Arctic warming and loss of sea-ice (Filbee-Dexter et al., 2018; Krause-Jensen et al., 2020; Krause-Jensen & Duarte, 2014). However, mapping their occurrence and tracking changes in distribution are challenging and costly tasks to undertake with traditional surveys that require long time-series monitoring and demand *in situ* identification. Molecular techniques could facilitate the identification of macroalgae remains in environmental samples from along Greenland's vast shoreline, and serve to document both a potential northward expansion of macroalgae and the potentially large role of macroalgae in supporting carbon sequestration and food webs (Krause-Jensen et al., 2018; Queirós et al., 2019). However, this requires new developments in fingerprinting macroalgae within sediments as existing techniques such as stable isotopes have had minimal success at discerning the provenence of organic carbon because of overlapping signals from various end-members (Geraldi et al., 2019).

A novel approach to identify macroalgae in environmental samples is based on DNA metabarcoding (Geraldi et al., 2019; Ortega et al., 2020). Environmental DNA (eDNA) represents DNA extracted from environmental samples (e.g., soil, water, and air) and metabarcoding of eDNA enables us to get a wide palette of taxonomic information about the environmental sample (Taberlet et al., 2018; Thomsen & Willerslev, 2015). An environmental sample will contain both intracellular DNA, that is, DNA inside active or dormant cells, and extracellular DNA that has been released to the environment from decaying cells or sloughed material (i.e., tissue, feces, or other secretion types) (Ceccherini et al., 2009; Levy-Booth et al., 2007; Pietramellara et al., 2009).

Most eDNA studies focus on metabarcoding of animals or land plants (Deiner et al., 2017), while only a few studies have explored the use of eDNA to track the export of macroalgae to the open ocean (Ortega et al., 2019) or the contribution of marine plants and macroalgae to sediment pools or food webs, with various success (del Carmen Gomez Cabrera et al., 2019; Nalley et al., 2021; Ortega et al., 2020; Queirós et al., 2019; Reef et al., 2017). The use of eDNA combined with sediment chronologies would also enable the identification of community changes over time (Bálint et al., 2018), as demonstrated by documented invasion of seagrass species in the Eastern Mediterranean (Wesselmann et al., 2021) and community changes in coral reefs of Australia (del Carmen Gomez Cabrera et al., 2019). Enhancing this approach with eDNA metabarcoding of Arctic macroalgae could provide information about past distribution patterns of macroalgae in the Arctic and how shifting climate regimes may have affected their distribution, as well as providing data for predictions of future responses to climate change.

Nevertheless, the lack of barcoding resources for resolving eDNA metabarcoding of macroalgae, including documented primers, restrict the use of the eDNA approach in macroalgal studies (Geraldi et al., 2019). While the complex evolution and phylogeny of macroalgae and lack of barcoding resources challenge the design of new primers (Baldauf, 2003; Krause-Jensen et al., 2018; Leliaert et al., 2014), recent studies have proposed the 18S rDNA as a suitable region for amplifying marine macrophytes in environmental samples (del Carmen Gomez Cabrera et al., 2019; Ortega et al., 2020; Queirós et al., 2019). However, in depth analyses of the resolution and specificity of these 18S primers are lacking, and much needed to develop metabarcoding of macroalgae for a range of applications and to understand both the opportunities and limitations of this method.

Here we study the primer performance of two 18S primers, recently applied to identify macroalgae from eDNA (Ortega et al., 2020; Queirós et al., 2019). We analyze *in silico* PCR results from both primers and we develop a local DNA barcode database of widespread Arctic and North Atlantic macroalgae based on one of these primers with a method developed by Ortega et al. (2020). Then we apply eDNA metabarcoding of both 18S primer pairs to identify the presence of macroalgal DNA in Arctic marine sediments. We use high-throughput sequencing and taxonomy assignment with existing DNA databases and with the addition of our newly developed barcodes of local species to evaluate whether they improve fingerprinting resolution. We demonstrate the methods on sediment samples collected near Disko Bay, W. Greenland, within and near macroalgal beds.

2 | MATERIALS AND METHODS

2.1 | Sampling and study site

To develop the barcode database, macroalgal samples were collected along the coast of Denmark and Southwest and East Greenland in connection with various research expeditions (Table 1). The samples include 44 individuals (37 species) of red, green, and brown algae. Visible epifauna and epiphytes were scraped off the tissue, and samples were dried in silica gel. Macroalgae were taxonomically identified by experts (see acknowledgements) based on, for example, Pedersen (2011) and Fletcher et al. (1987) and following species names in Algaebase (www.algaebase.org, Guiry, 2013).

Surface sediment samples for the eDNA analyses were collected in June 2016 from eight stations distributed in the Disko Bay area, W. Greenland, on the south coast of the Disko Island and along Kronprinsen Ejland located off the coast (Figure 1). The predominantly rocky shores have an abundant presence of macroalgae, including intertidal habitats dominated by fucoid species and subtidal kelps extending down to maximum depths deeper than 60 m in the

TABLE 1 List of 44 macroalgal tissue samples (37 species) with taxonomy and collection site

ID	Species	Family	Order	Class	Phylum	Collection site	*In silico* 18S-V7	*In silico* 18S-V9	NCBI/SILVA database
48	***Laminaria solidungula***	Laminariaceae	Laminariales	Phaeophyceae	Ochrophyta	East Greenland	No	No	Yes
49	***Hedophyllum nigripes****	Laminariaceae	Laminariales	Phaeophyceae	Ochrophyta	East Greenland	No	Yes	Yes
50	***Turnerella pennyi***	Furcellariaceae	Gigartinales	Florideophyceae	Rhodophyta	East Greenland	No	No	No
51	***Hedophyllum nigripes****	Laminariaceae	Laminariales	Phaeophyceae	Ochrophyta	Nuuk, Greenland	No	Yes	Yes
52	***Ascophyllum nodosum***	Fucaceae	Fucales	Phaeophyceae	Ochrophyta	Nuuk, Greenland	Yes	No	Yes
53	***Saccharina latissima****	Laminariaceae	Laminariales	Phaeophyceae	Ochrophyta	Nuuk, Greenland	No	Yes	Yes
54	***Fucus vesiculosus***	Fucaceae	Fucales	Phaeophyceae	Ochrophyta	Nuuk, Greenland	No	No	Yes
55	***Fucus evanescens***	Fucaceae	Fucales	Phaeophyceae	Ochrophyta	Nuuk, Greenland	No	No	Yes
56	***Alaria esculenta***	Alariaceae	Laminariales	Phaeophyceae	Ochrophyta	Nuuk, Greenland	No	Yes	Yes
57	***Agarum clathratum***	Agaraceae	Laminariales	Phaeophyceae	Ochrophyta	Nuuk, Greenland	Yes	No	Yes
58	***Palmaria palmata****	Palmariaceae	Palmariales	Florideophyceae	Rhodophyta	Denmark	Yes	Yes	Yes
59	***Halidrys siliquosa***	Sargassaceae	Fucales	Phaeophyceae	Ochrophyta	Denmark	No	No	Yes
60	***Desmarestia aculeata***	Desmarestiaceae	Desmarestiales	Phaeophyceae	Ochrophyta	Denmark	Yes	Yes	Yes
61	***Polysiphonia nigrescens***	Rhodomelaceae	Ceramiales	Florideophyceae	Rhodophyta	Denmark	No	No	No
62	***Dilsea carnosa****	Dumontiaceae	Gigartinales	Florideophyceae	Rhodophyta	Denmark	No	Yes	Yes
63	***Fucus serratus***	Fucaceae	Fucales	Phaeophyceae	Ochrophyta	Denmark	No	No	Yes
64	***Membranoptera alata****	Delesseriaceae	Ceramiales	Florideophyceae	Rhodophyta	Denmark	No	No	No
65	***Rhodomela confervoides***	Rhodomelaceae	Ceramiales	Florideophyceae	Rhodophyta	Denmark	Yes	Yes	Yes
66	***Phycodrys rubens***	Delesseriaceae	Ceramiales	Florideophyceae	Rhodophyta	Denmark	Yes	Yes	Yes
67	***Chondrus crispus***	Gigartinaceae	Gigartinales	Florideophyceae	Rhodophyta	Denmark	Yes	Yes	Yes
68	***Codium fragile***	Codiaceae	Bryopsidales	Ulvophyceae	Chlorophyta	Denmark	No	No	Yes
69	***Membranoptera alata****	Delesseriaceae	Ceramiales	Florideophyceae	Rhodophyta	Denmark	No	No	No
70	***Delessaria sanguinae****	Delesseriaceae	Ceramiales	Florideophyceae	Rhodophyta	Denmark	No	No	No
71	***Phyllophora pseudoceranoides***	Phyllophoraceae	Gigartinales	Florideophyceae	Rhodophyta	Denmark	No	No	No
72	***Saccharina latissima****	Laminariaceae	Laminariales	Phaeophyceae	Ochrophyta	Denmark	No	Yes	Yes
73	***Odonthalia dentata***	Rhodomelaceae	Ceramiales	Florideophyceae	Rhodophyta	Denmark	No	No	No
74	***Furcellaria lumbricalis***	Furcellariaceae	Gigartinales	Florideophyceae	Rhodophyta	Denmark	Yes	Yes	Yes
75	***Laminaria digitata***	Laminariaceae	Laminariales	Phaeophyceae	Ochrophyta	Denmark	No	No	Yes
76	***Delessaria sanguinae****	Delesseriaceae	Ceramiales	Florideophyceae	Rhodophyta	Denmark	No	No	No
77	***Dilsea carnosa****	Dumontiaceae	Gigartinales	Florideophyceae	Rhodophyta	Denmark	No	Yes	Yes
78	***Saccharina latissima****	Laminariaceae	Laminariales	Phaeophyceae	Ochrophyta	Denmark	No	Yes	Yes
79	***Ulva lactuca***	Ulvaceae	Ulvales	Ulvophyceae	Chlorophyta	Denmark	Yes	Yes	Yes
80	***Chaetomorpha linum***	Cladophoraceae	Cladophorales	Ulvophyceae	Chlorophyta	Denmark	No	No	Yes
81	***Palmaria palmata****	Palmariaceae	Palmariales	Florideophyceae	Rhodophyta	Northeast Greenland	Yes	Yes	Yes
82	***Chaetopteris plumosa***	Sphacelariaceae	Sphacelariales	Phaeophyceae	Ochrophyta	Northeast Greenland	No	No	No
83	***Coccotylus truncatus***	Phyllophoraceae	Gigartinales	Florideophyceae	Rhodophyta	Northeast Greenland	No	No	No
84	***Punctaria glacialis***	Chordariaceae	Ectocarpales	Phaeophyceae	Ochrophyta	Northeast Greenland	No	No	No
85	***Punctaria plantaginea***	Chordariaceae	Ectocarpales	Phaeophyceae	Ochrophyta	Northeast Greenland	No	No	No
86	***Pylaiella varia***	Acinetosporaceae	Ectocarpales	Phaeophyceae	Ochrophyta	Northeast Greenland	No	No	No
87	***Stictyosiphon tortilis***	Chordariaceae	Ectocarpales	Phaeophyceae	Ochrophyta	Northeast Greenland	No	No	No
88	***Dictyosiphon foeniculaceus***	Chordariaceae	Ectocarpales	Phaeophyceae	Ochrophyta	Northeast Greenland	No	Yes	Yes
89	***Polysiphonia arctica***	Rhodomelaceae	Ceramiales	Florideophyceae	Rhodophyta	Northeast Greenland	No	No	No
90	***Enteromorpha prolifera***	Ulvaceae	Ulvales	Ulvophyceae	Chlorophyta	Laksebugten, Greenland	No	Yes	Yes
91	***Scytosiphon complanatus***	Scytosiphonaceae	Ectocarpales	Phaeophyceae	Ochrophyta	Kronprinsens Ejland, Greenland	No	No	No

