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Rare inland reindeer lichens at Mima Mounds in southwest Washington State

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Abstract: Isolated populations of four reindeer lichen species and varieties co-occur in a unique relict prairie habitat at Mima Mounds Natural Area Preserve, southwest Washington State, USA. The prairie is the type locality for mima mounds, unusual geologic features providing topographical variation that influences vegetation patterns. Reindeer lichens (*Cladonia* subgenus *Cladina*) are usually more typical of northern boreal regions and are very rare in inland valley habitats of the western states outside of Alaska. Our study established distributional, ecological, chemotypic, and phylogenetic information for the target species. The species that at first appeared to be *C. arbuscula* was revealed by DNA sequences to be *C. ciliata* var. *tenuis*. We found that topography was not as important as recent fire history in explaining reindeer lichen distribution; in the future, prescribed fire is likely to benefit reindeer lichens so long as it

preserves pockets of refugia as propagule sources. We also detected moderate air pollution stress, which is projected to have impacts on lichen abundances and community compositions in the near future. Chemotype analysis revealed 6 reindeer lichen chemotypes, of which 2 are rare (*C. ciliata* var. *tenuis* and *C. portentosa* subsp. *pacifica* f. *decolorans*). Phylogenetic analyses supported previous species concepts, showing *C. portentosa* is distinct from the closely-related group that includes *C. rangiferina* and two varieties of *C. ciliata*. We synthesized our findings to provide a key for distinguishing the reindeer lichens of Mima Prairie. We suggested that rare inland reindeer lichens may benefit from small prescribed burns and sowing of propagules in disturbed areas, as well as continued monitoring and designation as state sensitive species.

Key words: *Cladonia*, *Cladina*, reindeer lichens, mima mounds, prescribed fire, forest encroachment, relict prairie, air quality, rare chemotype, phylogenetics, Puget Trough

Introduction:

The integrity of rare plant populations and habitat is of prime concern at sites established for the protection of unique geologic and biological features. The mandate of the Washington Natural Heritage Program calls for rare species protection through inventorying, mapping, and safeguarding rare species habitat; natural area preserves administered by the Washington Department of Natural Resources are established for the purpose of protecting natural lands and their biota (WDNR 2011b). The conservation of locally rare lichens and other non-vascular organisms is of particular interest because so very little is understood of their biology and distribution.

At Mima Mounds Natural Area Preserve in southwest Washington State, USA, disjunct populations of four reindeer lichen species and varieties (*Cladonia* subgenus *Cladina*) co-occur, although patterns in their abundance and spatial distribution have not been fully characterized. The namesakes of Mima Mounds Natural Area Preserve are mounded geologic features which provide topographical variation that influences vegetation patterns. Patterns of vascular and non-vascular vegetation are evident across several scales relative to microtopography, height and aspect upon mounds, and position along a successional gradient (del Moral and Deardorff

1976). Multiple factors influence vegetation differently at each scale, including edaphic characters, local hydrology, solar exposure, interactions with other species, and anthropogenic changes in air quality, grazing and fire regimes. Agencies currently lack an understanding of the ecological factors affecting reindeer lichens, inhibiting efforts to effectively protect rare lichen populations.

Frequent fire events are a major factor affecting lichen dominance in boreal regions to the north of Mima Mounds, where fully mature mats of reindeer lichens may take 80 to 120 years to establish after burning (Brodo et al. 2001), although recovery may occur in as little as 30 years (Holt et al. 2008). Changes in historical fire regime at Mima Mounds are expected to have an impact on the abundance of species, although little is known regarding the interactions of fire and *Cladonia* species at nearby locations. Additionally, changes in local air quality are expected to impact reindeer lichens at Mima Mounds due to lichens' absorptive nutritional mode and potential sensitivity to substrate conditions required for establishment.

Beyond ecological requirements, we also lack a clear understanding of the systematic, chemotypic, and phylogenetic relationships of reindeer lichens in the Pacific Northwest. Reindeer lichens are notably plastic in

morphology and are chemically diverse (e.g., Ahti 1984; Huovinen and Ahti 1986). In the late 20th century, reindeer lichens were placed in the genus *Cladina*, while recent evidence has recast them as a group nested within *Cladonia* (Stenroos et al. 2002). The variable morphology (e.g., terminal branching pattern) and cryptic distinguishing characters of reindeer lichens (e.g., color of pycnidial jelly) often deter easy identification.

In this study, we had four main objectives: 1) to characterize the local abundances of four taxa which are rare or only locally common in Washington and Oregon; 2) to identify the primary factors related to variation in their local distribution; 3) to clarify phylogenetic, chemotypic, and morphological distinctions between the four taxa; and 4) to inform management of reindeer lichens at Mima Mounds. We modeled their local distribution and habitat, analyzed systematic relationships based on DNA sequences, provided web-based tools for the chemical and morphological identification of species, and analyzed their response to invasive species and disturbance, especially prescribed fire. We expected that topography and fire were the chief factors influencing lichen distribution and abundance at Mima Mounds.

Study species

Reindeer lichens (*Cladonia* subg. *Cladina* spp.) are fruticose, terricolous mat-forming lichens which inhabit mostly higher-latitude regions around the globe. Reindeer lichens occupy an array of boreal and maritime habitats in both hemispheres, ranging from stabilized sand on the coasts, boreal forests, and nutrient-poor peat bogs of North America, to the acidic heathlands and coastlines of northern Europe, New Zealand and Australia (Table 1). They attain peak dominance in boreal forests where total lichen ground cover frequently exceeds 90 percent.

Terricolous lichens play critical ecosystem roles at the air–soil interface by regulating soil moisture, reflecting light, contributing organic matter, and trapping vascular plant seeds. Reindeer lichens can potentially affect the establishment of rare or invasive plants, either positively or negatively. They also provide important forage sources for specialist consumers (Brodo et al. 2001), as a variety of ungulates and smaller mammals have diets and life cycles which are dependent upon *Cladonia*. Lichen-feeding animals are vital trophic sources for owls, hawks, wolves, and other predator species of concern. While reindeer lichens are important as food sources for animals, they may also benefit wildlife in a therapeutic manner: protease compounds from *Cladonia rangiferina* have recently been associated with degradation of the prion agent that causes transmissible spongiform encephalopathies, of which chronic wasting disease (CWD) affecting Washington deer populations is one example (Johnson et al. 2011). Although not explicitly studied, reindeer lichens at Mima Mounds are assumed to contribute similar ecosystem functions.

The occurrence of reindeer lichens outside of their normal range and in association with curious landforms raises questions about their distribution, systematics, and ecology. In Washington and Oregon reindeer lichens are mostly restricted to low-elevation coastal or Cascade foothill sites (CNALH 2011; McCune and Geiser 2009). The occurrence of each of the four taxa at Mima Mounds is surprising and unusual in valley bottom prairies.

Study Site

Mima Mounds Natural Area Preserve is a remnant prairie located 20 km southwest of Olympia, in Thurston County, western Washington State, USA. The preserve area (~237 ha) is situated on a glacial outwash plain at the southern reaches of the Puget Trough at low

elevations (60–70 m). The locality receives abundant winter precipitation and at least some fog on more than 200 days annually due to its proximity to the Pacific coast (WRCC 2009). Though the study area shows a very slight southward-sloping trend, the extensive array of mounds promotes much small-scale variation in slope and aspect (Fig. 1). Mima mounds in the preserve are geologic features of approximately 1–3 m in height and often exceeding 8–10 m in diameter; a visitor might get the impression that squads of dump trucks had deposited glacial soil loads in hundreds of regular piles across the otherwise flat plain. The flat areas between the mounds are narrow enough that it is impossible to walk more than about 30 m in a straight flat line. Mounds are composed of fine soil interspersed with glacial cobble, and although inter-mound soils are somewhat rocky, water may collect seasonally between the mounds. The entire study area is characterized by Spanaway–Nisqually type soils, originating from sandy glacial outwash or volcanic ash over gravelly outwash (NRCS 2011). While the origin of the mounds has several competing hypotheses, there is no current consensus. Potential mound-forming agents include freeze–thaw processes (Ritchie 1953), rodent activity (Cox and Hunt 1990, Dalquist and Scheffer 1942), or seismic activity (Berg 1990).

Records of historical fire at Mima Mounds cover the period 1988 to 2011. Documented fires were prescribed burns conducted in early July through September of the years 1993, 1994, 1996, 2008, and 2009. Burns were prescribed to arrest the advance of forest trees and woody shrubs such as non-native Scot's broom (*Cytisus scoparius*); this was intended to enhance habitat for rare prairie-associated animals, especially butterflies. Information for burn intensity is sparse, but the area ecologist notes that the 2009 burn occurred at moderately high intensity in mid-September of that year (D. Wilderman, personal communication 2011). Burn temperature and intensity in Pacific Northwest prairies can vary

widely within a given burn, depending on the spatial patterning of shrubs, bunchgrasses, and other fuels (Hamman et al. 2011).

