**Plant and Fungal Systematics** 67(1): 17–23, 2022
DOI: https://doi.org/10.35535/pfsyst-2022-0002

ISSN 2544-7459 (print)
ISSN 2657-5000 (online)

# Two rare *Peltigera* species new to the Canadian Arctic, *P. islandica* and *P. lyngei*

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**Article info**

Received: 25 Apr. 2022
Revision received: 30 May 2022
Accepted: 30 May 2022
Published: 20 Jul. 2022

**Associate Editor**

Martin Kukwa

**Abstract**. *Peltigera islandica* and *P. lyngei* are rarely reported lichens. Previously, *P. islandica* was known from British Columbia, Estonia, and Iceland, and *P. lyngei* from Amchitka Island (Alaska), Gough Island (South Atlantic), Iceland, Siberia and Svalbard. Both species are reported here for the first time from the Canadian Arctic and from the second localities in North America. *Peltigera lyngei* is also reported for the first time from Canada. The identities of these species are confirmed morphologically, chemically, and with molecular data. Phylogenetic relationships are inferred using the ITS region. The widespread, but scattered, distribution of both species suggests that they may be underreported throughout their range.

**Key words**: Biogeography, *Peltigerales*, Arctic, Arviat, Kukluk/Bloody Falls Territorial Park, Nuvuk (proposed) Territorial Park, Sylvia Grinnell Territorial Park

## Introduction

*Peltigera* (*Peltigerales*, *Ascomycota*) is a cosmopolitan genus of relatively large foliose macrolichens (Martínez et al. 2003). It can be a taxonomically difficult genus with subtle morphological differences among some species, and many molecularly defined taxa appear to lack clear corresponding morphological characters (Magain et al. 2016; Miadlikowska et al. 2018). Consequently, some species may be overlooked in the field and have distributions that are poorly understood.

*Peltigera islandica* is an example of a species with a widespread, but scattered, distribution. It is known only from western Canada, Estonia, and Iceland (Jüriado et al. 2017; Manoharan-Basil et al. 2016). Since this species was recently described, new information about its range would not be surprising as more survey work is conducted. *Peltigera lyngei* is another species with a scattered distribution. It is known from Amchitka Island (Alaska), Gough Island (South Atlantic), Iceland, Siberia and Svalbard (Øvstedal et al. 2009; Dillman et al. 2012). However, it was described 90 years ago (Gyelnik 1932), so it is either a rare species or it has been overlooked, possibly both. Nevertheless, what we currently know about these two species is that they are rarely reported and only from widely dispersed localities.

During on-going lichen surveys in Nunavut, Canada, new localities for *P. islandica* and *P. lyngei* were discovered that are reported here for the first time from the Canadian Arctic. These records fill gaps in our understanding of their distribution and they illustrate the need for continued survey work in the Canadian Arctic to gather fundamental baseline biodiversity data in a quickly changing environment. The Arctic is warming faster than any other region on Earth (IPCC 2007; Kaufman et al. 2009), and knowing what species are present is essential for understanding the impacts of climate change.

## Materials and methods

### Metabolites, morphology, and deposition

Specimen morphology was examined microscopically using a stereoscope. Secondary metabolites were determined using thin layer chromatography following Culberson and Kristinsson (1970) and Orange et al. (2001) in solvents A, B', and C. Thallus fragments were extracted in hexane for ~5 min. All specimens examined have been deposited at the Canadian Museum of Nature (CANL) and duplicates at Duke University (DUKE).

### Distribution data

The global distributions of *P. islandica* and *P. lyngei* were determined by reviewing salient literature (e.g., monographs or large taxonomic treatments of *Peltigera*, major floristic studies that include *Peltigera*, and the original species descriptions) along with reviewing seven

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databases: 1) Canadensys (Canadensys 2022), 2) Canadian Museum of Nature (CMN 2022), 3) Consortium of North American Lichen Herbaria (CNALH 2022; contains digitized records from over 90 herbaria), 4) Biodiversity Institute of Ontario (FOIBIS 2022), 5) Global Biodiversity Information Facility (GBIF 2022), 6) NatureServe (NatureServe 2022), and 7) the New York Botanical Garden (NYBG 2022). Maps in Figures 2D and 3F were produced with SimpleMappr (Shorthouse 2010).