*Marks species that were sampled at several sites. In silico amplification and database availability in both SILVA and NCBI database is marked with purple, only SILVA is marked with blue, and only NCBI is marked with black. 23 (21) of the species are represented in NCBI (SILVA) with 18S rDNA/small subunit partial sequences. Yellow highlight: The species included in our final sequence database.

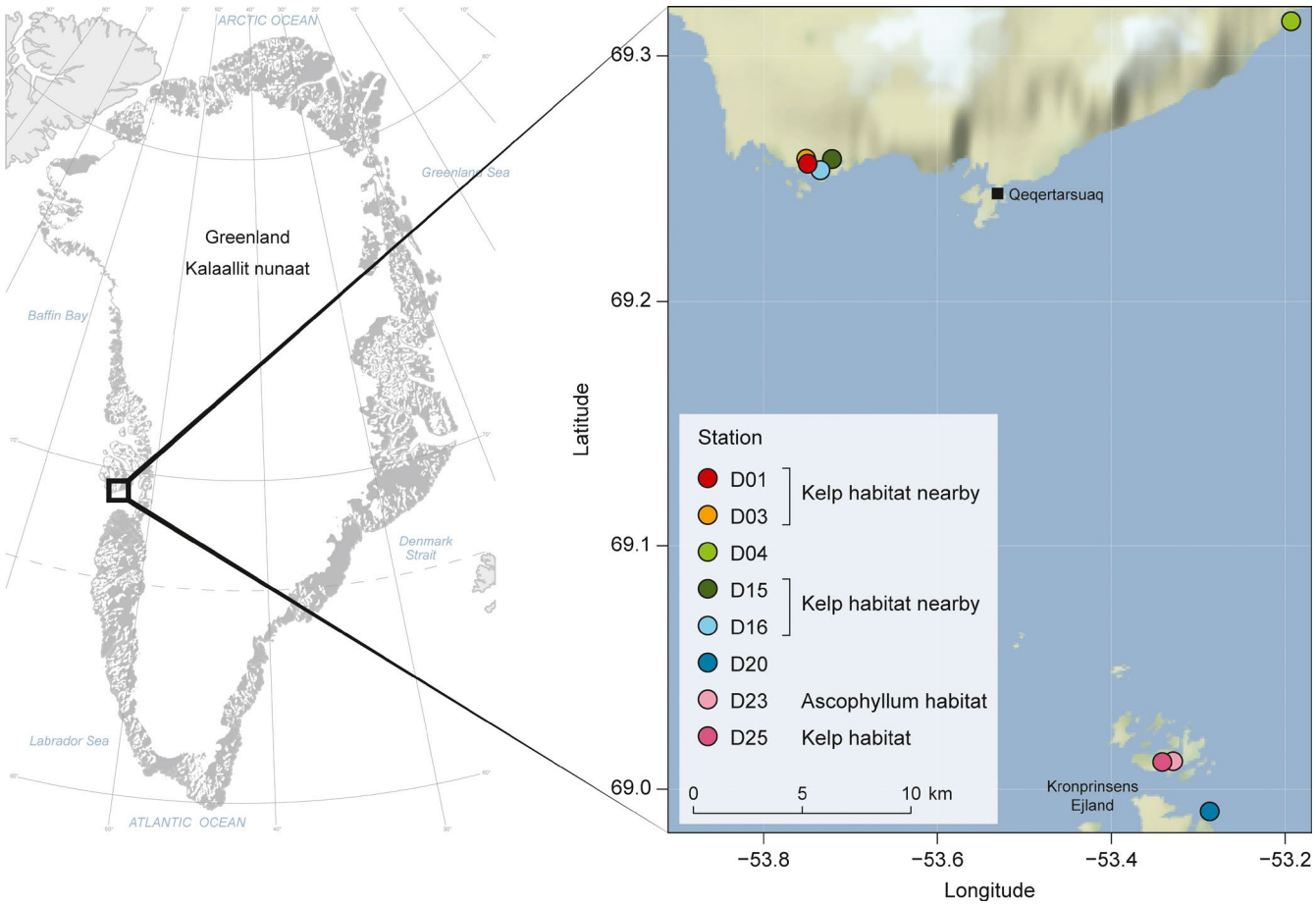

FIGURE 1 Map of sediment sampling stations in Disko Bay, Greenland

most clear-water sites, although icebergs also create barren areas (Krause-Jensen et al., 2019; Marbà et al., 2017; Pedersen, 2011). Samples were collected from six shallow stations (< 2 m depth) within and nearby macroalgae forests, and from two deep stations (75–85 m depth) (Table 2). One sediment core was collected at each station. In shallow settings, cores were collected using a hand-held kajak sediment sampler with acrylic tubes (5.2 cm inner diameter, www.kc-denmark.dk); while in deeper settings, the sediment was collected using a grap sampler. Surface sediments (upper 1 cm) were sampled for all stations, while deeper sediment layers (5–10 cm) were sampled only for three stations (Table 2). Shallow cores were sliced and processed back in the lab, while the deep sediment was processed on the sampling vessel. Sliced sediments were mixed thoroughly with an equal amount of DNAguard buffer to preserve DNA. The samples were stored at 5°C, and later at −80°C. To avoid contamination of samples during sampling and slicing of cores, we used sterile tools and gloves, but we were not able to include field controls.

2.2 | DNA extraction from tissue and sediment

Macroalgal tissue was homogenized using liquid nitrogen and grinded with a mortar and pestle, which were sterilized between samples with 10% bleach and 70% ethanol. DNA from 100 mg of grinded sample was extracted with the Nucleospin Plant II kit (Macherey-Nagel) following the manufacturers protocol.

