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First documented occurrences of *Cladonia krogiana* and *C. rangiformis* in North America

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ABSTRACT. – *Cladonia krogiana*, previously known only from Norway and the Czech Republic, is reported here for North America from two localities near the Bay of Fundy, New Brunswick, Canada. It occurs there on open, rocky banks of clear, free-flowing rivers, habitats similar to those in which it has been found in Norway. We also document the occurrence of *C. rangiformis* in North America, based on collections from two localities on the southwest coast of Conception Bay, on the Avalon Peninsula of the island of Newfoundland, Canada. It is possibly an accidental, but naturalized, introduction in this area, where European settlement began in the early 1600s. A molecular phylogenetic analysis confirmed the identity of one of the Newfoundland specimens. The IGS rDNA haplotype to which it belongs is the same as the most widely distributed haplotype of *C. rangiformis* in Europe and Macaronesia. Previous reports of *C. rangiformis* for continental North America are based on misidentifications. A 19th century collection reportedly made on the island of Bermuda, while correctly identified, is of uncertain provenance.

KEYWORDS. – Atlantic Canada, biogeography, Challenger Expedition, Cladoniaceae, hemiboreal zone, introduced species.

INTRODUCTION

Cladonia P. Browne is the most taxonomically diverse genus of macrolichens in North America, represented on this continent by more than 150 species (Esslinger 2019). Although most of these have fairly well-known distributions based on extensive collections, new species and major range extensions continue to come to light (e.g., Ahti & Stenroos 2008, Ahti et al. 2018, Lendemer & Hodkinson 2009). During field

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work in Atlantic Canada in recent years, we discovered populations of two *Cladonia* species, *C. krogiana* Løfall & Timdal and *C. rangiformis* Hoffm., that were not previously known to occur in North America. These occurrences were briefly noted in the treatment of Cladoniaceae in the Nordic Lichen Flora (Ahti & Stenroos 2013), which includes synoptic descriptions of the overall distributions of the species. Here, we substantiate the North American records of these two species and describe their habitats. In addition to reporting the occurrence of *C. rangiformis* on the island of Newfoundland, Canada, we review the status of a record from the island of Bermuda (Crombie 1884).

MATERIALS AND METHODS

Morphological characters and reactions of thalli with standard chemical reagents (C, K, P) were examined with a stereomicroscope. Secondary chemical products were analyzed with one- and two-dimensional thin layer chromatography (TLC) using standardized A, B', and C solvent systems (Culberson 1972, Orange et al. 2001). An extract of a specimen of *Lecanora vinetorum* Poelt & Huneck (Italy: Appiano (Eppen), 10 km SW of Bolzano, 14.ix.1973, *I.M. Brodo 20260* [CANL 49499]) was used as a control for chlorovinetorin (Elix 2014). Extracts of specimens of *C. krogiana* from Canada, Norway, and the Czech Republic were run on the same TLC plates.

DNA was extracted from a specimen of *C. rangiformis* from Newfoundland (*T. Ahti 67856 & S. R. Clayden*, H). The DNA extraction and PCR were carried out using the methods described in Stenroos et al. (2019). In order to confirm the identity of *C. rangiformis,* we tried to amplify three loci: ITS rDNA, rpb2, and IGS rDNA. Unfortunately, only a sequence from IGS rDNA was obtained (GenBank accession number MW574591). Although IGS rDNA has high values of intraspecific distances and in general is not a good barcode for *Cladonia*, in *C. rangiformis* the mean of intraspecific distances for this locus is lower than for ITS rDNA (Pino-Bodas et al. 2013). We therefore judged that IGS rDNA could be used to confirm the identity of the Canadian specimen.

In order to assess the phylogenetic placement of the Newfoundland specimen with respect to European *C. rangiformis* and the broader phylogeny of *Cladonia*, an analysis based on IGS rDNA was conducted. Eleven sequences of *C. rangiformis* were included, representing different haplotypes from the Azores, Madeira, the Netherlands and Spain. Additionally, 26 sequences were included in the alignment, representing 25 *Cladonia* species from different subclades of the *Cladonia* clade recognized by Stenroos et al. (2019). The sequences were aligned using MAFFT (Kuraku et al. 2013). The ambiguous regions were removed using Gblocks 0.91b (Castresana 2000) with the less stringent option, keeping 96.4% of the original positions. *Cladonia boryi* Tuck. was selected as outgroup based on Stenroos et al. (2019). Maximum likelihood (ML) analysis was implemented in RAxML 7.04 (Stamatakis 2006), with 1000 fast bootstrap replicates. JModelTest (Posada 2008) selected the TrNef+G model, but as this one is not implemented in RAxML it was replaced by GTRGAMMA, which usually is the best fitting model. The alignment and ML tree are available on Treebase at http://purl.org/phylo/treebase/phylows/study/TB2:S27817.

All IGS rDNA sequences for *C. rangiformis* available in GenBank were downloaded to infer a haplotype network (http://purl.org/phylo/treebase/phylows/study/TB2:S27818). These sequences were examined and a sequence of *C. rangiformis* from Spain was excluded because it contained several ambiguous positions that could have introduced artefacts in the network. In total, 42 sequences of *C. rangiformis* were analyzed. The haplotype network was generated under statistical parsimony with a confidence interval of 95%, using TCS 1.21 (Clement et al. 2000) and considering gaps as a 5th character state. We recognize that this analysis, based on a single locus and on a single specimen, cannot be used to infer the phylogeographic affinities of the Newfoundland population or broader population genetic structure in *C. rangiformis*. It is instead intended as a means of representing the known diversity and frequency of IGS haplotypes in this species.