### DNA extraction

DNA extraction was conducted at the Canadian Centre for DNA barcoding (CCDB) following CCDB protocols outlined by Ivanova et al. (2008, 2011). In summary, a small amount (~5 $mm^2$) of dry lichen tissue was removed from the sample using a stereoscope and fine tipped forceps while ensuring that there were no vegetative propagules (i.e., soredia or isidia) from other lichens and no lichenicolous fungi. A fragment of the lichen was placed into racked sterile mini tube strips with a 3.17 mm stainless steel bead in each tube and then sealed with a sterile cap strip. The fragment was then ground into fine powder using a Tissue Lyser (Qiagen, USA) with rack adapters at 28 Hz for 30 seconds, then rotated and ground for an additional 30 seconds. The ground material was then incubated with 2× CTAB buffer at 65°C for 1 hour and DNA was then extracted using semi-automated method employing glass fiber filtration (Fazekas et al. 2012; Ivanova et al. 2008). The final concentration of the eluted DNA was 20–40 ng/μL.

### PCR and sequencing

Fungal primers ITS-1F (Gardes & Bruns 1993) and ITS 4 (White et al. 1990) were used for amplification of the ITS1, 5.8S and ITS2 regions, commonly named ITS (Internal Transcribed Spacers 1 and 2). The PCR conditions for *rbcL* described by Kuzmina & Ivanova (2011) were followed for the ITS region. The thermocycle profile for the ITS region consisted of 94°C for 2 min, 40 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 1 min, with a final extension at 72°C for 5 min. PCR products were visualized on a 2% agarose gel using an E-Gel96® Pre-cast Agarose Electrophoresis System (Invitrogen). Bidirectional sequencing using the same PCR primers was done with the BigDye® Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Life Technologies) on an ABI 3730xl Genetic Analyzer (Applied Biosystems, Life Technologies) following Ivanova and Grainger (2007). Bidirectional sequences were assembled in CodonCode 4.2.2 and manually edited.

### Phylogenetic analyses

All newly generated sequences were subjected to BLAST searches using NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to confirm their putative identity. To validate the morphological and BLAST identifications of the specimens, their preliminary placement within the genus *Peltigera* was determined by the Evolutionary Placement Algorithm (EPA; Berger & Stamatakis 2011) based on the ITS sequences as implemented in the Tree-Based Alignment Selector toolkit (T-BAS version 2.1, available at http://tbas.hpc.ncsu.edu; Carbone et al. 2017, 2019) using first the global phylogeny of the genus *Peltigera* (Chagnon et al. 2019; Carbone et al. 2019) and subsequently the phylogenies for the *Polydactylon* and *Peltigera* + *Retifoveatae* sections (Magain et al. 2017, 2018; Carbone et al. 2019) as the reference trees. For each EPA analysis we implemented the GTR substitution model (Rodríguez et al. 1990) with gamma distribution parameter (GTRGAMMA) and calculated likelihood weights with a placement cut-off distance of 10. We also performed a follow up search of the best tree and bootstrap analyses (1000 replicates) (RAxML 8.2.12; Stamatakis 2006; Stamatakis et al. 2008) as implemented in T-BAS v.2.1 via the CIPRES Science Gateway v.3.3 (Miller et al. 2015) based on the multilocus datasets for sections *Peltigera* and *Polydactylon*. The newly added ITS sequences were realigned with MAFFT v.7.402 (Katoh & Standley 2010), and the GTRGAMMA nucleotide substitution model and backbone constraint on the multifurcating reference trees (where internodes with bootstrap support <70% were collapsed) were implemented. Based on the preliminary EPA placement of *P. lyngei* within the *Polydactylon* clade, we selected and downloaded from T-BAS the 8-locus alignments for the *Dolichorhizoid* clade, excluding the *scabrosella* group (Magain et al. 2017, Fig. 1). We adjusted the ambiguous regions manually and added the two ITS sequences for *P. lyngei*. We completed maximum likelihood search as implemented in IQ-TREE v. 2.1.3 (Nguyen et al. 2015) by estimating the best fit partitioning scheme and the substitution models (ModelFinder; Kalyaanamororthy et al. 2017) followed by inferring the best tree and bipartitions support (UFBoot; Minh et al. 2013) using the following command line: iqtree2 -s combined.phy -m MFP+MERGE -p codons.txt -bb 1000 -bnni -pre combined. The following three partitions and corresponding models were used in IQ-TREE ML search: HKY+F+R2 for ITS + beta-tubulin introns + beta-tubulin 3rd codon position + EFT2.1 introns + EFT 2nd codon position + EFT2.1 3rd codon position + RPB1 introns + RPB1 2nd codon position + RPB1 3rd codon position + COR1b + COR3 + COR16; HKY+F for beta-tubulin 1st codon position + beta-tubulin 2nd codon position + EFT2.1 1st codon position + RPB1 1st codon position; F81+F: for LSU. We also completed RAxML analyses (as implemented on the CIPRES portal) using the best partitioning scheme estimated by ModelFinder in IQ-TREE, GTRGAMMA substitution model across all partitions and performing 1000 bootstrap replicates.