DNA was extracted from sediment samples using an established method for marine sediments (Geraldi et al., 2020; Lever et al., 2015) (see also Appendix S1 for a detailed description). We included one extraction blank during the extraction of the eight sediment samples. DNA extractions were performed in an area physically separated from molecular work, and benches and instruments were cleaned with 10% bleach and 70% ethanol or RNaseZap (Invitrogen) before use to avoid contamination.

2.3 | In silico PCR

To analyze primer performance of 18S-V7 and 18S-V9 (Table 3), we performed *in silico* PCRs on 18S rDNA sequences in the SILVA database version-132 (November 4th 2018; Table 3) (Yilmaz et al., 2014), using the trim function from the insect package in R with default settings (Wilkinson et al., 2018). The SILVA *in silico* PCR dataset is available from https://github.com/ngeraldi/In-silico-tests. To briefly compare the SILVA database with the NCBI database (https://www.ncbi.nlm.nih.gov/), we also performed *in silico* PCR using PrimerBlast

TABLE 2 Field information on sediment samples from eight stations in the Disko Bay area, Greenland, along with DNA results from each station quantified as DNA (μg/μl)

	Stations							
	D1	**D3**	**D4**	**D15**	**D16**	**D20**	**D23**	**D25**
Depth (m)	1.5	1.5	75.5	1	2	85	2	0.5
Slice depths (cm)								
(a)	0–1	0–1	0–1	0–1	0–1	0–1	0–1	0–1
b)				5–6	5–7		5–10	
Comments from field	Saccharina latissima, Agarum clathratum, Alaria esculenta nearby	Saccharina latissima, Agarum clathratum, Alaria esculenta nearby		Saccharina latissima, Agarum clathratum, Alaria esculenta nearby	Saccharina latissima, Agarum clathratum, Alaria esculenta nearby	Greenish-brown organic mud	Ascophyllum nodosum habitat with Fucus vesiculosus interspersed	Saccharina latissima, Agarum clathratum, Alaria esculenta habitat
DNA (μg/μl)								
a)	1.85	5.23	0.30	1.37	1.24	0.405	2.78	1.01
b)				0.22	0.30		1.36	

TABLE 3 Table of primer pairs with reference and PCR protocols demonstrated in this study

Name	Forward primer	Reverse primer	Amplicon size	PCR protocol	References
18S-V7 18S-V7F- 18S-V7R	5'-TTTGTCTGSTTAATTSCG–3'	5'-CACAGACCTGTTATTGC–3'	123 bp	95°C for 15 min; 35 cycles (94°C –30 s, 53°C–45 s., 72°C –90 s); 72°C –10 min; hold at 4°C	Guardiola et al. (2015), Taberlet et al. (2018)
18S-V9 1380F–1510R	5'-CCCTGCCHTTTGTACACAC–3'	5'-CCTTCYGCAGGTTCACCTAC –3'	130 bp	95°C for 15 min; 40 cycles (94°C –30 s, 58°C–45 s, 72°C –90 s); 72°C –10 min; hold at 4°C	Amaral-Zettler et al. (2009)

(Ye et al., 2012, blasting the NCBI database) on our collected specimens, allowing one mismatch per primer (Table 1). However, in the following we only apply the use of the SILVA database, which is typically applied for evaluating taxonomic resolution and annotations for 18S sequences, due to its high level of quality control compared with the NCBI database (Hadziavdic et al., 2014; Harris, 2003; Kataoka & Kondo, 2019).

Taxonomic resolution (Bc) at species level was calculated by the number of sequence clusters with 97% identity match (with the DECIPHER package in R) within the SILVA *in silico* PCR dataset, divided by the total number of species available in SILVA. Taxonomic specificity (Bs) was calculated by the number of species amplified *in silico* PCR divided by the total number of species available in SILVA. In addition, we calculated taxonomic resolution at genus, family, order and class level based on clustering at a 99% identity match. Because the number of clusters often exceeded the number of, for example, genera, families, orders, or classes available in SILVA, the result of the standard calculation of taxonomic resolution (Bc) was often greater than 100%. We therefore present a second unit of taxonomic resolution for genus, family, order, and class level. This was calculated as the number of sequences within clusters (99% identity match) that only contain a single genus, family, order, or class, depending on the assessed taxon level, which was then divided by the total number of SILVA sequences amplified *in silico* PCR.

2.4 | Barcoding database preparation

2.4.1 | Barcoding PCR and sequencing

We targeted the 18S rDNA using the 18S-V7 primer pair (Table 3). PCR reactions contained 5 μl of QIAGEN Multiplex PCR Master Mix, 1 μl (1.0 μM) of each primer, 1 μl of DNA template, and 2 μl of PCR water. The PCR protocol for 18S-V7 was followed as detailed in Table 3. A PCR blank with DNA-free water and a mock sample, with a mix of algal DNA samples in different DNA concentrations (Appendix S2: Table S1) was included with the samples. PCR amplicons were prepared for sequencing with QIAquick PCR purification kit, and DNA concentration was measured with Qubit 2.0 Fluorometer (Invitrogen). The PCR station and materials were cleaned with RNaseZap (Invitrogen) and 70% ethanol and UV-sterilized before use.

We initially attempted SANGER sequencing; however, most of the sequencing failed, indicating either non-specific amplification and/or possible presence of DNA from epiphytic/endophytic species. To overcome this issue, we applied additional cleaning methods, such as a wash column (Amicon© Ultra 0.5 ml 10K membrane filter) and cleaning by gel, cutting out the expected amplicon size (3% agarose, Promega© Wizard SV gel and then QIAquick© PCR purification kit again). Also, we sterilized the macroalgal tissue with bleach and ethanol following Coombs and Franco (2003) and repeated DNA extractions. Nevertheless, Sanger sequencing failed for the majority of tissue samples, still indicating multiple nucleotides per site. Finally, we opted for sequencing of non-sterilized tissue (PCR amplicons also cleaned as described above) using Next-Generation Sequencing (NGS, Illumina-MiSeq). We followed the Illumina Metagenomic Sequencing Library Preparation protocol (Illumina) for indexing, and other preparation. Sequencing was conducted on an Illumina platform (MiSeq, Pair-end sequencing with a length of 301 bp) at KAUST Biological Core Lab with the Illumina MiSeq Reagent kit v3 (600 cycles).

2.4.2 | Barcoding bioinformatics and validation

The Illumina MiSeq sequencing output was demultiplexed using the Illumina protocol and primers were trimmed using Cutadapt 1.17 with default settings (Martin, 2011). The sequenced reads were assessed and filtered following default settings of the DADA2 1.8.0 pipeline in R version 3.5.1 (Callahan et al., 2016; RCoreTeam, 2017). The reads were quality-filtered, and forward and reverse reads shorter than 90 bp were removed. The reads were then dereplicated, sequencing errors were corrected and the forward and reverse reads were merged, forming sequence variants (SVs). Subsequently, we removed 1.1% of SVs identified as chimeras.

We then ran a cluster analysis following methods in Ortega et al. (2020) on the five most abundant SVs per alga sample (Appendix S2: Table S2) together with macroalgal sequences from the SILVA database. The SILVA sequences (65 SILVA sequences in total (Appendix S2: Table S3)) encompassed the same orders as represented among our algal tissue samples (Table 1), and included sequences from up to five species per family. SILVA sequences and SVs were aligned (MUSCLE) and subsequently clustered (Fast Tree 2.1.5; Appendix S3: Figure S1) to discard taxonomically odd SVs that did not cluster consistently near the class or order of closely related SILVA reference sequences. A PhyML tree using TN93 model and 1,000 replicates (Guindon et al., 2010) was run in GENEIOUS 11.1.4 (Biomatters Ltd.) with the remaining SVs and SILVA sequences to analyze the phylogenetic distance and relation between SVs and SILVA sequences. PhyML trees were edited in FigTree v1.4.3 (Rambaut, 2016). Finally, we calculated the identity between SVs within each algal sample with DECIPHER package in R. If the identity match between remaining SVs per alga sample was lower than 85%, the SVs were not included in our barcode database. SVs with read counts <1% of the total read count for the sample were not included, given they may be false positives and were unlikely to come from our target sequence.

Taxonomy was assigned to SVs in the blank samples with AssignTaxonomy from the DADA2 package with default settings in R.

