

## Tackling rarity and sample bias with large-scale biodiversity monitoring: a case study examining the status, distribution and ecology of the lichen *Cladonia rei* in Alberta, Canada

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**Abstract:** Species conservation depends on accurate data, but for many lichens existing collections are geographically biased and contain many taxonomic errors. It is unclear whether ‘non-expert’, systematic monitoring schemes can address these sources of error, particularly for taxonomically challenging lichens (e.g. species requiring chemistry for accurate identification). In this case study we use the Alberta Biodiversity Monitoring Institute (ABMI), a large-scale, systematic, multi-taxon monitoring programme, to better understand the ecology and distribution of a putative rare species, *Cladonia rei*. Collections of *C. rei* from Alberta dating from 1947 suggested the species was broadly distributed but rare, with seven accessioned specimens. We used comparative morphology, thin-layer chromatography and habitat modelling to compare historical records against more recent material from ABMI surveys. Contrary to the historical collections, ABMI samples suggest *C. rei* is almost entirely limited to the dry mixed grassland, northern fescue grassland and aspen parkland natural regions, and that within these ecosystems it is relatively common. The typical ecotype exhibited included a persistent primary thallus, podetia with a persistent basal cortex, and secondary squamules; typically they lacked cups, well-developed apothecia and fumarprotocetraric acid, and ramifications were sparse. *Cladonia rei* was consistently found in pastures and undisturbed grasslands that hosted relatively rich communities of epigeic lichens, thus it does not appear to act as a pioneer in Alberta or to commonly occupy the anthropogenic niches documented elsewhere. In summary, large-scale, systematic, non-targeted monitoring employing novices redressed issues of sample bias through almost 300 *C. rei* collections, simultaneously improving the ecological understanding of a putative rare species.

**Key words:** Alberta Biodiversity Monitoring Institute, Ascomycota, *Cladoniaceae*, cryptogamic crust, grassland, habitat modelling, species co-occurrence

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### Introduction

Knowledge of the distribution and ecology of rare species is critical for their conservation. However, available data on rare species, particularly those that are difficult to identify, are often scarce and may have several shortcomings, including geographical collection bias and high taxonomic error rates (Hamilton

*et al.* 2015). Macrolichens are a good example of this; while they are widely recognized to be excellent environmental indicators (e.g. Giordani *et al.* 2012), in many jurisdictions they are poorly documented. As a result, conservation ranks are often assigned tentatively due to insufficient or low quality data (e.g. Goward 1996; Government of Alberta 2014). Efforts to fill these knowledge gaps through targeted species-based approaches for lichens alone are often impractical due to limited resources. In this respect, large-scale, non-targeted monitoring is an opportunity to fill such gaps, particularly if such efforts are complemented by rigorous taxonomy and appropriate laboratory techniques. As compared to herbarium collections, geographically

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extensive, large-scale monitoring initiatives are less likely to suffer from geographical sampling biases because they typically employ random, systematic or stratified sampling, making them more appropriate for analyses of habitat preferences and population dynamics (e.g. Nielsen *et al.* 2009). However, large-scale monitoring programmes might not be efficient in providing data on rare or difficult-to-identify species because of a focus on breadth rather than depth of sampling, a resultant *de facto* emphasis on common species, and reliance on novice technicians conducting rapid assessments (Haughland 2012; Zhang *et al.* 2014). As such, it is important to examine the ability of these large-scale programmes to inform the conservation status of rare and/or taxonomically challenging species.

In this study we examine the ability of a large-scale biodiversity monitoring initiative in Alberta, Canada, to fill the information gap in the distribution and ecology of a rare, taxonomically challenging species, using *Cladonia rei* as our case study. The Alberta Biodiversity Monitoring Institute (ABMI) is designed around a systematic grid of permanent sites which are surveyed via rapid assessments for human disturbance, habitat metrics, and the occurrence and relative abundance of birds, mammals, oribatid soil mites, vascular plants, bryophytes and macrolichens (ABMI 2010, 2014; www.abmi.ca). *Cladonia rei* is a fruticose, difficult-to-identify lichen consisting of grey-green, typically sorediate podetia (Brodo *et al.* 2001; Ahti & Stenroos 2013). The morphology of *C. rei* is variable and podetia may produce secondary squamules, small cups or secondary proliferations (Paus *et al.* 1993; Dolnick *et al.* 2010; Pino-Bodas *et al.* 2010; Ahti & Stenroos 2013). This variability makes *C. rei* difficult to distinguish from similar, sympatric species, such as *C. subulata* and *C. coniocraea* (e.g. Goward 1999; Brodo *et al.* 2001; Spier & Aptroot 2007), and so relatively inexperienced technicians cannot target it amongst similar subulate *Cladonia* species. However, *C. rei* has a reliable chemical trait that can be used to confirm its identity in the laboratory; the presence of homosekikaic acid (Asahina 1938; Østhaugen 1976). This makes *C. rei* a good case study for the utility of

non-targeted monitoring to understand the ecology and distribution of taxonomically challenging species.

The few existing herbarium collections of *C. rei* from Alberta dating from 1947 suggested the species was broadly distributed but rare (Government of Alberta 2014), while in contrast, ecological studies by Looman (1964*a, b*) inferred that it could be a common inhabitant of grassland ecosystems. Here we use specimens and related data collected by the ABMI to validate the distribution of *C. rei* in Alberta, model its distribution using field and remote sensing-derived predictors, including other lichens and vascular plants, and look for predictable community assemblages that co-occur with *C. rei*. We then reassess its conservation status in the Province, and put the ecology and phenotypic variation of *C. rei* in Alberta into a global context.

## Methods

### Study area and survey design

Our study focused on the province of Alberta which covers an area of 661 648 km<sup>2</sup> or 7% of Canada's land mass. Alberta is ecologically diverse, encompassing ecosystems such as alpine and mountain environments, boreal forest, deciduous-dominated dry parkland and mixed-grass and fescue (*Festuca*) grasslands (Fig. 1, Government of Alberta 2006). We used data and specimens collected by the Alberta Biodiversity Monitoring Institute (ABMI) which samples a core, systematic, 20 × 20 km grid of 1656 permanent 1 ha sites (Fig. 1, www.abmi.ca), as well as a smaller number of 'off-grid' sites targeted to supplement existing environmental and anthropogenic disturbance gradients (Haughland 2012; Burton *et al.* 2014). ABMI core sites are grouped into blocks of 9 (3 × 3) sites, and each block is assigned systematically to 1 of 5 sampling sets with the goal of completing each set in order, given constraints imposed by natural disturbances such as large-scale forest fires, and regional and programme-wide funding. In our study we examined lichen collections from all 778 sites sampled by the ABMI between 2009–2013, representing a diversity of habitat types and anthropogenic disturbances (Fig. 1, Table 1).

### Field methods

Field sampling was conducted by technicians trained by the senior author between May and July of each year, and followed a modified floristic habitat (Newmaster *et al.* 2005) sampling scheme (detailed methods in ABMI 2010). In each of the 4 quadrants of