## Results and discussion

### Phylogenetic analyses

The two ITS sequences for the putative *P. islandica* (LICHN485-19, GenBank ON943472, and LICHN175-19, Genbank ON943469) blasted with 100% Query Cover and 100% Identity to the sequences of *P. islandica* from Estonia (LT852849) and Iceland (KJ413238). In the

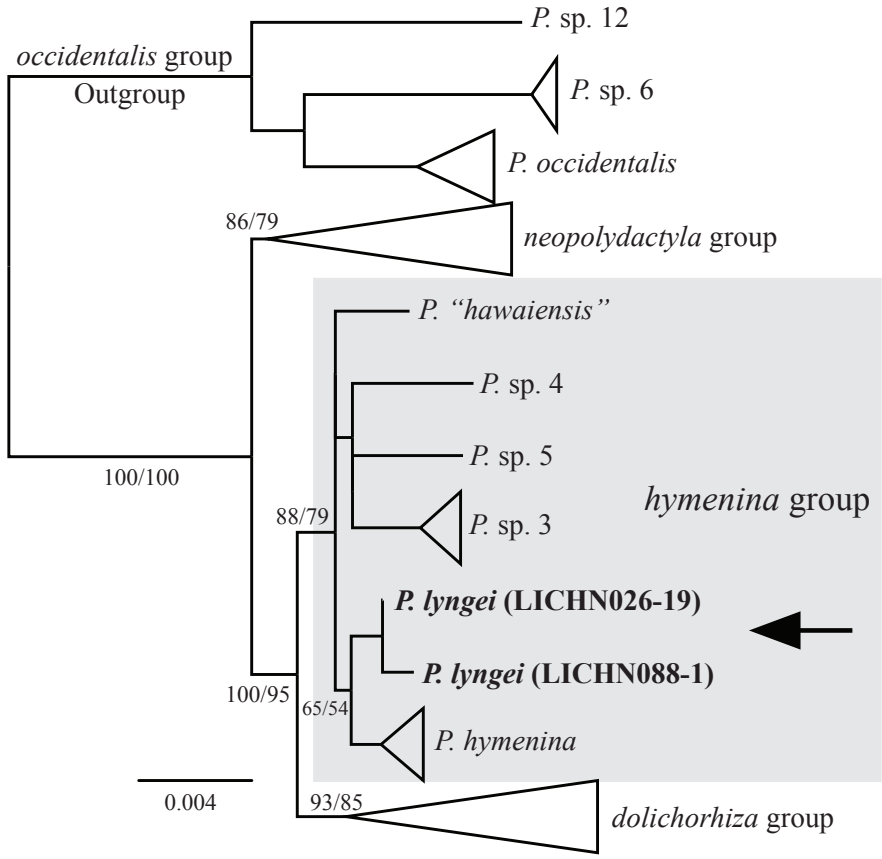


**Figure 1**. Phylogenetic placement of two Canadian specimens of *P. lyngei* in the *hymenina* group in the *Dolichorhizoid* clade based on the maximum likelihood analyses of the ITS region as implemented in IQ-TREE. Values below the internodes represent UFboot support (before the slash) and RAxML bootstrap support (after the slash) and are provided for the main clades including the sister relationship of *P. lyngei* with *P. hymenina*. Clades representing groups and species as defined in Magain et al. (2017) were collapsed.