2.5 | Metabarcoding of Arctic marine sediments

2.5.1 | PCR and sequencing for metabarcoding

We targeted the 18S rDNA in Arctic marine sediments using two primer pairs, 18S-V7 targeting the V7 region and 18S-V9 targeting

the V9 region (Table 3). For the three deeper (5–10 cm) samples, we only applied 18S-V7. PCR reactions contained 5 µl of QIAGEN Multiplex PCR Master Mix, 1 µl (1.0 µM) of each primer, 1 µl of DNA template, and 2 µl of PCR water. PCR protocols were followed as listed in Table 3 and performed with five replicates. PCR blanks were run in each PCR batch with DNAfree water and extraction blanks were also included. PCR replicates were pooled and visualized on 2% agarose gel to ensure successful amplification and expected amplicon size. Illumina Metagenomic Sequencing Library Preparation protocol (Illumina) was followed to clean and add indexes to the amplicons to distinguish samples after pooling and sequencing. Sequencing was conducted on an Illumina platform (MiSeq) at KAUST Biological Core Lab with the Illumina MiSeq Reagent kit v3 (600 cycles).

2.5.2 | Metabarcoding bioinformatics

The Illumina MiSeq sequencing output was demultiplexed using the Illumina protocol, adaptors and primers were trimmed using Cutadapt 1.17 with default settings (Martin, 2011). The sequenced reads were assessed following default settings of the DADA2 1.8.0 pipeline in R, also described earlier in the barcode preparation section. SVs with less than 10 reads were discarded as expected PCR errors.

Taxonomy was assigned with AssignTaxonomy from the DADA2 package with default settings, except for minboot, which was set at a more conservative value 70, unless other is specified. For 18S-V7 amplicons, taxonomy was assigned using the public SILVA database, both with and without including our local barcode database (31 species; Appendix S2: Table S2). For 18S-V9 amplicons, taxonomy assignment was performed using the SILVA database alone.

3 | RESULTS

3.1 | Primer performance in silico

Currently, the SILVA database holds 18s rDNA sequences from ca. 8% of the ca. 10.000 named marine macroalgal species (Table 4). Among the 37 species listed in Table 1, 20 were represented by partial 18S rDNA sequences in both SILVA and NCBI, while one was only available in SILVA and two only in NCBI. Nine of the species in Table 1, amplified *in silico* PCR with 18S-V7 (one with both NCBI and SILVA, three only with NCBI, and five only with SILVA), while 13 species amplified *in silico* with 18S-V9 (four with both SILVA and NCBI, eight only with NCBI, and one only with SILVA).

Within the SILVA database, 18S-V7 displayed both the highest taxonomic specificity (Bs: 91.9%) and resolution (Bc: 65.5%), of the two primer pairs. 18S-V9 displayed a much lower taxonomic specificity (52.5%), and a slightly lower resolution (63.2%) (Table 4). 18S-V7 had the highest taxonomic specificity within Florideophyceae (97.8%) and the lowest within Ulvophyceae (79.6%), while the taxonomic resolution was highest within Ulvophyceae (72.6%) and lowest within Phaeophyceae (39.3%). For 18S-V9, the highest taxonomic specificity was within Florideophyceae (61.7%) and the lowest within Phaeophyceae (32.4%), while the resolution was very similar between the classes, ranging 54.6–66.7%. At higher taxonomic levels (genus, family, order, and class), the taxonomic resolution improved in the products from both primers (note that taxonomic resolution was calculated differently at genus, order, and class level: see methods) (Figure 2). The resolution gradually increased in both primer products as the taxonomic level decreased. At class level, all sequences clustered according to class in both primers (Figure 2). At genus, family and order level, the resolution was generally higher for 18S-V9 than for 18S-V7, but note that the total number of sequences available was considerably lower for 18S-V9 (Figure 2). Similar to the species taxonomic resolution, the taxonomic resolution was overall lower of Phaeophyceae in 18S-V7 compared with 18S-V9 (Figure 2).

3.2 | Arctic and North Atlantic barcoding database

Based on metabarcoding and subsequent filtering and clustering analyses, we included 49 short 18S rDNA barcodes (18S-V7) from 31 species of marine macroalgae in our barcode database (Appendix S2: Table S2), spanning 20 families within 11 orders. Within this local barcode database, the taxonomic resolution at species level (Bc) was 61.3% in total, but 44.4% and 84.6% for Phaeophyceae and Florideophyceae, respectively (data not shown).

TABLE 4 Taxonomic specificity (*Bs*) and resolution (*Bc*) of three major classes of macroalgae using two different primer pairs *in silico* PCR on the SILVA database (Yilmaz et al., 2014)

		Primer pair							
		18S-V7				18S-V9			
	Total no. species with 18S rDNA available in SILVA	*Bs*		*Bc*		*Bs*		*Bc*	
Class	*N*	*N*	%	*N*	%	*N*	%	*N*	%
Phaeophyceae	68	61	89.7	24	39.3	22	32.4	12	54.6
Florideophyceae	499	488	97.8	286	58.6	308	61.7	194	63
Ulvophyceae	225	179	79.6	130	72.6	78	34.7	52	66.7
All three classes combined	792	728	91.9	477	65.5	408	52.5	258	63.2

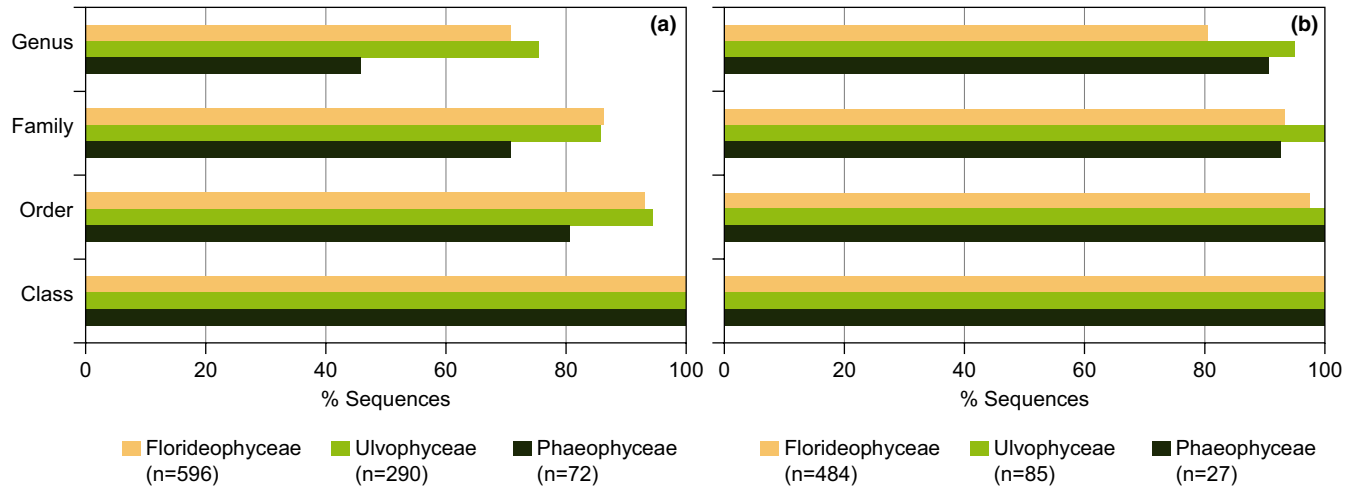

FIGURE 2 Barplots displaying the fraction of *in silico* PCR sequences from a) 18S-V7 and b) 18S-V9 within clusters (99% identity match) that only contain one genus, family, order, or class, depending on what taxon level is being assessed. This is divided in three sub-bars for each of the three classes of macroalgae with the total number of sequences given in ()

Before we performed the cluster analysis, we had 81.5 ± 7.2 (mean ± SE) unique SVs per alga sample. Then, assuming that our target SVs accounted for the majority of the total reads, we only included the five most abundant SVs in the cluster analysis, as they, on average, accounted for 90.1±1.4% of the total number of reads (Appendix S3: Figure S2). 96.3% of the remaining 1,252 less abundant SVs had 10 reads or less. Blanks contained 10 SVs (17,255 reads), and four of those (17,104 reads) were assigned to a fungi of the order Malasseziales; one SV with 10 reads was assigned to Florideophyceae.

A cladogram of only the 65 SILVA macroalgal sequences (*in silico* PCR amplicon of 18S-V7) formed rather distinct clades (Appendix S3: Figure S3), so we expected the target SVs from our algae samples to also cluster accordingly. Florideophyceae SILVA sequences were restricted within a single clade that branched into smaller clades, largely corresponding to the different taxonomic levels as the order rank branches presented subclades that represented the family level. For example, a Ceramiales clade was split into a Ceramiaceae and a Delesseriaceae clade (Appendix S3: Figure S3). All Phaeophyceae SILVA sequences, except from *Saccharina japonica*, were restricted within a single clade that similarly branched into smaller clades mimicking the order ranks but to a higher degree than the Florideophyceae clade. The distribution of Ulvophyceae SILVA sequences across the cluster was inconsistent, and did not form a single clade. At the order rank, Ulvales clustered within a sinlge clade, but Cladophorales clustered in multiple clades (Appendix S3: Figure S3).

