

Evidence of substrate endemism of lichens on Fox Hills Sandstone: Discovery and description of *Lecanora lendemeri* as new to science

Erin A. Tripp^{1,2,5}, Caleb A. Morse³, Kyle G. Keepers¹, Carly Anderson Stewart¹, Cloe S. Pogoda¹, Kristin H. White¹, Jordan R. Hoffman⁴, Nolan C. Kane¹ and Christy M. McCain¹

¹ University of Colorado, Dept. of Ecology & Evolutionary Biology, UCB 334, Boulder, CO 80309, U.S.A.; ² University of Colorado, Museum of Natural History, UCB 350, Boulder, CO 80309, U.S.A.; ³ University of Kansas, Biodiversity Institute, R.L. McGregor Herbarium, Lawrence, KS 66047, U.S.A.; ⁴ New York Botanical Garden, 2900 Southern Blvd., Bronx, NY 10458, U.S.A.

ABSTRACT. Recent lichenological investigations of Fox Hills Formation sandstone outcrops in Colorado resulted in the discovery of three populations that represent an undescribed member of the *Lecanora dispersa* group (= *Myriolecis*). This new species is different from all others in the group in its production of usnic acid, which yields apothecia that are yellowish-green in color in fresh field material. The new species, here formally described as *Lecanora lendemeri*, is further characterized by its relatively large ascospores, endolithic thallus, presence of POL+ granules, and apparent restriction to this sandstone formation. We conducted molecular phylogenetic analyses to place the new species into the context of other members of *Lecanora* using new shotgun sequence data generated for this study in tandem with previously published rDNA data, and found that the new species is resolved as nested within the *L. dispersa* group, which was a strongly supported clade in our analysis. Using IUCN criteria including a known occurrence of only three populations, the largest of which is under conservation threat, we herein formally rank this new species as Endangered. This discovery comes on the heels of several other recent lichen discoveries on Fox Hills Sandstone, all species that are, so far as known, restricted to this rock type, suggesting that substrate endemism may be a common element of the biotic communities of the Fox Hills Formation. From the results of this and prior studies, it is clear that sandstone outcrops serve as important, yet still incompletely documented, habitats for cryptogamic diversity. This discovery further highlights the significance of conservation areas, even tiny units (e.g., 40 ha or less) that represent mere islands in a sea of urban development, such as in the Front Range of Colorado.

KEYWORDS. Colorado, edaphic, endemism, Fox Hills, *Lecanora dispersa* group, lichen, new species, sandstone, substrate, *Myriolecis*.



Exposed rock surfaces represent one of the most widely available and ecologically important substrates for lichens and other biotic crust communities throughout western North America (WNA). Sandstone formations, in particular, are both abundant and geologically diverse throughout WNA, as evidenced by the many sandstone-rich, iconic landmarks of the West ranging from Arches, Bryce, Canyonlands, and Grand Canyon National

Park to Grand Staircase-Escalante National Monument and Monument Valley Navajo Tribal Park. Sandstones of the Arikaree, Aztec, Cedar Mesa, Coconino, Dakota, Entrada, Morrison, Potsdam, Navajo, and Wave Formations represent but a few of those present within WNA. Biological constituents on outcrops of these sandstone formations are in part determined by geological attributes specific to each, including age, degree of weathering, chemistry (e.g., calcium carbonate-rich or not) and clay content, permeability, water holding capacity, exposure, aspect, and orientation combined with other

⁵ Corresponding author's e-mail:
erin.tripp@colorado.edu

DOI: 10.1639/0007-2745-122.2.246

microhabitat features including the biotic environment (Hirschfeld 2013a,b; Tripp 2015).

A lesser-known sandstone formation, but one that has been the focus of recent lichenological investigation, is that of the Fox Hills Formation (Tripp 2015, 2016; Tripp & Lendemer 2015; Westberg et al. 2011). Fox Hills Sandstone is Late Cretaceous in age, occurs from Colorado to Alberta, and is best developed in portions of Colorado, Montana, North Dakota, South Dakota and Wyoming (Dobbin & Reeside 1929; Henderson 1920; Roehler 1993). Biologically, Fox Hills has been understudied owing in part to a limited number of instances in which this rock type is exposed at the surface (Dobbin & Reeside 1929). Fox Hills Sandstone is associated with the Laramie Formation, dates to ~67 mybp, is typically low in clay content, and is composed primarily of quartz and is non-calcareous (Hirschfeld 2013a,b; MacPhail et al. 1970; Weimer 1973). Exposed surfaces of Fox Hills Sandstone are extremely fragile and susceptible to weathering, a process that is slowed by the process of “case hardening” imposed by interactions between biotic crusts and minor clay content (Hirschfeld 2013a,b; Tripp 2015).

Earlier studies documented that Fox Hills Sandstone harbors several rare plants and invertebrates (including fossil forms), likely in part attributable to substrate specialization (Byars 1936; Clark et al. 2001; Dobbin & Reeside 1929; Henderson 1920; Weber 1949). More recently, several new lichenological discoveries have been made on this substrate, including two new species of *Candelariella* (Tripp & Lendemer 2015; Westberg et al. 2011) and one of *Lecidea* (Tripp & Lendemer 2015). To date, all known occurrences of these species are from Fox Hills Sandstone, suggesting a close relationship between geographical range limits and substrate specificity, in this case, a sandstone formation characterized by nutrient limitation. Conservation needs of these restricted-endemic substrate specialists is further exacerbated by the limited extent in which Fox Hills Sandstone is outcropped across central and western North America (Dobbin & Reeside 1929).

Fieldwork in preparation for Tripp (2015, 2016) resulted in the discovery of an unusual species of *Lecanora* with an endolithic thallus, belonging most likely to the *L. dispersa* group sensu Śliwa (2007a) (= *Myriolecis* Clements; see Discussion below) owing

to its endosubstratal thallus, apothecia with prominent thalline margins, absence of atranorin, and KOH-insoluble crystals. This discovery was based on a collection consisting of limited material, deriving from a lichenological inventory of a large, 40 ha outcropping of Fox Hills Sandstone (hereafter, “White Rocks Boulder,” Fig. 5C&D) that is located within the city limits of Boulder, Colorado, and is under permanent conservation protection by the City of Boulder’s Open Space Program (Fig. 1; Tripp 2015, 2016). At the time of the inventory, paucity of material prevented further analysis, including molecular phylogenetic work. In late 2016, the first author initiated additional lichen inventory work on a nearby but much smaller parcel of Fox Hills Sandstone. This site (hereafter, “White Rocks Niwot,” Fig. 5A), which is near the town of Niwot, Colorado and approximately 4 linear km north White Rocks Boulder, is much smaller in geographical size (~2 ha of outcrops). It occurs on Boulder County Open Space, but is not under conservation protection (Fig. 1). Inventory of White Rocks Niwot resulted in the discovery of several sizeable populations of this unusual *Lecanora*. Based on its production of usnic acid, which gives the apothecial margins a distinctly yellowish-green color in fresh material, in combination with other anatomical and morphological features, this species is distinct from all currently documented taxa within the *L. dispersa* group (Śliwa 2007). Below, we place this undescribed entity within a phylogenetic context using newly generated rDNA data derived from shotgun sequencing, then describe it as new to science.

METHODS

Voucher collection. Voucher specimens were collected by the first author at White Rocks Niwot (Fig. 5A) as well as White Rocks Boulder (Fig. 5C&D) between 2014 and 2016. A full set of all vouchers collected as part of floristic (lichenological) inventories of both sites is deposited at the University of Colorado Herbarium (COLO) (acronyms follow Thiers 2018), and many vouchers are deposited at the New York Botanical Garden William and Lynda Steere Herbarium (NY). Additional reference material was sought in COLO, but none was located. One additional Fox Hills Sandstone outcropping south of Ellicott, Colorado

