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Updates to the Marine Algal Flora of the Boulder Patch in the Beaufort Sea off Northern Alaska as Revealed by DNA Barcoding

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ABSTRACT. Since its discovery four decades ago, the Boulder Patch kelp bed community in the Beaufort Sea has been an important site for long-term ecological studies in northern Arctic Alaska. Given the difficulties associated with identifying species of marine algae on the basis of morphology, we sought to DNA barcode a portion of the flora from the area and update a recently published species list. Genetic data were generated for 20 species in the area. Fifty-five percent of the barcoded flora confirmed the morphological species identifications. Five barcoded species revealed what are likely misapplied names to the Boulder Patch flora; the updated names include *Ahnfeltia borealis*, *Phycodrys fimbriata*, *Pylaiella washingtoniensis*, *Rhodomela lycopodioides* f. *flagellaris*, and *Ulva prolifera*. The remaining four species require taxonomic work and possibly represent new records for the Boulder Patch. Our observations indicate that we need considerably more research to understand marine macroalgal biodiversity in the Arctic. Supplementing Arctic species lists using genetic data will be essential in establishing an accurate and reliable baseline for monitoring changes in ecosystem biodiversity driven by long-term changes in regional climate.

Key words: Arctic benthic algae; Alaskan Beaufort Sea; DNA barcoding; Boulder Patch

RÉSUMÉ. Depuis sa découverte il y a quatre décennies, le peuplement d'algues brunes de la Boulder Patch dans la mer de Beaufort représente un site important pour les études écologiques à long terme dans l'Extrême-Arctique, en Alaska. Étant donné les difficultés liées à l'identification des espèces d'algues marines en fonction de leur morphologie, nous avons cherché à effectuer le codage à barres de l'ADN d'une partie de la flore de la région et avons mis à jour une liste d'espèces récemment publiée. Des données génétiques ont été produites pour 20 espèces de la région. Les identifications morphologiques ont été confirmées dans le cas de 55 % des espèces de la flore dont le code à barres a été établi. Pour cinq espèces dont le code à barres a été généré, nous avons constaté que des noms erronés avaient probablement été attribués à la flore de la Boulder Patch. Les noms à jour sont les suivants : *Ahnfeltia borealis*, *Phycodrys fimbriata*, *Pylaiella washingtoniensis*, *Rhodomela lycopodioides* f. *flagellaris* et *Ulva prolifera*. Les quatre autres espèces nécessitent une classification taxonomique et représentent peut-être de nouveaux signalements d'espèces pour la Boulder Patch. Nos observations indiquent que beaucoup d'autres recherches sont nécessaires pour comprendre la biodiversité des macroalgues marines de l'Arctique. Il sera essentiel de compléter les listes des espèces de l'Arctique à l'aide de données génétiques pour établir des données de base précises et fiables afin de surveiller les changements dans la biodiversité de l'écosystème causés par des changements à long terme du climat régional.

Mots clés : algues benthiques de l'Arctique; eaux alaskiennes de la mer de Beaufort; codage à barres de l'ADN; Boulder Patch

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INTRODUCTION

The Boulder Patch kelp bed community of the Beaufort Sea lies in Stefansson Sound off the northern coast of Alaska. The boulders and cobbles are part of a Quaternary deposit that is unique to the Beaufort Sea inner shelf, which is otherwise composed of silty clays and mud in relatively shallow water (4 – 7 m) that is estuarine in character (Dunton et al., 1982). The surprising faunal and floral diversity of the Boulder Patch, in concert with its relative isolation, has attracted scientific interest since its discovery by geologists in the mid 1970s (Reimnitz and Ross, 1979). Subsequent ecological studies have focused on gaining an understanding of the community and the abiotic factors that influence productivity, kelp growth, community assemblages, and trophic structure (Dunton et al., 1982; Martin and Gallaway, 1994; Aumack et al., 2007).