Based on the cluster analysis between the 65 SILVA sequences and the five most abundant SVs per alga sample, at least one of the SVs from each of the brown and red algae samples clustered with their respective SILVA sequences according to class (Appendix S3: Figure S1), and were hence included in the final tree (Figure 3). None of the abundant SVs from the green algae samples (*Codium fragile*, *Ulva lactua*, *Chaetomorpha linum*, and *Enteromorpha prolifera*) clustered with their respective SILVA sequences according to class (Appendix S3: Figure S1), and were therefore excluded from the final tree and barcode database (Figure 3). With few exceptions, the remaining Phaeophyceae SVs were affiliated with the correct SILVA order, family, genus level (e.g., Fucaceae and Sargassaceae under Fucales), or with the corresponding species if the sequence was represented in SILVA (Figure 3). However, some unexpected clustering occurred, as the Ectocarpales subclade held many SVs from algae samples that belong to other Phaeophyceae orders. All the resulting Florideophycea SVs were affiliated with the correct SILVA order, family, genus or species level. Indeed, Florideophyceae presented more diversity than Phaeophyceae, resulting in many subclades (Figure 3).

Twenty-six of the 31 species in our final local barcode database are represented by a single SV, while five species are represented by two to three very similar SVs each (>85% identity match; Appendix S3: Table S4). *Ascophyllum nodosum* and *Odonthalia dentata* were left out of the barcode database, because their SVs were less than 85% identical. Four species had been collected more than once, in both Greenland and Denmark (*Saccharina latissima*, *Membranoptera alata*, *Delessaria sanguinae* and *Dilsea carnosa*, Table 1), and the final SVs obtained from the three individuals of *S. latissima* were identical, and so were the two individuals of *M. alata*. For *D. sanguinae*, the SVs were 99% identical and for *D. carnosa*, they were 85% identical (Appendix S2: Table S4). The final sequences included in our local barcode database are listed at genus level in the NCBI (accession code: MK453128-MK453194, www.ncbi.nlm.nih.gov), for example, *Laminaria* sp.; and all the sequences are available at PANGEA (www.pangaea.de).

3.3 | Fingerprinting macroalgae in Arctic marine sediments

Despite our efforts in adding sequences to the databases, macroalgae are still poorly represented in DNA databases. Accordingly, the primer performance *in silico* PCR revealed the limited variability

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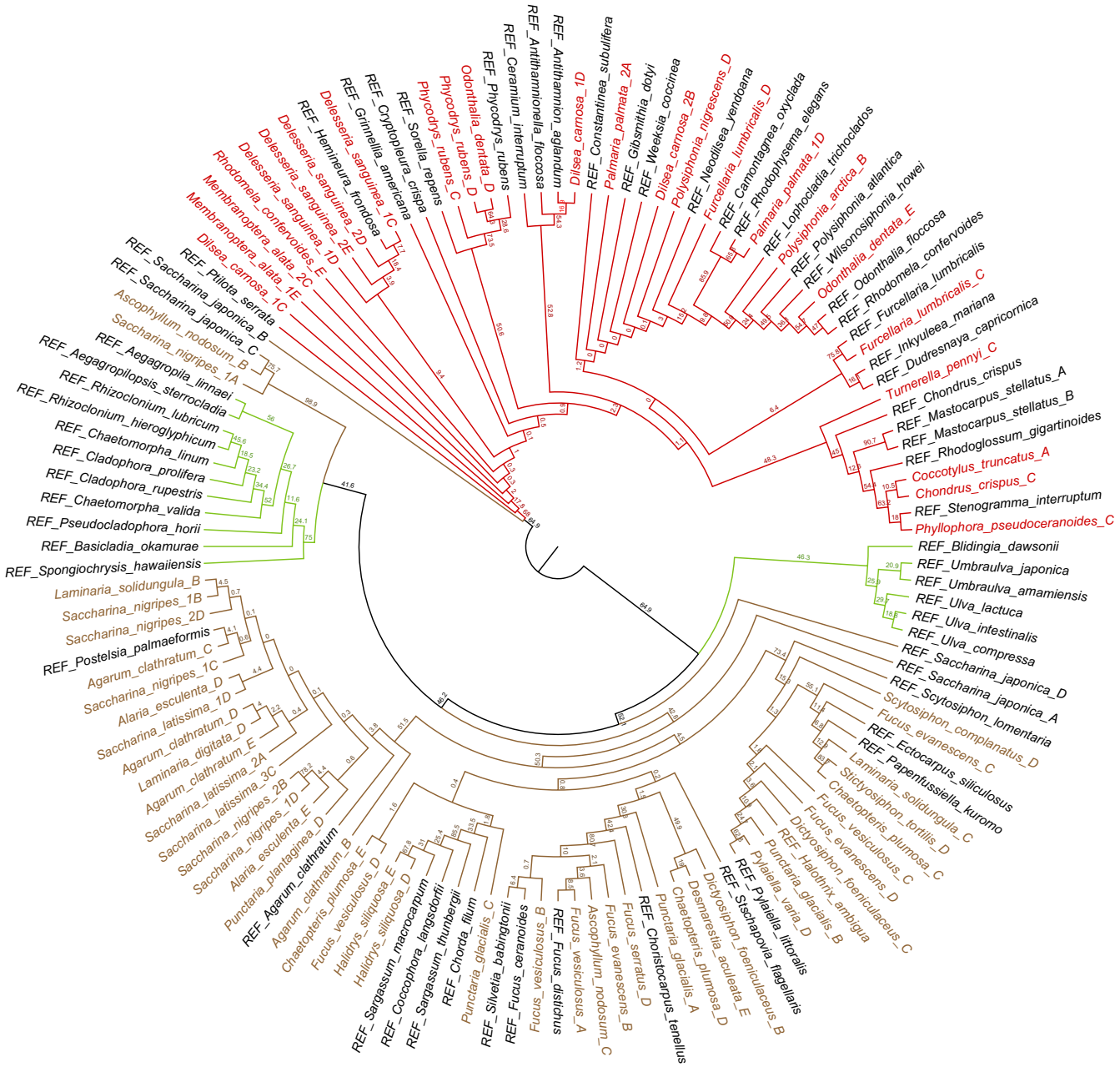

FIGURE 3 Maximum-likelihood (ML) cladogram of chosen SVs and 65 SILVA reference sequences extracted from the SILVA database (18S rDNA (v7) (123 bp), 18S-V7 primer pair). Numbers annotate the bootstrap proportion per clade in percent

among species within genera and families (Table 4 and Figure 2). Therefore, we disregarded any assignments at higher resolution than family level in the sediment samples.

With the 18S-V7 primer pair, we found 1,111 unique SVs (1,023,647 reads) in surface sediment samples, and 2.1% of SVs were assigned as macroalgal taxa with SILVA combined with our newly developed database. We detected macroalgal DNA in all sampled sediments except those retrieved from the deepest station (85 m, D20), and identified seven families within six orders of macroalgae (five brown and two red). These five families (Laminariaceae, Furcellariaceae, Chordariaceae, Palmariaceae, and Sphacelariaceae) and two orders (Palmariales and Sphacelariales) were resolved based on our local barcoding database (Table 5a), covering 44% of the total read counts. Nearly all (97%) of the macroalgal reads were assigned to three families of brown algae (Fucaceae, Laminariaceae, and Sphacelariaceae) and 57% of those reads were assigned to taxa within our newly developed barcode database (Figure 4a,c, Table 5a). These three families where identified in all shallow stations with one exception as Fucaceae was not identified at D4. Laminariaceae was also identified in one of the deep stations (D3).