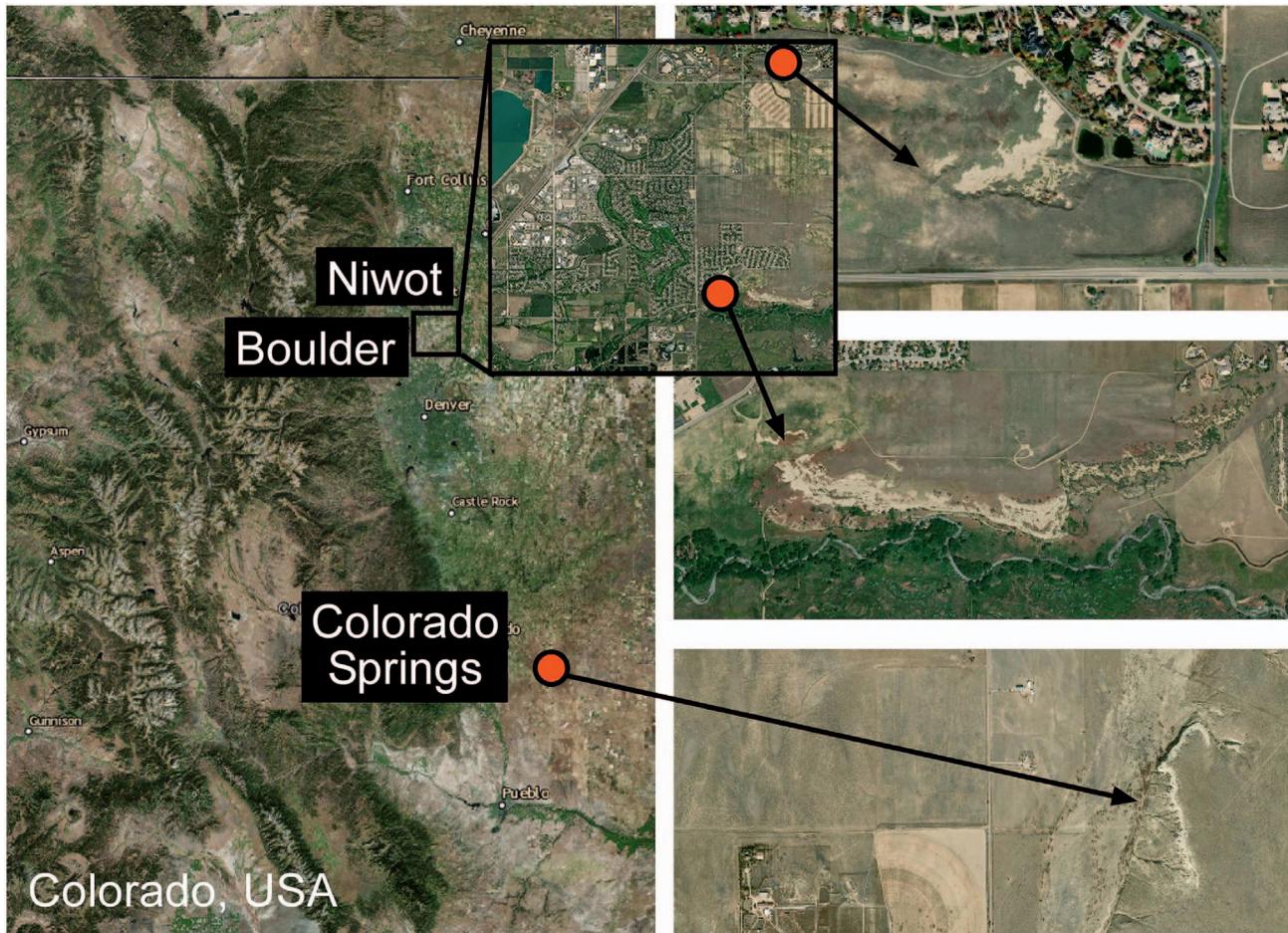


Figure 1. Satellite image of the greater Boulder/Denver /Colorado Springs area delimiting (1) “White Rocks Boulder” (middle right) – a large [100 acre] Fox Hill Sandstone outcropping within the City of Boulder, located on Boulder City Open Space, which was previously the focus of lichenological inventory work (Tripp 2015; Tripp 2016; Tripp & Lendemer 2015); (2) “White Rocks Niwot” (top right) – a much smaller [5 acre] outcropping of Fox Hills Sandstone located on Boulder County Open Space, which was the focus of more recent lichen inventory work that yielded discovery of ample populations of the new species; and (3) Bohart Ranch (bottom right), an outcropping east of Colorado Springs that is currently managed by The Nature Conservancy. The first two sites are nested within a sea of urban development (the Boulder–Denver–Longmont urban triangle). White Rocks Boulder is under permanent conservation protection that limits foot traffic or other human forms of disturbance. White Rocks Niwot is currently not protected and is open to the public and human foot traffic.

(Bohart Ranch, **Fig. 5B**; vicinity of Colorado Springs) was visited on separate occasions by two of the authors (EAT and CAM), the former in an effort to find additional populations of this and other unusual species and the latter as part of a general inventory of the site. Voucher material collected by CAM is deposited at the University of Kansas McGregor Herbarium at (KANU).

Morphological, anatomical and chemical study. Field photographs of the new species were taken using a Nikon D7100 digital SLR with a 105 mm 1:1 macro lens and ring flash. Vouchered specimens were further examined using compound

and dissecting microscopes at COLO and KANU. Anatomical structures such as ascospores were studied using hand-prepared sections cut with a razor blade then mounted in water or 10% KOH (hereafter K). Solubility of POL+ granules was evaluated in 25% K and 65% HCl. Images were captured using imaging systems at the two herbaria. Size ranges in the descriptions of the new species are expressed as simple ranges, or, where samples were sufficiently large, as one standard deviation around the mean, bounded by the largest and smallest values and followed by the sample size (n), i.e., (smallest value–)x-SD–x+SD(–largest value) (n=).

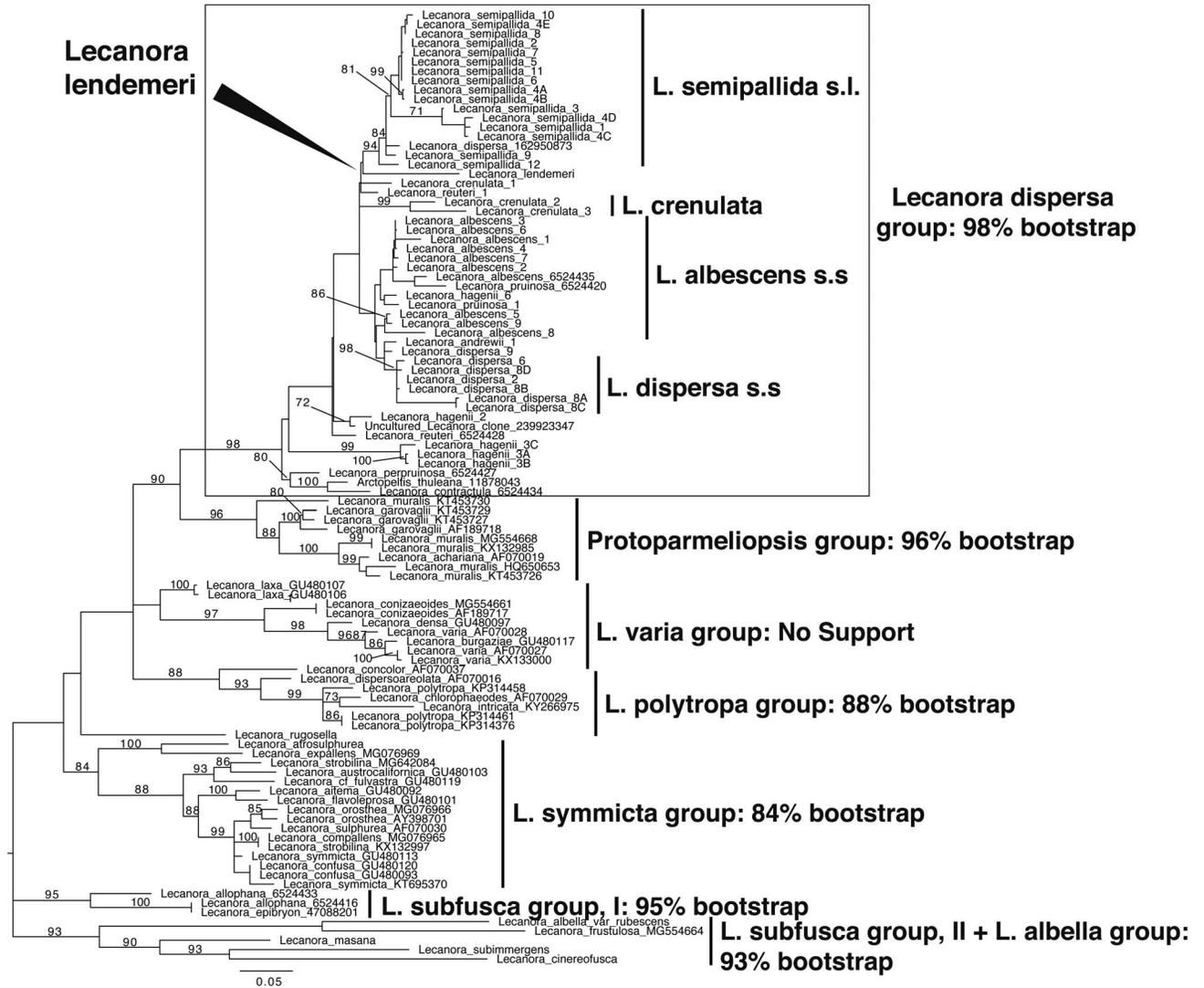


Figure 2. Best tree resulting from maximum likelihood phylogenetic analysis showing the new species *Lecanora lendemeri* as included within the *L. dispersa* group with strong bootstrap support (98%). ML analysis was based on new sequence data (derived from shotgun sequencing, including of the new species) analyzed together with existing sequence data (downloaded from GenBank and derived from various sources, see methods). Major clades of *Lecanora* highlighted and bootstrap values ($\geq 70\%$) shown.