The Mima Mounds flora is dominated by graminoids (native and non-native), including *Holcus lanatus*, *Anthoxanthum odoratum*, and species of *Agrostis*, *Festuca* and *Carex*. Dense patches of mosses occur intermittently and include species of *Racomitrium*, *Dicranum*, and *Polytrichum*. The native woody sub-shrub *Arctostaphylos uva-ursi* (bearberry) occasionally forms extensive closed mats in eastern portions of the study area among remnant tree stumps; many trees (mostly *Pseudotsuga menziesii*) have been felled in recent decades in a successful effort to reverse forest encroachment onto the prairie. Non-native *Cytisus scoparius* is nearly ubiquitous in the shrub layer across the site but has also been much reduced by management activities including cutting, burning, and herbicide applications. The lichen flora of the site includes at least eighteen *Cladonia* taxa, of which four are the subject of this study: *Cladonia ciliata* (two varieties), *C. portentosa*, and *C. rangiferina*. *Cladonia ciliata* here includes *C. ciliata* s. str. which lacks usnic acid, and the usnic-containing chemotype *C. ciliata* var. *tenuis* (Flörke) Ahti in Poelt & Vězda (Ahti 1984). One other regionally rare *Cladonia*, *C. novochlorophaea* (Brodo and Ahti 1996), is also known from the mounds (Hennings 1310, 1383, DUKE!; Hammer 1995 as *C. merochlorophaea* var. *novochlorophaea*), and was relocated in 2009 (McCune 29914, OSC; Nelson 4249, herb. Nelson). Nomenclature follows McCune and Geiser (2009) for lichens, Norris and Shevock (2004) for bryophytes, and Hitchcock and Cronquist (1973) for vascular plants.

Materials and Methods:

Field Methods

Five teams (2-3 technicians each) sampled four target species and varieties of reindeer lichens (Table 1) at Mima Mounds Natural Area Preserve

in late April and early May of 2011. We arbitrarily selected specimens for chemotypic and phylogenetic analyses. Vouchers are in OSC and McCune's research herbarium.

We used a restricted random sampling design to quantify lichen presence; the field team sampled 106 plots at random intervals along 12 parallel transects that ran north-south through the whole preserve (Fig. 2). Transects were separated by 100 m. Each plot was a circle with a radius of 3 m (area 28.3 m²), yielding a total surveyed area of 299.7 m². Within plots, we recorded presence/absence of the four target taxa in addition to trees and shrubs, and noted the presence or absence of *Arctostaphylos* and *Cytisus*. We also recorded abiotic factors, including aspect in relation to mounds, and noted any evidence of burning or tree cutting (stumps).

For the air quality analysis, we conducted an ocular survey of all epiphytic macrolichens in a plot approximately 140 m south of the public parking area at the preserve (Fig. 2), following the protocol of McCune and Geiser (2009: xxvii). The circular plot (radius 40 m) was established in a *Pseudotsuga menziesii*–*Acer circinatum*–*Gaultheria shallon* forest (46.903°, -123.047°; elev. 74 m). The western plot boundary was 4 m from the prairie–forest boundary.

We generated all maps and plot coordinates using the NAD83 geodetic reference system and ArcGIS 9.3.1 (ESRI, Redlands, CA 2009). Additionally, we included aerial imagery (WAGDA 1998), and fire boundaries (WDNR 2011a). We deposited all data files and GIS layers in Scholars Archive at Oregon State University (<http://hdl.handle.net/1957/25765>).

Laboratory methods

For phylogenetic analyses we extracted, amplified, and sequenced DNA from two-week old specimens of each of the four target taxa (Table 2). To extract DNA, we used the Fast Prep protocol (MP Biomedicals, Solon, OH, USA). We

ground lichen thalli with a drill in 200 µL of CIS-VF buffer, then added approximately 300 µL of CIS-VF buffer to attain a total volume of 500 µL. We transferred samples to tubes for two Fast-Prep machine cycles of 20 seconds (s) each. Samples were then incubated for 1 hour at 55–65°C and centrifuged for 6 min at 12 g (where 1 g = 1 equivalent standard gravitational acceleration). Supernatant was transferred into new centrifuge tubes and approximately equal volumes of chloroform were added. After thorough mixing, samples were centrifuged for 10 min at 12 g. We then transferred 300 µL of supernatant for each sample to new centrifuge tubes, and removed contaminants using the GeneClean/GlassMilk Procedure (MP Biomedicals, Solon, OH, USA). First we added 900 µL of NaI solution and 10 µL of GlassMilk, mixed, and then incubated samples for 5 min at room temperature before centrifuging again for 1 min at 12 g. We then discarded the supernatant and resuspended the pellet in 500 µL of New Wash before centrifuging samples for 20 min at 12 g; we repeated this step one time. After removing and discarding the final supernatant, the pellet was dried in a 55°C oven for 1 hour. To elute DNA, we added 30 µL of water to each sample and centrifuged for 30 s at 12 g. Eluted product was transferred to fresh tubes and freezer-stored.

To amplify the ITS1–5.8S–ITS4 region, we used the primers ITS1-F (fungal-specific) and ITS4 (White et al. 1990). We used 2 µL of DNA extraction for amplification using the GenScript TAQ kit (GenScript USA Inc., Piscataway, NJ, USA), and we followed the RPB2A thermocycling protocol for all samples: 94°C for 120 s (1x), 94°C for 40 s (10x), 52°C for 45 s (1x), 72°C for 150 s (1x), 94°C for 40 s (35x), 47°C for 45 s (1x), 72°C for 150 s (1x), and finally 72°C for 60 s (1x). Following thermocycling, we cleaned 25 µL of amplified product using the QIAquick PCR purification kit (Qiagen, Valencia, CA, USA). Product content was quantified using a Qubit fluorometer and the Quant-iT dsDNA BR assay

kit (Invitrogen, Eugene, OR, USA). We sequenced dsDNA with the Sanger direct sequencing method using the BigDye Terminator 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) with the ABI Prism 3730 Genetic Analyzer (Applied Biosystems); all sequencing was completed at the Center for Genome Research and Biocomputing (CGRB, Oregon State University, Corvallis, OR, USA).

For the secondary chemistry analysis of the four target species, we followed the thin-layer chromatography (TLC) procedure of Culberson et al. (1981), using solvent systems C and B' (Culberson and Johnson 1982). Aluminum-backed silica gel plates (Merck 5554/7 Silica gel 60 F254) were cut in half and spotted with lichen substances extracted in acetone at room temperature. We used at least three specimens from each of the four target taxa.

Data Analysis

We used nonparametric multiplicative regression (NPMR) in HyperNiche 2 (MjM Software, Glenden Beach, OR, USA, 2004) to regress lichen occurrence against multiple environmental variables in 106 plots. We modeled the local mean with a Gaussian kernel, using the “free search” option to maximize the fit, as expressed in a \log_{10} -likelihood ratio (logB). This likelihood ratio compares the fitted model with a naive model where the probability of occurrence is, for every site, the overall frequency of occurrence. Model fitting optimizes selection of variables and their tolerances (smoothing parameters). Tolerances define how broadly the estimate for a given point is based on the surrounding sample space. Models were constructed with a forward stepwise procedure, avoiding overfitting by using leave-one-out cross-validation in the model fitting phase, along with the default controls for minimum average neighborhood size, minimum improvement in fit, and minimum ratio of data

points to predictors. We retained the best model for each species for each number of predictors up to the stopping point as determined by the overfitting controls. Supporting figures were plotted with the software R 2.14.1 (R Development Core Team 2011).

Our phylogenetic analysis combined our sequences with selections from GenBank (Table 2). These included some closely related North American species not known from Mima Mounds (*C. mitis* and *C. arbuscula*), along with *C. uncialis* as an outgroup. We aligned sequences with Geneious Pro 5.4.3 with a 65% similarity cost matrix, gap open penalty, and gap extension penalty of 12 and 3, respectively, followed by minor manual adjustment, then trimmed to full length ITS1, 5.8S, and ITS2 sequences, leaving 589 sites, with some samples having a short section of missing data at one end. We then used Geneious Pro with PhyML (Guindon and Gascuel 2003) to construct a maximum likelihood tree using a GTR substitution model, a transition/transversion ratio of 4, and 1,000 bootstrap replications.

For the air quality analysis, we estimated annual nitrogen deposition rates following the method of McCune and Geiser (2009: *xxvii* and Table 2 therein). In this method, species found in the study plot are assigned to oligotrophic and eutrophic categories; mesotrophic species are ignored. Nitrogen deposition is then estimated by averaging the peak nitrogen deposition values for the remaining species.