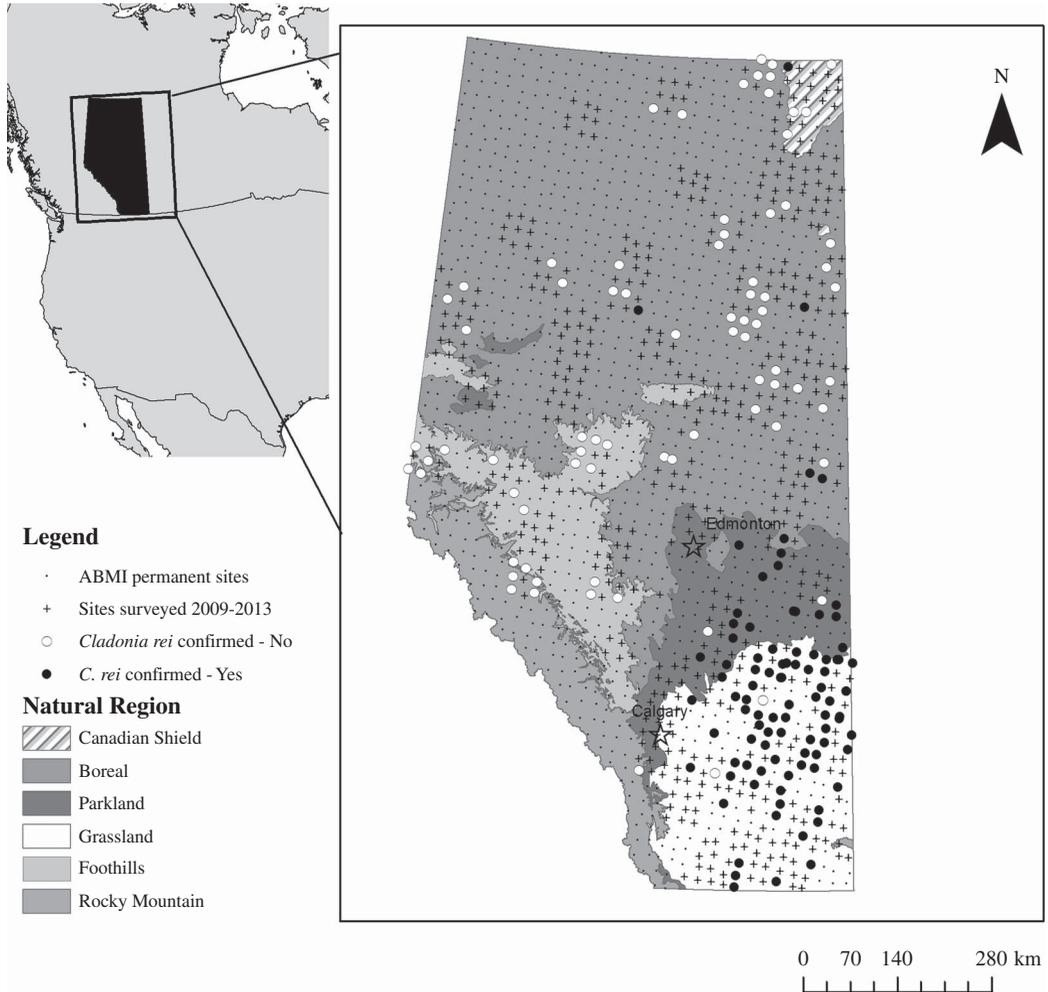


FIG. 1. *Cladonia rei* distribution in Alberta, Canada resulting from the Alberta Biodiversity Monitoring Institute sampling effort. The map shows all 1656 on-grid ABMI sites, and all ABMI on- and off-grid sites surveyed from 2009–2013. For the latter, sites where *C. rei* was originally suspected but later determined to be absent are marked with open circles and sites where *C. rei* was confirmed to be present are marked with closed circles.

each 1 ha study site, a 25 × 15 m plot was established in the outermost corner (Fig. 2). Technicians estimated the amount and type of anthropogenic disturbance per plot. Microhabitats were assigned to 1 of 5 strata (trees, shrubs and vertical structures; logs and stumps; rocks and cliffs; upland and disturbed soils; wetland substrata). At each plot, the collector first spent up to 25 min searching within the boundaries of the plot, collecting unique macrolichen and calicioid morphotypes from microhabitats in the first 3 strata. In each quadrant, the collector then spent 10 min surveying microhabitats and collecting unique macrolichen and calicioid morphotypes from the latter 2 strata on 2 belt transects of 2 × 25 m (Fig. 2). In total, macrolichen and calicioid samples collected from each quadrant included those from

a plot and those from 2 belt transects (a maximum 35 min of survey effort and 5 composite collection bags), and samples from each site included those from all 4 quadrants (a maximum of 2.3 h of survey effort and 20 composite collection bags). All lichen samples were collected for identification in the laboratory. Samples are stored at the Royal Alberta Museum for future accessioning.

### Chemical analyses and morphometrics

Spot tests and morphology were used initially to separate putative *C. rei* from similar *Cladonia* species. Specimens identified as putative *C. rei* (PD+ red or PD-, K-, KC-, UV+ dull white or UV-) were examined using thin-layer chromatography (TLC) to confirm

TABLE 1. *The Alberta Biodiversity Monitoring Institute (ABMI) is designed around a systematic grid of 1656 sites. Here we show the sampling effort, anthropogenic disturbance samples, and hypothesized and confirmed Cladonia rei detections by natural regions, as represented in the 2009–2013 subset analysed herein. “-” indicates value not applicable.*

Natural Region	Sampling effort		Anthropogenic disturbance (% quadrants with disturbance in a given category/mean % quadrant disturbed)								<i>Cladonia rei</i> detections	
	Number of ABMI sites surveyed	% of ABMI sites surveyed from each natural region	Cultivation	Forestry	Industrial features	Linear features	None	Other	Pasture	Human settlement	Number of sites with putative <i>C. rei</i>	Number of sites confirmed postTLC
Grassland	225	49	34/96	0/-	1/39	7/26	7/-	4/28	45/98	2/74	78	76
Parkland	65	30	37/88	<1/30	1/10	7/28	14/-	2/25	36/88	4/81	15	13
Boreal forest	375	25	5/97	11/92	4/92	12/32	57/-	3/63	6/80	2/63	52	5
Foothills	77	16	3/68	22/80	11/88	20/41	27/-	11/24	4/100	0/-	15	0
Rocky Mountain Shield	16	13	0/-	0/-	0/-	3/23	81/-	3/5	13/88	0/-	9	0
Shield	20	80	0/-	0/-	0/-	0/-	0/-	0/-	0/-	0/-	4	1
Total	778	38	16/95	7/88	4/87	11/33	39/-	4/39	18/93	2/72	173	95

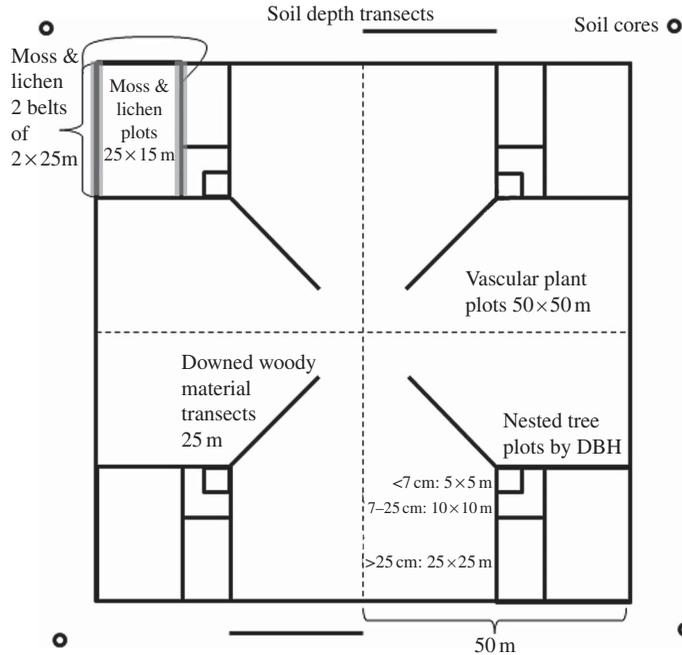


FIG. 2. Overview of lichen collection protocols as well as other key Alberta Biodiversity Monitoring Institute terrestrial protocols, together with their survey dimensions (from 2009 onwards, detailed in ABMI 2010). The 1 ha site is divided into  $50 \times 50$  m squares or quadrants. A summary of the survey protocols is given within the diagram; protocols were repeated in each of the quadrants; soil depth was an exception, it was measured along 2 transects north and south of the 1 ha plot. DBH = diameter at breast height.

the presence of homosekikaic acid (following Orange *et al.* (2010), on  $10 \times 20$  cm plates, and boiling the acetone-specimen mixtures 3 times in a water bath prior to spotting as per I. M. Brodo, pers. comm.). A collection from Ontario was used as the initial standard for homosekikaic acid and fumarprotocetraric acid (1965, I. M. Brodo, 6476 (PMAE)). For 35% of samples we used solvent systems A, B' and C; however, once it was determined that solvent C was sufficient to distinguish homosekikaic acid from other likely secondary metabolites (such as barbatic or squamatic acid), we typically used solvent C only. Ambiguous results were re-run in all three solvents.

All putative *C. rei* specimens underwent TLC at the beginning of our study. The results from each round of TLC were used to refine our species concept, our understanding of *C. rei*'s distribution and to decide which specimens required TLC going forward. During the latter TLC rounds, we analyzed  $\geq 1$  specimen from every site within the Grassland and Parklands Natural Regions; if that specimen was confirmed, further TLC was not conducted on putative *C. rei* from that site. If the representative specimen was incorrect, all additional putative *C. rei* specimens from the site underwent TLC. In contrast, all putative *C. rei* specimens from the remaining natural regions of Alberta (mountains, foothills, boreal and shield) underwent TLC.

Using a haphazard selection of verified samples, we recorded the height of the tallest podetium in each sample as well as the presence of primary squamules, secondary squamules, cups, pycnidia, apothecia, secondary proliferations and substratum. Measurements are presented as the mean ( $\bar{x}$ )  $\pm$  1 standard deviation (SD), followed by the range (smallest–largest observed values) and the sample size ( $n$ ).

### Additional specimens examined

We located seven accessioned *C. rei* specimens from Alberta herbaria (ALTA and PMAE). Additional sources searched included: CFB (data provided by G. Pohl in 2013), UBC (UBC Herbarium 2016), UAC (B. Smith, pers. comm.) and the Consortium of North American Lichen Herbaria (2016). All accessioned material of *C. rei* from ALTA and PMAE (regardless of collection location) was examined and underwent TLC, as did unaccessioned specimens tentatively identified as *C. rei* from surveys in Alberta and British Columbia with T. Goward and T. Ahti (Supplementary Material Appendix 1, available online). In addition, we examined PMAE collections of *Cladonia subulata*, *C. cornuta*, *C. coniocraea* and *C. macilenta* (no *C. acuminata*, *C. verruculosa*, *C. glauca*, or *C. norvegica* from Alberta were accessioned); for each species, a range of specimens

chosen to represent the geographical range and morphological variation present also underwent TLC in the three solvent systems.

### Habitat variables for modelling

We compiled variables reflecting anthropogenic and natural ecological gradients at small scales (field data) and at larger scales (remote sensing data) to test previously documented ecological patterns for *C. rei*. Variables for each quadrant were derived from field data and summarized at the site scale by averaging or aggregation (Table 2).