absence of *P. lyngei* sequence data in GenBank, BLAST results for our ITS sequences (LICHN026-19, Genbank ON943470, and LICHN088-19, GenBank ON943471) were inconclusive with 100% Query Cover and 96–94% Identity with the individuals of multiple species from the *Polydactylon* section, e.g., *Peltigera hymenina*, *P. neopolydacyla*, and *P. pacifica*. In addition to a few nucleotide differences, the ITS of the putative *P. lyngei* contains a 22 base pairs long insertion in the ITS1 compared to the sequences of the most similar species. The EPA analyses using the genus *Peltigera* reference phylogeny placed *P. islandica* in the section *Peltigera*, *P. canina* clade (Clade 9 in Magain et al. 2018) and *P. lyngei* in the section *Polydactylon*, *Dolichorhizoid* clade (Magain et al. 2017) (Fig. 1). Although, their respective placements did not receive high support based on the ITS sequences alone using the EPA and maximum likelihood (RAxML) tools as implemented in T-BAS, we feel confident about the identity of *P. islandica* based on the morphology, BLAST results, and the presence of the species specific unique nucleotide motif (16 nucleotides) in the hypervariable region of the ITS1 (Miadlikowska et al. 2003; Magain et al. 2018). Maximum likelihood analyses grouped our two collections of *P. lyngei* together and placed them sister to *P. hymenina* within the *hymenina* group in the *Dolichorhizoid* clade; however, this phylogenetic relationship does not have strong support (Fig. 1). Morphologically, *P. lyngei* resembles *P. malacea* because of its almost veinless lower surface and *P. scabrosa* because of its scabrous upper surface (Vitikainen 1994). Therefore, *P. lyngei* was assumed to have a close affinity to *P. malacea* in section *Peltidea* or *P. scabrosa* in section *Polydactylon*, *Scabrosoid* clade (see discussion under *P. lyngei* in Vitikainen 1994). The revealed phylogenetic placement of this species in Section *Polydactylon*, *Dolichorhizoid* clade, *hymenina* group, is somewhat surprising. While other scabrid species do occur in the *Dolichorhizoid* clade (i.e., *Peltigera pulverulenta*, *P. scabrosella*, and some morphotypes of *P. truculenta*), there are no other scabrid species in the *hymenina* group. Moreover, species in the *hymenia group* mostly occur around the Pacific Ocean in temperate and tropical regions – *Peltigera hymenina* (Ach.) Delise is the only exception (Magain et al. 2017; Martínez et al. 2003). Additional loci should be sequenced and the type material for *P. lyngei* should be included to confirm the identity and phylogenetic placement of this morphospecies.

## New reports

***Peltigera islandica*** T. Goward & S.S. Manoharan-Basil (Fig. 2)

**Notes**. *Peltigera islandica* was recently described from Canada and Iceland (Manoharan-Basil et al. 2016). In Canada, it is previously known from one collection in British Columbia (H. O'Brien HOB020708-66-1-4,