Based on our results from examining all of the available evidence, we produced a field guide for target species at Mima Mounds (<http://lichens.science.oregonstate.edu/Mima/lichenkeys.html>). This website also provides information on other species present at Mima Mounds, as well as further background for our study.

Results:

Ecological correlates of abundance

Two reindeer lichen taxa (*Cladonia portentosa* and the usnic chemotype of *C. ciliata*) were distributed widely across the study area; two taxa (*C. rangiferina* and the usnic-deficient *C. ciliata*) were distributed widely but occurred less frequently (Fig. 3; Table 3). Fires did not appear to differentially affect study species. Each burn varied in season and extent (Fig. 4), and the most recent fire had very low overall reindeer lichen frequency; on the other hand, areas that were burned from 1993 to 1996 had frequent and large reindeer lichen patches in 2011 (Fig. 5).

Reindeer lichens were more frequent on north and east sides of mounds and less frequent on south and west sides of mounds (Fig. 6); furthermore, field crews noted apparently higher abundance of the target species on north sides of mounds. However, we found no statistically significant support for our expectation that topography would be an influential predictor of lichen occurrence, and we observed only weak relationships between the other measured environmental predictors and occurrence of the study species (Table 3). Presence of live *Cytisus* was weakly associated with a lower occurrence of one species, the usnic chemotype of *Cladonia ciliata* ($p = 0.042$; Table 3).

Phylogeny and identification

Our DNA sequence data demonstrated that the usnic acid-containing species from Mima Mounds (other than *C. portentosa*) was *C. ciliata* var. *tenuis*, rather than *C. arbuscula* as we had initially assumed. Monophyletic groups were strongly supported for *Cladonia portentosa* and *C. ciliata* (Fig. 7). *Cladonia arbuscula* and *C. mitis* were closely-related sister groups (represented here only by GenBank sequences); and *C. ciliata* and *C. rangiferina* likewise were sister groups (Fig. 7).

Cladonia ciliata var. *tenuis* (containing usnic acid) and *C. ciliata* var. *ciliata* (lacking usnic acid) obviously differed in color in the field (Fig. 8). However, they were not clearly resolved in the phylogenetic tree, rather falling intermixed into a single monophyletic group (Fig. 7).

Chemotypes

We detected a total of six chemotypes: one chemotype in *C. rangiferina*, two for *C. portentosa*, and three for *C. ciliata* (Table 4). The latter two species each show an unusual chemotype; perlatolic acid unaccompanied by usnic acid in *C. portentosa* (*C. portentosa* subsp. *pacifica* f. *decolorans* (Ahti) Ahti; Huovinen and Ahti 1986) and fumarprotocetraric acid unaccompanied by protocetraric acid in *C. ciliata* var. *ciliata* (Sample 15 in Fig. 9). Protocetraric acid is usually lower in concentration than fumarprotocetraric acid in *C. ciliata*, and is often present in only trace amounts (Huovinen and Ahti 1986), although we detected it easily with TLC (Fig. 9). The absence of protocetraric acid in *C. ciliata* is, therefore, perhaps a result of detection limits of TLC rather than a clear qualitative difference.

Based on differences in microchemistry, spot tests can be used to identify the four reindeer lichen taxa described herein, including the usnic-present vs. usnic-absent chemotypes of *C. ciliata* (Table 4). In practice, we found the P test (*p*-phenylenediamine) to be unambiguous for separating *C. portentosa* from the other species, while the K test (potassium hydroxide) for separating *C. rangiferina* from *C. ciliata* was less distinct. Morphologically, however, *C. rangiferina* was distinguishable in the field from usnic-deficient *C. ciliata* by the coarser appearance of *C. rangiferina*, grayish rather than purplish brown tones when solarized, and its tendency to have secund branch tips (i.e., swept in one direction).

Air quality

We observed 17 epiphytic macrolichen species in our plot in the adjoining forest. Many of these species are eutrophic, known to be tolerant of elevated nitrogen concentrations (McCune and Geiser 2009). The estimated average nitrogen deposition value for our forest plot adjacent to Mima Mounds was $4.14 \text{ kg ha}^{-1} \text{ yr}^{-1}$ (70% confidence interval $\pm 1 \text{ kg ha}^{-1} \text{ yr}^{-1}$). We also observed morphologies associated with elevated nitrogen and moderate air pollution stress. The most abundant species (present on greater than 50% of trees) was *Platismatia glauca*, which frequently displayed hyper-production of reproductive propagules. Many *Usnea* and *Hypogymnia* individuals exhibited reduced, thickened thalli. *Usnea* individuals appeared stunted, with compact, dense branching. Many *Usnea* also exhibited a reddish-brown tint or had lost their cortex, leaving only the medullary cord. One individual of *Menegazzia subsimilis* supported abundant external algal growth.

Discussion: Many factors determine patterns of reindeer lichen distribution at Mima Mounds. The evidence supported our expectation that fire would be a major influence on Mima Mounds reindeer lichens. The recent reintroduction of fire has helped to curtail the successional advance of woody shrubs and trees; terricolous lichens likely benefit from low-intensity, patchy burns that maintain open grassy areas, prevent conversion to forest, and prevent displacement of lichens by mosses. Small unburned prairie patches likely serve as propagule sources for recolonization by lichens.

In the only other study of prescribed fire effects on reindeer lichens in the Pacific Northwest, Holt and Severns (2005) found nearly complete loss of reindeer lichens following fire, although some patchy survival occurred. Similarly, a nearby area not open to the public has had frequent and thorough prescribed fires and supports very few reindeer lichens (J. Ponzetti, personal communication 2012). In contrast, we found that

reindeer lichens seemed to recover substantially in 15 years following less extensive prescribed fires, though we lacked pre-fire measures of abundance for comparison. While our findings suggest that occasional prescribed fire does not fully eradicate rare reindeer lichens, we caution against over-simplifying the inference. Indeed, Holt and Severns (2005) concluded that “maintenance of fire refuges for *Cladina mitis* is essential for future survival of extant colonies and as a source population for lichen colonization.” At Mima Mounds, as in the Willamette Valley wetlands of Holt and Severns (2005), prescribed burning is needed to retain the open prairie vegetation. With this in mind, we suggest that fire prescriptions should avoid widespread, homogenous, high-intensity burning which could retard the regeneration of lichen mats by several decades (Johansson and Reich 2005; Snytkin 1996). Future monitoring of reindeer lichen response to fire is needed to assess pre- and post-fire abundance; studies might also benefit from measuring lichen cover rather than presence–absence as in our study.

Although we expected that local-scale distributions of the four reindeer lichen taxa would be associated with microtopography, this relationship did not emerge strongly. The continued future viability of reindeer lichen populations at inland valley sites may, however, rely on variations in mound topography that regulate moisture loss and direct solar radiation. The mounds create variability in moisture of fuels during prescribed burning, with the wetter areas presumed to result in lower fire intensity and greater pockets of lichen survival. Furthermore, there may be direct physiological effects of mound topography on lichens. Near the south edge of their ranges, northern species of reindeer lichens may not tolerate heat loads and desiccation on exposed southern aspects. If temperatures in the region increase, the consistently cooler, moister north sides of mounds may act as microrefugia for reindeer lichens at Mima Mounds.

Causes for the landscape-scale distributions of these isolated reindeer lichen populations are even less clear. Dispersal limitations and agricultural, urban, and suburban development may account for the isolation of the Mima Mounds populations. Sexual reproductive structures (apothecia) were not observed for the four taxa at this locality, and are generally absent even in more favorable habitats; specialized reproductive propagules (when found elsewhere) are relatively small and are more likely to disperse long distances than asexual thallus fragmentation. Dispersal limitation may also explain why we did not find three other Pacific Northwest reindeer lichens (*Cladonia arbuscula*, *C. mitis*, and *C. stygia*) at Mima Mounds, despite their having habitat requirements similar to the study species.

We noted extensive rodent activity on mound-tops; if fragmentation is the primary means of reproduction within the Mima site, rodents and other small mammals may play a role in extending the range of fragment dispersal over moderate distances. Given this scenario, ideal management practices for lichens would seek to maintain not only the viability of lichen populations, but also that of small mammals at Mima Mounds.

It is possible that reindeer lichens were historically more frequent in the Puget Sound region, but were reduced by recent land use changes. Fire suppression in minimally managed areas and consequent afforestation would reduce habitat for reindeer lichens. Conversion of prairies to agricultural, urban, and suburban land uses further restricts available habitat. Near Mima Mounds, habitat has been severely limited by direct destruction of mounds, as many have been flattened, quarried, or built upon.