Long-term and nearly continuous studies in the Boulder Patch have provided a valuable baseline dataset for documenting biological responses to regional climatic change. Assessments of benthic diversity based on years of intensive sampling have also provided species inventories

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for infaunal and epilithic fauna (Dunton and Schonberg, 2000) and for benthic algae (Wilce and Dunton, 2014). These databases hold great promise for monitoring future changes in the Western Arctic since species from southern marine communities are expected to invade northward areas as perennial ice cover diminishes and isotherms retreat (Müller et al., 2009; Jueterbock et al., 2016). Accurate species inventories from multiple Arctic locations are critical to monitoring these changes as they relate to local flora and fauna. However, correct species identifications are critical to the accuracy and reliability of such baseline data. Marine algae can be extremely difficult to identify from morphology alone because of phenotypic plasticity, convergent evolution, and cryptic species diversity (Saunders, 2005). It is therefore important to corroborate morphological species identifications of marine algae with genetic data. Given the ecological importance of the Boulder Patch and its value for assessing shifts in benthic species composition and distribution, we sought to DNA barcode some of the flora reported by Wilce and Dunton (2014; DNA barcoding: Hebert et al., 2003; Saunders, 2005). This note represents updates to the Boulder Patch flora in light of DNA barcoding efforts. We feel these updates are important considering the ongoing ecological work at the Boulder Patch, the overall difficulty of obtaining specimens from Arctic locations, and the limited number of genetically verified species records for Arctic algae.

METHODS

We used scuba equipment to collect marine algae from several sites in the Boulder Patch area (Fig. 1) during two summer seasons (20–21 August 2014 and 20–25 July 2015). A portion of each specimen (approx. 1 cm^2) was preserved in silica gel (not as pressed material) and brought back to the University of New Brunswick for DNA extraction (Saunders and McDevit, 2012) and amplification of the 5 end of the cytochrome c oxidase subunit I gene (COI-5P) in red algae (Saunders and Moore, 2013) and brown algae (Saunders and McDevit, 2012), and *tufA* in green algae (Saunders and Kucera, 2010). These specimens are currently stored at the University of New Brunswick. Partial ribulose-1, 5-biphosphate carboxylase large subunit (*rbc*L-3P) or full-length reads of *rbc*L were amplified and sequenced for some specimens of brown algae for which COI-5P either did not amplify or was contaminated, and in specimens for which additional markers were needed to resolve taxonomy (Saunders and McDevit, 2012). Successful PCR products (see online Appendix 1: Table S1 for a list of specimens collected and Genbank accession numbers) were sent to Genome Quebec for forward and reverse sequencing. All genetic data were edited in Geneious version 8.0 (http://www.geneious.com; Kearse et al., 2012).

RESULTS AND DISCUSSION

We generated genetic data for two species of Chlorophyta, nine species of Ochrophyta, and nine species of Rhodophyta (Table 1). For 11 (55%) of the species barcoded, genetic data were consistent with their name assignment based on morphology, though two of these remain only tentatively confirmed. The remaining 45% represented five species requiring name updates, two species of uncertain genetic assignment, and two species representing new records for the Boulder Patch (Table 1). We elaborate briefly on the updated species names below; however, we emphasize that these records may represent names misapplied by Wilce and Dunton (2014) or otherwise represent new records for the Boulder Patch (if both species identified occur in the area). In cases where sample sizes are large (e.g., > 20 specimens barcoded in *Ahnfeltia borealis* D.Milstein & G.W.Saunders, *Phycodrys fimbriata* [Kuntze] Kylin, and *Rhodomela lycopodioides* f. *flagellaris* Kjellman), if the congener identified by Wilce and Dunton (2014) does occur in the Boulder Patch, it is likely a rare member of the flora.