RESULTS AND DISCUSSION PART 1: *CLADONIA KROGIANA*

Cladonia krogiana has a squamulose, non-podetiate thallus, and is unique among *Cladonia* species in producing the xanthone chlorovinetorin (5,7-dichloro-3-*O*-methylnorlichexanthone) as a major secondary metabolite. Recent molecular phylogenetic studies show that it is most closely related to the temperate North American endemic *C. robbinsii* A. Evans (Stenroos et al. 2019). It was described from southeastern Norway, where it occurs in “open, exposed sites at the borders of conifer or mixed forests near lakes or major rivers” (Løfall & Timdal 2002).

Figure 1. Habitat and morphology of *Cladonia krogiana*. **A and B**, rocky river banks at the two known localities in New Brunswick, Canada (A, New River, New River Beach Provincial Park; B, Forty Five River, Fundy National Park). **C**, morphology of *C. krogiana* from New River locality (*McMullin 7183*, CANL). Scale bar = 1 mm.

We found this species at two localities in New Brunswick through targeted collecting of mainly squamulose *Cladonia* species in a range of habitats (Figure 1A & B). When tested chemically with standard reagents, the collections stood out among other such species in lacking cortical or medullary reactions. The presence of barbatic acid and chlorovinetorin was confirmed with TLC.

The morphology of the populations in New Brunswick and Norway are similar. The thalli consist of small mats of crowded, ascending to erect, strap-shaped to deeply-lobed squamules up to 8 mm long (mostly < 6 mm), 3 mm wide (mostly < 2 mm), and 0.1–0.3 mm thick (Figure 1C). They are grey-green to olive-brown above, and mostly recurved in their upper one to two millimeters, revealing the compact ecorticate lower surface, which varies from chalky white to brownish grey or brown, often with a violaceous hue. Appressed, cobwebby, white hyphae are patchily present on the lower surface, evident especially where they overlie otherwise grey- to brown-pigmented areas. Pycnidia and apothecia were not detected. The squamules are brittle, and it is likely that fragmentation is the primary means of reproduction.

Cladonia krogiana can be distinguished morphologically from most other mainly squamulose *Cladonia* species occurring in northeastern North America (see Lendemer & Hodkinson 2009). However, secondary product chemistry assessed with spot-tests or TLC is decisive. Among the squamulose species occurring on open rocky outcrops, only *C. robbinsii* also lacks P+ yellow, orange, or red depsides or depsidones, instead producing usnic and barbatic acids. Although the phylogenetic analysis by Stenroos et al. (2019) indicated a sister relationship between *C. krogiana* and *C. robbinsii*, it is highly unlikely that these two lichens are chemotypes of a single species. They also differ in morphology and ecology, and they have discrete geographical distributions. *Cladonia robbinsii* has squamules that are 10–20 mm long, and it sometimes produces podetia. It occurs on soil over rock, and in old fields and natural grasslands (Brodo et al. 2001, Hinds & Hinds 2007, Lewis 2014, Thomson 1968). In the Northeast, it is unknown north of southern New England (Hinds & Hinds 2007). In contrast, *C. krogiana* has squamules that are 8 mm or less in length, and it is not known to produce podetia (Løfall & Timdal 2002; present study). All of its known occurrences are on rock outcrops with thin soil or moss cover, and it is restricted to cooler, wetter ecoclimatic regions than those in which *C. robbinsii* occurs.

One specimen collected at the New River locality by T. Ahti (*Ahti 60678*, H) was believed initially to represent *C. krogiana*, but proved to belong instead to *C. verticillata* (Hoffm.) Schaer. Its identity as *C. verticillata* was confirmed by DNA analysis (R. Pino-Bodas, unpublished, GenBank accession number MW674634). In fact, this collection turned out to be a mixture of *C. verticillata* and *C. krogiana*, the latter now cited below as *Ahti 60678a*. Besides containing fumarprotocetraric acid (P+ red), the basal squamules of the co-occurring *C. verticillata* are lighter in colour on their lower surfaces and slightly larger than those of *C. krogiana*, and they have poorly developed podetia.

The two known occurrences of *C. krogiana* in New Brunswick are within ten kilometers of the Bay of Fundy, and separated by approximately 135 kilometers. Both are on open, rocky (granitic) banks of swift, clear, free-flowing rivers bordered by predominantly coniferous forests of *Picea rubens* Sarg. and *Abies balsamea* (L.) Mill., with or without *Thuja occidentalis* L. (Figure 1A & B). At the New River locality, the lichen was found growing mainly on thin gravelly soil over flat rock, one to three meters above the summer water level. Associated species include the vascular plants *Sibbaldia tridentata* (Aiton) Paule & Soják, *Fragaria virginiana* Duchesne, *Sisyrinchium montanum* Greene, *Solidago bicolor* L., and *S. puberula* Nutt.; the mosses *Hedwigia ciliata* (Hedw.) P. Beauv. and *Polytrichum piliferum* Hedw.; and the lichens *Cladonia arbuscula* (Wallr.) Flot., *C. borealis* S. Stenroos, *C. pyxidata* (L.) Hoffm., *C. strepsilis* (Ach.) Grognot, *C. uncialis* (L.) F. H. Wigg., *C. verticillata*, *Stereocaulon dactylophyllum* Flörke, *S. glaucescens* Tuck., and *Xanthoparmelia conspersa* (Ach.) Hale. On the Forty Five River, *C. krogiana* was found directly over rock, with *C. pleurota* (Flörke) Schaer., *C. pyxidata*, and the mosses *Andreaea rupestris* Hedw. and *Paraleucobryum longifolium* (Hedw.) Loeske.