Chemistry was studied using standard spot tests ([10%]K, C, KC, P, UV) following Brodo et al. (2001) supplemented by thin layer chromatography (TLC) using solvents A, B', and C following Culberson & Kristinsson (1970; COLO) or Orange et al. (2001; KANU).

Conservation assessment. We used data on population occurrences and habitat features of documented specimens to provide a first and preliminary conservation assessment of the new species following IUCN (2014) guidelines. We used satellite imagery provided by Google Earth Pro (2018) to assess habitat quality and conditions.

GeoCAT was used to calculate Area of Occupancy (AOO) as well as Extent of Occurrence (EOO) per IUCN (2014) recommendations.

Genome sequencing. DNA was extracted using a Qiagen Dneasy Plant Kit following standard protocols, with two small changes at the beginning and near the end of the protocol: first the protocol was modified to incubate the powdered apothecial tissue for 10 min at 65°C in lysis buffer to aid in cell degradation, and an additional 100% ethanol wash was added before final drying of the membrane prior to elution. This DNA (DNA) sample was prepared for sequencing using the Nextera® XT

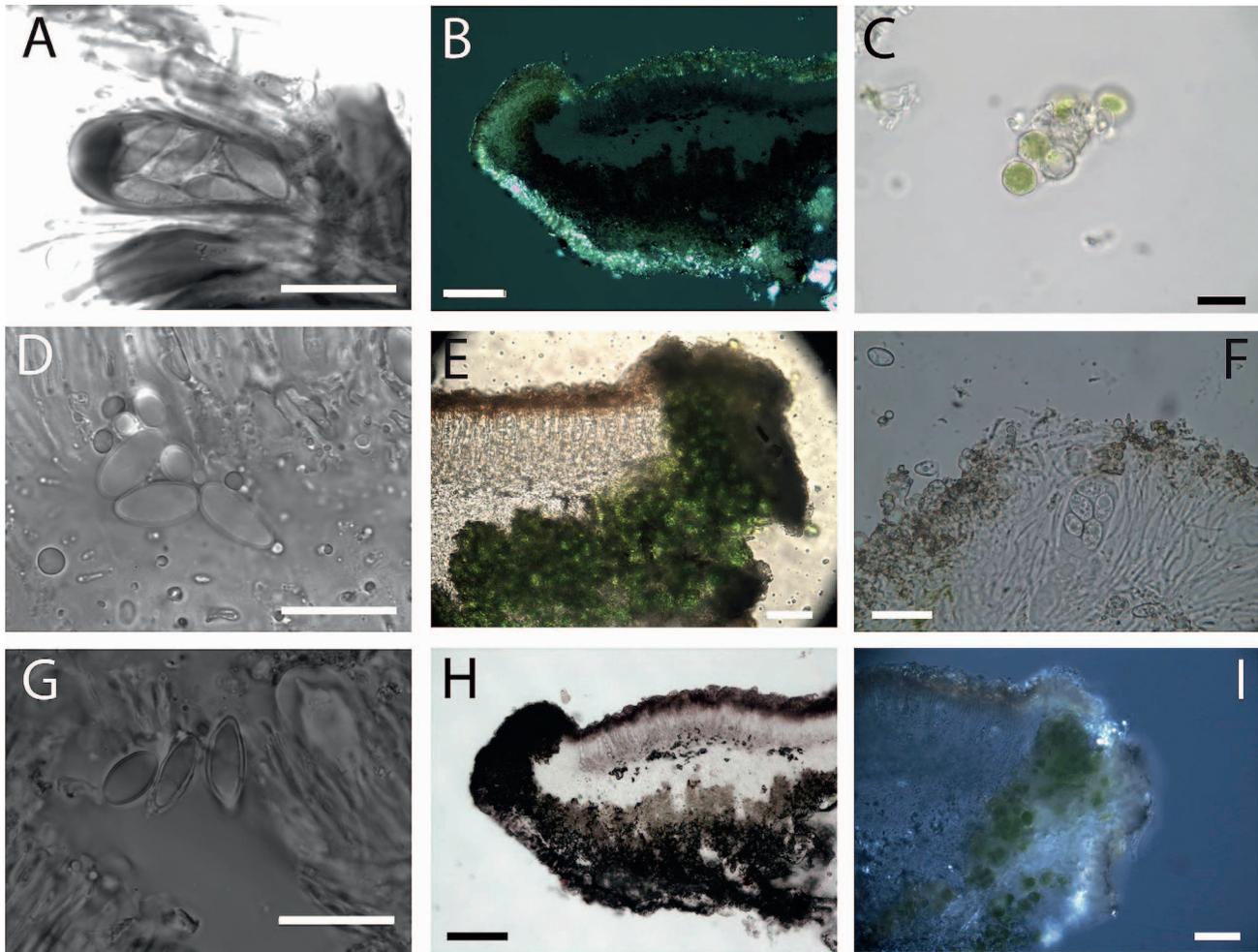


Figure 3. Micromorphological features and variation in *Lecanora lendemeri*. **A.** Ascus, treated with K, 1000 \times , scale=20 μ m, *C. Morse 15869*. **B.** Apothecia section, 100 \times , scale=100 μ m, POL light, *C. Morse 15869*. **C.** Trebouxoid photobiont, 100 \times , scale=15 μ m, *E. Tripp 5806*. **D.** Ascospores, 1000 \times , scale=20 μ m, *C. Morse 15869*. **E.** Apothecia section, 40 \times , scale=50 μ m, *E. Tripp 5806*. **F.** Ascus and paraphyses, 100 \times , scale=30 μ m, *E. Tripp 5806*. **G.** Ascospores, 1000 \times , scale=20 μ m, *C. Morse 15869*. **H.** Apothecia section, 100 \times , scale=100 μ m, *C. Morse 15869*. **I.** Apothecia section, 40 \times , scale=50 μ m, POL light, *E. Tripp 5806*.

DNA library prep kit. DNA samples were diluted to the appropriate concentration confirmed using a Qubit 3.0 fluorometer. Samples were uniquely identified by dual index adapters, Nextera[®] i5 and i7. The amplified DNA underwent solid phase reversible immobilization (SPRI) paramagnetic bead size selection using an epMotion 5075TMX automated liquid handling system. Sample quality control (QC) was conducted to ensure the appropriate sample concentration of 1.8-2.1pM (Qubit 3.0 fluorometer) and DNA fragment size (Agilent 2100 Bioanalyzer) before normalizing the loading concentration. After passing QC, this prepared library was processed for paired end 150 base pair reads on the Illumina NextSeq[®] sequencer housed at the

University of Colorado's BioFrontiers Sequencing Facility, with Illumina PhiX v.3 utilized as a control.