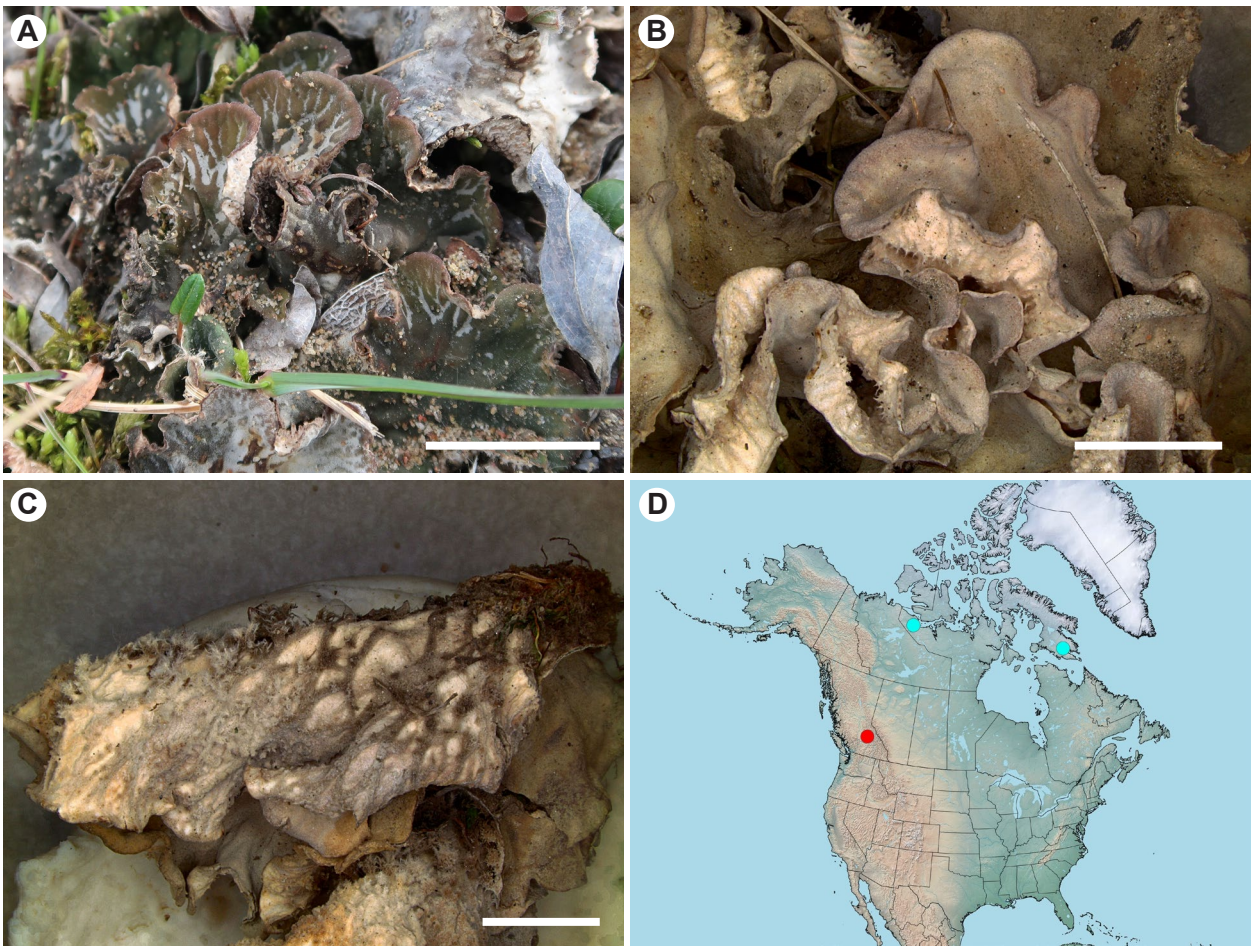


**Figure 2**. *Peltigera islandica*. A–C, McMullin 20779, CANL. A – Wet thallus; B – Dry thallus; C – Lower surface; D – North American distribution. Scales: A = 2.0 cm; B–C = 5.0 mm. In maps, blue dots = new records, red dots = previous collections.

DUKE). Here, we report it for the first time from Nunavut. We found no chemical substances with TLC, which corresponds with the negative spot test results reported by Manoharan-Basil et al. (2016). *Peltigera islandica* is distinguished from other species of *Peltigera* by its *Nostoc* primary photobiont, laminal tomentum, lobes 5–10 mm wide with downturned tips, and an emerald green upper surface when wet. Specimens from Nunavut have the hypervariable ITS1 region (Miadlikowska et al. 2003; Magain et al. 2018) identical to the motif found in other specimens of *P. islandica* from Iceland and British Colombia (as *P*. sp. 20 in Magain et al. 2018), which is unique to this species (GGGTTCGTATGTGCCC; Magain et al. 2018; Manoharan-Basil et al. 2016). The ITS sequence for one of the two specimens (McMullin et al. 20779) has only partial ITS1 sequenced and the first seven base pairs of the 16 base pairs motif are missing.