At both localities, some of the thalli of *C. krogiana* are subject to immersion during spring freshet. On the Forty Five River, the thalli are evidently flooded more frequently, following heavy rains. The species was discovered at this locality in mid-October 2006, three days after a rainfall of at least 40 mm, at which time some of the thalli were underwater at the river's edge. Average annual precipitation at climate stations near the Bay of Fundy in New Brunswick ranges from 1295 to 1510 millimeters; about 80% of this total falls as rain, with rainfalls of 25 millimeters or more occurring 12.4 days per year on average (Environment Canada 2020). These habitats are similar to those of *C. krogiana* in Norway (Løfall & Timdal 2002). Nonetheless, this species is not a member of a semi-aquatic community, unlike lichens such as *Ionaspis lacustris* (With.) Lutzoni and *Rhizocarpon lavatum* (Fr.) Hazsl.

In Norway, *C. krogiana* was originally reported from eight localities, all in Østfold (Løfall & Timdal 2002). Since 2002, it has been found additionally in the neighbouring or nearby southeastern landscapes of Akershus, Buskerud, Oppland, and Telemark. In all, it is currently known from 27 localities (1×1 km grid-cells) in Norway (Global Biodiversity Information Facility 2021). Most of the occurrences are on rocky shores of large lakes and rivers; two are on coastal rocks five to ten meters above sea level. One of the localities is on a rock outcrop about 50 meters from a small lake in a meadow kept open by careful, long-term, seasonal burning for farming (B. P. Løfall, unpublished data). A geographically and ecologically outlying occurrence of *C. krogiana* is known from open semi-native pine forest on ultramafic rock in the Ransko National Nature Reserve, Žďár Hills of the Czech Republic, where it was found in 2003 (Palice et al. 2018).

The bioclimatic contexts of the Canadian and Norwegian occurrences of *C. krogiana* are quite similar. The Norwegian localities are in an area of transition between the hemiboreal (boreonemoral) and southern boreal zones (Moen 1999), while the Fundy coastal region in New Brunswick is in the hemiboreal zone (Clayden 2010). Along a gradient of oceanic to continental conditions, reflecting differences in the seasonal distribution of heat and moisture, the Norwegian localities are in oceanic to intermediate sectors: O_2, O_1, and OC (Moen et al. 1999) following the subdivisions introduced by Ahti et al. (1968) and extended throughout the circumboreal zone by Tuhkanen (1984). The localities in New Brunswick are in somewhat more continental (borderline O_1–OC) sectors (Clayden 2010). The more oceanic O_2 bioclimatic sector has no representation in eastern Canada (Clayden et al. 2011, Tuhkanen 1984).

It is likely that additional occurrences of *C. krogiana* will be found on the lower reaches of rivers emptying into the Bay of Fundy. It might also be present in coastal eastern Maine, near the Atlantic coast of Nova Scotia, or in southern Newfoundland. Given the connections among the climate, vegetation, and lichen biotas of these northeastern coastal regions and those of the spruce-fir zone of higher elevations in the Appalachian Mountains (Clayden 2010, Clayden et al. 2011, Gowan & Brodo 1988, Lendemer et al. 2013), open rocky stream banks in the latter areas might also provide conditions suitable for *C. krogiana*. Its discovery in open pine woods over ultramafic bedrock in an upland locality in the Czech Republic (Palice et al. 2018) suggests that its ecological and geographical ranges could be much wider still. Isolated occurrences of other oceanic lichens are known from several Czech localities with ultramafic rock, but the ecological basis of this association remains unclear (Palice et al. 2018; Z. Palice, pers. comm.).