Genome assembly and rDNA database. Sequenced reads were filtered using Trimmomatic-0.36 to trim adapters from reads, and with parameters "LEADING:3 TRAILING:3 MINLEN:100" (Bolger et al. 2014). Filtered reads were then assembled using SPAdes 3.9.0 with parameters "-careful -k 21,33,65,81" (Bankevich et al. 2012). Ribosomal DNA complexes for each assembly were identified by conducting a BLAST search of the rDNA complex of the Trebouxoid photobiont of *Cladonia uncialis* against each assembly. The algal rDNA was used in the search rather than a mycobiont sequence to avoid bias in BLAST hit

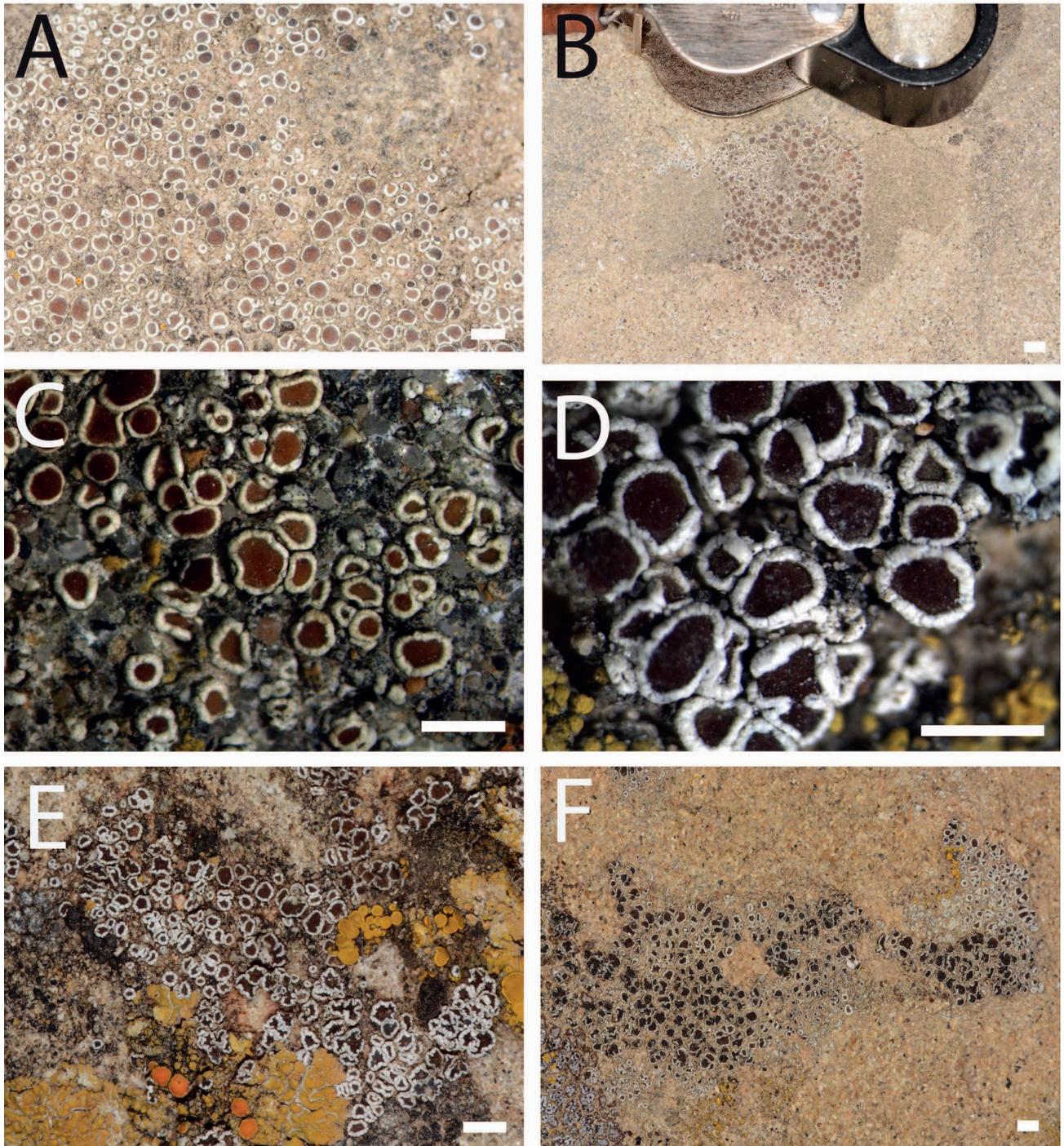


Figure 4. Macromorphological features and variation in *Lecanora lendemeri*. **A.** Population at White Rocks Niwot (Type), apothecia, scale=1mm, *E. Tripp 5806*. **B.** Population at White Rocks Niwot (Type), apothecia, scale=2mm, *E. Tripp 5806*. **C.** Population at Bohart Ranch, apothecia, scale=1mm, *C. Morse 15869*. **D.** Population at White Rocks Niwot (Type), apothecia, scale=1mm, *E. Tripp 5806*. **E.** Population at White Rocks Boulder, apothecia, scale=1mm, *E. Tripp & D. Clark 4823*. **F.** Population at White Rocks Boulder, apothecia, scale=2mm, *E. Tripp & D. Clark 4823*. Note that lime coloration typical of apothecial margins (apparent in the color online pdf) is best seen in material that is damp, a state not reflected in any of these photographs.

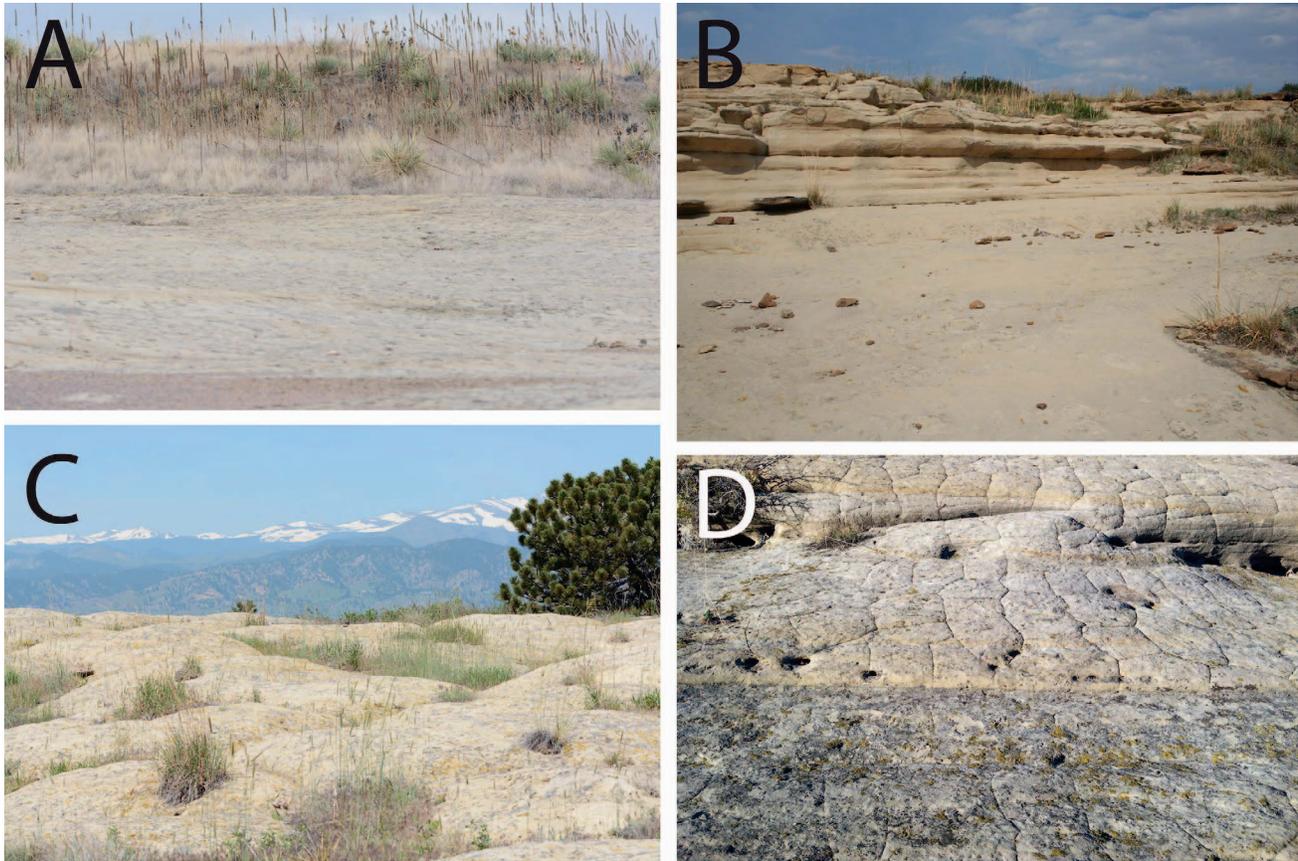


Figure 5. Site features typical of locations in which *Lecanora lendemeri* has been collected. **A.** Habitat, White Rocks Niwot (Type Locality), very small parcel of Fox Hills Sandstone outcropping that hosts large population of *Lecanora lendemeri* but is currently not under any conservation mandate; photo: E. Tripp. **B.** Habitat, Bohart Ranch, Fox Hills Sandstone outcropping east of Colorado Springs that is currently protected and managed by The Nature Conservancy; photo: C. Morse. **C.** Habitat, White Rocks Boulder, Fox Hills Sandstone outcropping overlooking Western Continental Divide, site closed to the public for conservation prioritization but open to guided visits with Open Space biologists; photo: E. Tripp. **D.** Habitat, White Rocks Boulder, Fox Hills Sandstone outcropping showing typical “turtleback” formations in upper rock surface as a function of freeze-thaw cycles and associated water and mineral content; photo: E. Tripp.