Ulva prolifera O.F. Müller (previously identified as *Ulva flexuosa* Wulfen by Wilce and Dunton, 2014) is a species with genetically verified collections in Pacific, Arctic, and Atlantic basins but has only recently been DNA-barcoded from Canadian Arctic waters (Hudson Bay; Saunders and McDevit, 2013). *Pylaiella washingtoniensis* C.C. Jao (previously identified as *Pylaiella littoralis* [Linnaeus] Kjellman) is similarly reported in all three oceans and has again only recently been DNA-barcoded in the Arctic (Hudson Bay and Baffin Island; Saunders and McDevit, 2013; Küpper et al., 2016). *Pylaiella littoralis* is also reported from the Arctic, but occurs as three genetic groups, one in the Pacific and two in the Atlantic (one of the Atlantic groups is confirmed in the Arctic; Saunders and McDevit, 2013; Küpper et al., 2016). Substantial taxonomic work remains for species of *Pylaiella*.

In the red algae, *Ahnfeltia borealis* (previously identified as *Ahnfeltia plicata* [Hudson] Fries) is a recently described species occurring in Hudson Bay Arctic waters (Milstein and Saunders, 2012), and although *A. plicata* is reported from the Arctic, it is a rare member of sub-Arctic flora (only two genetically verified collections from Churchill, Manitoba; Saunders and McDevit, 2013) as compared to *A. borealis*. *Phycodrys rubens* (Linnaeus) Batters (updated to *Phycodrys fimbriata*) had been previously reported from the Arctic, but subsequent studies revealed that these specimens were assignable to *P. fimbriata* (Lindeberg and Lindstrom, 2010; Saunders and McDevit, 2013). Other genetically verified records of *Phycodrys* from Alaska include an *rbc*L sequence from St. Lawrence Island that matches *P. fimbriata* (published as *Phycodrys riggii* N.L. Gardner; Lin et al., 2001), and *P. riggii* from Southeast Alaska (van Oppen et al., 1995). Bona fide *Phycodrys rubens* is also rare in the Atlantic provinces of Canada and the New England states (only nine genetically verified