The use of the SILVA database alone led to more reads being assigned, but with a severely impaired taxonomic resolution, with

TABLE 5 Taxa identified (minboot = 70) in surface sediments (0–1 cm) with primer pair (a) 18S-V7 and (b) 18S-V9 and their proportion of the total macroalgae read count across all surface samples

Primer pair	Order	Family	Genus	Proportion of total macroalgae reads %	Identified in station							
					D1	D3	D4	D15	D16	D20	D23	D25
a) 18S-V7	Fucales	Fucaceae	Fucus	49.60	x			x	x		x	x
	Laminariales	Laminariaceae*	Saccharina	40.35	x	x	x	x	x		x	x
	Sphacelariales	Sphacelariaceae*	Chaeopteris	6.95	x		x	x	x		x	x
	Ectocarpales	Chordariaceae*	Punctaria	0.38							x	x
	Gigartinales	Furcellariaceae*	Turnerella	0.27							x	
	Fucales	Sargassaceae	Halidrys	0.08			x					
	Palmariales	Palmariaceae*	Palmaria	0.02					x			
Total				**97.65**								
b) 18S-V9	Ectocarpales	Chordariaceae	Halothrix	27.00	x	x	x	x	x		x	x
	Fucales	Fucaceae	Fucus	15.17	x	x		x	x		x	x
	Fucales	Fucaceae	Fucus	6.69	x	x			x		x	x
	Ectocarpales	Ectocarpaceae	Ectocarpus	3.27	x	x		x	x		x	x
	Gigartinales	Furcellariaceae	Furcellaria	0.58		x	x	x			x	
	Laminariales	Akkesiphycaceae	Akkesiphycus	0.28	x	x	x		x		x	x
	Corallinales	Porolithaceae	Porolithon	0.26							x	
	Ulothricales	Ulothricaceae	Pseudothrix	0.24	x			x	x		x	x
	Ulvales	Ulvellaceae	Ulvella repens	0.16				x				x
	Palmariales	Rhodophysemataceae	Rhodophysema	0.08		x						
	Ceramiales	Rhodomelaceae	Micropeuce	0.04					x			
	Ulvales	Ulvaceae	Ulva	0.03				x				
	Ulvales	Ulvaceae	Ulvaria	0.01								x
	Sphacelariales	Lithodermataceae	Heribaudiella	0.01								x
Total				**53.82**								

*Marks families assigned with our local barcode library. Brown text is Phaeophyceae, red text is Florideophyceae and green text is Ulvophyceae.

only 58.2% of the Phaeophyceae reads being assigned past order level, compared with 99% when we included our newly developed barcode database in the assignment (Figure 4c). In addition, when we included our newly developed barcode database, 38% of the reads assigned with the SILVA database alone were assigned with greater taxonomic resolution. We observed that 93% of the 28,771 additional reads assigned with the SILVA database alone, were not assigned past order level (7%: Phaeophyceae, 86%: Ectocarpales, 7%: Ectocarpaceae). None of the families or orders identified in the samples were detected in the blanks, but we found very low reads of SVs assigned to the macroalgal families/orders, Dictyotaceae/Dictyotalesand Galaxaurineae/Nemaliales.

In deeper sediment layers (5–10 cm depth), the amount of SVs assigned to macroalgae was only 1.08% (424 reads) out of the total, which is half of that recovered from surface layers (2.01%, both with 18S-V7 and SILVA and our newly developed database combined) (Table 6). Across the three stations, we identified six families (four red and two brown) (Table 6). Two of the families, Fucaceae and Laminariaceae, were also found in surface sediments. Note that the number of reads associated to each SV was very low, as the maximum number of reads was 152 reads in one SV assigned to Solieriaceae and the next highest was 138 reads in another SV assigned to Fucaceae.

With the 18S-V9 primer pair, we found 2,088 unique SVs (664,920 reads) in the surface sediment samples and 14.3% of SVs were assigned to macroalgal taxa with SILVA. 18S-V9 identified macroalgal DNA at all stations except at the deepest station (D20), and identified 14 families within 11 orders of macroalgae (four red, four green, and six brown) (Figure 4b,d, Table 5b). A majority (52%) of the total macroalgae reads were assigned to three families (Chordaricaceae, Fucaceae, and Ectocarpaceae) of brown algae (Table 5b), while 31.7% of the total macrolgae reads were not assigned past class level. Five of the identified families were detected in the blanks, although at lower read counts.

3.4 | Validation of taxonomic assignment

To validate the taxonomic assignments in the sediment samples and the potential of quantifying macroalgal DNA with metabarcoding, we sequenced a mock sample containing known volumes of DNA from a range of macroalgal species (Appendix S2: Table S1). With 18S-V7, the initial assignment indicated that we had five species in the mock sample, of which four were originally added to the mock sample (Appendix: Table S1a). However, adjusting the assignments for the lowered taxonomic resolution of the primer product, we limited the identification to five families within four orders, of which three families within four orders were represented in the mock. We found no significant positive correlation

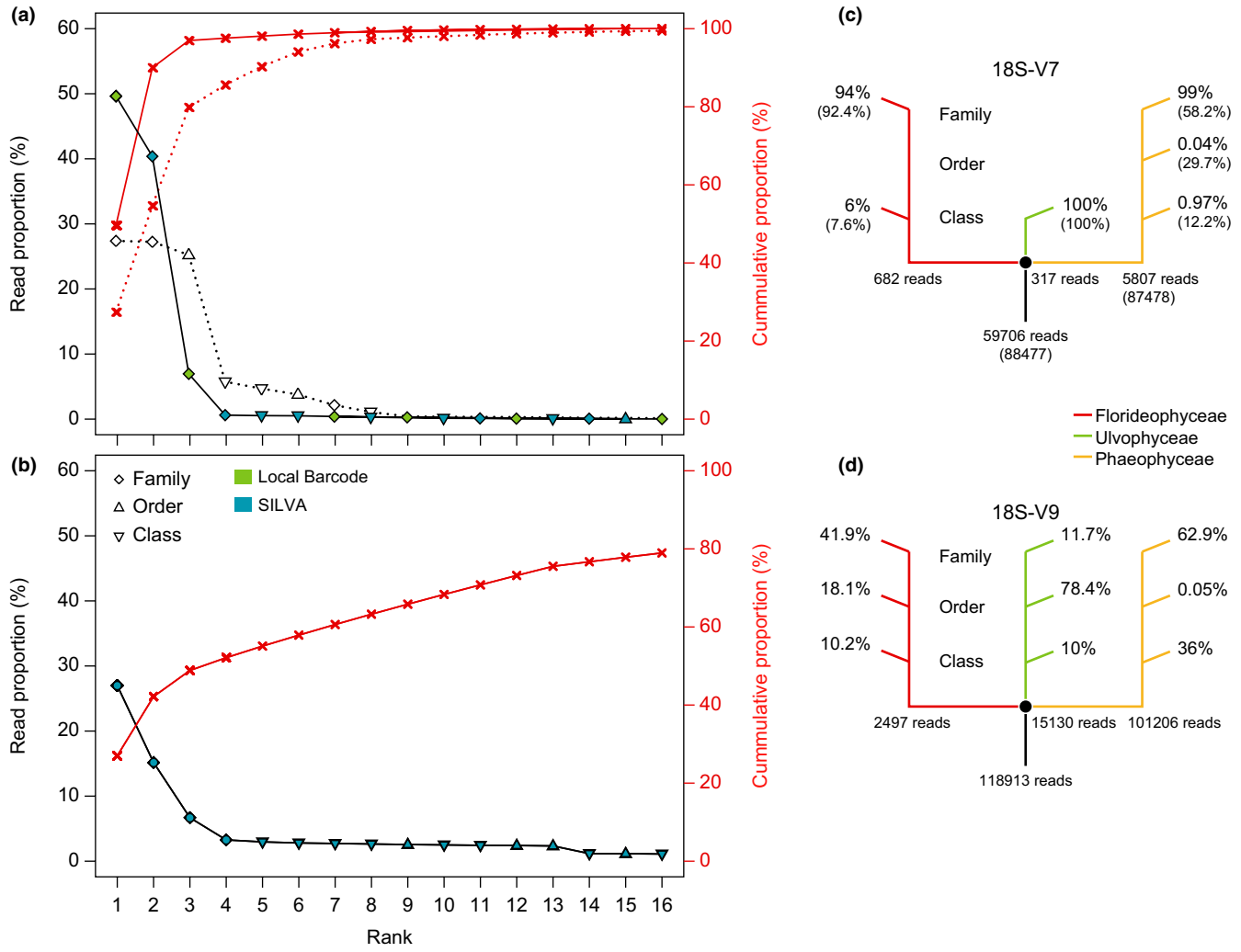

FIGURE 4 Rank abundance and cumulative proportion of reads in percent for both (a) the 18S-V7 and (b) the 18S-V9 output assigned as macroalgae taxa by either our local barcode database or the SILVA database. Dotted lines depict ranked assignments without using our local barcode database. Trees display the level of taxonomic assignment of red, green and brown algal taxa given as the proportion of reads in percent for both (c) the 18S-V7 and (d) the 18S-V9 output reads. Values within brackets depict proportions of taxonomic assignments obtained without using our barcode database

(spearman ran order correlation) between DNA concentration and read counts for the identified species (Rs[4] = −0.6, $p > 0.05$), nor at genus (Rs[4] =0, $p > 0.05$), family (Rs[5] = −0.3, $p > 0.05$), or order level (Rs[5] =0.2, $p > 0.05$), suggesting that metabarcoding with these primers is more of a qualitative measure than quantitative for these macroalgae. When assigning taxonomy with amplicons this size, it is recommended to apply a minimum bootstrap threshold (minBoot) of 60 (Porter & Hajibabaei, 2018). We tested different bootstrap levels in the 18S-V7 mock sample (50, 70, 90). There were few changes in the number of reads/SVs assigned with taxonomy between 50 and 70 bootstrap threshold, while increasing the bootstrap threshold from 70 to 90 had dramatic effects, as only half of the SVs were assigned at a 90 compared with 70 bootstrap threshold (Appendix: Table S1a).