length due to phylogenetic similarity. To identify relatively complete rDNA complexes from the BLAST tables, contigs were required to have two or greater distinct hits and the span of the hits was required to be greater than 1000bp in length. Sequences were parsed from the assemblies based on the nucleotide positions of the BLAST hits and oriented with the 18S in the 5' direction. The complete rDNA complex of the new species plus rDNAs of five other previously sequenced *Lecanora* spp. were assembled as part of this study. These five additional taxa are not members of the *Lecanora dispersa* group (*L. albella* var. *rubescens* (Imshaug & Brodo) Lumbsch [Lendemer 46417], *L. cinereofusca* H.Magn. [Lendemer et al. 45937], *L. masana* Lendemer & R.C.Harris [Lendemer et al. 46185], *L. rugosella* Zahlbr. [Lendemer et al. 46180], and *L.*

subimmergens Vainio [Lendemer & Tripp 49057]) and were sampled to help delimit phylogenetic placement of the new species as well as serve as outgroups. Voucher specimens of these five taxa are deposited at NY. The resulting lengths of the assembled rDNA contigs ranged from 4.7 to 9.7 kb.

Matrix preparation. Existing sequences from taxa within the *Lecanora dispersa* group were available from previous phylogenetic study of that group (Śliwa et al. 2012; <http://purl.org/phylo/treebase/phylovs/study/12681>). Because Śliwa et al. (2012) utilized the ITS1+5.8S+ITS2 locus, we truncated our rDNA contigs to reflect only this locus using a web BLAST against *L. semipallida* (JQ993757) from Śliwa et al. (2012). Sequences were aligned using default parameters in the MEGA7

(Kumar et al. 2016) implementation of clustalW (Larkin et al. 2007) and then the alignment was manually adjusted in PhyDE (<http://www.phyde.de/index.html>). To this matrix and using GenBank, we incorporated sequences from several other lineages of *Lecanora* (the *Lecanora polytropa*, *L. symmicta*, *L. varia* and *Protoparmeliopsis* groups) to explore the possibility of alternative phylogenetic placement of the putatively undescribed taxon. Using this final matrix (deposited in Zenodo, record #2281314; new GenBank sequences deposited under the following accession numbers: MK573924, MK573925, MK573926, MK573927, MK573928, MK573929), relationships among species were inferred in MEGA7 under maximum likelihood and a GTR+ Γ +I model of sequence evolution (Tavaré 1986) using default search parameters, and 1000 bootstrap replicate searches were conducted to assess branch support.

RESULTS

Fieldwork associated with this study resulted in the discovery of three locations in which populations of the new species occur. The first, White Rocks Boulder, was discovered during inventory work at that site, as documented in Tripp (2015, 2016). The second, White Rocks Niwot, was discovered subsequently; populations at the latter site were qualitatively deemed to be much more extensive than at the former site, despite the fact that the latter is only 5% of the total surface area of the former. The third, Bohart Ranch (**Fig. 5B**), was discovered during the site visit by CAM, who noted that the lichen was locally common at the base of the bluff.

The morphological features of the new species described below, which we will call *Lecanora lendemeri*, suggest a clear affinity to the *Lecanora dispersa* group (Śliwa 2007a). Like many other species in the *L. dispersa* group, *L. lendemeri* has an endosubstratal thallus and apothecia with prominent thalline margins. Although it lacks atranorin like all members of the group, *L. lendemeri* consistently produces usnic acid and lacks xanthenes, giving it a unique chemical profile as compared to other species in the *L. dispersa* group.

Our phylogenetic analysis (**Fig. 2**) recovered (1) a strongly supported monophyletic *Lecanora dispersa* group (98% ML bootstrap [BS]), which includes the new species *Lecanora lendemeri*. Within

this group, our analysis recovered several major groups (e.g., *L. semipallida* s.l., *L. albescens* s.str., *L. dispersa* s.str.), but bootstrap support for these various entities was typically low or non-existent, likely as a function of limited utility of the ITS1+5.8S+ITS2 locus in phylogenetic studies at the species level. The new entity, *L. lendemeri*, was resolved proximal to the *L. semipallida* s.l. lineage, but BS support was low. Outside of the *L. dispersa* complex, our analysis resolved several other lineages of *Lecanora* including a monophyletic *Protoparmeliopsis* group (96% BS, and sister to the *L. dispersa* group), a monophyletic *L. varia* group (not supported), a monophyletic *L. polytropa* group (88% BS), a monophyletic *L. symmicta* group (84% BS), and the *L. subfusca* group I (95%) as well as *L. subfusca* group II + *L. albella* (93% BS).

Based on a known Extent of Occurrence (EOO) of 145 km² and a known Area of Occupancy (AOO) of 12.0 km², the new species is here classified as “Endangered” following IUCN criteria: B1: EOO that is <5000 km² and AOO that is <500 km², coupled with (a) severely limited number of locations (≤ 5) and (b) continuing decline estimated or projected in (i) extent of occurrence, (ii) area of occupancy, and (iii) quality of habitat, as a function of the unprotected and exceptionally fragile Fox Hills Sandstone present at the White Rocks Niwot population, which is deemed to be the largest and most substantial. It is worth noting that the EOO and AOO of this species very narrowly exceeded the thresholds that yield a ranking of Critically Endangered.

DISCUSSION

Phylogenetic analysis of our assembled matrix placed *Lecanora lendemeri* within the *L. dispersa* group with strong bootstrap support (**Fig. 2**). The *L. dispersa* group has traditionally been characterized as including species with an endolithic or endophloedal thallus, apothecia with white thalline margins, and granules in the cortex, epithecium, or algal layer of the amphithecium. Members lack atranorin or usnic acid but produce chlorinated xanthenes and rarely the depsidone pannarin or related substances, or lack chemistry, and are typically calciphilous (Śliwa 2007a,b; Śliwa et al. 2012; Zhao et al. 2015). The morphologically similar *Lecanora varia* group s.l., members of which are shown in **Fig. 2** to be more distantly related to the *L.*

dispersa group, has been characterized by production of the dibenzofurans, isousnic, or usnic acids, along with other substances (Śliwa & Wetmore 2000). The outgroup in **Fig. 2** is composed of members of the third large group of non-lobate species of *Lecanora*, the *L. subfusca* group (= *Lecanora* s.str.), which has been traditionally defined by the production of K-insoluble crystals in the amphithecium and atranorin as a major substance. Phylogenetic studies (Arup & Grube 1998; Pérez-Ortega et al. 2010; Zhao et al. 2015) have generally supported traditionally defined species groups within *Lecanora*, although the morphologically heterogeneous *L. varia* group has been shown previously to include several unrelated lineages (referred here and by Pérez-Ortega et al. 2010 to *L. polytropa*, *L. saligna*, *L. symmicta* and *L. varia* groups; see **Fig. 2**). Our results agree with those studies in resolving, with strong support, the *L. dispersa* group (here including *L. lendemeri*) as sister to the lobate genus *Protoparmeliopsis* Choisy (= *Lecanora muralis* group), members of which produce usnic acid as a major secondary compound.

While the *Lecanora dispersa* group for its part has long been recognized as distinct within *Lecanora*, results of Śliwa et al. (2012) led to expanding its circumscription to include four taxa with lobate thalli (*L. contractula* Nyl., *L. pruinosa* Chaub., *L. reuteri* Schaer., and *Arctopeltis thuleana* Poelt [= *L. thuleana* (Poelt) Śliwa]). New combinations were then proposed in *Myriolecis* for this expanded concept of the *L. dispersa* group (Zhao et al. 2015). However, this expanded concept was based on sequence data from a minority (10/30) of the taxa presently included in *Myriolecis* (see Śliwa et al. 2012). Moreover, to date, phylogenetic studies have not sampled several morphologically similar taxa that have been discussed alongside the *L. dispersa* group but excluded from it (Śliwa 2007a,b; Śliwa & Wetmore 2000) due to their chemistry—namely, *L. elenkinii* Mereschk., *L. thallophila* H.Magn., and *L. utahensis* H.Magn., all with isousnic acid; “*Lecanora* sp. 4” of Śliwa & Wetmore (2000) with usnic acid; and *L. flotoviana* Spreng. with usnic acid ± psoromic acid. While the new taxon is morphologically similar to members of the *L. dispersa* group, it is also characterized by its preference for non-calcareous substrates and production of usnic acid. This, in combination with our results presented in **Fig. 2**, suggests that the circumscription of *Myr-*

iolecis may not be as straightforward as previously conceived. Pending the evaluation of the phylogenetic position of these other taxa, we prefer to treat the new species as a member of *Lecanora* in the broad sense, and retain nomenclature adopted in Śliwa (2007a,b) for purposes of comparison below.