Air quality has also had an impact on epiphytic lichen communities in both urban and forest systems in the Puget Sound region (Geiser and Neitlich 2007; Johnson 1979). Our findings are

consistent with moderate nitrogen deposition and moderate air pollution stress at Mima Mounds. The altered morphologies of many individuals indicate environmental eutrophication from atmospheric deposition. Continuously increasing nitrogen deposition, in the form of nitrogen oxides and ammonia, is of particular ecological concern for lichens because of their absorptive mode of nutrition and pH-dependent establishment. Mima Mounds is located in an area previously evaluated as having exceeded the critical load value for nitrogen deposition, defined as the maximum deposition level at which the maintenance of a relatively steady ecosystem state is possible (Geiser et al. 2010). Although these air pollution bioindication protocols were not specifically constructed for use in terricolous lichen communities, we propose that further deterioration of air quality may negatively affect the abundance of reindeer lichens at Mima Mounds.

Our phylogenetic reconstruction supported groups that accord with accepted taxa. Other recent multiple-loci analyses (for example, those of the many subspecies of *C. arbuscula* at other sites) showed much genetic variation which was not confined within subspecific taxa (Myllys et al. 2003; Piercey-Normore et al. 2010). Similarly, Stenroos et al. (2002) suggested that the delineation of *C. ciliata* and *C. rangiferina* into separate clades within subgenus *Cladina* should be abandoned, a view supported by our finding that these two are highly related sister groups.

Four of six chemotypes that we observed accord with those commonly stated in the literature, while two are relatively rare. Whether the local chemotypic variation within species is ecologically or geographically patterned is unknown. However, some chemical variation within species has previously been associated with differences in macroclimate, as compared between oceanic and inland habitats (Goward and Ahti 1997).

In the field, we were able to distinguish the four species and varieties of reindeer lichens at Mima Mounds. A more challenging problem, however, is field separation of the two chemically identical and morphologically similar species *C. arbuscula* (which we did not find at Mima Mounds) and *C. ciliata* var. *tenuis*. Because of their similarity, it is quite likely that *C. ciliata* var. *tenuis* has been misreported in western North America as *C. arbuscula*, a common and circumboreal species. Despite those similarities, the two are clearly distinct in our phylogenetic reconstruction (Fig. 7). *Cladonia ciliata* has red pycnidial jelly (which may be difficult to find!) and branching in pairs, while *C. arbuscula* has clear pycnidial jelly and more frequent branching in threes or fours (Ahti 1961). Prof. T. Ahti (personal communication 2011) suggested the following method:

“In the absence of pycnidial jelly I simply try to estimate (count of e.g. 20 branchings) the percentage of pairs. Also, under the microscope several successive very distinct pairs on older axes are diagnostic for *C. ciliata*. And *C. ciliata* is more slender in habit and the tips tend to be more nutant (drooping). Also the red P+ reaction of *C. ciliata* tends to be more instant (higher fumarprotocetraric acid contents). Using several characters I can usually identify a specimen, but difficult specimens (especially if small) do exist.”

In conclusion, we found four regionally rare to uncommon reindeer lichen taxa, *Cladonia ciliata*, *C. ciliata* var. *tenuis*, *C. portentosa*, and *C. rangiferina*, at Mima Mounds Natural Area Preserve. These taxa are abundant, conspicuous components of this unique prairie. Based on the rarity of these four taxa in the Willamette-Puget trough, we recommend that they be categorized as “sensitive” on the Washington Natural Heritage Program list, which currently lists only *Cladonia portentosa* as “recommended for monitoring” (WNHP 2010). Furthermore, we recommend that permanent plot monitoring be employed in order to maintain viable populations

at this site. Monitoring permanent plots would track changes in the abundance of these species that could be due to human intervention, interspecific competition, and ongoing natural successional processes.

Key to reindeer lichens known or expected to occur at Mima Prairie, Washington

- 1a. KC+Y (usnic acid present); lichen has yellowish tint; mostly common at Mima Prairie
 - 2a. P-, K-, UV+; color a clear pale yellowish green, sometimes with slightly brownish tips; branching dichotomous so that a main axis is not apparent; sometimes forming colonies like heaps of cotton-puffs
..Cladonia portentosa subsp. pacifica
 - 2b. P+R, K-, UV-; color darker, often brownish to violet tinged from above, may or may not have brown tips; branching lateral from a main axis; colonies never appearing like heaps of cotton
 - 3a. Branching slender, terminal branches in pairs (sometimes drooping); pycnidial jelly red.....
**Cladonia ciliata var. tenuis**
 - 3b. Branching robust, in terminal 3's or 4's; pycnidial jelly clear; occurrence possible but not currently documented at Mima Prairie.....**Cladonia arbuscula**
- 1b. KC- (lacking usnic acid); lichen not yellow, instead brownish or gray (though side view sometimes faintly yellowish green when moist); occasional to rare at Mima Prairie
 - 4a. Strong P+R and K+Y; relatively coarse, tall with a main stalk axis, branch tips often swept in one direction; color whitish to grayish (violet-tinged dark gray).....**Cladonia rangiferina**
 - 4b. P+R, K-; more finely and densely branched than *C. rangiferina*, branch tips not turned in one direction; color dark brown to grayish brown; rare.....**Cladonia ciliata var. ciliata**

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Literature cited

- Ahti, T. 1961. Taxonomic studies on reindeer lichens (*Cladonia*, subgenus *Cladina*). *Annales Botanici Societatis Zoologicae Botanicae Fennicae 'Vanamo'* 32(1): i–iv + 1–160.
- Ahti, T. 1984. The status of *Cladina* as a genus segregated from *Cladonia*. *Nova Hedwigia*, Beihefte 79:25–61.
- Berg, A. W. 1990. Formation of Mima mounds: A seismic hypothesis. *Geology* 18:281.
[http://dx.doi.org/10.1130/0091-7613\(1990\)018<0281:FOMMAS>2.3.CO;2](http://dx.doi.org/10.1130/0091-7613(1990)018<0281:FOMMAS>2.3.CO;2)
- Brodo, I. M. and T. Ahti. 1996. Lichens and lichenicolous fungi of the Queen Charlotte Islands, British Columbia, Canada. 2. The *Cladoniaceae*. *Canadian Journal of Botany* 74:1147–1180.
<http://dx.doi.org/10.1139/b96-139>
- Brodo, I. M., S. D. Sharnoff and S. Sharnoff. 2001. *Lichens of North America*. Yale University Press, New Haven.
- Christensen, S. N. and I. Johnsen. 2001. The lichen-rich coastal heath vegetation on the isle of Anholt, Denmark – description, history and development. *Journal of Coastal Conservation* 7:1–12.
<http://dx.doi.org/10.1007/BF02742462>
- CNALH. 2011. Dynamic Checklists: Taxon filter: *Cladoniaceae*. Retrieved May 12, 2011, from web site: Consortium of N. Amer. Lichen Herbaria.
<http://symbiota.org/nalichens/checklists/dynamicmap.php?interface=checklist>.
- Cox, G. W. and J. Hunt. 1990. Form of Mima mounds in relation to occupancy by pocket gophers. *Journal of Mammalogy* 71(1):90–94.
<http://dx.doi.org/10.2307/1381323>
- Culberson, C. F., W. L. Culberson and A. Johnson. 1981. A standardized TLC analysis of β -orcinol depsidones. *Bryologist* 84(1):16–29.
<http://dx.doi.org/10.2307/3242974>
- Culberson, C. F. and A. Johnson. 1982. Substitution of methyl tert-butyl ether for diethyl ether in the standardized thin-layer chromatographic method for lichen products. *Journal of Chromatography* 238:483–487.
[http://dx.doi.org/10.1016/S0021-9673\(00\)81336-9](http://dx.doi.org/10.1016/S0021-9673(00)81336-9)
- Dalquist, W. W. and V. B. Scheffer. 1942. The origin of the Mima Mounds of western Washington. *Journal of Geology* 50:68–84.
<http://dx.doi.org/10.1086/625026>
- del Moral, R. and D. C. Deardorff. 1976. Vegetation of the Mima mounds, Washington State. *Ecology* 57(3):520–530.
<http://dx.doi.org/10.2307/1936436>
- Ferry, B. W. and E. Lodge. 1996. Distribution and succession of lichens associated with *Prunus spinosa* at Dungeness, England. *Lichenologist* 28(2): 129–143.
- Geiser, L. H., S. E. Jovan, D. A. Glavich, and M. K. Porter. 2010. Lichen-based critical loads for atmospheric nitrogen deposition in Western Oregon and Washington Forests, USA. *Environmental Pollution* 158:2412–2421.
<http://dx.doi.org/10.1016/j.envpol.2010.04.001>