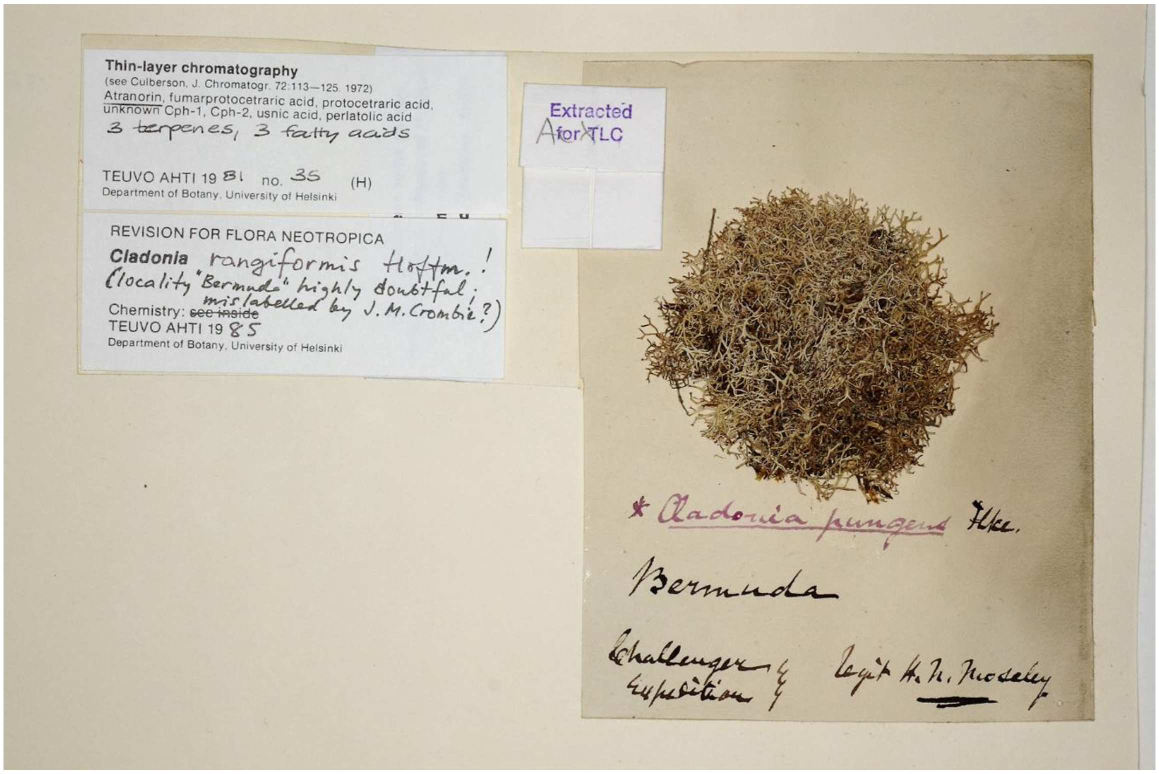
Cladonia krogiana is red-listed in Norway and categorized as NT, "near threatened" (Timdal et al. 2015). The NT designation indicates that it is not considered to be at risk of extinction, but is close to meeting some of the criteria that would place it in a category with a specified extinction risk. Damming of rivers for electric power generation is identified as a potential threat to "moisture-demanding [red-listed] species growing on rock in stream ravines" in Norway (Timdal et al. 2015). Both of the known occurrences in New Brunswick are in protected areas. Many rivers in Atlantic Canada have been dammed, but most small and medium-sized rivers in this region remain free-flowing.

Specimens examined. – **CANADA.** NEW BRUNSWICK. Albert Co.: Fundy National Park, Forty Five River, ca. 300 m below covered bridge near NE corner of park, 220 m, 15.x.2006, *S.R. Clayden 16523* (NBM). Charlotte Co., New River Beach Provincial Park, New River, ca. 100 m S (downriver) of bridge on Route 780, 40 m, 24.viii.2004, *S.R. Clayden 13046* (NBM), 22.ix.2006, *S.R. Clayden 16519* (NBM), 1.v.2011, *W.R. Buck 57753* (NY), *S. R. Clayden 21916* (NBM), *R.T. McMullin 7183* (CANL), 6.ix.2014, *T. Ahti 60678a & S.R. Clayden* (H). **CZECH REPUBLIC.** E Bohemia, Žďárské vrchy Mts., Ransko: Ranský Babylon, ca. 2 km SW of Staré Ransko, 670 m, 2.x.2003, *Z. Palice et al. 11510* (PRA). **NORWAY.** Østfold: Moss, Mossesundet like vest for Molbekktjernet, 3 m, 14.ix.2003, *B.P. Løfall bpl-L10443 & A. Ognedal* (NBM); Aremark, Kasa, 115 m, 18.vi.2003, *B.P. Løfall bpl-L10289* (NBM); Askim, Kykkelsrud, 60 m, 28.iv.2005, *B.P. Løfall 10973* (H, O; vouchers for DNA analyses in Stenroos et al. 2019).

RESULTS AND DISCUSSION PART 1: *CLADONIA RANGIFORMIS*

Cladonia rangiformis occurs widely in temperate western Eurasia and North Africa (Burgaz et al. 2020, Litterski & Ahti 2004). Previous reports for continental North America (e.g., Fink 1935, Thomson 1968) are based on misidentifications (Ahti 1962, Litterski & Ahti 2004). Crombie (1884) reported *C. rangiformis* (as *C. pungens* Flörke) from the island of Bermuda, based on a specimen said to have been collected there by Henry N. Moseley (Figure 2). One of us (Ahti) has examined this collection and confirmed Crombie's identification; however, the provenance of the specimen remains uncertain.

Moseley was a naturalist on the Challenger Expedition from 1872–1876, and was on Bermuda from 5–21 April and 27 May–12 June 1873 (Moseley 1879). His lichen collections from several oceanic islands and other localities visited during the five-year expedition were identified and reported by Stirton (1874, 1880) and Crombie (1877, 1884; see also Hemsley 1885). The first mention of *C. rangiformis* in this sequence of papers ("Bermuda … On the ground. Sterile") was by Crombie (1884). Three other *Cladonia* species were reported for Bermuda in Stirton's and Crombie's earlier papers on Moseley's collections. The 1884 list was prefaced by the note: "The following Lichens from different localities visited by the 'Challenger,' which were inadvertently placed in the packets containing Mosses & C., have to be added to those previously enumerated by me in Linn. Soc. Journ., Bot. vol. xvi, pp. 211-231."