The occurrence of a previously unrecognized usnic acid-bearing species within the *Lecanora dispersa* group is perhaps no more surprising than is the discovery of evolutionary gains of lobate thalli in other lineages or the discovery of usnic acid as a major substance within other species groups in *Lecanora* (e.g., within the *L. subfusca* group [Kirika et al. 2012; Papong et al. 2012; see also Brodo 1984; Lumbsch 1994]). Results presented in **Fig. 2**, together with other phylogenetic studies, support more recent arguments (Gudlerley 1999; Lumbsch 1995; Lumbsch et al. 1995) that usnic acid-containing species should be accepted within *Lecanora* s.str. Inclusion in future phylogenetic analyses of additional species morphologically similar to members of the *L. dispersa* group, but with usnic acid or other substances (see, e.g., Bertrand & Roux 2011), may shed additional light on the evolution of secondary metabolites within this taxonomically difficult group of lichens.

From this study and others, it is clear that sandstone outcrops serve as important and still incompletely documented habitats for cryptogamic diversity. Yet, many of these locations lack established conservation protection. Indeed, the largest and most extensive populations of the new species occur at a site that is unprotected, fragile, and human-impacted, i.e., White Rocks Niwot. Whereas the City of Boulder limits human impacts on the White Rocks Boulder site by mandating visitation through guided tours only, the Boulder County White Rocks Niwot site is open to the public, immediately abuts a large, relatively recent development/neighborhood (and thus a modern threat to this population that was lacking in earlier decades), and is negatively impacted by human foot and bicycle traffic on a daily basis. The third population, on Fox Hills outcroppings at Bohart Ranch, is currently under conservation easement and is managed by The Nature Conservancy. In sum, two of the three populations (White Rocks Boulder and Bohart Ranch) are relatively secure for the time being whereas the third (White Rocks Niwot) needs

immediate conservation action to ensure longevity of these individuals.

IUCN protocols applied to *Lecanora lendemeri* yielded a conservation assessment of Endangered for this new species. However, both the EOO (145 km²) and AOO (12 km²) only narrowly exceeded thresholds that would otherwise have resulted in a ranking of Critically Endangered, which requires an EOO of <100 km² and an AOO of <10 km². Thus, the small EOO, small AOO, immediate conservation threats to the Niwot site, and apparent restricted-endemic specificity to an already rare substrate seem to suggest that *Lecanora lendemeri* may be, in practical terms, more endangered than the IUCN assessment of “Endangered” conveys.

Discovery of an extensive new population of this previously unrecognized species at a tiny outcropping that occurs within a sea of urban development (White Rocks Niwot), plus one additional site in which rare Fox Hills Sandstone is outcropped (Bohart Ranch), calls further attention to the importance of biodiversity reservoirs (Tripp 2015), even miniature ones that barely exceed 2 ha in size, amidst massively reworked human landscapes. Sandstone outcroppings such as White Rocks Niwot should be the focus of conservation action by local and regional land management agencies, and it is hoped that local communities understand the severity of this message and support such actions. This is especially vital for substrates/habitats that are themselves extremely rare or endangered, such as outcropped Fox Hills Sandstone in western North America.

TAXONOMY

Lecanora lendemeri E.Tripp & C.A.Morse, *sp. nov.*

Figs. 2–4

MYCOBANK MB 829939

Saxicolous member of the lichen genus Lecanora, with largely endolithic thallus; with reddish brown to yellowish-brown, epruinose discs and yellowish-green, epruinose margins due to the presence of usnic acid; with coarse, POL+ granules above and between paraphyses tips, soluble in 25% KOH and insoluble in 65% HNO₃; with corticate margins with POL+ granules, insoluble in 25% KOH and ± soluble in 65% HNO₃; and with ascospores (11–)12.5–15.5(–17.5) × (5–)5.5–7(–8) µm; most

similar to L. dispersa but differing in having larger ascospores, expanded paraphyses tips, and usnic acid.

TYPE: U.S.A. COLORADO: Niwot, small outcropping of Fox Hills Formation sandstone 0.25 km N of Mineral Road and 0.3 km W of Somerset Drive, immediately adjacent to (SW of) community development, mixed grass relict prairie with *Bouteloua gracilis*, *Pascopyrum smithii*, *Toxicodendron rydbergii*, *Bromus tectorum*, 40°05'21.88"N, 105°09'15.46"W, 5281 ft. elevation, 24 June 2016, E. Tripp 5806 (holotype, COLO!; isotypes, H!, KANU!, NY!).

Description. Crustose lichen with primarily indistinct, endolithic thallus, rarely with inconspicuous, dispersed-areolate thallus, these creamy white, 0.1–0.2 mm in diameter. Apothecia dispersed or more typically crowded, sessile to slightly stipitate and raised above substrate, (0.3–)0.5–0.9(–1.3) mm in diameter (n=54). Discs primarily pale reddish-brown to yellowish-brown, epruinose, mostly plane, sometimes becoming weakly convex on mature apothecia and infrequently excluding the margins. Thalline margins yellowish-green when moist, dull yellow when dry, and creamy white in older herbarium specimens, epruinose, sublustrous, thick and raised above the disc except for most mature apothecia, narrowed by expanding discs on most mature apothecia, entire, weakly incised, or conspicuously cracked, typically a progression associated with apothecium age. Amphithecium 130–185 µm thick (measured from lateral side of outer wall of amphithecium to outer edge of hymenium), corticate, with gelatinous hyphae and photobiont cells filling the area internal to the lower part of the cortex; cortex ± uniform, ca. 35–60 µm thick, abundantly interspersed with POL+ granules, these present in almost all sections but apparently lacking from two sections from the type specimen, insoluble in 25% K and slowly ± soluble in 65% HNO₃; algal layer below hypothecium uneven, ~53–138(–175) µm tall. Hypothecium (37–)84–149 µm thick, hyaline, composed of slender, intricately interwoven hyphae ca. 1.3–2.1 µm wide, free in 10% K. Hymenium 61–114 µm high, hyaline, POL–. Epithecium 14–32 µm high, yellowish-brown, with coarse, POL+ granules above and between paraphyses tips, soluble in 25% K and insoluble in 65% HNO₃. Paraphyses 1.3–2.8 µm wide (distal cells

occasionally widened to 4 μm), often branched, especially distally, sometimes anastomosing, hyaline (although appearing brown due to epithelial pigment in gel surrounding paraphyses), free in 10% K. Asci clavate, *Lecanora*-type, 8-spored, 36–58 \times 19–24 μm long. Ascospores hyaline, simple, often slightly pointed at one or both ends, oblong-ellipsoid to ellipsoid, (11–)12.5–15.5(–17.5) \times (5–)5.5–7(–8) μm , (1.6–)2.0–2.6(–3.0) times longer than wide ($n=57$, measured in both water and 10% K). Pycnidia not seen. Photobiont trebouxoid, (8–)11.5–15(–17.5) μm in diameter.

Chemistry. Usnic acid detected via Thin Layer Chromatography. Spot tests: Apothecial margins K+ yellow, KC+ yellow, C–, PD–; discs K–, KC–, C–, PD–; apothecia UV–.

Etymology. It is a tremendous honor to name this new species after our friend and colleague, James Lendemer. James is among the most talented and productive lichenologists of the 21st Century. His exceptional work ethic, knowledge and drive has yielded an extraordinary record of scholarship, helping to usher in a new era lichen taxonomy, ecology and evolution, and establishing a long-lasting legacy in North American lichenology. For two decades, James has been a staunch advocate not only for the discovery but also the conservation of the North American lichen biota. He has shared his passion and excitement for these organisms through hundreds of talks and seminars delivered to a broad variety of individuals, ranging from the general public to hobbyists to professionals. James is also a selfless teacher: he has been an important mentor to many, including the authors of this paper. Dedication of this new species to James is particularly fitting because James's first love is the tiniest of crustose lichens that go unnoticed by others. But, as James says, "shove it under the scope!"