With 18S-V9, the initial assignment indicated that we had four species in the mock sample, none of which were originally added to the mock sample. But, again adjusting for the low taxonomic resolution of the primer product, we limited the identification to four families within three orders, of which one family and three orders were represented in the mock (Appendix: Table S1b). We found no significant positive correlation (spearman rank order correlation) between DNA concentration and read counts at the order level (Rs[4] =0.32, $p > 0.05$). Note, that we only mention assignments to species level in relation to mock samples, since we found it informative due to knowing the exact species added to the mock sample.

4 | DISCUSSION

4.1 | A Barcoding database to resolve eDNA for macroalgae

We created a DNA barcode database including 31 (18 brown and 13 red) widespread Arctic and North Atlantic macroalgal species based on a small section (123 bp) of the V7 region in the 18S rDNA gene. For 23 of these species, this region was not available in the SILVA

TABLE 6 Taxa identified (minBoot=70) to SVs in the second layer sediment samples (5–10 cm) with primer pair 18S-V7 and their read count. Macroalgae SVs take up 0.33% of the total read count within second layer samples. Red text is red algae (Florideophyceae) and brown text is brown algae (Phaeophyceae)

Taxa assigned					Station			
Phylum	**Class**	**Order**	**Family**	**Genus**	**D15**	**D16**	**D23**	**Total # reads**
Rhodophyta	Florideophyceae	Gigartinales	Solieriaceae	NA	43	61	48	**152**
Ochrophyta	Phaeophyceae	Fucales	Fucaceae	Fucus	-	23	115	**138**
Rhodophyta	Florideophyceae	Ceramiales	Rhodomelaceae	NA	14	14	23	**51**
Ochrophyta	Phaeophyceae	Laminariales	Laminariaceae	Saccharina	-	32	17	**49**
Rhodophyta	Florideophyceae	Bonnemaisoniales	Bonnemaisoniaceae	Bonnemaisonia	-	20	-	**20**
Rhodophyta	Florideophyceae	Ceramiales	Ceramiaceae	Callithamnion	5	-	10	**15**

nor NCBI database (Table 1), despite all being relatively abundant species in the North Atlantic and the Arctic regions (Table 2). This highlights the poor representation of macroalgae in existing barcode databases and explain why very few studies have been published on this topic (Ortega et al., 2020; Queirós et al., 2019; Reef et al., 2017). Our local database spans 20 families within 11 orders, and two of the families (Alariaceae and Sphacelariaceae) and one of the orders (Sphacelariales) were not represented with this region in the public databases.

Our results show the positive impact of our local barcode database in enabling the use of eDNA to identify North Atlantic and Arctic macroalgae, thereby complementing existing tracing methods such as isotopic (Geraldi et al., 2019; Reef et al., 2017; Renaud et al., 2015) and fatty-acid analyses (Filbee-Dexter et al., 2018) to improve our capacity to resolve macroalgal distribution patterns, connectivity and contribution to carbon sequestration and food webs based on traces left in the environment.

Brown and red algae are well represented in our local barcode database, but we could not include the green algae as they delivered inconsistent results, indicating that the applied primer pair (18S-V7) will not identify green algae, despite the relatively good results *in silico* PCR. Surprisingly, the SILVA sequences of neither *Chaetomorpha linum* nor *Ulva lactuca* matched the samples sequenced here, meaning that either our or SILVA's sequences for these species maybe misidentified. This suggests that it is unlikely that one primer pair will be able to resolve all groups of macroalgae equally. This is not surprising, considering the complex evolutionary diversity of macroalgae (Baldauf, 2003; Charrier et al., 2008), including species as different as fungi and elephants (Krause-Jensen et al., 2018). Similar challenges with identification of green algae have been documented by Ortega et al. (2020) for 18S-V7. Other primer pairs that amplify both brown and red algae also often perform poorly on green algae, which has been attributed to the high incidence of introns in green algae DNA (Saunders & Kucera, 2010). Interestingly, we identified green algae in sediment samples with 18S-V9, but not with 18S-V7, indicating that 18S-V9 may have a larger capacity for identifying all three classes of macroalgae. However, this was not evident based on the *in silico* PCR, and 18S-V9 did not identify green algae in the mock sample of DNA with species of both red, green and brown algae, while 18S-V7 did identify green algae in the mock sample. This discrepancy could also suggest errors in the existing green algae reference sequences.

We obtained the barcodes for our database with Next-Generation sequencing (NGS) because Sanger sequencing, the method typically used to retrieve barcodes (Kress et al., 2015; Saunders & McDevit, 2012), was unsuccessful even after attempting both tissue sterilization and PCR preparation methods designed to isolate and purify target DNA. Initially, we obtained multiple SVs per sample with NGS, revealing the presence of several different sequences within samples, most likely from the epiphytic and endophytic organisms living in and on macroalgal tissue. However, on average, the five most abundant SVs included 90% of all reads, and corresponded to the targeted organisms as shown by clustering. We suspect that DNA from both epiphytic and endophytic organisms may have caused Sanger sequencing to fail, even though we extracted DNA from tissue samples following recommendation from existing barcoding studies (Le Gall & Saunders, 2007; Mcdevit & Saunders, 2009; Saunders & McDevit, 2012). To completely avoid epiphytic and endophytic organisms, some barcoding studies have made unialgal cultures in the laboratory from samples collected in the field by the Germling Emergence Method (Küpper et al., 2016; Peters et al., 2015). However, this method is time-consuming and requires precise knowledge on reproductive period and settling dynamics, which are often quite complex, varying both within and between areas (Ørberg et al., 2018). Also, cultivation of macroalgae is time consuming and not all macroalgal species can be cultivated in the laboratory, limiting the feasibility of the method for barcoding a wide range of macroalgae.

4.2 | Fingerprint of macroalgae in Arctic marine sediments

We identified macroalgal DNA in Arctic marine sediments using two primer pairs that target 18S rDNA. Our local barcoding database

enhanced the identification of macroalgal taxa in sediment samples. Half of the macroalgal reads were identified using our barcoding database, which added three families to the otherwise 16 families identified in surface sediments. Moreover, our local database improved the taxonomic assignment of recovered sequences for both brown and red algae relative to the information available in the SILVA database.

We detected the presence of macroalgal DNA in all sediment samples taken within or nearby macroalgae habitats. In addition to previous studies using similar 18S rDNA primers (Queirós et al., 2019), this confirms that macroalgae leave traceable DNA in the surface sediment below their habitat and in the nearby surface sediment. We also identified macroalgal DNA in older, sub-surface sediments, which has not been documented before. This metabarcoding method represents an advance in our capacity to use eDNA approaches to interrogate Arctic sediment archives for the presence of macroalgae, although it is constrained by the taxonomic resolution of the amplicon in general.

The kelp forests in the Disko Bay area are dominated by *Saccharina latissima*, *Agarum clathratum*, and *Alaria esculenta* (Krause-Jensen et al., 2019; Pedersen, 2011), which are all within the order of Laminariales but belong to their own separate families (Laminariaceae, Agaraceae, and Alariaceae). *Saccharina latissima* was the most abundant kelp in the areas we sampled, and thus we expected to find more sequences assigned to Laminariaceae in the sediment, which was also the case, but we did not detect Agaraceae or Alariaceae at all. With this in mind, we found that the taxonomic resolution of the 18S-V7 product was reduced in brown algae, suggesting that assignments to order level might be the more conservative approach when it comes to brown algae, while green and red algae displays relatively higher taxonomic resolution according to our *in silico* PCR results.

The intertidal macroalgal habitats in the Disko bay area are dominated by fucoid species, such as *Ascophyllum nodosum* and Fucus spp. interspersed. They belong to the same family, Fucaceae, and we detected Fucaceae in the sediment with both primers.