#### *Variables measured in the field*

Lichen richness (grouped by substratum affinity: epigeic, both obligate and occasional, and non-epigeic) was included in the model to ascertain whether *C. rei* was more common in diverse cryptogamic crust communities or depauperate lichen assemblages, possibly behaving as a pioneer (e.g. Osyczka & Rola 2013). We included native and non-native vascular plant richness to reflect biotic condition and potentially disturbed soils such as intense grazing in pastures (see Fig. 2, ABMI 2010). Since we observed *C. rei* growing on *Selaginella densa* in many of our samples, we included the occurrence of *S. densa* in our models. Soil pH was also included because Paus *et al.* (1993) concluded that *C. rei* was more common on slightly acidic soils (*c.* 5.9 on average), Looman (1964a) on circumneutral soils, while Ahti & Stenroos (2013) indicate that more basic soils and calcareous rock may be the native substratum of *C. rei*. We examined total soil organic carbon, which typically decreases when soil has been intensively cultivated and is higher in more fertile, biologically active and moisture retentive soils (e.g. Parton *et al.* 1987; Havlin *et al.* 1990). We also considered soil litter, a proxy for the amount of decomposing organic matter available above the ground. High plant productivity and/or low grazing levels may increase the amount of litter and, concomitantly, diminish established epigeic lichen communities (e.g. Zhang *et al.* 2013).

#### *Variables derived from remote sensing*

Remote sensing-derived variables are less reflective of fine-scale microhabitats because of their large grain, but large-scale processes can influence species distributions and their inclusion permits models to be mapped across areas not previously surveyed for testing larger-scale habitat associations. We compiled data from geographic information system (GIS) layers for soils, land cover and anthropogenic disturbance (Supplementary Material Appendix 2, available online).

#### *Geographic and climatic variables*

Alberta's categories of Natural Regions and Subregions (Fig. 1; Government of Alberta 2006), in conjunction with latitude and longitude, are good

proxies for climate variables and other underlying spatial gradients so we included these as site level variables. We also examined climatic variables estimated from the Alberta Climate Model (Alberta Environment 2005) which reflect average conditions from 1961–1990.

### Data Analysis

We conducted logistic regression to relate the occurrence of *C. rei* to habitat and spatial variables. Analyses considered the presence/absence of *C. rei* at both site and quadrant scales because each scale represents potentially different ecological processes and thus results might be scale-specific. Of the suite of potential variables representing local habitat conditions, biotic factors (co-occurring flora), climate and geography, we selected 13 habitat variables based on their ecological significance and independence from other variables (Table 2). Variables such as tree density, basal area and canopy cover were excluded after TLC and redetermination but prior to modelling because the realized distribution of *C. rei* was almost exclusively in the Grassland and Parkland Natural Regions where these variables were not applicable. Other variables were excluded because they were highly correlated ( $P > 0.7$ ). For example, the richness of native vascular plants was highly correlated with total vascular plant richness ( $r = 0.96$ ), as was the richness of lichens with the richness of epigeic lichens (obligate and occasional,  $r = 0.87$ ) and non-epigeic lichen richness ( $r = 0.71$ ). Climate variables were correlated with geography and not considered further; for example, latitude was highly negatively correlated with mean annual temperature ( $r = -0.95$ ), potential evapotranspiration ( $r = -0.85$ ) and mean coldest month temperature ( $r = -0.85$ ), whereas longitude was highly negatively correlated with mean annual precipitation ( $r = -0.73$ ) and positively correlated with mean warmest month temperature ( $r = 0.7$ ). Prior to analyses, the final set of variables was examined for outliers and checked for collinearity using variance inflation factors (Quinn & Keough 2002; Zuur *et al.* 2009). We explored linear and polynomial relationships between occurrence probability and the continuous variables. Accordingly, we incorporated quadratic terms for soil pH and latitude in our multiple regression models. Finally, we assessed the relative importance of each of the predictor variables using standardized regression coefficients in the global model (Schielzeth 2010); to obtain the standardized coefficients, continuous predictive variables were standardized as z scores prior to regression. The logistic regression analysis was performed using the glm function in R (version 3.2.2; R Development Core Team 2015).

### Species co-occurrence analyses

We used the approach described by Azeria *et al.* (2009) to identify species groups within our lichen and vascular plant assemblages as well as the association of *C. rei* to those groups. The method applies null-model analysis (Gotelli 2000) to first obtain the species co-occurrence beyond that expected by chance alone (e.g. a common species will co-occur with many species simply

by being more common) and then applies hierarchical clustering to identify species groups. Null models are used to ascertain whether observed patterns of species co-occurrence are beyond those expected if species were randomly distributed (Connor & Simberloff 1979; Gotelli & Graves 1996; Sanderson 2000; Azeria *et al.* 2009). In this study we employed two types of null models, the fixed–fixed (FF) and the fixed–equiprobable (FE), which have been shown to have reasonably low rates of Type I and II errors (Gotelli 2000). Both null models maintain species occurrence frequencies from the observation matrix (fixed row totals). The FF also maintains the total number of species at sites (i.e. fixed column totals), while the FE considers sites to be colonized equiprobably (Gotelli 2000). We applied the two null models because the FF null model effectively reveals segregated co-occurrences (negative associations) but is conservative in detecting aggregated distributions (positive associations; Wilson 1987; Azeria 2004). The converse is the case for the FE null model (Gotelli & Graves 1996; Azeria 2004). We thus used the FF and FE null models simultaneously (Azeria *et al.* 2012) to detect significant positive and negative associations of *C. rei* with other lichen species. The null communities (using FF and FE null models) were generated by a quasi-swap algorithm (Miklós & Podani 2004) using the function ‘permatfull’ in vegan for R (Oksanen *et al.* 2015).

The co-occurrence analysis was conducted at the site scale using species presence/absence data. The plant assemblage was restricted to 249 species occurring in 10 or more sites. As the lichen assemblage was relatively poor, we used a lower threshold (5 sites) to include 63 species or species groups in the analysis. A non-metric multi-dimensional scaling analysis (NMDS) was carried out on the lichen species matrix to visualize the species groups.

## Results

ABMI field surveys resulted in 70 181 lichen specimens, including 27 870 *Cladonia* specimens. Of those, we identified 435 putative *C. rei* specimens (Table 1). We analyzed 212 ABMI specimens by TLC, including all putative *C. rei* samples from the mountain, boreal, foothills and shield Natural Regions (Fig. 1, Table 1). Using the presence of homosekikaic acid (either in the sample or in samples from the same site) we confirmed 293 samples, 93% of which were located in the Grassland and Parkland Natural Regions (Table 1, Fig. 1, Supplementary Material Appendix 1, available online). Most *C. rei* was found growing on upland soils including upland vegetation and vegetation debris (90% of confirmed samples), followed by rock (6%), wetland substrata (2%), logs and stumps (1%) and on the bases of trees and

other vertical substrata (<0.6%). The sole *C. rei* specimen from the shield Natural Region occurred in undisturbed habitat, while four of the five boreal sites were pastures or adjacent to pastures.

None of the seven accessioned *C. rei* collections from Alberta were determined to be *C. rei* (Supplementary Material Appendix 1, available online). Of the c. 200 accessioned Alberta collections of similar species examined (such as *C. subulata*, *C. coniocraea*, *C. cornuta* and *C. macilenta*), 86 of which underwent TLC, one collection of *C. coniocraea* was redetermined to *C. rei* (Supplementary Material Appendix 1, available online). Of the *C. rei* collections from outside of Alberta, three accessioned *C. rei* (from Saskatchewan, Iowa and Minnesota respectively) contained homosekikaic acid alone and displayed a similar phenotype to the Alberta material (Supplementary Material Appendix 1, available online).

## Chemistry and morphology

We considered the presence of homosekikaic acid as a diagnostic trait; fumarprotocetraric acid was never found as an accessory metabolite. The only accessory metabolite occasionally detected was sekikaic acid. Approximately half of the specimens had detectable fluorescence, typically a dull white, which we found easiest to detect under short-wave (254 nm) rather than longwave light (365 nm). The mean maximum podetium height was  $16 \pm 9$  mm (range 2–58 mm,  $n=72$ ; Fig. 3). The podetia rarely proliferated or proliferated very sparingly (16% with proliferations,  $n=86$  for all further morphometrics; Fig. 3A–C), sometimes resulting in forked tips but almost never giving rise to cups with subulate marginal proliferations (Fig. 3C). Specimens rarely became scyphose; 11% of podetia ended in a narrow or funnel shaped cup. The lower third to half of the podetia were typically grass green and corticate, with farinose to granular patchy brown and green soredia forming towards the terminus (Fig. 3D–H). Primary squamules were often persistent (67%) and secondary squamules were