Ecology and distribution. So far as known, *Lecanora lendemeri* is restricted to three separate outcroppings of non-calcareous Fox Hills Sandstone, where it occurs on highly fragile, primarily horizontal rock surfaces. It is currently known only from these three populations, all of which occur within north-central Colorado, U.S.A. The two populations present in Boulder County occur within 4 km distant from one another.

Notes. Following Śliwa (2007a), eleven members of the *Lecanora dispersa* group produce POL+

granules in their amphithecial cortex, but only members of the *L. semipallida* group produce crystals in the epithecium that are soluble in 25% K. Members of this group, which includes *L. fugiens* Nyl., *L. invadens* H.Magn., *L. semipallida* H.Magn., *L. wetmorei* Śliwa and *L. zosteræ* var. *beringii* (Nyl.) Śliwa, all differ from *L. lendemeri* in their secondary chemistry, either by producing xanthenes or no secondary metabolites. In addition to this, *L. fugiens*, *L. invadens* and *L. wetmorei* all have pruinose apothecial discs and/or margins and shorter ascospores; *L. semipallida* has pale discs and shorter ascospores; and *L. zosteræ* var. *beringii* is calcicolous and has an arctic alpine distribution (Śliwa 2007a).

In the field, *Lecanora lendemeri* is most likely to be confused with *L. dispersa*, which is a geographically widespread and morphologically highly variable taxon that grows on a wide variety of substrates, including siliceous rocks. *Lecanora dispersa*, however, has consistently smaller ascospores (8–12 \times 4.5–6 μm), paraphyses tips that are not expanded apically, and typically produces 2,7-dichlorolichexanthenes (sometimes in addition to pannarin) or no compounds.

Rarely, sections through apothecia of the type specimen appear to lack POL+ granules in the amphithecial cortex. It is unclear, based on current material, how variable this feature is across individuals and populations, but available data indicate that the amphithecia typically produce POL+ granules. Nonetheless, it is thus possible that *Lecanora lendemeri* could be confused with several species that lack POL+ granules. Among these, *L. lendemeri* differs from *L. zosteræ* in growing on non-calcareous sandstone rather than on calcareous rocks or organic substrates, typical of the latter species, in containing usnic acid rather than lacking any lichen products, and in having an interspersed and uniform cortex rather than non-interspersed, gelatinous, and greatly expanded at the base. *Lecanora crenulata* (Ach.) Hook., *L. agardhiana* Ach., and *L. perpruinosa* Fröberg differ from the new species in their exclusive occurrence on calcareous rocks and in having smaller ascospores. *Lecanora percrenata* H.Magn., although similar to *L. lendemeri* in its cracked margins, typically has discs that are nearly black in color (vs. reddish-brown in *L. lendemeri*) and also lacks any lichen substances; however, in the absence of a chemical study, the two species are

otherwise morphologically comparable and *L. percrenata* similarly has relatively large ascospores. *Lecanora flowersiana* H.Magn. is also morphologically very similar to the new species, and may be especially confusing without close investigation given the two species occur together at both White Rocks Boulder and White Rocks Niwot; the two are best distinguished through spot tests and/or TLC given that *L. flowersiana* lacks lichen products. Two other species are less likely to be confused for *L. lendemeri* because they either have margins that are more or less concolorous with the discs, smaller ascospores, and apothecia in groups of two or three (*L. persimilis* (Th. Fr.) Arnold) or have smaller ascospores and lack lichen substances (*L. hagenii* (Ach.) Ach.).

Specimens with distinctly yellowish apothecial margins may also be confused with members of the *Lecanora polytropa* or *L. varia* groups in the field. In North America, the *L. polytropa* group includes approximately seven taxa, excluding members referred to the *L. filamentosa* or *L. symmicta* groups by Perez-Ortega et al. (2010) and to *Protoparmeliopsis* by Kondratyuk et al. (2014) and Zhao et al. (2015). These species—*L. atrosulphurea* (Wahlenb.) Ach., *L. chlorophaeodes* Nyl., *L. intricata* (Ach.) Ach., *L. jamesii* J.R.Laundon, *L. polytropa* (Ehrh.) Rabenh., *L. soralifera* (Suza) Räsänen and *L. stenotropia* Nyl.—differ from *L. lendemeri* in their secondary chemistry, producing aliphatic acids, xanthenes, and/or zeorin in addition to usnic acid, and most produce a well-developed, epilithic thallus and/or smaller ascospores (Edwards et al. 2009; Ryan et al. 2004).

Śliwa & Wetmore (2000) included 16 North American taxa in a treatment of the *Lecanora varia* group, most of which differ from the new species in the solubility of amphithecial granules in K or their preferred substrate. One taxon, referred to by Śliwa & Wetmore (2000) as “*Lecanora* sp. 4,” was characterized by those authors as being similar to members of the *L. dispersa* group but producing usnic acid as a secondary metabolite. “*Lecanora* sp. 4” of Śliwa & Wetmore (2000) differs from *L. lendemeri* in producing pruinose apothecial discs (and sometimes margins), shorter ascospores, and in being corticolous.

Śliwa (2007a,b) excluded several additional species that are morphologically similar to the *Lecanora dispersa* group but that produce isousnic or usnic acid as a secondary metabolite. Among

these, *L. flotoviana* Spreng. is similar to *L. lendemeri*. *Lecanora flotoviana*, however, has a distinct epilithic, areolate thallus of flattened rosettes, brown to blackish discs, and occurs on calcareous rocks or on other lichens. According to Śliwa (2007a,b), *L. flotoviana* sometimes also produces psoromic acid and, so far as known, is restricted to Europe. It is noteworthy that some previous phylogenetic studies of the *L. dispersa* group have included an accession originally determined as *L. flotoviana* (GenBank accession 6524436, as *L. flotowiana*). Śliwa et al. (2017) regarded this sequence as representing *L. semipallida* with the comment that the voucher was likely misidentified, but there is no indication that those authors were able to examine the specimen itself. Consequently, we have not included this sequence in our study. However, it is clear from the results presented here that future studies of phylogenetic relationships of members of the *L. dispersa* group should include a broader sample of *Lecanora* species, including additional taxa with usnic acid, as well as explore next-gen methods of phylogenetic reconstruction based on the limited utility of most Sanger loci.

Paratypes. U.S.A. COLORADO: Boulder Co., *E. Tripp* & *D. Clark* 4823 (COLO!, NY!). El Paso Co., *C. Morse* & *D. Ladd* 15869 (KANU!).

ACKNOWLEDGMENTS

Most of the authors of this manuscript are collaborators on a National Science Foundation Dimensions of Biodiversity project (together with James Lendemer) and we gratefully acknowledge James's many contributions to this research endeavor and more generally to good times in lichenology. We are indebted to our friends and colleagues at Boulder County Open Space and City of Boulder's Open Space and Mountain Parks who encouraged this work and facilitated the acquisition of permits and site visits. Mac Kobza, Jennifer Kesler (Boulder County) and Lynn Riedel (City of Boulder) have been especially helpful. We additionally thank Lynn Riedel and Dina Clark (COLO), who first inspired fieldwork on Fox Hills Sandstone by the first author. At COLO, Dina Clark, Tim Hogan, Ryan Allen, and numerous students have provided extensive support to the first author. Irwin Brodo and one anonymous reviewer provided comments that improved an earlier version of the manuscript. We thank the following sources of financial support that made this work possible: a research grant from Boulder County Open Space to EAT, a research grant from City of Boulder's Open Space and Mountain Parks to EAT, and an NSF Dimensions of Biodiversity award to three of the present authors (EAT, CMM, NK, University of Colorado: Award #1542629) as well as to James Lendemer (New York Botanical Garden: Award #1432629). CAM thanks Doug Ladd for companionship in the field and The Nature Conservancy of Colorado for

permission to collect at the Bohart Ranch. CAM's fieldwork was made possible by anonymous contributions to the McGregor Herbarium Endowment Fund.