- Geiser, L. H. and P. N. Neitlich. 2007. Air pollution and climate gradients in western Oregon and Washington indicated by epiphytic macrolichens. *Environmental Pollution* 145:203–218.
<http://dx.doi.org/10.1016/j.envpol.2006.03.024>
- Goward, T. 1999. *The Lichens of British Columbia: Illustrated Keys: Part 2 – Fruticose Species*. Ministry of Forests Research Program. Crown Publications, Victoria, British Columbia.
- Goward, T. and T. Ahti. 1997. Notes on the distributional ecology of the *Cladoniaceae* (lichenized ascomycetes) in temperate and boreal western North America. *Journal of the Hattori Botanical Laboratory* 82: 143–155.
- Guindon, S. and O. Gascuel. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* 52(5): 696–704.
<http://dx.doi.org/10.1080/10635150390235520>
- Hamman, S. T., P. W. Dunwiddie, J. L. Nuckols and M. McKinley. 2011. Fire as a restoration tool in Pacific Northwest prairies and oak woodlands: challenges, successes, and future directions. *Northwest Science* 85(2):317–328.
<http://dx.doi.org/10.3955/046.085.0218>
- Hammer, S. H. 1995. A synopsis of the genus *Cladonia* in the northwestern United States. *Bryologist* 98:1–28.
<http://dx.doi.org/10.2307/3243636>
- Hitchcock, C. L., and A. Cronquist. 1973. *Flora of the Pacific Northwest: An illustrated manual*. University of Washington Press, Seattle.
- Holt, E. A. and P. M. Severns. 2005. The effects of prescribed burning on wet prairie lichen communities. *Natural Areas Journal* 25:130–136.
- Holt, E. A., B. McCune and P. Neitlich. 2008. Grazing and fire impacts on macrolichen communities of the Seward Peninsula, Alaska, U.S.A. *Bryologist*. 111:68–83.
[http://dx.doi.org/10.1639/0007-2745\(2008\)111\[68:GAFIOM\]2.0.CO;2](http://dx.doi.org/10.1639/0007-2745(2008)111[68:GAFIOM]2.0.CO;2)
- Huovinen, K. and T. Ahti. 1986. The composition and contents of aromatic lichen substances in the genus *Cladina*. *Annales Botanici Fennici* 23:93–106.
- Johansson, P. and P. B. Reich. 2005. Population size and fire intensity determine post-fire abundance in grassland lichens. *Applied Vegetation Science* 8(2):193–198.
<http://dx.doi.org/10.1111/j.1654-109X.2005.tb00645.x>
- Johnson C. J., J. P. Bennett, S. M. Biro, J. C. Duque-Velasquez, C. M. Rodriguez, R. A. Bessen, and T. E. Rocke. 2011. Degradation of the disease-associated prion protein by a serine protease from lichens. *PLoS ONE* 6(5): e19836.
<http://dx.doi.org/10.1371/journal.pone.0019836>
- Johnson, D. W. 1979. Air pollution and the distribution of corticolous lichens in Seattle, Washington. *Northwest Science* 53:257–263.
- McCune, B., and L. Geiser. 2009. *Macrolichens of the Pacific Northwest*. 2nd ed. Oregon State University Press, Corvallis.
- Myllys, L., S. Stenroos, A. Thell, and T. Ahti. 2003. Phylogeny of bipolar *Cladonia arbuscula* and *Cladonia mitis* (Lecanorales, Euascomycetes). *Molecular Phylogenetics and Evolution* 27(1):58–69.
[http://dx.doi.org/10.1016/S1055-7903\(02\)00398-6](http://dx.doi.org/10.1016/S1055-7903(02)00398-6)
- Norris, D. H. and J. R. Shevock. 2004. Contributions toward a bryoflora of California: II. A key to the mosses. *Madroño* 51(2):131–269.
- NRCS. 2011. Web Soil Survey. Retrieved May 12, 2011, from web site: Natural Resources

Conservation Service, United States Department of Agriculture.

<http://websoilsurvey.nrcs.usda.gov/>

Piercey-Normore, M. D., T. Ahti, and T. Goward. 2010. Phylogenetic and haplotype analyses of four segregates within *Cladonia arbuscula* s.l. *Botany* 88:397–408.

<http://dx.doi.org/10.1139/B10-027>

R Development Core Team. 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Web site: <http://www.R-project.org>.

Ritchie, A. M. 1953. The erosional origin of the mima mounds of southwest Washington. *The Journal of Geology* 61(1):41–50.

<http://dx.doi.org/10.1086/626035>

Snytkin, G. V. 1996. Fire in Ecosystems of the Far Northeast of Siberia. Pp. 170–210 in: J. G. Goldammer and V. V. Furyaev, eds. *Fire in Ecosystems of Boreal Eurasia*. Kluwer Academic Publishers, Netherlands.

Stenroos, S., J. Hyvönen, L. Myllys, A. Thell, and T. Ahti. 2002. Phylogeny of the genus *Cladonia* s.lat. (*Cladoniaceae*, Ascomycetes) inferred from molecular, morphological, and chemical data. *Cladistics* 18:237–278.

<http://dx.doi.org/10.1111/j.1096-0031.2002.tb00151.x>

WAGDA. 1998. Washington State Geospatial Data Archive. Retrieved April 13, 2011, from web site: Washington State Geospatial Database, University of Washington Libraries.

http://wagda.lib.washington.edu/data/geography/wa_state/

WDNR. 2011a. Available GIS Data: Wildfire and Prevention. Retrieved April 23, 2011, from web site: Washington Dept. of Natural Resources. <http://fortress.wa.gov/dnr/app1/dataweb/dmmatrix.html>

WDNR. 2011b. Supplement to 2011 Natural Heritage Management Plan: Land Management Designations: Their Role in Protecting Natural Biological Diversity in Washington. Retrieved May 12, 2011, from web site: Washington Department of Natural Resources.

http://www1.dnr.wa.gov/nhp/refdesk/plan/land_mgmt_desig.pdf

WRCC. 2009. Climatological Summary, Olympia Airport (KOLM) Washington. Retrieved May 12, 2011, from web site: Western Regional Climate Center, Desert Research Institute, Reno, Nevada. <http://www.wrcc.dri.edu/summary/olm.wa.html>

White, T. J., T. Bruns, S. Lee, and J. W. Taylor. 1990. Amplification and Direct Sequencing of Fungal Ribosomal RNA genes for Phylogenetics. Pp. 315–322 in: Innis, M. A., D. H. Gelfand, J. J. Sninsky, and T. J. White, eds. *PCR Protocols: A Guide to Methods and Applications*: 315–322. Academic Press, Inc., New York.

WNHP. 2010. Working List of Rare Lichens. Retrieved May 12, 2011, from web site: Washington Natural Heritage Program. <http://www1.dnr.wa.gov/nhp/refdesk/lists/lichens.html>

Table 1. Overview of study species' known habitats and distributions. Sources include Brodo et al. (2001), Ferry and Lodge (1996), Goward (1999), Christensen and Johnson (2001), and McCune and Geiser (2009); PNW = Pacific Northwest of North America.

Study species	Habitats	Substrate	World distribution
<i>Cladonia portentosa</i> subsp. <i>pacifica</i> (incl. f. <i>decolorans</i> lacking usnic acid)	Exposed maritime sites such as sand dunes, seaside cliffs, and deflation plains	Sandy soils, humus soils or noncalcareous outcrops	N. American west coast from Alaska to northern California, rarely more than several kilometers inland
<i>Cladonia rangiferina</i>	Full sun or sheltered, but with high diffuse light; often rocky talus or rock outcrops in PNW	Rocky or sandy soil, mossy rock, thin soil, humus or sand	Circumpolar; widespread in Canada and northern United States (Pacific Northwest and Rocky Mts.)
<i>Cladonia ciliata</i> s. str.	Maritime; coastal heath or shrublands	Mossy soil, acidic sandy soils; acidic soils with mosses	Circumpolar with gaps; Europe; Canada; northwest United States
<i>Cladonia ciliata</i> var. <i>tenuis</i>	Maritime; coastal heath or shrublands	Mossy soil, acidic sandy soils; acidic soils with mosses	Circumpolar with gaps; Europe; Canada; northwest United States; nearest reported occurrence is British Columbia

Table 2. GenBank accessions for ITS1, 5.8S, and ITS2 used in our phylogenetic analysis. Mima Mounds specimens were deposited in OSC.