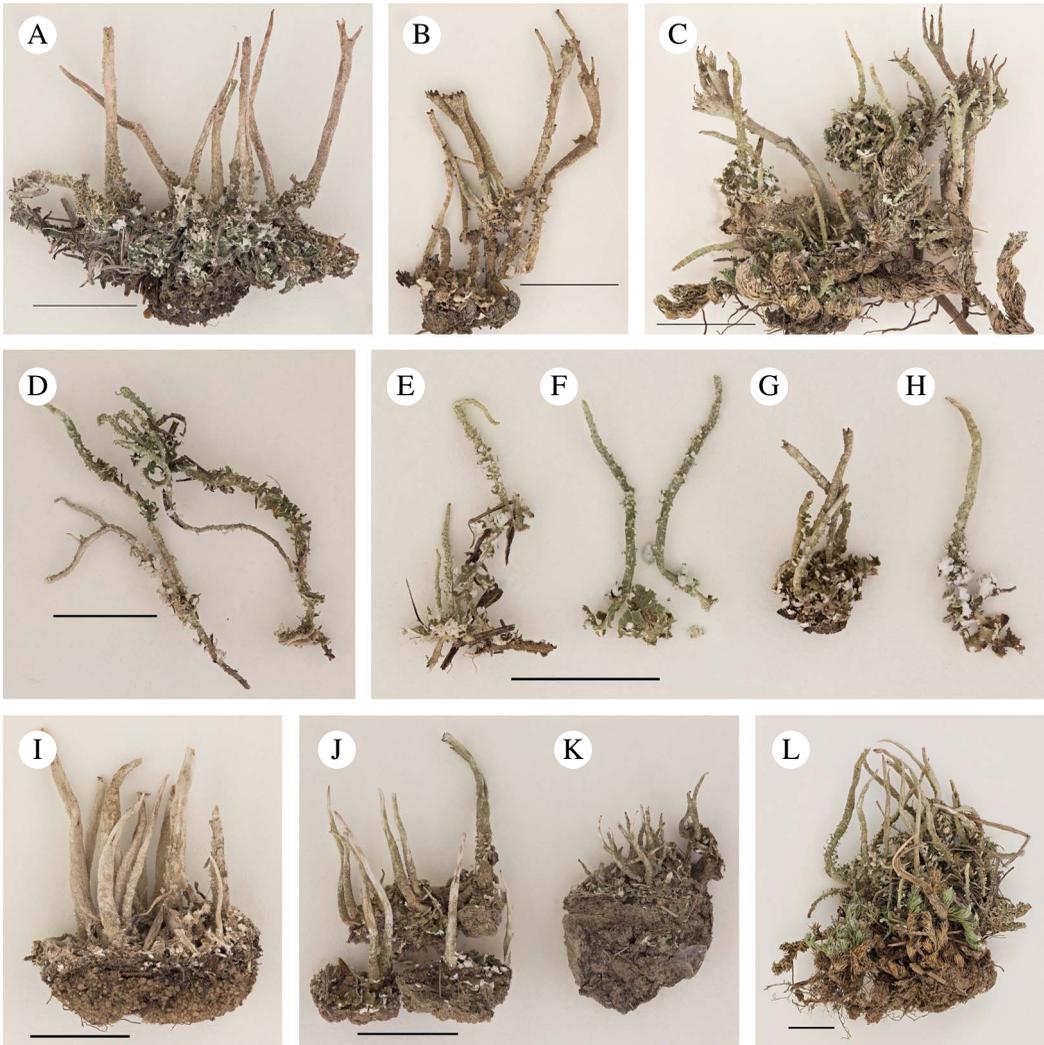


FIG. 3. Representative *Cladonia rei* collections from Alberta. The grassland ecotype most commonly encountered is depicted in samples D–H and L, with unbranched podetia and secondary squamules. The cupped or proliferating phenotypes depicted in A–C are extremely rare, as are the non-squamulose, almost entirely sorediate forms depicted in I–K. Scales = 1 cm.

ABMI collections pictured: A, on *Selaginella densa*, Grassland Natural Region (GNR), 2015, site 1458, 51.0°N, –112.1°W, 600355; B, on *S. densa*, GNR, 2012, site 1368, 51.6°N, –110.8°W, 84639; C, on *S. densa*, GNR, 2014, site 1503, 50.5°N, –111.1°W, 428022; D, on organic debris, GNR, 2012, site 1506, 50.4°N, –110.3°W, 69394; E, on *S. densa*, Parkland Natural Region, 2011, site OG-ABMI-1090-1, 53.4°N, –111.5°W, 306021; F, on soil, GNR, 2012, site 1395, 51.3°N, –110.3°W, 84652; G, on soil, GNR, 2009, site OG-ABMI-1341-1, 51.6°N, –110.6°W, 154859; H, GNR, 2012, site OG-ABMI-1498-1, 50.7°N, –112.5°W, 90376; I, on soil, GNR, 2010, site 1500, 50.6°N, –111.9°W, 251534; J, on soil, Boreal Forest Natural Region (BFNR), 2014, site 922, 54.3°N, –111.9°W, 652277; K, on soil, BFNR, 2013, site OG-ABMI-561-1, 56.5°N, –115.0°W, 76165; L, on *S. densa*, GNR, 2013, site 1519, 50.4°N, –112.0°W, 15214. Numbers reference unique records in the ABMI lichen database, latitudes and longitudes are hazed to within a 5.5 km radius of the actual site to protect site locations.

common on the lower half of the podetia (66%; Fig. 3D–H & L). Relatively large apothecia, as depicted in Brodo *et al.* (2001), were never found and small, inconspicuous apothecia or pycnidia were observed in 35 specimens (41%). The ABMI specimen from the northern Canadian Shield Natural Region was phenotypically consistent with *C. rei* confirmed in this study from eastern North America, British Columbia and Europe, albeit lacking fumarprotocetraric acid (Supplementary Material Appendix 1, available online).

### Habitat models

Similar coefficient estimates in magnitude and direction resulted from both scales of analysis (Tables 2 & 3). Here we present the site results; while the quadrant model might represent habitat at a more lichen appropriate scale, the habitat models for site level analyses are more conservative and samples are independent. Variables measured in the field, remotely derived habitat variables and geographical variables all had significant predictive capacity. The occurrence of *C. rei* was higher at more epigeic lichen rich sites, with each additional species detected increasing the likelihood of detecting *C. rei* by a factor of c. 2.7 (Tables 2 & 3). *Cladonia rei* was more common where *Selaginella densa* was recorded, supporting our observations in the laboratory. While native vascular plant richness was on average twice as high at sites with *C. rei* (Table 2), neither native nor non-native richness co-varied significantly with *C. rei* occurrence. *Cladonia rei* was more likely at sites with more extensive litter cover. It also occurred more often at grassland sites with more productive soil types, probably in part because it was also detected predominantly in pastures, typically areas utilized for their productivity. The probability of detecting *C. rei* in a pasture site were 16 times greater than that of detection in a site dominated by alienating disturbances (which alter the soil long term, including cultivated fields, industrial activity, linear features such as powerlines and roads, and human settlements). Undisturbed or 'intact' sites were also more likely to contain *C. rei* than sites with disturbance but the effect

was not as strong (10 times more likely) or significant at  $\alpha = 0.05$  (Table 3). *Cladonia rei* exhibited a quadratic relationship with soil pH, occurring at intermediate but slightly acidic pH levels; however, the mean difference in pH between occupied and unoccupied sites was small (Tables 2 & 3).

Within the regions modelled, *C. rei* was more likely to be found at intermediate to high latitudes, which corresponded to lower mean annual temperatures, colder winters and lower evapotranspiration. Geographically, *C. rei* was more likely to be found in the Northern Fescue Natural Sub-region of the grasslands, a zone that transitions to the Parkland Natural Region to the north. We found no relationship between *C. rei* presence and longitude, that is the gradient represented by the Rocky Mountains region with its colder mean monthly temperatures and higher mean annual precipitation in the west and the drier, more arid grasslands to the east. Additionally, there was no relationship between *C. rei* presence and soil total organic carbon. Figure 4 represents the extrapolated final model of *C. rei* distribution based on landscape level variables.

### Species associations

Three groups emerged from our analyses of lichen co-occurrence (Fig. 5, Supplementary Material Appendix 3, available online). Group 1 was largely composed of epiphytic and epixylic species common on deciduous *Populus balsamifera*, *P. tremuloides*, coniferous *Picea glauca* and downed wood, and included some epigeic and epixylic *Peltigera* species. Group 1 was also the most stable grouping and membership was largely robust to analytical methods. Group 2 and 3 species were more loosely structured and in general members of each group showed more positive than negative associations with each other. Group 2 contained a diverse assemblage of grassland genera, including vagrant and semi-vagrant *Cetraria* and *Xanthoparmelia* species and *Phaeophyscia constipata*, together with epigeic *Cladonia*, and epilithic *Rhizoplaca* and *Xanthoria* species. Group 3 was dominated

TABLE 2. Summary of the covariates in *Cladonia rei* habitat models as well as the ecological arguments for their inclusion. For quantitative data the range, mean and standard deviation are provided; for categorical or binary data the percentages are provided. Where applicable, summaries are presented for both quadrants (Q) and sites (S) where *C. rei* was confirmed present (181 quadrants, 86 sites) as well as not detected (927 quadrants, 191 sites).