LITERATURE CITED

- Arup, U. & M. Grube. 1998. Molecular systematics of *Lecanora* subgenus *Placodium*. *Lichenologist* 30: 415–425.
- Bankevich, A., S. Nurk, D. Antipov, A. A. Gurevich, M. Dvorkin, A. S. Kulikov, V. M. Lesin, S. I. Nikolenko, S. Pham, A. D. Prjibelski, A. V. Pyshkin, A. V. Sirotkin, N. Vyahhi, G. Tesler, M. A. Alekseyev & P. A. Pevzner. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *Journal of Computational Biology* 19: 455–477.
- Bertrand, M. & C. Roux. 2011. *Lecanora oyensis* sp. nov., une nouvelle espèce de lichen maritime. *Bulletin Association Française de Lichénologie* 36: 107–117.
- Bolger, A. M., M. Lohse & U. Usadel. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 1: 2114–2120.
- Brodo, I. M. 1984. The North American species of the *Lecanora subfusca* group. *Beihefte zur Nova Hedwigia* 79: 63–185.
- Brodo, I. M., S. D. Sharnoff & S. Sharnoff. 2001. *Lichens of North America*. Yale University Press, New Haven.
- Byars, L. F. 1936. An ecological study of the ants of Boulder County, Colorado. MA Thesis, Department of Biology, University of Colorado, Boulder.
- Clark, D. A., C. Crawford & W. F. Jennings. 2001. Draft baseline plant survey of White Rocks and Surrounding Area in Eastern Boulder County. Unpublished report prepared for the City of Boulder Open Space and Mountain Parks Department.
- Culberson, C. F. & H. Kristinsson. 1970. A standardized method for the identification of lichen products. *Journal of Chromatography* 46: 85–93.
- Dobbin, C. E. & J. B. Reeside Jr. 1929. The contact of the Fox Hills and Lance Formations. Professional Paper 158-B. U. S. Geological Survey, Department of the Interior, Washington, D.C.
- Edwards, B., A. Aptroot, D. L. Hawksworth & P. W. James. 2009. *Lecanora*. Pages 465–502. In: C. W. Smith, A. Aptroot, B. J. Coppins, A. Fletcher, O. L. Gilbert, P. W. James & P. A. Wolsley (eds.), *The Lichens of Great Britain and Ireland*. British Lichen Society, London.
- Google Earth Pro 2018. V 7.3.2.5495. 2018. Colorado, USA. DigitalGlobe 2018. <http://www.earth.google.com> [accessed November 30, 2018.]
- Guderley, R. 1999. Die *Lecanora subfusca*-Gruppe in Süd- und Mittelamerika. *Journal of the Hattori Botanical Laboratory* 87: 131–257.
- Henderson, J. 1920. The Cretaceous formations of northeastern Colorado and the foothills formations of north-central Colorado. *Colorado Geological Survey Bulletin* 19, 98 pp.
- Hirschfeld, S. 2013a. Unique Geology of White Rocks. Information Report to City of Boulder, Open Space and Mountain Parks.
- Hirschfeld, S. 2013b. Unique Geology and Biota of the Niwot/Somerset White Rocks on Boulder County Parks and Open Space. Information Report to City of Boulder, Open Space and Mountain Parks.
- IUCN Standards and Petitions Subcommittee. 2014. Guidelines for using the IUCN Red List Categories and Criteria. Version 11. Prepared by the Standards and Petitions Subcommittee.
- Kirika, P., S. Parnmen & H. T. Lumbsch. 2012. Two new species of *Lecanora* sensu stricto (Lecanoraceae, Ascomycota) from east Africa. *MycKeys* 3: 37–47.
- Kondratyuk, S. Y., J. Kim, A. S. Kondratyuk, M. H. Jeong, S. H. Jang, M. Pirogov & J. S. Hur. 2014. First data on molecular phylogeny of the genus *Protoparmeliopsis* M. Choisy (Lecanoraceae, Ascomycota). *Modern Phytomorphology* 5: 63–68.
- Kumar, S., G. Stecher & K. Tamura. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1870–1874.
- Larkin, M. A., G. Blackshields, N. P. Brown, R. Chenna, P. A. McGettigan, H. McWilliam, F. Valentin, I. M. Wallace, A. Wilm, R. Lopez, J. D. Thompson, T. J. Gibson, & D. G. Higgins. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics* 23: 2947–2948.
- Lumbsch, H. T. 1994. Die *Lecanora subfusca*-Gruppe in Australasien. *Journal of the Hattori Botanical Laboratory* 77: 1–175.
- Lumbsch, H. T. 1995. A new species in the *Lecanora subfusca* group containing usnic acid in addition to atranorin. *Lichenologist* 27: 161–167.
- Lumbsch, H. T., G. B. Feige & J. A. Elix. 1995. A revision of the usnic acid containing taxa belonging to *Lecanora* sensu stricto (Lecanorales: lichenized Ascomycota). *The Bryologist* 98: 561–577.
- MacPhail, D. D., H. L. Young & D. I. Netoff. 1970. White Rocks Natural Area Study. Unpublished manuscript, Department of Geography, University of Colorado, Boulder.
- Orange, A., P. W. James & F. J. White. 2001. *Microchemical Methods for the Identification of Lichens*. British Lichen Society, London.
- Papong, K., K. Boonpragob, S. Parnmen & H. T. Lumbsch. 2012. Molecular phylogenetic studies on tropical species of *Lecanora* sensu stricto (Lecanoraceae, Ascomycota). *Nova Hedwigia* 96: 1–13.
- Pérez-Ortega, S., T. Spribille, Z. Palice, J. A. Elix & C. Printzen. 2010. A molecular phylogeny of the *Lecanora varia* group, including a new species from western North America. *Mycological Progress* 9: 523–535.
- Roehler, H. 1993. Stratigraphy of the Upper Cretaceous Fox Hills Sandstone and adjacent parts of the Lewis Shale and Lance Formation, east flank of the Rock Springs Uplift, southwest Wyoming. Professional Paper 1532. U. S. Geological Survey, Department of the Interior, Washington, D.C.
- Ryan, B. D., H. T. Lumbsch, M. I. Messuti, C. Printzen, L. Sliwa, and T. H. Nash III. 2004. *Lecanora*. Pages 176–286. In: T. H. Nash III, B. D. Ryan, C. Gries & F. Bungartz (eds.), *Lichen Flora of the Greater Sonoran Desert Region. II. Lichens Unlimited*, Tempe. Sliwa, L. 2007a. A revision of the *Lecanora dispersa* complex in North America. *Polish Botanical Journal* 52: 1–70.
- Śliwa, L. 2007b. *Lecanora semipallida*, the correct name for *L. xanthostoma*, and a reappraisal of *L. flotviana* (Lecanoraceae, Ascomycotina). *Polish Botanical Journal* 52: 71–79.
- Śliwa, L., J. Miadlikowska, B. D. Redelings, K. Molnar & F. Lutzoni. 2012. Are widespread morphospecies from the *Lecanora dispersa* group (lichen-forming Ascomycota) monophyletic? *The Bryologist* 114: 265–277.
- Śliwa, L. & C. M. Wetmore. 2000. Notes on the *Lecanora varia* group in North America. *The Bryologist* 103: 475–492.
- Tavaré, S. 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. *Lectures on Mathematics in the Life Sciences* 17: 57–86.

- Thiers, B. M. 2018 [continuously updated]. Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. <http://sweetgum.nybg.org/science/ih/>.
- Tripp, E. A. 2015. Lichen inventory of White Rocks Open Space (Boulder, Colorado). *Western North American Naturalist* 75: 301–310.
- Tripp, E. A. 2016. Field Guide to the Lichens of White Rocks Open Space. University Press of Colorado, Boulder, Colorado, 170 p.
- Tripp, E. A. & J. C. Lendemer. 2015. *Candelariella clarkiae* and *Lecidea hoganii*: two lichen species new to science from White Rocks Open Space, City of Boulder, Colorado. *The Bryologist* 118: 154–163.
- Weber, W. A. 1949. The Flora of Boulder County, Colorado. Unpublished report, University of Colorado Museum, Boulder, Colorado.
- Weimer, R. J. 1973. A guide to the uppermost Cretaceous stratigraphy, central Front Range, Colorado: deltaic sedimentation, growth faulting and early Laramide crustal movement. *The Mountain Geologist* 10: 53–97.
- Westberg, M., C. A. Morse & M. Wedin. 2011. Two new species of *Candelariella* and a key to the Candelarieales (lichenized Ascomycetes) in North America. *The Bryologist* 114: 325–334.
- Zhao, X., S. D. Leavitt, Z. T. Zhao, L. L. Zhang, U. Arup, M. Grube, S. Pérez-Ortega, C. Printzen, L. Śliwa, E. Kraichak, P. K. Divakar, A. Crespo & H. T. Lumbsch. 2015. Towards a revised generic classification of lecanoroid lichens (Lecanoraceae, Ascomycota) based on molecular, morphological and chemical evidence. *Fungal Diversity* 78: 293–304.

manuscript received December 17, 2018; accepted March 2, 2019.