Species	Collection information	GenBank Accession
<i>C. arbuscula</i>	Georgia, USA Ahti 58505	AY170773
<i>C. arbuscula</i>	Massachusetts, USA Ahti 56659	AY170774
<i>C. arbuscula</i>	Nova Scotia, Canada Ahti 57256	AY170776
<i>C. ciliata</i>	Ireland, 2000 Rikkinen s.n.	AF458310
<i>C. ciliata</i>	Portugal, 1997 Ahti & Burgaz 55883	AF458311
<i>C. ciliata +usnic</i>	Mima Mounds, McCune 31095	JQ695927
<i>C. ciliata +usnic</i>	Mima Mounds, Class 18, McCune 31127	JQ695917
<i>C. ciliata +usnic</i>	Mima Mounds, Class 20, McCune 31129	JQ695916
<i>C. ciliata +usnic</i>	Mima Mounds, Class 29, McCune 31133	JQ695915
<i>C. ciliata s.str.</i>	Mima Mounds, McCune 31136	JQ695924
<i>C. ciliata s.str.</i>	Mima Mounds, McCune 31138	JQ695925
<i>C. ciliata s.str.</i>	Mima Mounds, McCune 31142	JQ695926
<i>C. ciliata s.str.</i>	Mima Mounds, Class 25	JQ727669
<i>C. mitis</i>	Sweden, Thell 9915	AY170756
<i>C. mitis</i>	Canada NWT, Gorin s.n.	AY170760
<i>C. mitis</i>	Argentina, Stenroos 5500	AY170761
<i>C. mitis</i>	Finland, Myllys 218	AY170792
<i>C. portentosa</i>	United Kingdom	FR799167
<i>C. portentosa</i>	Germany, 1999 Thell 9930	AF458302
<i>C. portentosa</i>	United Kingdom	FR799168
<i>C. portentosa</i>	Mima Mounds, Class 8, McCune 31123	JQ695923
<i>C. portentosa</i>	Mima Mounds, Class 19, McCune 31128	JQ695921
<i>C. portentosa</i>	Mima Mounds, Class 24, McCune31131	JQ695922
<i>C. rangiferina</i>	South Korea Hur 040220	DQ394367
<i>C. rangiferina</i>	Finland, Stenroos 5173	AF458306
<i>C. rangiferina</i>	Guyana, Stenroos 4867	AF458307
<i>C. rangiferina</i>	Mima Mounds, Class 2, McCune 31122	JQ695920
<i>C. rangiferina</i>	Mima Mounds, Class 23, McCune 31130	JQ695919
<i>C. rangiferina</i>	Mima Mounds, Class 30, McCune 31134	JQ695918
<i>C. uncialis</i>	Newfoundland, Ahti 57167	AF455248
<i>C. uncialis</i>	Finland, Stenroos 5207	AF456391

Table 3. Nonparametric regression of species occurrences on environmental predictors; f = prevalence (proportion of plots containing a taxon); $\log B$ = log likelihood ratio (measures model fit); p = p (Type I error), based on the proportion of 10,000 randomizations that yielded $\log B \geq$ observed fit; $N = 106$ plots for all models.

Species	f	# predictors	$\log B$	p	Major environmental predictors
<i>Cladonia ciliata</i> usnic chemotype	0.64	1	1.86	0.042	Live <i>Cytisus</i> presence
		2	7.02	0.067	Aspect, <i>Cytisus</i> presence
<i>Cladonia ciliata</i> usnic deficient	0.14	1	4.82	0.066	Aspect
		2	7.38	0.140	Aspect, <i>Cytisus</i> presence
<i>Cladonia portentosa</i>	0.64	1	0.20	0.365	<i>Cytisus</i> presence
		2	2.22	0.808	Aspect, <i>Cytisus</i> presence
<i>Cladonia rangiferina</i>	0.09	1	4.63	0.22	Aspect
		2	8.16	0.09	Aspect, <i>Cytisus</i> presence

Table 4. Secondary chemical compounds detected in study species using thin-layer chromatography for lichens. Standard spot test and UV reactions are indicated for each compound. Presence denoted by (+), not detected denoted by (o).

Species	Proto-cetraric acid (P+ red)	Fumarproto-cetraric acid (P+ red)	Usnic acid (KC+ yellow)	Perlatolic acid (UV+ blue-white)	Atranorin (K+ yellow)
<i>Cladonia ciliata</i> var. <i>ciliata</i>	+	+	o	o	o
<i>Cladonia ciliata</i> var. <i>ciliata</i>	o	+	o	o	o
<i>Cladonia ciliata</i> var. <i>tenuis</i>	+	+	+	o	o
<i>Cladonia portentosa</i> subsp. <i>pacifica</i>	o	o	+	+	o
<i>Cladonia portentosa</i> subsp. <i>pacifica</i> f. <i>decolorans</i>	o	o	o	+	o
<i>Cladonia rangiferina</i>	+	+	o	o	+



Figure 1. Mima mounds, March 2010, with *Cladonia portentosa* and *C. ciliata* var. *tenuis* in the left foreground. Adjoining *Pseudotsuga* forest is visible in the background; *Cytisus* shrubs are invading in the left background behind the person in blue.

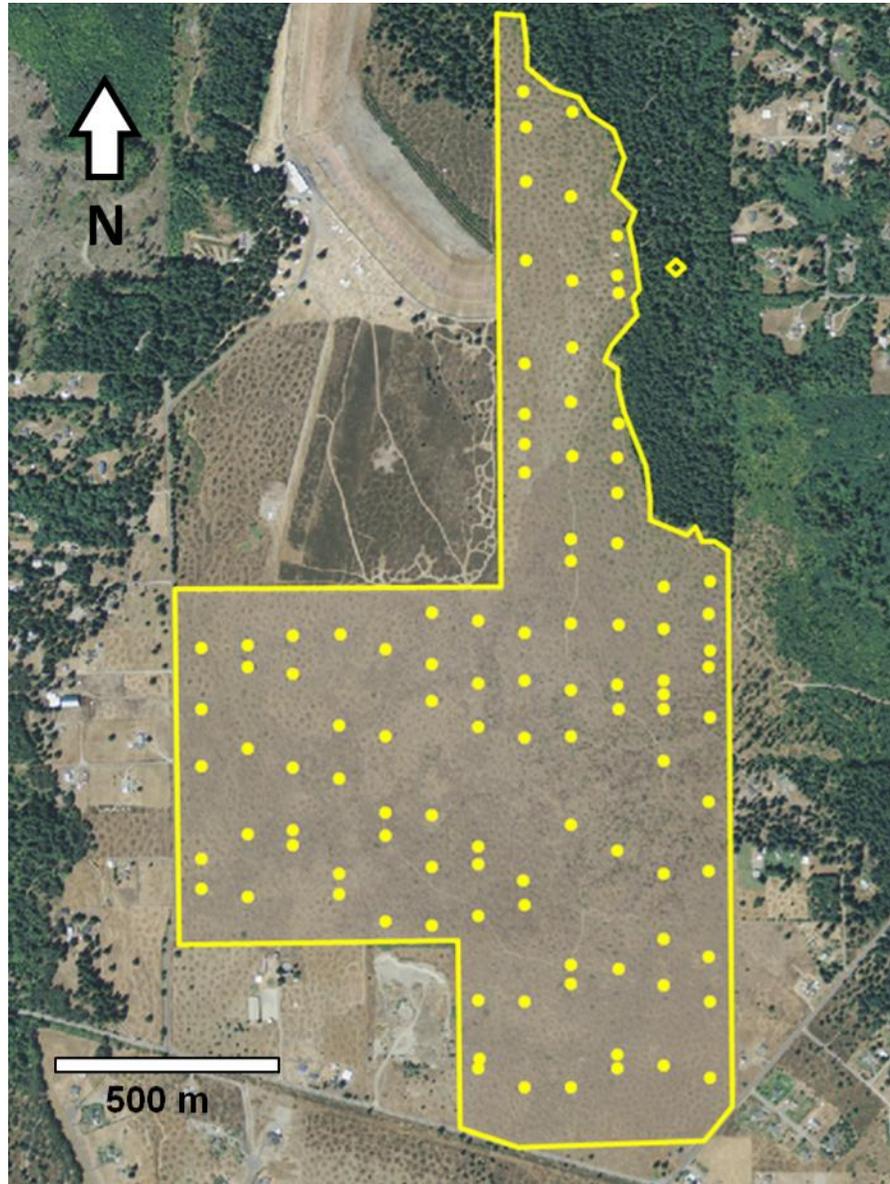


Figure 2. Sample plot locations at Mima Prairie, WA. Boundary of reindeer lichen survey area is delineated by yellow line. Reindeer lichen plots (N = 106 plots, denoted by filled circles) were circles with a 3 meter radius, randomly placed along linear north-south transects and surveyed for presence/absence of four target taxa of reindeer lichens as well as for several environmental factors. The single air quality plot (denoted by open diamond, forest at upper right) was surveyed for epiphytic lichen species.