Covariates	Hypothesized sign of the slope of relationship with p(detection) (the model-predicted probability of detecting <i>C. rei</i> )	Scale	$\bar{x} \pm 1$ S. D. (min-max) or proportion	
			<i>C. rei</i> present	<i>C. rei</i> not detected
<b>Field derived habitat predictors</b>				
Soil pH	-: previous studies found it to occur on neutral to acidic soils but others have hypothesized its native substratum is basic	Q S	6.4 ± 0.5(5.3–8.1) 6.5 ± 0.5(5.7–8.0)	6.7 ± 0.9(4.6–9.6) 6.8 ± 0.8(4.8–8.4)
Total organic soil carbon (% dry weight)	+: total organic carbon decreases when soil has been tilled, which is not conducive to epigeic lichens	Q S	3.2 ± 2.4(0.2–12.8) 3.3 ± 2.1(0.3–9.0)	3.1 ± 2.6(0.1–15) 2.9 ± 2.1(0.2–9.0)
Pasture (% area)	?: while pasture could mimic historical herbivore grazing, it is an anthropogenic disturbance often tied to increased non-native vascular plant richness and decreased biotic crust richness	Q S	82 ± 38(0–100) 79 ± 37(0–100)	40 ± 48(0–100) 33 ± 44(0–100)
Undisturbed area (% area)	-: as a pioneer may be less likely to occur in undisturbed areas	Q S	16 ± 36(0–100) 16 ± 35(0–100)	12 ± 31(0–100) 11 ± 27(0–100)
Litter cover (% area)	-: increased litter likely decreases all epigeic lichens	Q S	54 ± 26(5–95) 52 ± 23(8–93)	34 ± 27(0–95) 31 ± 23(0–91)
<b>Field derived biotic predictors</b>				
Epigeic lichen richness (number of species)	-: if a pioneer, may be less likely to co-occur with more-lichen rich communities	Q S	6 ± 3(1–15) 9 ± 4(1–21)	1 ± 2(0–12) 2 ± 3(0–14)
<i>Selaginella densa</i> (presence)	+: laboratory observations suggest <i>S. densa</i> acts as a lichen substrate	Q S	56 51	18 12
Native vascular plants (number of species)	-: if a pioneer, may be less likely to co-occur with rich native vascular plant communities	Q S	34 ± 12(6–77) 47 ± 18(8–93)	16 ± 16(0–69) 22 ± 22(0–91)
Non-native vascular plants (number of species)	+: if a pioneer, it may co-occur with non-native plants which are more common at disturbed sites	Q S	5 ± 4(0–25) 10 ± 5(1–30)	8 ± 3(0–26) 12 ± 7(0–38)
<b>Remotely derived habitat predictors</b>				
Grassland cover (% of 1 ha)	+: if common in open areas will be negatively associated with other land cover classes such as shrublands	S	76 ± 35(0–100)	23 ± 39(0–100)
Productive soil type (% of 1 ha)	-: if productive soils are more likely to be cultivated, expect it to occur on less productive soil types	S	51 ± 47(0–100)	17 ± 35(0–100)
<b>Geographic data</b>				
Natural subregions, roughly listed from north to south (categories by site) (% of sites)	?: subregions reflect a suite of biogeoclimatic variables			
	Central Parkland		16	24
	Foothills Parkland		0	3
	Northern Fescue Grass.		24	8
	Dry Mixedgrass		49	36
	Foothills Fescue Grass.		2	13
	Mixedgrass		8	17
Latitude (decimal degrees)	?: co-varies with a suite of biogeoclimatic variables	S	51.4 ± 1.0(49.0–53.6)	50.9 ± 1.3(49.0–54.1)
Longitude (decimal degrees)	?: co-varies with a suite of biogeoclimatic variables	S	-111.5 ± 0.9 (-113.4 – -110.0)	-112.2 ± 1.2 (-114.4 – -110.0)

TABLE 3. Summary of models estimating the probability of detecting *Cladonia rei* at a site ( $\alpha = 0.05$ ,  $df = 235$ , pseudo  $r^2 = 62\%$ ) and a quadrant ( $df = 1066$ , pseudo  $r^2 = 47\%$ ): variables presented are model coefficients ( $\beta$ ), standard errors (SE),  $z$ -score values,  $P$  values (Wald  $z$  statistic) and percent change in odds ratios per unit increase in significant covariates (% $\Delta$ OR). Alienating disturbances include those that alter the soil over the long term and include industrial disturbances, crop fields, residential and urbanized areas, and linear features such as roads and railways. The superscript <sup>2</sup> indicates quadratic variables included to account for polynomial relationships.

Variable	Site					Quadrant				
	$\beta$	SE	$z$ -value	$P$ -value	% $\Delta$ OR	$\beta$	SE	$z$ -value	$P$ -value	% $\Delta$ OR
Intercept	-4.5	1.6	-2.7	0.006		-4.5	0.87	-5.2	<0.001	
Field-derived habitat predictors										
Soil pH	-0.91	0.37	-2.5	0.01	15	-0.63	0.18	-3.4	0.001	20
Soil pH <sup>2</sup>	-0.16	0.31	-0.51	0.6		-0.10	0.16	-0.65	0.5	
Total organic soil carbon	-0.10	0.30	-0.33	0.7		-0.33	0.156	-2.1	0.04	27
Litter cover	0.73	0.30	2.4	0.02	76	0.37	0.14	2.6	0.01	53
Site condition levels (Alienating Disturbance = Contrast)										
Pasture	2.8	1.1	2.6	0.008	595	1.7	0.63	2.7	0.006	210
Undisturbed	2.3	1.2	1.9	0.06	353	1.3	0.69	1.9	0.05	137
Field-derived biotic predictors										
Presence <i>Selaginella densa</i>	0.62	0.30	2.1	0.04	68	0.36	0.12	3.1	0.002	53
Epigeic lichen richness	0.97	0.35	2.7	0.006	97	0.77	0.13	6.2	<0.001	79
Native vascular plants	0.00	0.02	0.011	>0.9		0.012	0.011	1.0	0.3	
Non-native vascular plants	0.32	0.33	0.98	0.3		-0.19	0.18	-1.7	0.3	
Remotely derived habitat predictors										
% Grassland cover	0.52	0.40	1.3	0.2		0.51	0.20	2.6	0.01	62
% Productive soils	0.55	0.25	2.2	0.03	64	0.27	0.12	2.2	0.03	48
Geographic data										
Natural Subregions (Parkland = Contrast)										
Dry Mixedgrass Grass.	0.91	1.4	0.68	0.5		0.054	0.70	0.076	0.9	
Foothills Fescue Grass.	0.52	1.5	0.35	0.7		-0.37	1.0	-0.37	0.7	
Mixedgrass Grass.	1.08	1.6	0.68	0.5		0.049	0.79	0.062	>0.9	
Northern Fescue Grass.	2.80	1.2	2.3	0.02	607	0.91	0.54	1.7	0.09	
Latitude	1.34	0.50	2.7	0.007	141	0.98	0.28	3.6	<0.001	98
Latitude <sup>2</sup>	-0.34	0.24	-1.4	0.2		-0.15	0.15	-1.03	0.3	
Longitude	-0.095	0.37	-0.26	0.8		0.019	0.19	0.10	0.9	

by generalist epigeic and occasionally epixylic *Cladonia* and *Peltigera* species, as well as *Cetraria arenaria*. *Cladonia rei* clustered with group 3 under both null models (Supplementary Material Appendix 3, available online, Figs 5 & 6). Within Group 3 the species with the strongest associations with *C. rei* were *C. cornuta* ssp. *cornuta*, *C. gracilis* ssp. *turbinata*, *C. multififormis*, *C. subulata*, *C. chlorophaea* and *C. pocillum*.

Five groups emerged from our analyses of vascular plant co-occurrence with *C. rei* (Table 4, Supplementary Material Appendix 3, available online). *Cladonia rei* clustered with group 1, which was a mixed ecological group including species common in mesic fescue grasslands, deciduous parklands and

dry pine (*Pinus* spp.) forests and included low shrubs (e.g. *Rosa woodsii*, *Symphoricarpus occidentalis*), grassland species (e.g. *Festuca hallii*, *Carex inops*, *Thermopsis rhombifolia*) and boreal/parkland transition plants (e.g. *Achillea millefolium*). Group 2 was characteristic of native dry mixed grasslands further south, and included *Artemisia* spp., *Selaginella densa* and many grassland specialists (e.g. *Opuntia polyacantha*, *Gaura coccinea*). Group 3 represented cultivated fields with agronomic species such as canola (*Brassica rapa*) and wheat (*Hordeum vulgare*), and weedy communities found in disturbed areas (e.g. *Matricaria discoidea*, *Capsella bursa-pastoris*), whereas group 5 contained grassland plants and exotic weeds common