**Specimens examined**. CANADA. Nunavut. Kitikmeot Region: Kukluk/Bloody Falls Territorial Park, on the western shore of Bloody Falls along the Coppermine River, ~14 km SW of Kugluktuk, in a clearing ~25 m west of the shore, 9 VII 2019, saxicolous, R.T. McMullin 21927 & M. Kuzmina (CANL). Qikiqtaaluk Region: Sylvia Grinnell Territorial Park, on the eastern shore of the Sylvia Grinnel River at the northernmost edge of the park, tundra, glacial marine delta with sand, silt, boulders, and gravel, 10 VII 2018, terricolous, R.T. McMullin et al. 20779 (CANL).

***Peltigera lyngei*** Gyeln. (Fig. 3)

**Notes**. Although the sequences we generated do not match any known species, the secondary metabolites and morphology are consistent with *P. lyngei*, for which no previous reference sequences exist. *Peltigera lyngei* was described from Svalbard (Gyelnik 1932) and reported from North America for the first time from Labrador (Kallio & Kärenlampi 1966), but Dillman et al. (2012) revised that specimen to *P. malacea*. Dillman et al. (2012) also revised two collections from 1962 (R.J. Reich 18 and 257, OULU, both as *P. malacea*) that are the only previous reports of *P. lyngei* in North America. Both collections are from Amchitka Island in Alaska. However, Dillman et al. (2012) did not provide TLC results, morphological characters, or DNA sequence data to support their determinations. Additional study of those specimens is recommended. Here, we report *P. lyngei* for the first time in Canada from Nunavut. It is distinguished from *Peltigera malacea* by its scabrose upper surface without tomentum and from *P. scabrosa* and *P. scabrosella* by its mostly veinless lower surface and absence of zeorin (Holtan-Hartwig 1988; Vitikainen 1994). We detected gyrophoric acid, methyl gyrophate, tenuiorin, and three triterpenoids (not zeorin) with TLC, which corresponds with previous studies that included a TLC analysis of the holotype (Holtan-Hartwig 1988; Vitikainen 1994).