Thin-layer chromatography
(see Culberson, J. Chromatogr. 72:113—125. 1972)
Atranorin, fumarprotocetraric acid, protocetraric acid, unknown Cph-1, Cph-2, usnic acid, perlatolic acid
3 terpenes, 3 fatty acids
TEUVO AHTI 19 81 no. 35 (H)
Department of Botany, University of Helsinki

Extracted for TLC

REVISION FOR FLORA NEOTROPICA
Cladonia rangiformis Hoffm.!
(locality "Bermuda" highly doubtful; mislabelled by J. M. Crombie?)
Chemistry: ~~see inside~~
TEUVO AHTI 19 85
Department of Botany, University of Helsinki

* Cladonia pungens Flke.
Bermuda
Challenger Expedition
legit H. N. Moseley

Figure 2. Specimen of *Cladonia rangiformis* of uncertain origin, reportedly collected by H. N. Moseley on the island of Bermuda during the Challenger Expedition (BM000764738).

No subsequent collections of *C. rangiformis* are known from Bermuda (Imshaug 1957, Riddle 1916; S. LaGreca, pers. comm.). In this light, it seems likely that the Moseley collection in question was mislabeled. It might have originated, for example, from the Cape Verde Islands, the Azores, or the Canaries, where Moseley also collected lichens during the Challenger Expedition, and where *C. rangiformis* is known to occur (Litterski & Ahti 2004, Pino-Bodas et al. 2017a). Still, it is possible that this species was accidentally introduced to Bermuda and either did not persist or has been overlooked there in recent decades.

We (Clayden, Pitcher) discovered *C. rangiformis* at two localities along the southwest coast of Conception Bay in eastern Newfoundland, Canada, in 2007 (Figures 3 & 4). We returned to the Bay Roberts locality with a group of North American and European lichenologists during the 2007 Tuckerman Workshop (Pitcher & Clayden 2007), and additional collections of *C. rangiformis* were made at that time. The thalli were very distinct in the field, and are morphologically and chemically typical of *C. rangiformis*: K+ yellow, P–, containing atranorin and rangiformic acid—confirmed with TLC. Among other densely-branched *Cladonia* species occurring in northeastern North America, the most similar morphologically is *C. furcata* (Huds.) Schrad.; however, *C. furcata* contains fumarprotocetraric acid (K–, P+ red) and lacks fatty acids.

In the phylogenetic tree based on maximum likelihood analysis, the specimen from Newfoundland is nested within a well-supported clade including other specimens of *C. rangiformis* from Europe and Macaronesia (Figure 5). Other morphologically similar species occurring in North America, such as *C. furcata* and *C. multiformis* G. Merr., were included in the analysis but were found to be not closely related to *C. rangiformis*. This result is consistent with the phylogenetic analysis of *Cladonia* by Stenroos et al. (2019), who included these species among a much broader taxonomic sampling of the genus.

In Eurasia and North Africa, *C. rangiformis* occurs mainly, but not exclusively, in calcareous habitats. In the Mediterranean region it is equally frequent on acidic and basic substrata (Burgaz et al. 2020); its northernmost occurrences, in the British Isles and Fennoscandia, are on seacoasts (Ahti & Stenroos 2013, Litterski & Ahti 2004). At both localities where we found it in Newfoundland, it formed scattered small clumps in low treeless vegetation on thin soil over rock, within a few hundred meters of the seashore. The extensive bedrock exposures in this area are mainly late Precambrian siliceous sediments and volcanics. Near

Figure 3. Habitat of *Cladonia rangiformis* (semi-natural, rocky, coastal heathland) near Bay Roberts, Newfoundland, Canada.

Harbour Main, Cambrian slates with thin inter-bedded limestone are exposed along with more acidic Precambrian rocks (King 1988).

This was the first area of Newfoundland settled by Europeans, and its coastal vegetation has been much-modified by burning, pasturing, and activities related to the fisheries (Meades 1983). The fishing settlement of Cupids (originally Cuper's Cove), mid-way between Bay Roberts and Harbour Main, was established in 1610, and has been continuously inhabited since that time (Gilbert 2013). By the 1620s, as many as 16 ships involved in the fisheries were sailing annually between Bristol, UK, and the island of Newfoundland (Cadigan 2009). These and other ships brought large quantities of ballast, which were generally dumped on shore, rather than in the sea; they were probably a vehicle for the introduction of many vascular plants, as well as a few bryophytes and lichens (Ahti 1983).

The occurrences of *C. rangiformis* reported here plausibly originate from propagules accidentally introduced from Europe. At both localities, non-native grasses and other weedy flowering plants (e.g., *Hieracium* species) are present. Also, several collections of the moss *Homalothecium sericeum* (Hedw.) Schimp. were made at the Bay Roberts locality during the 2007 Tuckerman Workshop (*B.H. Allen 28698, 28701*, MO; *R.A. Pursell 12817*, MO). This species may, likewise, be an introduction from Europe (Ahti 1983). Its occurrences on the island of Newfoundland, including others around Conception Bay, are mainly in "grazed meadows along the seaside" (Crum & Anderson 1981). It is unknown elsewhere in North America.