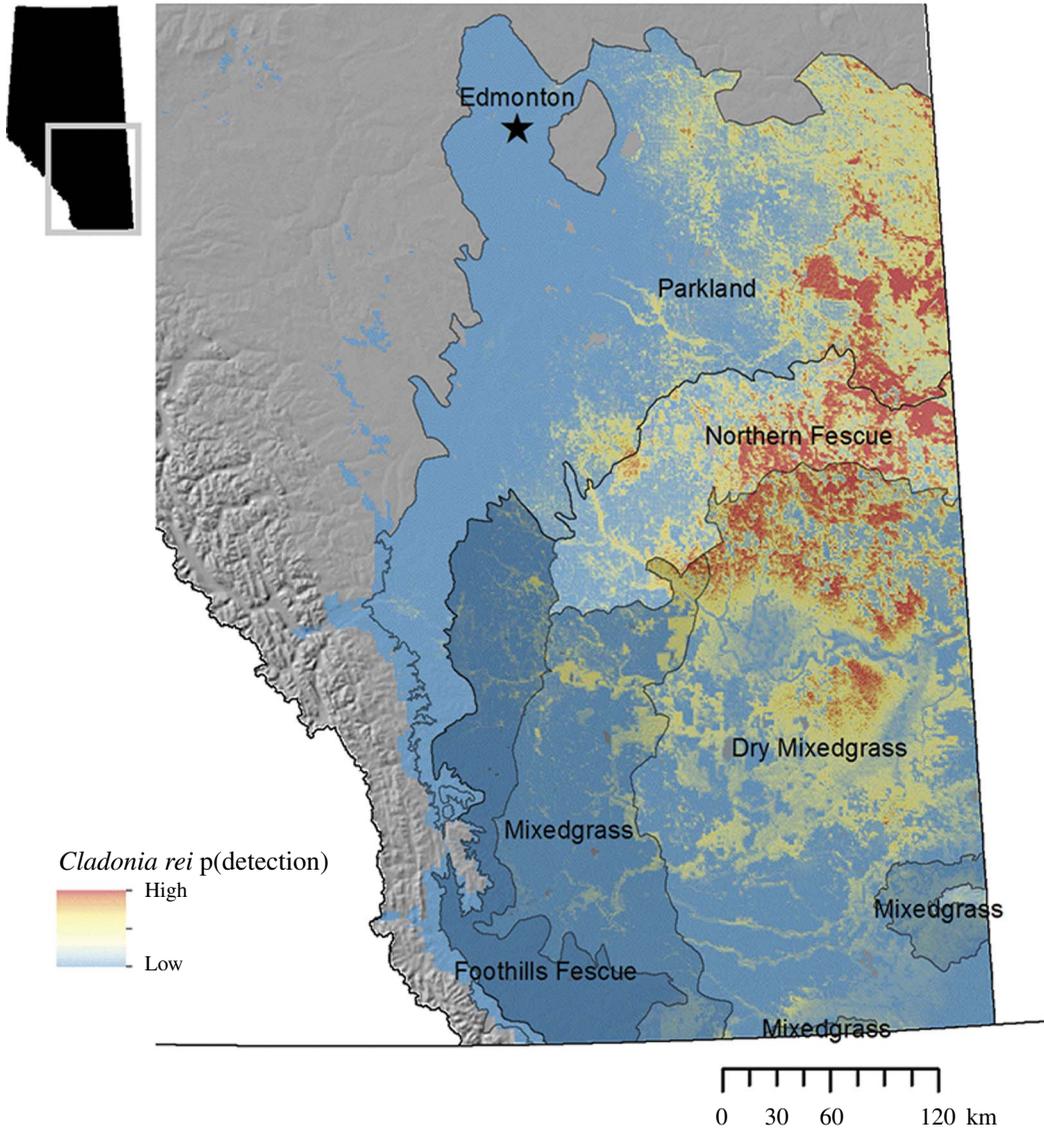


FIG. 4. Final site-scale habitat model for *Cladonia rei*, based on the landscape-level remotely-derived and geographic variables that could be extrapolated across space.  $p$  (detection) is the model-predicted probability of detecting *Cladonia rei*, from high (close to 1, coloured red) to low (close to 0, coloured blue). Model extrapolation is limited to the Grassland and Parkland Natural Regions of Alberta. Regions are labelled as either the Natural Region (Parkland) or Subregions within the Grassland Natural Region (all other labels).

in the margins of prairie and saline wetlands (e.g. *Beckmannia syzigachne*, *Taraxacum officinale*, *Rumex crispus*). Group 4 appeared to be a geographical grouping, with parkland species (e.g. *Agrimonia striata*), upland exotics (e.g. *Cirsium arvense*) and native plants (e.g. *Lathyrus venosus*) found in the dry

boreal subregions. At the individual species level, we found positive associations between *C. rei* and most plants in groups 1 and 2, negative associations with most group 3 plants, and very few significant associations with group 4 and 5 plants (Supplementary Material Appendix 3, available online).

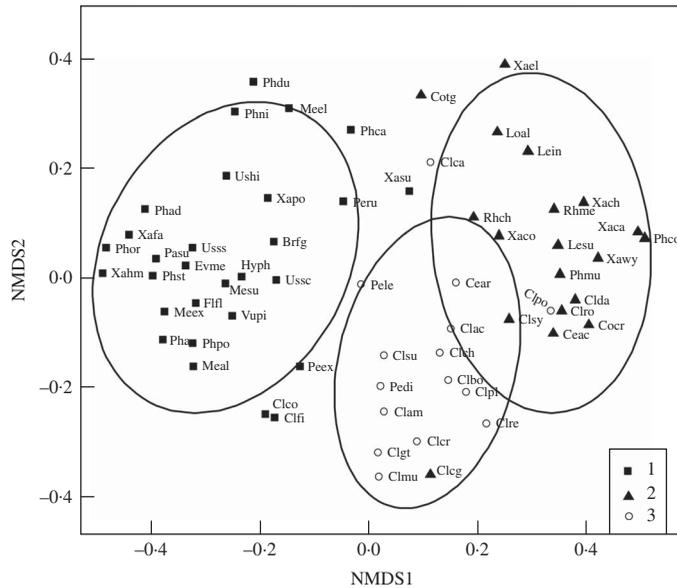


FIG. 5. Non-metric multidimensional scaling ordination plot of associations between *Cladonia rei* and other lichens from southern Alberta, as determined by the site-scale species co-occurrence analyses. Symbols are as follows: group 1 (squares), mainly epiphytic lichens common on *Populus balsamifera*, *P. tremuloides* and *Picea glauca*; group 2 (triangles), grassland genera including vagrant and semi-vagrant *Cetraria* and *Xanthoparmelia*, epigeic *Cladonia* epilithic *Rhizoplaca* and *Xanthoria*; group 3 (circles), generalist epigeic and occasionally epixylic *Cladonia* (including *C. rei*) with *Peltigera* species and *Cetraria arenaria*.

**Brfg** - *Bryoria fuscescens* grp. (includes cf. *vrangiana*), **Ceac** - *Cetraria aculeata*, **Cear** - *Cetraria arenaria*, **Clac** - *Cladonia acuminata*, **Clam** - *Cladonia arbuscula* ssp. *mitis*, **Clbo** - *Cladonia borealis*, **Clca** - *Cladonia cariosa*, **Clch** - *Cladonia chlorophaea* s.s., **Clcg** - *Cladonia chlorophaea* grp. (suite of PD-, granular sorediate species including *C. grayi*, *C. merochlorophaea*), **Clco** - *Cladonia coniocraea*, **Clcr** - *Cladonia cornuta* ssp. *cornuta*, **Clda** - *Cladonia dahliana* (psoromic acid chemotype of *C. symphylicarpa*), **Clfi** - *Cladonia fimbriata*, **Clgt** - *Cladonia gracilis* ssp. *turbinata*, **Clmu** - *Cladonia multififormis*, **Clpl** - *Cladonia pleurota*, **Clpo** - *Cladonia pocillum*, **Clre** - *Cladonia rei*, **Clro** - *Cladonia robbinsii*, **Clsu** - *Cladonia subulata*, **Clsy** - *Cladonia symphylicarpa*, **Cocr** - *Collema crispum* s. lat., **Cotg** - *Collema tenax* grp. (mostly infertile), **Evme** - *Evernia mesomorpha*, **Fifi** - *Flavopunctelia flaventior*, **Hyph** - *Hypogymnia physodes*, **Lein** - *Leptogium intermedium*, **Lesu** - *Leptogium subtile* grp. (including *L. subtile*, cf. *nanum* and *L. tenuissimum*), **Loal** - *Lobothallia alphoplaca*, **Meal** - *Melanelixia albertana*, **Mesu** - *Melanelixia subaurifera*, **Meel** - *Melanohalea elegantula*, **Meex** - *Melanohalea exasperatula*, **Pasu** - *Parmelia sulcata*, **Pedi** - *Peltigera didactyla*, **Peex** - *Peltigera extenuata*, **Pele** - *Peltigera lepidophora*, **Peru** - *Peltigera rufescens*, **Phpo** - *Phaeocalicium populneum*, **Phco** - *Phaeophyscia constipata*, **Phni** - *Phaeophyscia nigricans*, **Phor** - *Phaeophyscia orbicularis*, **Phad** - *Physcia adscendens*, **Phaa** - *Physcia aipolia* & *alnophila*, **Phca** - *Physcia caesia*, **Phdu** - *Physcia dubia*, **Phst** - *Physcia stellaris*, **Phmu** - *Physconia muscigena*, **Rhch** - *Rhizoplaca chrysoleuca*, **Rhme** - *Rhizoplaca melanophthalma*, **Ushi** - *Usnea hirta*, **Ussc** - *Usnea scabrata*, **Usss** - *Usnea substerilis* & *subfloridana*, **Vupi** - *Vulpicida pinastri*, **Xafa** - *Xanthomendoza fallax*, **Xahm** - *Xanthomendoza hasseana* & *montana*, **Xaca** - *Xanthoparmelia camtschadalis*, **Xach** - *Xanthoparmelia chlorochroa*, **Xaco** - *Xanthoparmelia coloradoensis*, **Xasu** - *Xanthoparmelia subdecipiens*, **Xawy** - *Xanthoparmelia wyomingica*, **Xael** - *Xanthoria elegans*, **Xapo** - *Xanthoria polycarpa*.