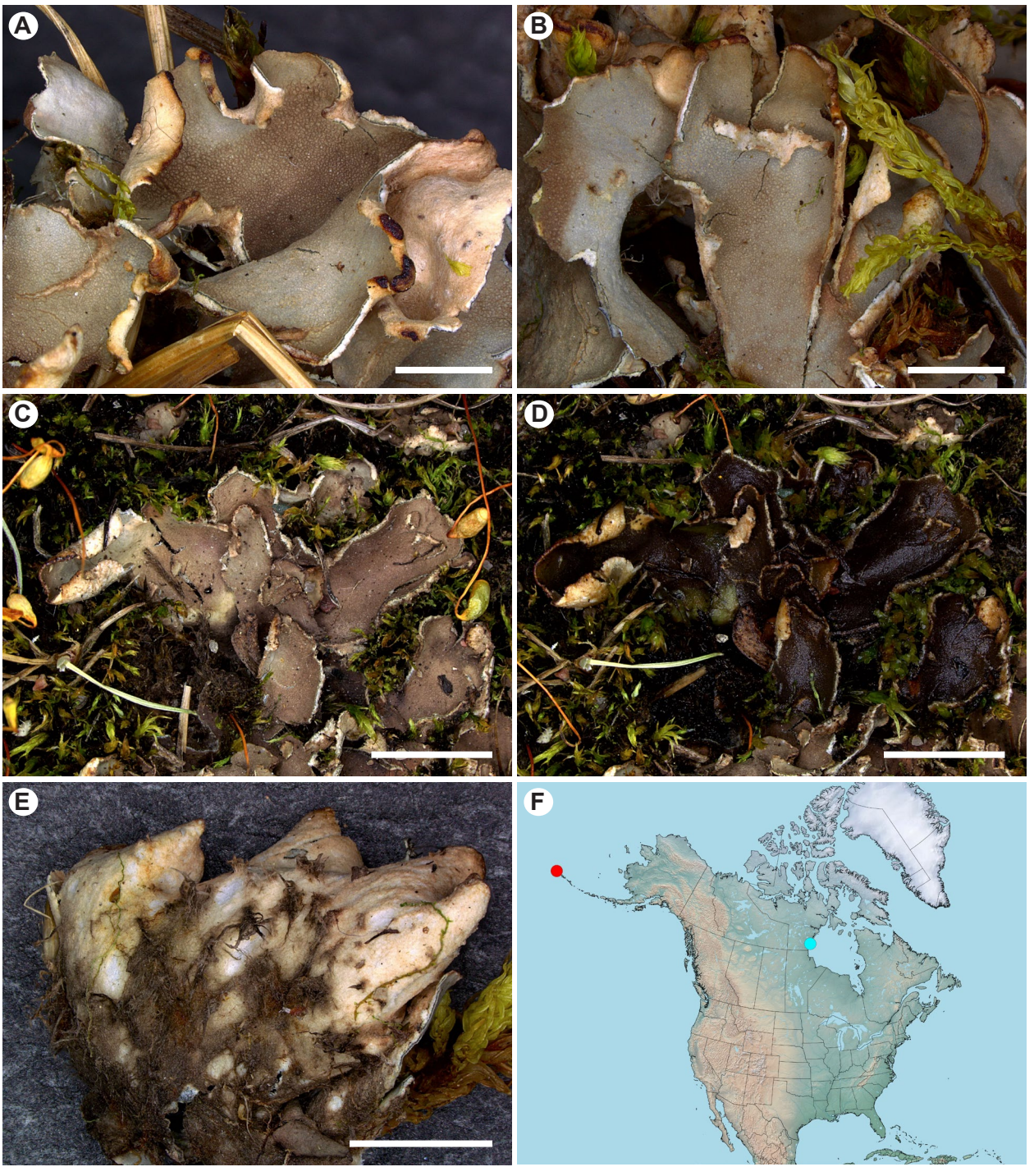


**Figure 3**. *Peltigera lyngei*. A–B – Dry thallus (McMullin 17790, CANL, DUKE); C – Dry thallus; (McMullin 17818, CANL, DUKE); D – Wet thallus (McMullin 17818, CANL, DUKE); E – Lower surface (McMullin 17790, CANL, DUKE); F – North American distribution of *P. lyngei*. Scales: A = 2.0 mm; B = 3.0 mm; C–D = 5.0 mm; E = 4.0 mm. In map, blue dots = new records, red dots = previous collections.

All records of *P. lyngei* are from Arctic and subarctic regions, except for reports from Gough Island (South Atlantic) (Øvstedal et al. 2009). However, a specimen (M. Gremmen 99-348, H) from Gough Island identified by O. Vitikainen as *P. lyngei* was shown to represent *P. truculenta* in a phylogenetic analysis (Magain et al. 2017) (specimen number P3016). Therefore, it is possible that all of the reports from the Southern Hemisphere correspond with *P. truculenta*.

**Specimens examined**. CANADA. Nunavut. Kivalliq Region: Arviat, Nuvuk proposed Territorial Park, ~300 m ESE of Hudson Bay Post cairn on north coast, 08.vii.2016, terricolous, R.T. McMullin et al. 17790 (CANL). Arviat, Nuvuk proposed Territorial Park, ~130 m W of the E end of the peninsula, adjacent to a small pond, 06.vii.2016, terricolous, R.T. McMullin et al. 17818 (CANL).

## Acknowledgements

We gratefully acknowledge: David Beamer, Geoffrey Levin, Jeffery Saarela, Lynn Gillespie, Maria Kuzmina, Paul Sokoloff, Ruth Kaviok, and Samantha Godfrey for accompanying TM in the field; Joavie Nookiguak, Leo Ikakhik, and Shayne Ubluriak for being polar bear monitors; Scott LaGreca and Niels van

Miltenburg for performing TLC analyses; and the Centre for Biodiversity Genomics, Government of Nunavut Department of Environment, Nunavut Parks and Special Places, Ontario Parks, for providing collecting permits or financial and/or in-kind support. This work was a part of the Arctic BIOSCAN project. JM acknowledges the National Science Foundation (NSF) award BEE 1929994.

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