At Bay Roberts, these introduced species are part of a semi-natural rocky heath community with *Empetrum nigrum* L., *Vaccinium* species, *Sibbaldia tridentata*, *Juniperus communis* L., grasses, bryophytes, and lichens. Although no obviously calciphilous vascular plants were noted at either locality, a few such lichens were collected at both, indicating localized calcareous influences. At the locality near Harbour Main, the habitat was dominated by grasses, with sporadic thalli of calciphilous lichens including *Bacidia bagliettoana* (A. Massal. & De Not.) Jatta, *Cladonia acuminata* (Ach.) Norrl., and *Scytinium imbricatum* (P. M. Jørg.) Otálora, P. M. Jørg. & Wedin. At Bay Roberts, *Catapyrenium cinereum* (Pers.) Körb., *Cladonia acuminata*, *Cladonia pocillum* (Ach.) Grognot, and *S. imbricatum* were present (collections at NBM).

Only a few cases of apparently non-native but naturalized species have been reported among the lichens of Atlantic Canada (Ahti 1983; Clayden 2010). However, inferences about the anthropogenic origin

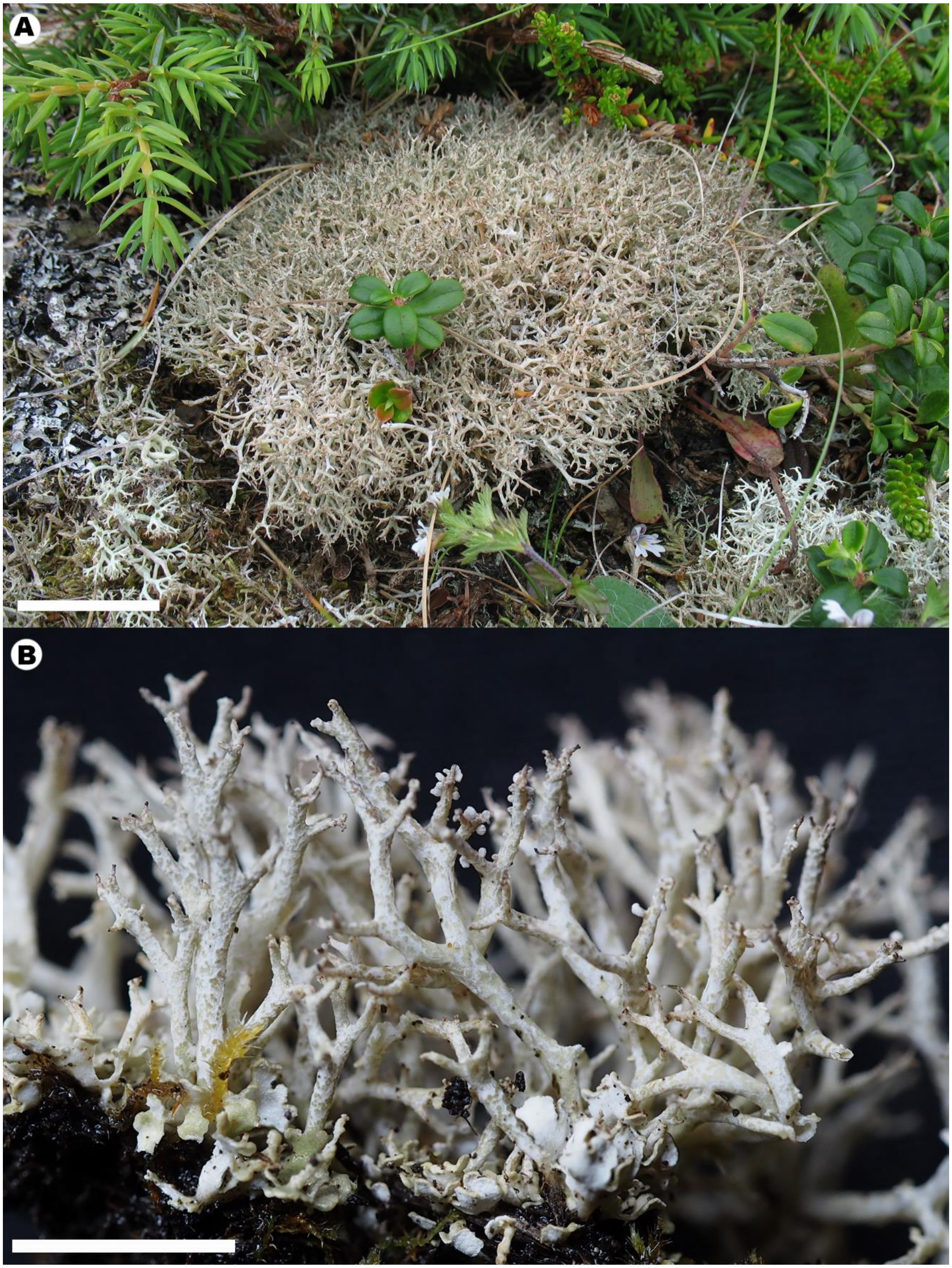


Figure 4. Habit and morphology of North American *Cladonia rangiformis*. **A**, habit (photographed in situ; *Clayden 17854 & Pitcher*, NBM). **B**, detail of podetia with maculate-areolate surface, and both podetial and basal squamules, the latter of which usually not persistent in this species (*Clayden 17787 & Pitcher*, NBM). Scale bars: A = 2 cm, B = 1 cm.