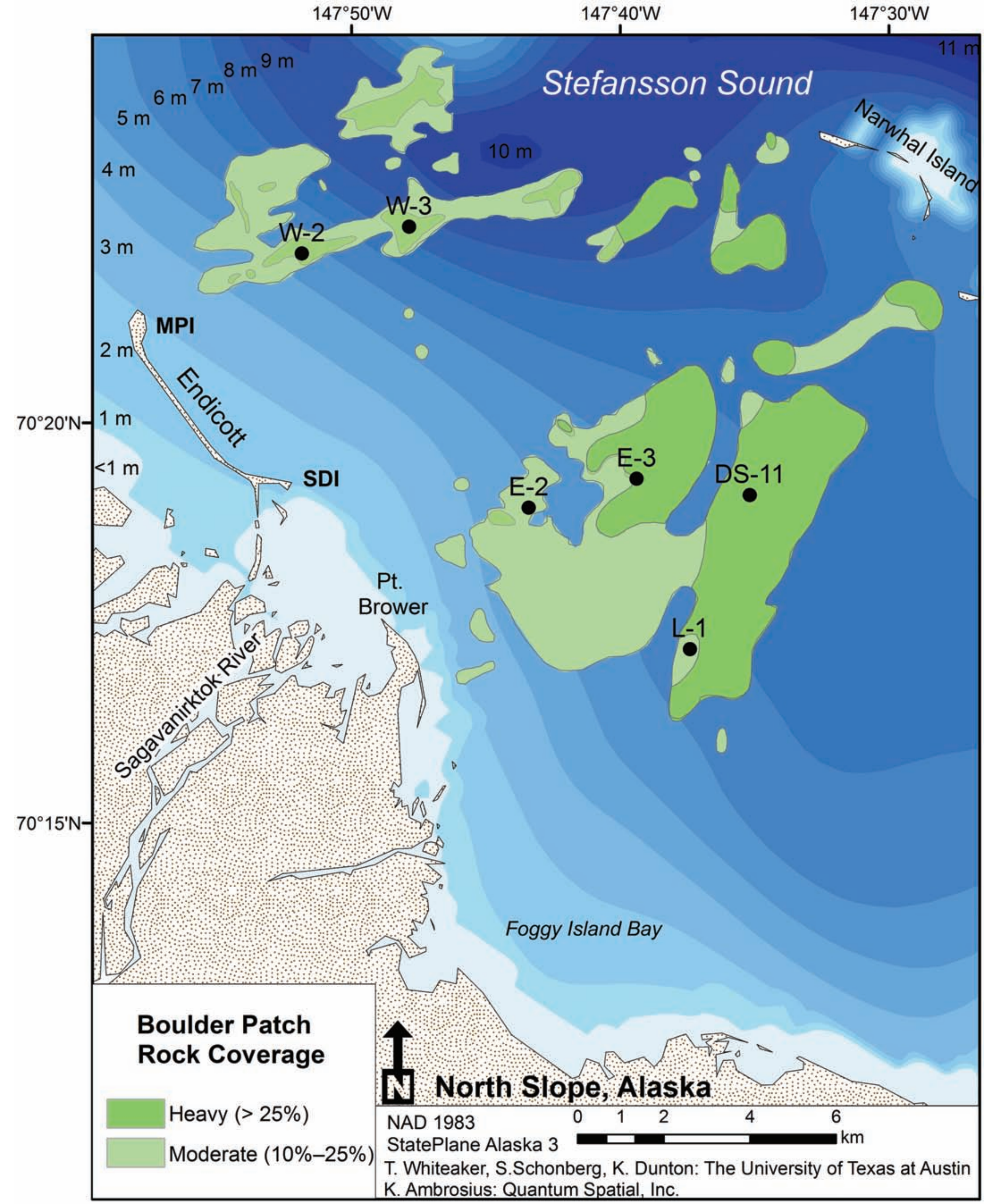

FIG. 1. Map of the Boulder Patch in Stefansson Sound off the North Slope of Alaska. Green shaded areas show the extent of the Patch as calculated from geological surveys conducted in 1980 and 1997 (Toimil and England, 1980; Coastal Frontiers and LGL, 1998). Light green represents zones where boulders cover 10% to 25% of the area, while dark green indicates zones with more than 25% boulder cover.

TABLE 1. Summary of results from a DNA barcode survey of marine flora of the Boulder Patch in the Beaufort Sea off northern Alaska. See Figure 1 for site locations.