### Conservation status

Using the NatureServe Status Calculator (2015), we calculated a rank of “apparently secure” for *C. rei* in Alberta. The rank of “apparently secure” is a technical rank of S4 on a 5 point scale ranging from S1 (critically

imperilled) to S5 (secure), versus the then-assigned rank of S2. The metrics used in that calculation were as follows: area of occupancy of 100–500 4 km<sup>2</sup> grid cells, number of occurrences equal to 81–300, population size of 2500–10 000 (assuming a minimum of 25 thalli per occupied site based on field

TABLE 4. Vascular plants significantly positively or negatively associated with *Cladonia rei* in both co-occurrence models (see Supplementary Appendix 3). Asterisks (\*) indicate species recognized by Looman (1964a, b) as associates of the *Cladonietum nemoxynae* phytoassociation in Saskatchewan grasslands.

(-) Dissociates		(+) Associates			
Crops	<i>Brassica napus</i>	Exotic weeds	<i>Potentilla argentea</i>	Native forbs cont'd	<i>Potentilla hippiana</i>
	<i>B. rapa</i>		<i>Tragopogon dubius</i>		<i>P. pennsylvanica</i>
	<i>Hordeum vulgare</i>	Native forbs	<i>Achillea millefolium</i>	<i>Ratibida columnifera</i>	<i>Selaginella densa</i>
	<i>Triticum aestivum</i>		<i>Agoseris glauca</i>	<i>Silene drummondii</i>	
	<i>Phleum pratense</i>		<i>Anemone patens</i>	<i>Sisyrinchium montanum</i>	
Exotic weeds	<i>Amaranthus retroflexus</i>		<i>Antennaria parvifolia</i>	<i>Solidago missouriensis</i>	<i>Sphaeralcea coccinea</i>
	<i>Avena fatua</i>		<i>Arabis holboellii</i>	<i>Symphyotrichum ericoides</i>	<i>S. falcatum</i>
	<i>Capsella bursa-pastoris</i>	<i>Arnica fulgens</i>	<i>Thermopsis rhombifolia</i>	<i>Turritis glabra</i>	
	<i>Chenopodium album</i>	<i>Artemisia frigida</i>	<i>Xanthisma spinulosum</i>		
	<i>Cirsium arvense</i>	<i>A. ludoviciana</i>			
	<i>Echinochloa crusgalli</i>	<i>Astragalus laxmannii</i>	Native graminoids	<i>Agrostis scabra</i>	
	<i>Elymus repens</i>	<i>A. pectinatus*</i>		<i>Avenula hookeri</i>	
	<i>Fallopia convolvulus</i>	<i>Campanula rotundifolia</i>	<i>Carex inops</i>		
	<i>Galium aparine</i>	<i>Cerastium arvense</i>	<i>C. obtusata</i>		
	<i>Kochia scoparia</i>	<i>Chamaerhodos erecta</i>	<i>Bouteloua gracilis*</i>		
	<i>Matricaria discoidea</i>	<i>Comandra umbellata</i>	<i>Deschampsia cespitosa</i>		
	<i>Plantago major</i>	<i>Drymocallis arguta</i>	<i>Elymus trachycaulus</i>		
	<i>Salsola tragus</i>	<i>Erigeron caespitosus</i>	<i>Festuca hallii</i>		
	<i>Sonchus asper</i>	<i>E. glabellus</i>	<i>Hesperostipa comata</i>		
	<i>Stellaria media</i>	<i>Gaillardia aristata</i>	<i>H. curtisetia*</i>		
<i>Thlaspi arvense</i>	<i>Gaura coccinea</i>	<i>Koeleria macrantha</i>			
<i>Trifolium hybridum</i>	<i>Geum triflorum</i>	<i>Nassella viridula</i>			
Native forbs	<i>Oxytropis deflexa</i>	<i>Grindelia squarrosa</i>	<i>Pascopyrum smithii</i>		
	<i>Persicaria lapathifolia</i>	<i>Gutierrezia sarothrae</i>			
Wetland plants	<i>Gnaphalium palustre</i> <i>Typha latifolia</i>	<i>Heterotheca villosa</i>	Native shrubs and succulents	<i>Artemisia cana</i>	
		<i>Heuchera parvifolia</i>		<i>Opuntia fragilis</i>	
		<i>Lygodesmia juncea</i>		<i>Rosa woodsii</i>	
		<i>Orthocarpus luteus</i>		<i>Symphoricarpos occidentalis</i>	
		<i>Oxytropis monticola</i>			
		<i>Penstemon procerus</i>			
		<i>Phlox hoodii</i>			

observations), many occurrences with excellent or good viability, moderate generalist with some key requirements scarce, an overall low threat impact due to its ability to persist with the dominant disturbance in its range (cattle-grazing), and the low likelihood that a large percentage of current pasture land will be converted to crops in the next decade.

### Discussion

Our study suggests that large-scale monitoring employing novice collectors, combined with rigorous taxonomy, can redress pseudo-rarity due to geographically biased sampling for at least some taxonomically challenging species. With >50 000 accessioned lichen collections

in herbaria, we confirmed one *Cladonia rei* from Alberta. Conversely, samples from large-scale monitoring resulted in almost 300 collections, largely from the south-eastern quarter of the province. In combination with TLC, the ABMI samples improved our understanding of the ecology and habitat preferences of a taxonomically challenging species. These data show that *C. rei* is neither acting as a pioneer species nor commonly associating with industrial or anthropogenically altered habitats in Alberta. Instead, the habitat models and species co-occurrence analyses show that *C. rei* occupies an intermediate niche in pastures and grasslands of the deciduous parkland of central Alberta and the mixed grass and fescue grasslands

found to the south, as originally inferred by Looman (1964*a, b*). Below we explore these conclusions in more detail.

### Tackling pseudo-rarity

Previous studies have shown that non-targeted data collection by novices can provide robust community level information (McCune *et al.* 1997). Here we show that these initiatives can also provide valuable species level data, even for taxonomically challenging species that require chemical verification in the laboratory. ABMI specimens show that *C. rei* is not imperiled in Alberta as herbarium collections suggest (Government of Alberta 2014), but rather is common where it occurs. For *C. rei*, the disparity between the perceived and actual abundance is in part due to the tradition of exclusive reliance on validated reports and specimens in herbaria to assess status. Agencies are often challenged to incorporate resources such as ecological research or industrial environmental impact assessments unless collections are accessioned (Whitehead *et al.* 2015). Thus, the widespread presence of *C. rei* (as *Cladonia nemoxyna*) in the grasslands apparently first observed by Looman (1964*a, b*) while studying the neighbouring province of Saskatchewan, could not be considered because no Alberta collections were accessioned. Similarly, it is challenging for conservation agencies to incorporate ABMI data in part because of the lag between reporting and accessioning collections (this lag is common across large-scale monitoring programmes, such as the Forest Inventory and Analysis Lichen Indicator in the United States, USDA Forest Service 2017). It is vital that these programmes ensure specimens are conserved and available for verification and inevitable future taxonomic revisions to realize their full value in informing conservation.

### Documenting species' distributions and informing ecology

Geographically unbiased, large-scale sampling can provide more complete ecological gradients to test species habitat associations.

Most studies within the boreal and temperate biomes show *C. rei* to be an occupant of anthropogenic habitats such as lawns, gravel piles, road and railway embankments and industrial areas (Ahti & Stenroos 2013; Osyczka & Rola 2013; Rola *et al.* 2014; but see Looman 1964*a, b*). The large-scale monitoring dataset used here is the first to show *C. rei* to be more abundant in undisturbed grasslands and native pastures than in concurrently surveyed industrial and heavily altered anthropogenic habitats. In fact, the area occupied by *C. rei* coincides closely to a region characterized as 'High Value Landscape', or HVL, by the Prairie Conservation Forum in Alberta (ABMI 2015). The HVL has 2–3 times less anthropogenic disturbance than the area outside the HVL and is managed with the priority of preserving large patches of native vegetation and biodiversity corridors (ABMI 2015).

One hypothesis that could reconcile previous studies with our findings is that dry deciduous forest edges and grasslands are the native habitat of *C. rei* and anthropogenic habitats most closely approximate those conditions; however, some aspects of its distribution remain puzzling. For example, despite surveys across many disturbed sites, it is unclear why *C. rei* is apparently absent or rare in anthropogenic habitats in Alberta. It is also not apparent why it is absent from other grassland ecosystems in North America (e.g. the Columbia Basin grasslands of Washington and Oregon (Looman 1964*b*; Hammer 1995; McCune & Rosentreter 2007; Root *et al.* 2011) or the interior grasslands of southern British Columbia (Goward & Ahti 1997; Goward 1999; UBC 2016)). Assuming these issues are not due to taxonomic discrepancies, more data on the species' distribution and habitat affinities across its southern range (e.g. Macaronesia, East Africa, Asia and New Zealand; Archer & Bartlett 1986; Goward 1999; Ahti & Sohrabi 2006; Ahti & Stenroos 2013; Ahti *et al.* 2016) might help answer these questions. In the interim, we hypothesize that *C. rei* is absent from more southerly, open, arid grasslands because it has higher moisture requirements than many other grassland macrolichens and it has the ability to adapt to lower light levels.

For example, *C. rei* is one of only a small number of lichen species the first author has observed persisting under thick, dead grass in grassland grazing exclosures.