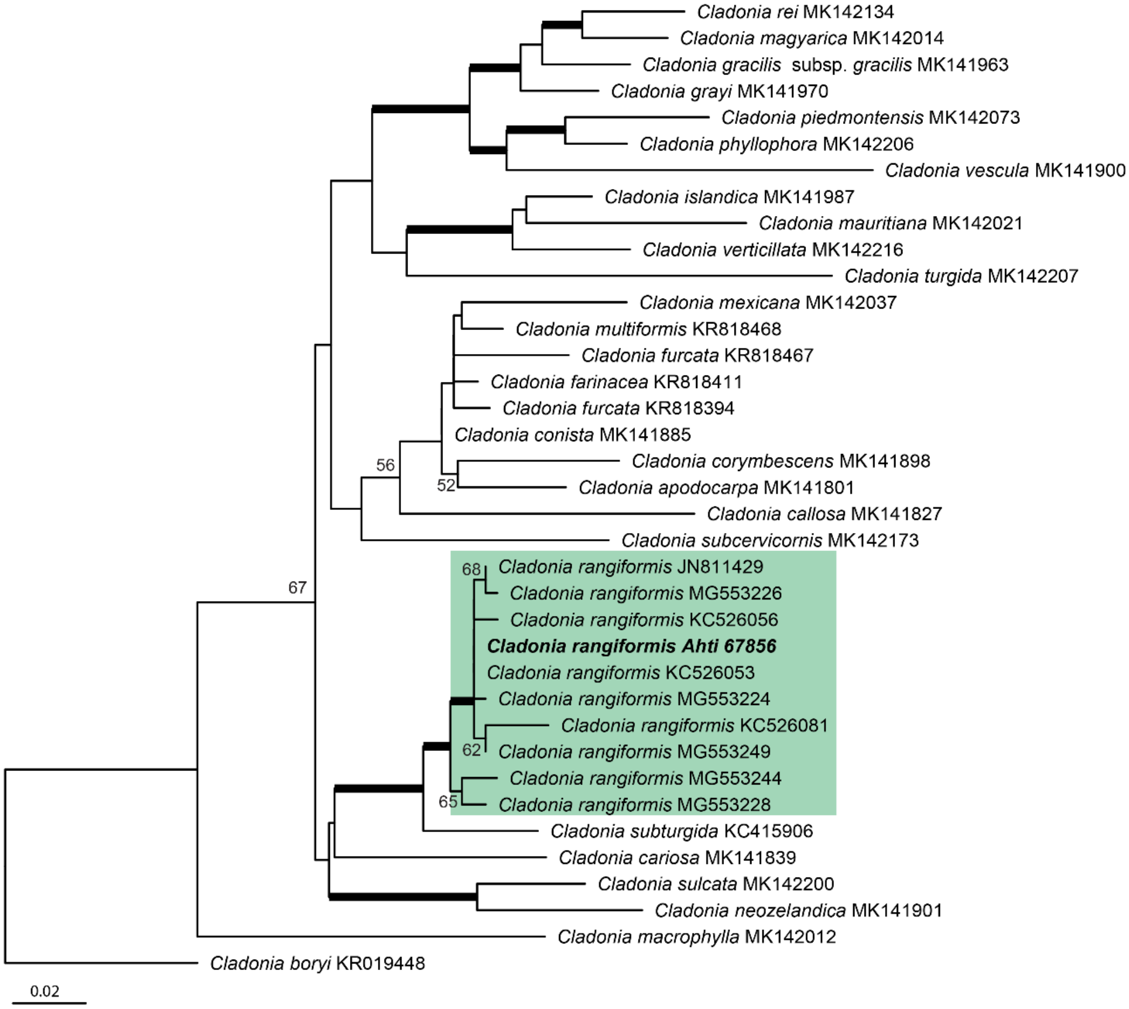


Figure 5. Phylogenetic tree of the clade *Cladonia* according to Stenroos et al. (2019) based on ML analysis of the IGS rDNA region, -lnl = 2470.892. Branches supported by bootstrap values ≥70% are in bold. Bootstrap values ≥50% for ML at the branches. The newly sequenced specimen from Newfoundland is indicated in bold. The GenBank accession number for each sequence is provided after the species name.

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of such populations have not been evaluated with molecular methods. In our analysis of IGS rDNA sequences, eight *C. rangiformis* haplotypes were found connected in a unique network with 95% confidence (Figure 6). A single widely distributed haplotype contained 66% of all sequences (28 sequences). The specimen from Newfoundland belongs to this haplotype. Three of the IGS rDNA haplotypes were restricted to Spain, one to Greece, and another to the Azores. Two haplotypes were shared among specimens from different countries. One of these was found in Greece, Iran and Spain, the other in the Azores, Spain and the Netherlands.

Although our data are very limited, based on few specimens and a single locus, they are congruent with those obtained for *C. rangiformis* by Pino-Bodas et al. (2017b) with three loci (ITS rDNA, LSU rDNA and IGS rDNA): the populations of this species are not geographically structured with respect to the three loci examined. Also, as expected, these data are not conducive to testing the hypothesis of a recent European origin for the Newfoundland occurrences. Population genetic analyses based on more variable markers (such as microsatellites or RadSeq data) might resolve this uncertainty. For now, the possibility that *C. rangiformis* colonized Newfoundland by long-distance dispersal cannot be rejected, and the probable timeframe of its establishment on the island remains uncertain.