The majority of macroalgal reads were assigned to families that have been observed in the area of Disko Bay, such as mentioned in the above. Out of the 19 families detected with metabarcoding in the sediment, 13 have been observed in Disko Bay (Boertmann et al., 2013; Pedersen, 2011). Two of the remaining families (Ulvellaceae and Rhodophysemataceae) have only been observed further south of the study site, 67°N, and could potentially occur at low abundance in the study area. The last four families (Akkesiphycaceae, Lithodermataceae, Sargassaceae, and Porolithaceae) have not been observed on the west coast of Greenland so far (Boertmann et al., 2013; Pedersen, 2011). Nevertheless, these four families account for only 0.08% and 0.55% of the macroalgal reads from 18S-V7 and 18S-V9, respectively. Optimally, eDNA studies should include field controls. We were not able to include field controls, and contamination while collecting the samples cannot be ruled out. Yet, our macroalgae results match observations at sites, which serve as independent controls and indicate that field contamination was unlikely to have affected our findings.

The overall taxonomic resolution (Bc) of both primer products is quite similar but 18S-V7 displays a generally lower resolution within Phaeophyceae, and 18S-V9 displays higher resolution at genus, family, order and class level. However, the number of sequences available in SILVA and taxonomic specificity is quite low for 18S-V9 compared with 18S-V7. The mock sample analysis confirmed both the difference in specificity and the difference in availability of resources between the two primers. Although there may be more 18S-V9 sequences available in NCBI than in SILVA (Table 1) and species assignments may change with the addition of NCBI as a reference database, SILVA has a higher level of quality control in sequence entries compared with NCBI and is therefore commonly used in this type of taxonomic assignments. Applying both primers concurrently as we demonstrate in this study has advantages. Together they can strengthen the conclusions, but each additional primer multiplies the costs and efforts of the analyses.

Applying both primers concurrently as we demonstrate in this study has its advantages. Together they can strengthen the conclusions, but each additional primer multiplies the costs and efforts of the analyses.

4.3 | eDNA as a promising tool to delineate the ecological role of macroalgae

Despite the growing importance of macroalgae as key primary producers in the North Atlantic and in the Arctic, resolving key questions on their ecological role remain elusive, including key questions such as: (1) What is the distribution patterns of macroalgae – past, present and future – and what regulates them? (Bartsch et al., 2012; Bolton, 1994; Lüning, 1990) and (2) What is the fate of macroalgae and their contribution in food webs and carbon sequestration? (Dierssen et al., 2009; Duarte & Cebrian, 1996; Fischer & Wiencke, 1992; Harrold et al., 1998). These questions require mapping, predictions of distribution patterns and tracing the fate of macroalgal production, as changes in distribution and fate of the production will greatly impact both food webs and carbon budgets (Krause-Jensen & Duarte, 2016; Queirós et al., 2019). The advent of assessing eDNA provides a tool that can address these questions, but its application remains limited to date.

We show for the first time how it is possible to detect the presence of macroalgae with eDNA, not only in surface sediments but also in older sub-surface sediments beyond their habitat. Macroalgae have been reported to travel far from their source area (Dierssen et al., 2009; Harrold et al., 1998; Krause-Jensen & Duarte, 2016), and a recent assessment based on metagenomics reported a widespread presence of macroalgal genes across the open ocean, including the deep sea (Ortega et al., 2019). Our results show that metabarcoding of eDNA in sediment samples can reveal the presence of adjacent macroalgae species, enhanced by including local species in the reference barcode database. Our sediment eDNA approach can also benefit monitoring of macroalgae, and may provide insights in the past and present distribution of macroalgae in the Arctic, where data

are limited and direct surveys are challenging. To be specific, we may better address the question of northward expansion of macroalgae, as the presence of macroalgal DNA in environmental samples north of their distribution limits would support the current trend observed in the northward expansion of macroalgae (Krause-Jensen et al., 2020). Moreover, the assessments of eDNA can enhance tracing the role of macroalgae in food webs, already a useful tool applied in diet analyses (de Sousa et al., 2019).

The detection of macroalgal DNA in deeper (5–10 cm) sediment layers is consistent with evidence that DNA can persist for a long time in sediments (Harrison et al., 2019). Therefore, assessments on current distribution patterns should rely on surface (0–1 cm) sediment samples, while past distribution patterns may be observed from deeper sediment layers (Bálint et al., 2018), particularly when combined with geochemical methods to establish chronologies (del Carmen Gomez Cabrera et al., 2019; Marba et al., 2018; Wesselmann et al., 2021). Dated sediment layers can resolve past climate environments (Jensen, Kuijpers, Koc & Heinemeier, 2004; Georgiadis et al., 2018), which, when coupled with macroalgal eDNA analyses, could allow linking past distribution patterns of macroalgae to past changes in the climate, which may help inform projections of future macroalgae distributions patterns with climate change in the Arctic (Krause-Jensen & Duarte, 2014).

Identifying macroalgae with metabarcoding of eDNA remains challenging, both due to the lack of DNA resources for assigning taxonomy, but also due to potential dilution of macroalgal sequences when using primer pairs that target a wide array of taxa. Sequencing machines are ultimately limited in the number of reads they deliver, and if macroalgal DNA is present in very low amounts in an otherwise DNA rich sample, macroalgal amplicons may be present but the likelihood of sequencing them may be very small, resulting in false negatives for the presence of macroalgal taxa. Hence, the detection limit for macroalgae with metabarcoding in DNA rich samples may vary. False positives may also result from tag-jumping, where reads switch tags and are associated with the wrong sample after metabarcoding accounting for up to 0.01% of the reads (Schnell et al., 2015). Tag-jumping can be reduced by using individual tags; however, it also increases costs.

The combined use of the barcode resource generated here and eDNA metabarcoding allowed the detection of macroalgae in Arctic marine sediments. However, whether metabarcoding can be used as a tool to quantify their contribution to carbon sequestration in marine sediments is inconclusive based on these results. We found no relation between DNA concentration and read counts in mock samples, but we show that families, for example, Laminaricaceae, observed in high abundancies at the sampling sites or nearby generally display higher read counts in sediment samples. Indeed, a recent experimental study showed a positive relationship between read counts and organic carbon contributions from macroalgae in sterilized marine sediment (Ortega et al., 2020). This relationship is likely specific to both the primer pair and the target taxa, and requires further study.

Many studies have assessed the relation between reads and biomass in other groups of animals (Bista et al., 2018; Thomsen et al., 2016) and developed tools that correct biases that affect quantification by reads (Thomas et al., 2016). Nevertheless, qPCR is currently the most accurate method for quantifying eDNA and relating eDNA to the abundance or biomass of organisms, because the concentration of target DNA is measured during amplification as opposed to after PCR in which the final concentration of amplicons may not relate to their initial concentration. However, as qPCR or related methods simply measure the DNA of the target group, it requires species specific primers pairs, and currently we only know of one successful primer pair for a species, which is not relevant in Greenland (Anglès d'Auriac et al., 2021) and development of such primers is challenging because of the limited number and coverage of reference sequences. Conclusively, our results provide an encouraging basis to apply eDNA metabarcoding as a tool to delineate the ecological role of Arctic macroalgae and trace past and present distribution patterns.

ACKNOWLEDGEMENTS

This research was supported by the Independent Research Fund Denmark (8021-00222 B, 'CARMA') to DK-J and King Abdullah University of Science and Technology through baseline funding provided to CMD. We thank Susse Wegeberg, Ole Geertz-Hansen, Karsten Dahl, Peter Stæhr and Michael Bo Rasmussen for help with collection and identification of algae and Wajitha J. Raja Mohamed Sait and Nadia Haj Salah for help with extraction and sequencing. We thank Pierre Taberlet for suggesting the use of primer 18S-V7 and MiSeq sequencing for DNA barcoding. We further thank the suggestions and advice provided by anonymous reviewers and the editor to improve the manuscript. SBO was supported by funding from Independent Research Fund Denmark (8021-00222 B, 'CARMA').

CONFLICT OF INTEREST

Authors have no conflict of interest to disclose.

AUTHOR CONTRIBUTIONS

CDM and DK-J conceived the idea of the study and all authors contributed to the design and methods of the study. SBO and AO extracted DNA from tissue samples. SBO, NRG and RD-R prepared samples for and performed sequencing. NRG performed in silico PCR. NRG, RD-R, AO, CDM, DK-J, and SBO performed analysis and interpretation of the data. SBO prepared the first draft of the manuscript along with figures and tables. SBO incorporated comments from all authors to form the final manuscript. All authors have approved the submitted version of the manuscript.

DATA AVAILABILITY STATEMENT

The raw data underlying the main results of the study are available on the data Dryad repository (www.datadryad.org) at https://doi.org/10.5061/dryad.1jwstqjw9.

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SUPPORTING INFORMATION

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How to cite this article: Ørberg SB, Krause-Jensen D, Geraldi NR, Ortega A, Díaz-Rúa R, Duarte CM. Fingerprinting Arctic and North Atlantic Macroalgae with eDNA – Application and perspectives. Environmental DNA. 2021;00:1–17. https://doi.org/10.1002/edn3.262