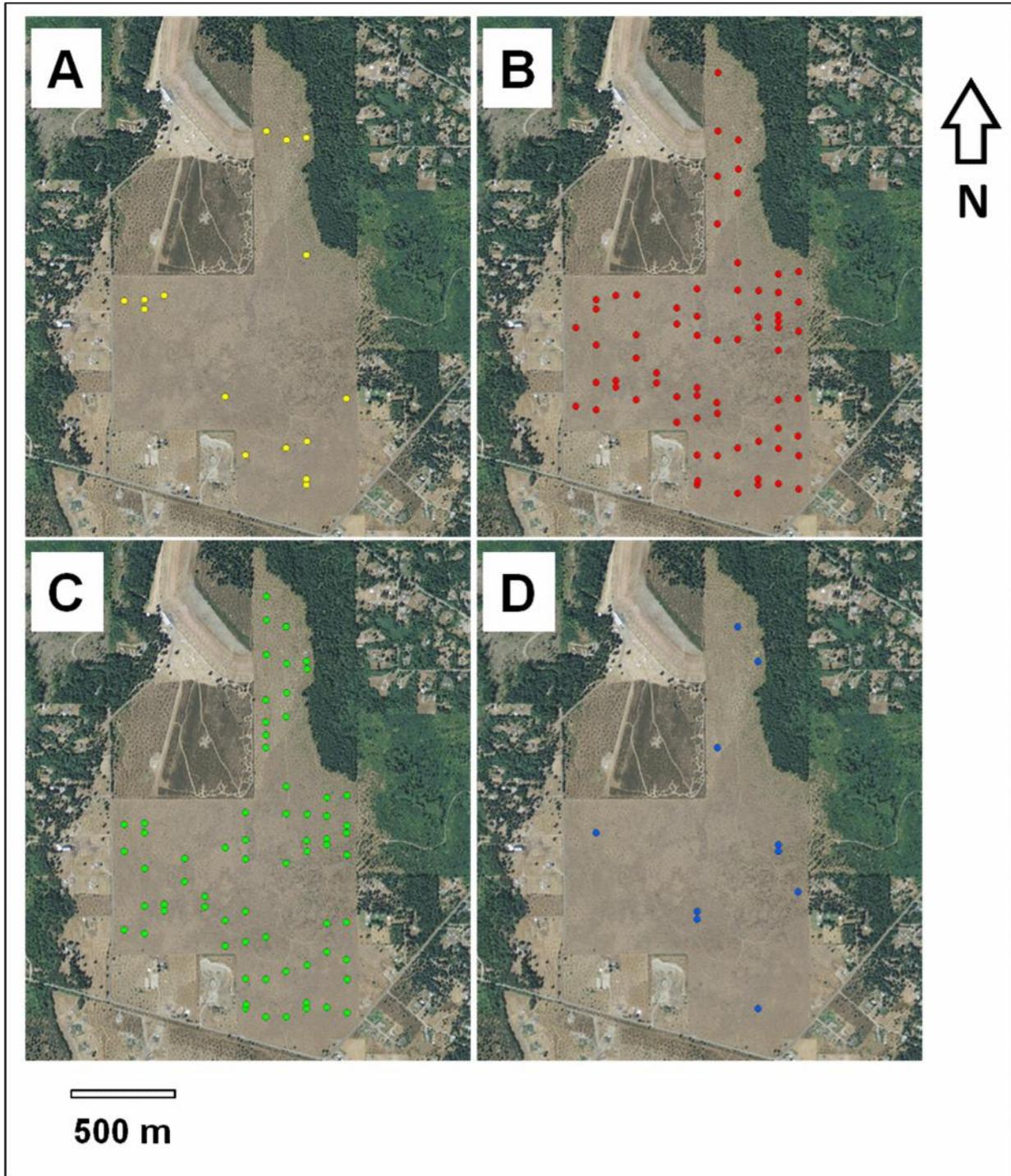


Figure 3. Spatial distributions of reindeer lichens at Mima Mounds, with presences at 106 sample points denoted by filled circles, absences not depicted. Key: A = *Cladonia ciliata* usnic chemotype, B = *C. ciliata*, C = *C. portentosa*, and D = *C. rangiferina*.

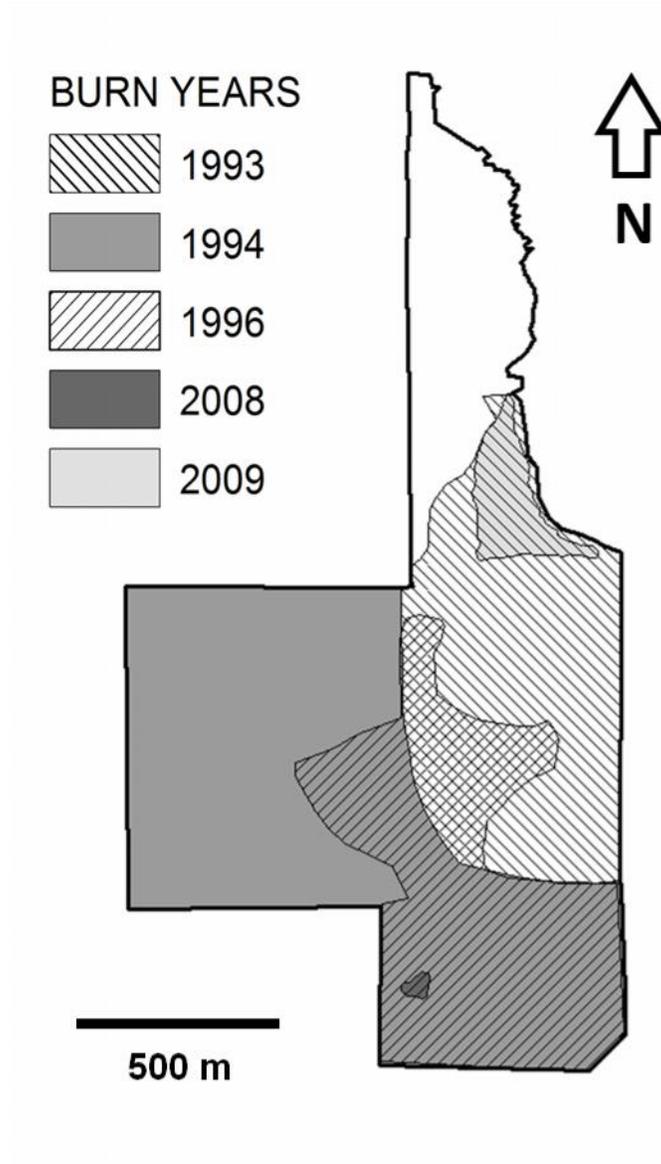


Figure 4. Extent of prescribed fires recorded at Mima Mounds Natural Area Preserve from 1988 to 2011. Random plot assignment resulted in no plots sampled from the smallest (2008) burn.

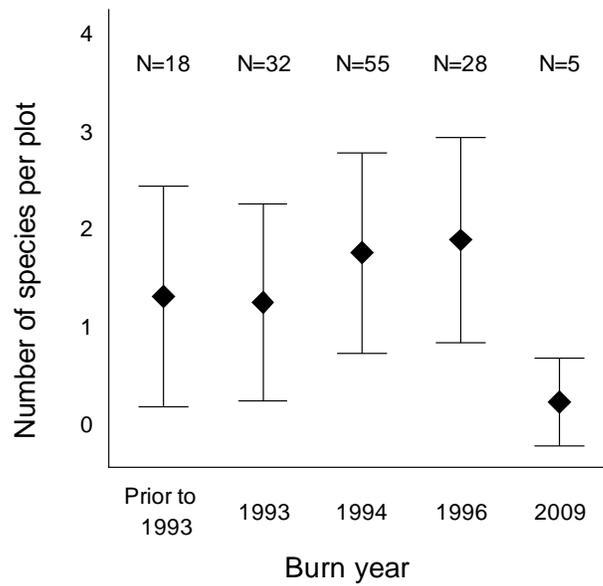


Figure 5. Reindeer lichen occurrence in relation to time since most recent burn (burn year). For each burn year, bars indicate \pm one standard deviation from the mean (diamond). Sample sizes sum to greater than 106 due to some plots experiencing more than one fire.

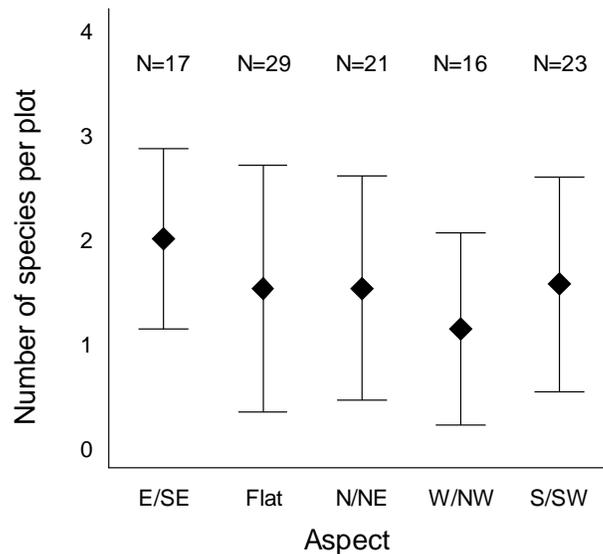


Figure 6. Reindeer lichen occurrence in relation to directional aspects upon mima mounds. For each burn year, bars indicate \pm one standard deviation from the mean (diamond). Aspect grouping codes: E = east, W = west, N = north and S = south.