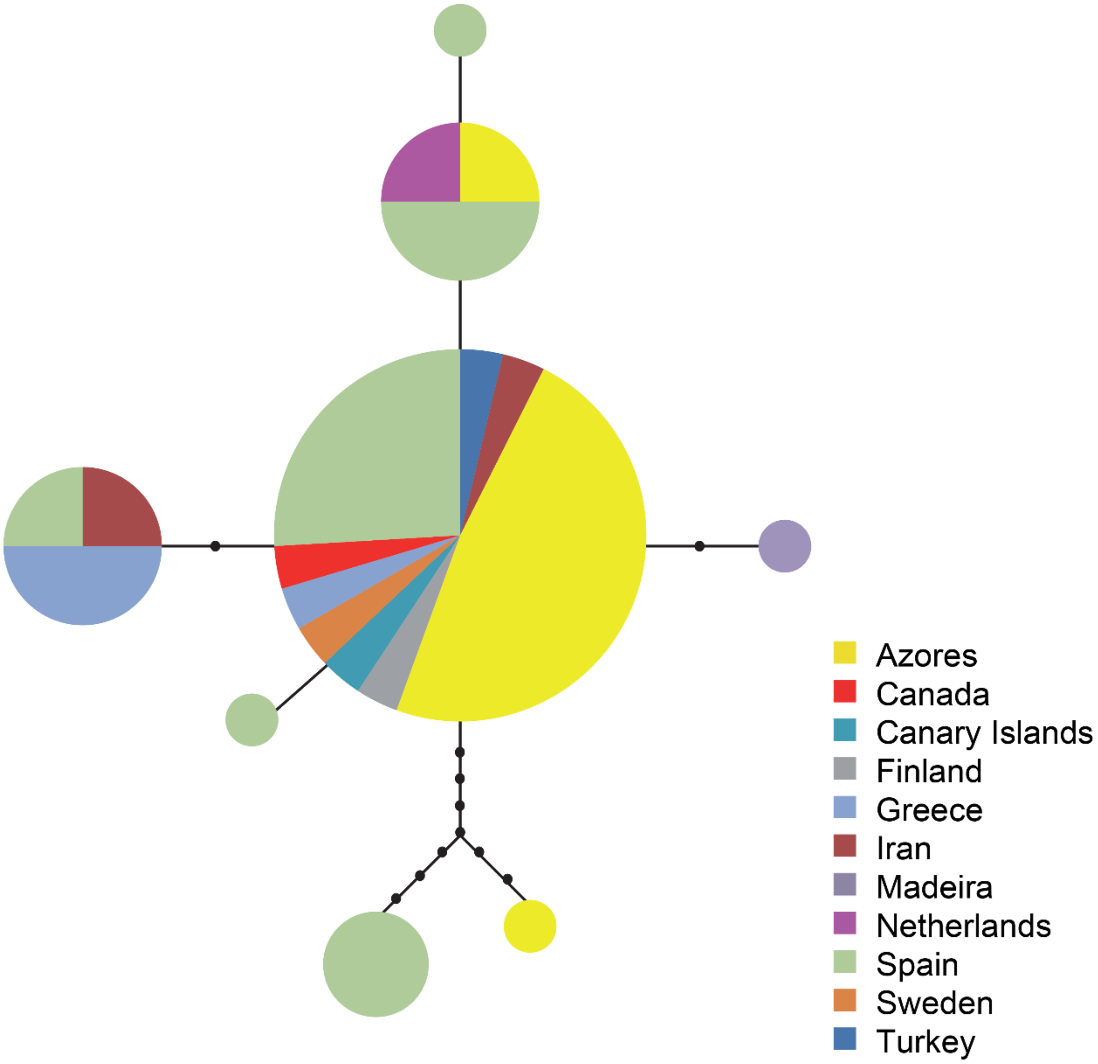

Figure 6. Parsimony network of *Cladonia rangiformis* based on IGS rDNA sequences. Each circle represents a haplotype; the circle size is proportional to haplotype frequency. Small black circles represent haplotypes not observed in the data. The haplotype colours represent the countries where the specimens were collected.

Specimens examined. – **"BERMUDA"[?]:** "Challenger Expedition", locality, substratum, and date not specified, *H.N. Moseley* (BM, barcode BM000764738). **CANADA.** NEWFOUNDLAND AND LABRADOR: Island of Newfoundland, Avalon Peninsula, Conception Bay, Gasters Bay, Goat Shore, ca. 2 km NNW of town of Harbour Main, elev. 5 m, 17.vi.2007, *S.R. Clayden et al. 17787* (NBM); Conception Bay, Bay Roberts, East Shoreline Heritage Walk, between Mad Rock and Long Point, elev. 10 m, 1.ix.2007, *S.R. Clayden 17854 & M. Pitcher* (NBM), *S.R. Clayden 17855 & M. Pitcher* (NBM), 1.ix.2007, *T. Ahti 67856* (H), *T. Ahti 67872* (H), *R.C. Harris 54002* (NY), *E. Kneiper 07-0168* (NY), *J.C. Lendemer 10311* (NY), *M. Pitcher 109* (herb. Pitcher).

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