The large-scale, multi-taxon dataset presented here also provided the opportunity to test previous studies of *C. rei* associations. Groupings in data-driven classifications are contingent on the degree of heterogeneity in the data, the pool of species included, the completeness of the data and the spatial scale of the study (e.g. Legendre & Legendre 1998), so differences between studies are expected. It is of interest therefore that our results support the phytosociological findings of Looman in nearby temperate grasslands (1964*a, b*) more than 50 years after his surveys and despite disparity in the survey scale. Of the “recognition species” Looman (1964*a*) commonly found with *C. rei* that were also recorded by ABMI, all but two were positively associated species in our co-occurrence analyses (Fig. 6). Our co-occurring species also overlap in part with

Paus *et al.*'s (1993) *Cladonietum rei* association, common in disturbed habitat in Central Europe (Fig. 6), but aside from *C. rei*, the shared species largely behave as generalists in our region (see <http://species.abmi.ca/pages/species/lichens.html> for distribution maps of lichen species, based on ABMI collections).

Taken together, our data suggest that rather than acting as a bare soil pioneer in Alberta, *C. rei* occurs more commonly with a relatively rich epigeaic lichen community, including *Cladonia fimbriata*, *C. chlorophaea*, and *C. robbinsii* (Fig. 6). In Alberta, this community occurs primarily in the south-eastern grasslands with slightly acidic soils (supporting the earlier findings of Paus *et al.* 1993) that are relatively productive and overlain with litter, as well as on *Selaginella densa* mats.

### Grassland ecotype

In Alberta, the majority of *C. rei* specimens fit what we have tentatively called the grassland ecotype, characterized by the absence of

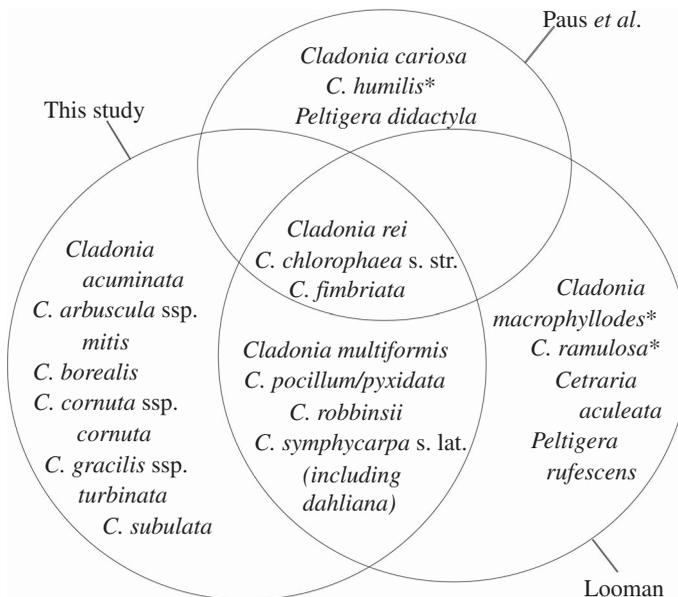


FIG. 6. Lichen species found to be positively associated with *Cladonia rei* in this study compared with those of Looman's phytoassociation *Cladonietum nemoxyinae* (1964*a*) for the grasslands of Saskatchewan, Canada and the *Cladonietum rei* of Paus *et al.* (1993) for anthropogenic habitats in Central Europe. Species within all three circles are associates in all three studies, while those included in a single circle are associates in one study only. \* = species absent from ABMI's collections.

fumarprotocetraric acid, cups or apothecia as well as sparse proliferations, a persistently corticate base, a persistent primary thallus and typically abundant secondary squamules. Considered individually, these traits have been documented by other authors (e.g. Paus *et al.* 1993; Goward 1999; Ahti & Stenroos 2013). The review of herbarium specimens and literature from North America and Europe (Fig. 3, Supplementary Material Appendix 1, available online), highlighted characteristics of *C. rei* commonly documented outside of our study area but rarely observed in the ABMI collections. These include 1) presence of fumarprotocetraric acid (60% in Pino-Bodas *et al.* (2010) and 53% Paus *et al.* (1993) vs. 0% here), 2) sparse secondary squamules and an evanescent primary thallus (35% with basal squamules in Pino-Bodas *et al.* (2010) vs. 67% here), 3) brownish soredia to the base of the podetium (38% of specimens with a corticate base in Paus *et al.* (1993)), 4) cups (forming “frequently and even on young podetia” in Ahti & Stenroos (2013) vs. 11% here), and 5) well-developed apothecia present in part (e.g. Brodo *et al.* (2001) vs. absent here). The grassland ecotype may be the result of development in the relatively shady, moist boundary layer of grassland vegetation with abundant litter, within an otherwise arid, grassland environment. The persistence of primary squamules and relatively abundant secondary squamules may optimize photosynthetic area in such habitats. It is unclear why fumarprotocetraric acid is absent from *C. rei* within our study area.

### Potential sources of error and unexplained variance

There are potential challenges when making autecological inferences from coarse-scale biodiversity surveys. The scale of ABMI surveys is 100–1500 times larger than other studies of *C. rei* communities (ABMI methods: 375 m<sup>2</sup> per plot, 100 m<sup>2</sup> per belt transect, for a total area surveyed of 1700 m<sup>2</sup> per site vs. 0.1–1.0 m<sup>2</sup> in Looman (1964a) and Rola *et al.* (2014)). Larger plots capture more microhabitats and

consequently more species, but they also capture more ecological variation and may obfuscate fine scale phytosociological or ecological patterns. ABMI sites are placed without regard to the homogeneity of the environment whereas most ecological studies place plots in homogeneous patches of vegetation. Depending on how the mean patch size of a landscape compares with ABMI’s survey area, this can introduce additional variation. A further information gap is the under-representation of the mountain Natural Region in the ABMI dataset (Table 1, Fig. 1). Historically, lichenologists have surveyed this region but survey effort likely remains an issue given that traditional haphazard surveys often don’t include all meso- and microhabitats. While all samples of putative *C. rei* from Alberta’s mountains were redetermined as other *Cladonia* species (Supplementary Material Appendix 1, available online), further sampling is required. Detection error is also likely to be unequal across sites; however, it is not likely to be driving our results given that technicians had similar levels of experience and that overall lichen detection is probably lowest in the grasslands where most *C. rei* collections were made (D. L. Haughland, pers. obs.). Finally, there is likely to be some error in our site classifications; for example, some undisturbed grassland sites might have been grazed or over-seeded historically even though the site lacks visually perceptible or remotely sensed signals of disturbance at the time of the survey.

### Recommendations

Many lichen collectors do not have ready access to TLC; it may be one of the major factors impeding taxonomic accuracy for lichenologists without laboratories. Some herbaria routinely conduct TLC on incoming challenging genera such as *Cladonia* but they appear to be in the minority as only 4% of *C. rei* specimens had TLC data in the CNALH (2016). Solutions include TLC services from a few central laboratories at a low per specimen cost; more work on spot test development for a wider variety of lichen substances (e.g. FeCl<sub>3</sub> test for homosekikaic acid (Huneck &

Yoshimura 1996)); more resources for herbaria so they can accession and TLC more material; adoption of a qualifier, such as *ante TLC*, or some analogous system to indicate specimens in taxonomically challenging groups identified without TLC, allowing that uncertainty to be incorporated into conservation ranking.

Wider use of standardized, geographically unbiased sampling would probably be beneficial in testing many ecological assumptions across longer ecological gradients; however, our aim is not to be prescriptive. Instead we outline some practices that have benefited the ABMI lichen programme. Key amongst these is the adoption of a standardized sampling method across all habitat types, focusing on effort per unit area and a standardized set of microhabitats. It is a basic premise yet one that is often challenged, even in a rapid survey, because technicians search both depauperate and biodiverse microhabitats in order to document absence as well as presence. The standardized plot size was ascertained experimentally using accumulation curves of occupied microhabitats rather than species (Haughland 2012). Lastly, we have sacrificed detailed information for individual collections to increase field sampling efficiency so that the method fits within the suite of protocols conducted by the ABMI. These practices do not negate observer effects however, and the percentage of the total species pool sampled by each technician varies (see also McCune *et al.* 1997). The benefit of inclusion within a larger monitoring programme is access to sites across the entire province and access to supporting ecological information.

## Conclusion

Understanding the spatial distribution of rare or common species is critical because of the insight it lends to studies of dispersal, gene flow, speciation rates, adaptive plasticity, threats to species from climate change or other anthropogenic activities, biodiversity richness, hotspots and conservation prioritization. North America has received more survey effort than many regions but gaps still remain in our understanding of lichen ecology and

distribution (e.g. Goward & Ahti 1997). Programmes such as the ABMI are one tool to narrow these knowledge gaps. The ABMI is unique in North America because of its systematic sampling of a diversity of habitats (both forested and unforested) and anthropogenic activities. However, programmes that share design elements exist in other areas, including the United States, United Kingdom, Switzerland, New Zealand, parts of Australia and South America (e.g. reviewed in Haughland 2012; Herzog & Franklin 2016). These programmes can circumvent comparative methodological issues, geographical sampling biases, and with concerted effort, taxonomic uncertainty, that otherwise might confound both meta-analyses and smaller scale studies documenting species autecology and assessing conservation status.

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## SUPPLEMENTARY MATERIALS

To view supplementary material for this article, please visit <https://doi.org/10.1017/S0024282918000099>

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