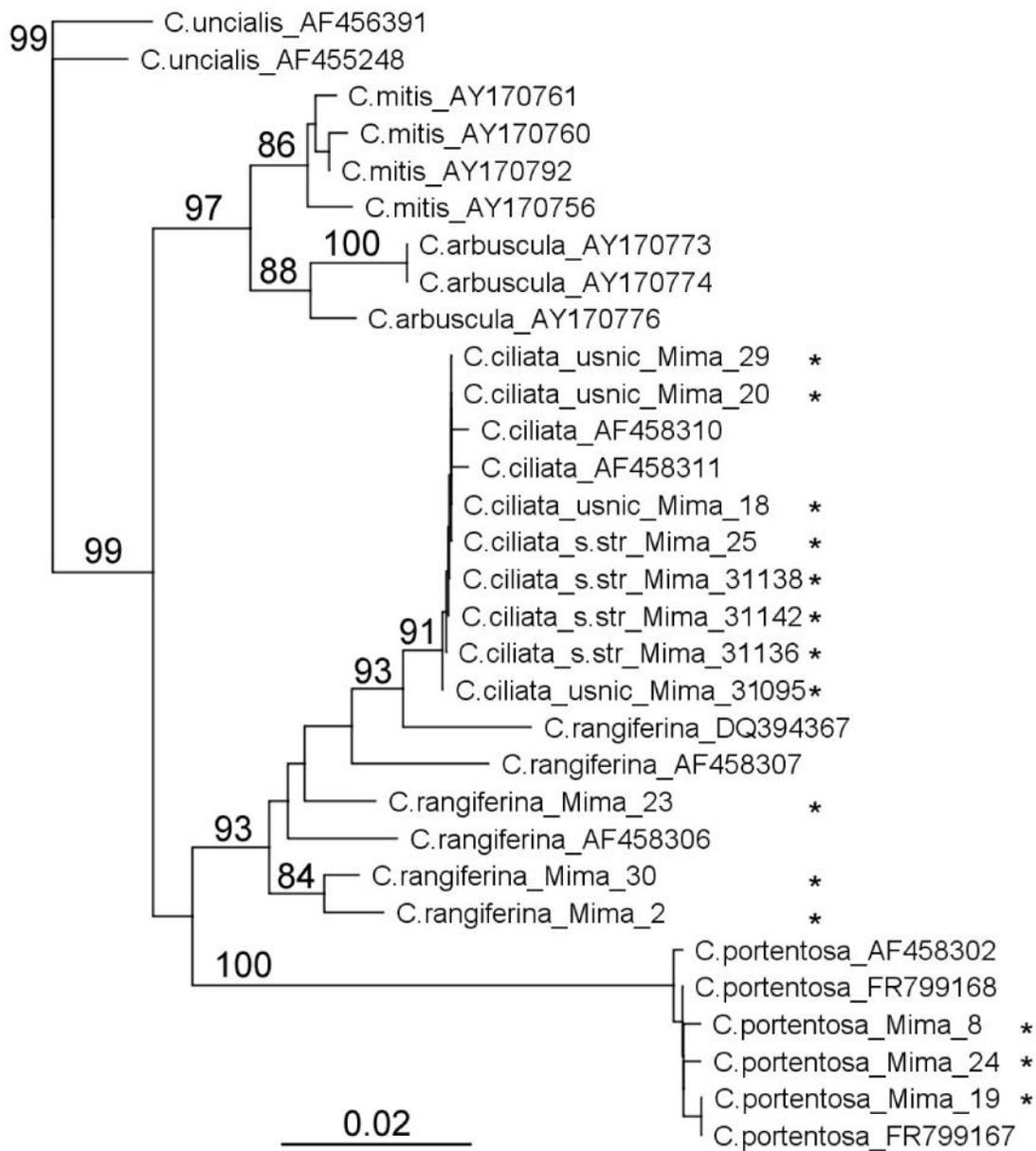


Figure 7. Phylogenetic relationships of Mima Mounds reindeer lichen samples (*) with a selection of related species from GenBank. The maximum likelihood tree is based on ITS data using PhyML then rooted with *C. uncialis*. The two dominant chemotypes of *C. ciliata* (with and without usnic acid) are indicated by “usnic” in the sample name; “*C. ciliata* s. str.” lacks usnic acid. Branch lengths are based on number of substitutions per site. Bootstrap values > 85% are indicated by branches.



Figure 8. *Cladonia ciliata* var. *tenuis*, containing usnic acid, and *C. ciliata* var. *ciliata*, lacking usnic acid, growing in adjacent clumps at Mima Mounds.

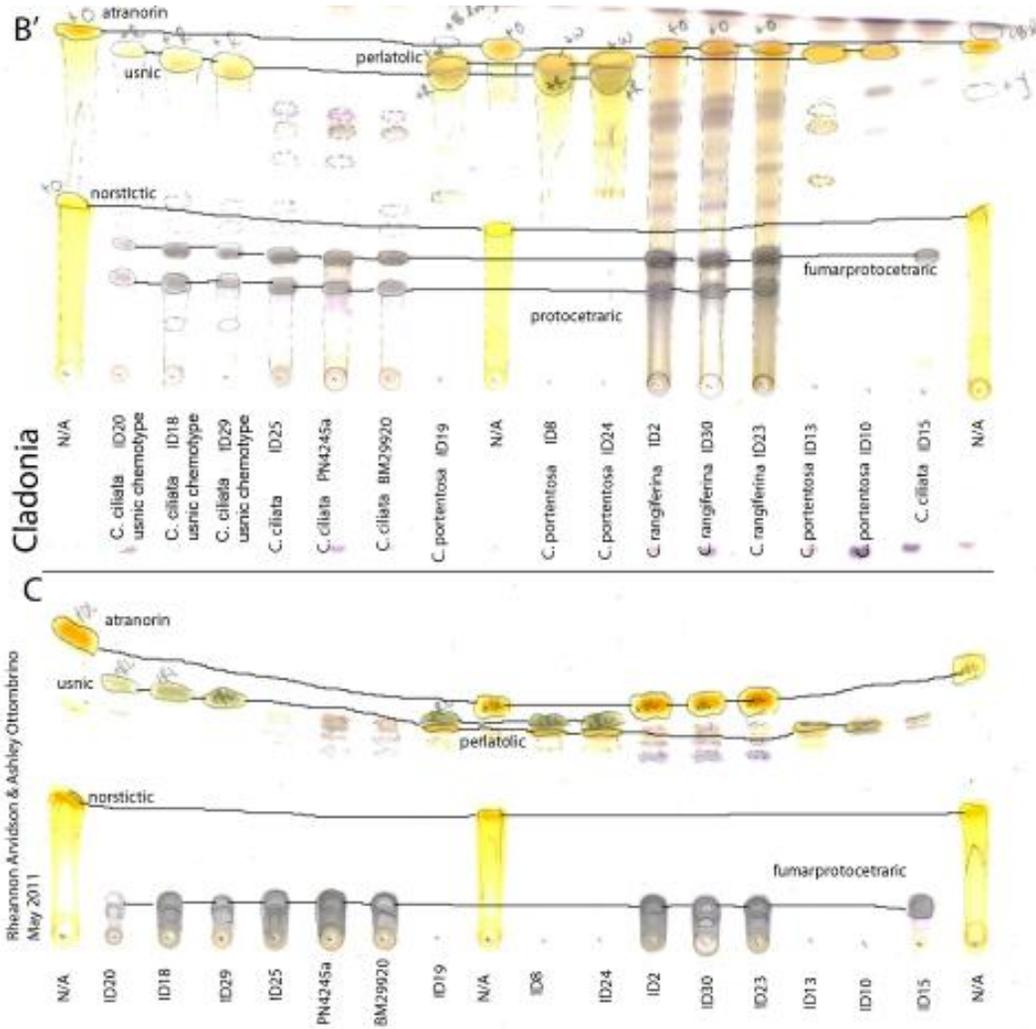


Figure 9. Scan of thin-layer chromatography (TLC) plate (approximately 2/3 size), showing secondary chemical products from six chemotypes in three Mima Mounds reindeer lichen species. Contrast was enhanced slightly after scanning. Upper half shows results in solvent B' of Culberson & Johnson (1982); lower half is for the same samples in solvent C of Culberson et al. (1981). The lanes marked "N/A" are norstictic acid and atranorin controls provided by *Parmotrema hypotropum*.