Morphological listing	Collection site(s)	Molecular listing
Chlorophyta:		
Blidingia spp.	Narwhal Is.	Confirmed (tentative): taxonomy of group needs study. Two species listed in Wilce and Dunton (2014) with a single genetic group confirmed (*Blidingia* sp._5GWS, n = 1).
Ulva flexuosa Wulfen	Endicott Is.	Updated: *Ulva prolifera* O.F.Müller (n = 2).
Ochrophyta:		
Alaria esculenta (Linnaeus) Greville	DS-11	Confirmed (n = 3).
Battersia arctica (Harvey) Draisma, Prud'homme & H.Kawai	E-2	Confirmed (n = 1).
Chaetopteris plumosa (Lyngbye) Kützing	E-3	Uncertain: cryptic complex (n = 2); material needed from type locality, but may represent a new species (recorded in Table S1 as *Chaetopteris* sp. _2plumosa).
Laminaria solidungula J.Agardh	DS-11, E-3, W-3	Confirmed (n = 7).
Lithoderma fatiscens Areschoug	E-3	Confirmed (tentative; recorded in Table S1 as *Lithoderma* sp. _2GWS): taxonomy of group needs study (n = 1).
Pylaiella littoralis (Linnaeus) Kjellman	Endicott Is.	Updated: *Pylaiella washingtoniensis* C.C.Jao (n = 2).
Saccharina latissima (Linnaeus) C.E.Lane, C.Mayes, Dreuhl, & G.W.Saunders	DS-11, E-3	Confirmed (n = 7).
Sorapion kjellmanii (Wille) Rosenvinge	DS-11, E-3, W-3	Uncertain or new: Tilopteridalean sp._2GWS (n = 4), which may account for records of this species. Taxonomic work is needed.
	E-3	Uncertain or new: Tilopteridalean sp._3GWS (n = 2), which may account for records of this species. Taxonomic work is needed.
Rhodophyta:		
Ahnfeltia plicata (Hudson) Fries	DS-11, E-2, E-3, W-3	Updated: *Ahnfeltia borealis* D.Milstein & G.W.Saunders (n = 23).
Coccotylus truncatus (Pallas) M.J.Wynne & J.N.Heine	DS-11, E-2, E-3, L-1, W-3	Confirmed (n = 31).
Dilsea socialis (Postels & Ruprecht) Perestenko	DS-11, E-3, W-3	Confirmed (n = 28).
Odonthalia dentata (Linnaeus) Lyngbye	DS-11, E-3, W-2, W-3	Confirmed (n = 24).
Phycodrys rubens (Linnaeus) Batters	DS-11, E-3, L-1, W-3	Updated: *Phycodrys fimbriata* (Kuntze) Kylin (n = 33).
Phymatolithon foecundum (Kjellman) Düwel & Wegeberg	DS-11, E-3	Confirmed (n = 2). Current name *Leptophytum foecundum* (Kjellmann) Adey.
Phymatolithon tenue (Rosenvinge) Düwel & Wegeberg	W-3	Confirmed (n = 1). Considerable nomenclatural confusion surrounds the correct name for this genetic group (Guiry and Guiry, 2017).
Rhodomela lycopodioides (Linnaeus) C.Agardh	DS-11, E-3, W-3	Updated or new: *Rhodomela lycopodioides* f. *flagellaris* Kjellman (n = 29).
	E-2	Updated or new: *Rhodomela* cf. *virgata* Kjellman (n = 1).

records, with all records of *Phycodrys* from the Canadian Arctic assignable to *P. fimbriata*; Saunders and McDevit, 2013). In short, *Phycodrys rubens* is relatively rare compared to *P. fimbriata* on Northwest Atlantic coasts, but it is common in the Northeast Atlantic, where *P. fimbriata* does not appear to occur. *Rhodomela lycopodioides* f. *flagellaris* (previously identified as *Rhodomela lycopodioides* [Linnaeus] C.Agardh) was previously reported only from the Arctic (Hudson Bay) along with several other genetic groups, including one that reportedly matches the features of *Rhodomela lycopodioides* sensu stricto (Saunders and McDevit, 2013). We did not, however, verify *Rhodomela lycopodioides* sensu stricto in our Boulder Patch collections. We did recover a second species of *Rhodomela*, *Rhodomela* cf. *virgata* Kjellman, that also occurs in Churchill (Saunders and McDevit, 2013) and has since been collected in the Northwest Atlantic (G.W. Saunders and T.B. Bringloe, unpubl. data). Considerable taxonomic work is needed in *Rhodomela*.

Substantial DNA barcoding work remains to be done in the Boulder Patch. If the same proportion of mismatches between morphological and DNA species identifications found in our sampling holds true for all other species on the list from Wilce and Dunton (2014), then nearly half of the remaining identifications may also be uncertain. A concerted effort to continue DNA barcoding in both freshly collected benthic algae and archived algal specimens from the Boulder Patch is a high priority. The long history of ecological study in the area (Dunton et al., 1982; Wilce and Dunton, 2014) has provided baseline information that will permit us to monitor impending ecological and species shifts due to climate change that may subsequently affect biodiversity. However, such monitoring requires accurate species lists, which highlights the urgent need to corroborate morphological identifications of marine algae with genetic data before such changes occur.

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APPENDIX 1

Table S1 is available as a supplementary file to the online version of this article at:
http://arctic.journalhosting.ucalgary.ca/arctic/index.php/arctic/rt/suppFiles/4679/0

TABLE S1. List of specimens collected, in alphabetical order by species name, showing specimen ID, Genbank accession number, date sampled, and name and location of collection site.

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