"Clayton's Variety" of Lecanora pringlei: What is it and where is it?

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March, 2013

Funded by
Interagency Special Status/Sensitive Species Program
Introduction

*Lecanora pringlei* was first described as *Lecidea pringlei* by Tuckerman (1883), from a collection in the Sierra Nevada and by a second collection from the east slope of the Cascade Mountains in Washington. It is currently placed in the genus *Lecanora* (Lamb 1939) of which most species are crustose. However, in *L. pringlei*, the areoles become tall and stipitate, broadening towards the top, so that mature specimens are fruticose. It has been suggested that this species, along with a few others could be segregated into a separate genus (Nash et al. 2004).

The thallus is shiny and light cream to yellowish green, but when exposed to sun it becomes dark olive to black. Thus a thallus when seen in the field looks like a rounded cushion made of a group of black areoles, but when pulled apart the lower extent of each areole is often nearly white. The cortex is shiny and gives the thallus a waxy look. Apothecia are black and immersed in the top of the areoles, often indistinguishable from the rest of the thallus, but at other times filling the whole areole so that the apothecia look stalked with a definite black margin. Spores are hyaline, simple and broadly ellipsoid 8-10x3-5 µm (Nash et al. 2004).

The habitat for this species is rock cliffs and outcrops, especially in soil along cracks. It is found on igneous rock, usually andesite (Scot Loring, pers. comm.). In southern Oregon, it grows from about 5500' up to the alpine zone (Scot Loring, pers. comm.). It is found on many of volcanic peaks in the Cascade Range in Washington and Oregon.

In the late 1990's the bryologist Clayton Newberry reported seeing an unusual form of *Lecanora pringlei*. It seemed to be a *Lecanora* and appeared similar to *L. pringlei* in its 3-dimensional habit of a somewhat fruticose, stalked thallus with apothecia on the upper surface with a poorly defined margin, especially on larger, older apothecia (Scot Loring, pers. comm.). Multiple differences, however, made Newberry think it was different from typical *L. pringlei*. First, it appeared at low elevations, as low as 1300', was generally smaller in width and height, growing on a variety of rock substrates (including serpentine) and varied in color from brown to red-brown to grey (S. Loring, pers. comm.). Unfortunately, no collections of this different-looking lichen exist.

The purpose of this study was to determine if there is a variety or form of *Lecanora pringlei* in southwestern Oregon that is reddish brown and grows at elevations lower than 5500 ft., on a variety of rock substrates. In addition, if such a lichen is found, we were to determine if it is a rare lichen.

Methods

All *Lecanora pringlei* vouchers held by the USDA Forest Service/USDI Bureau of Land Management Interagency Special Status/Sensitive Species Program (ISSSSP) were examined (23 specimens). All collections of *L. pringlei* at the Herbarium of Oregon State University (OSC) and in the personal herbaria of Dr. Bruce McCune and Dr. Daphne Stone were also examined (33 specimens, Appendix 1.). In addition, to assess low elevation sites, most sites below 4000' in southwestern Oregon where *L. pringlei* was
reported on BLM land but where specimens were not collected, were revisited in fall 2011 in an attempt to find some of the unusual-looking thalli.

Other species that were collected because of their superficial similarity to *L. pringlei* were sequenced to verify their affinities. These sequences were compared to other sequences in GenBank. Sequenced specimens will be annotated and submitted to OSC.

Well-preserved thalli of *Lecanora pringlei* without any visible damage or fungal infection were chosen for DNA isolation. Small fragments of lobes, including the mycobiont and the photobiont, from fresh or dried herbarium collections (up to 4 years old) were sampled for DNA extraction. Sampled specimens were Oregon collections made by Dr. Bruce McCune and Dr. Daphne Stone as well as by surveyors working for the Bureau of Land Management.

Approximately 0.5 mg of lichen thalli was placed in a 2.0 mL microfuge tube with 1 mL CTAB extraction buffer (2% cetyltrimethyl ammonium bromide, 100mM Tris pH 8.0, 20 mM Na2-EDTA, 1.4 M NaCl, 1% polyvinylpolypyrrolidone, 1% 2-mercaptoethanol) and 3 mm glass beads. The sample was agitated for 30" at 4200 rpm in a mini beadbeater (Biospec products, Bartlesville, OK). After mixing the sample was incubated at 65° C for 2 hours. The crude DNA extract was then purified in 24:1 chloroform:isoamyl alcohol followed by further extraction using a DNeasy tissue extraction kit (Qiagen, Valencia CA) following the manufacturer's instructions.

Nuclear ribosomal large subunit DNA was amplified from the template DNA using primers LROR and LR7 (Vilgalys and Sun 1994) and ITS primers ITS4 (White et al. 1990) and ITS1-F (Gardes and Bruns 1993). PCR reactions (50 uL) were carried out in a 1X buffer, 200 µM dNTP, 0.4M forward and reverse primers, 0.05 U/uL RedTaq DNA polymerase (Sigma, St. Louis), 0.05% blocking powder (Schleicher & Schuell Blocking Powder # 78034), and 20-200 ng template DNA. Amplification was carried out for 35 cycles of 30s at 95° C, 30s at 52° C and 90s at 72° C using a PTC 100 thermal cycler (MJ Research, Watertown, Mass). PCR products were prepared for DNA sequencing by addition of 1 µL EXOSAP-IT (USB, Cleveland OH) and incubated overnight at room temperature, followed by 15 min. at 80° C. Cycle sequencing of both forward and reverse strands was carried out in an ABI capillary sequencer (Applied Biosystems, Foster City, CA). Contigs were assembled and edited with the Staden software package (1996) and aligned using ClustalX (Thompson et al 1997).

Photomicrographs were taken by the author using a Canon Powershot digital camera held against the ocular on compound microscopes and stereoscopes. The photos were cropped and scales added using Photoshop.

**Results**

**Sites visited**

Six sites that are at lower elevations than normal for *Lecanora pringlei*, but where *L. pringlei* had been reported, were visited (Table 1).
Table 1. Low elevation sites where *Lecanora pringlei* was reported, visited during study.

<table>
<thead>
<tr>
<th>Sites visited</th>
<th>TRS</th>
<th>elevation, ft.</th>
<th>L. pringlei found</th>
<th>County</th>
<th>UTM NAD83 Zone 10 T</th>
</tr>
</thead>
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<tr>
<td>Rattlesnake Rocks</td>
<td>T34S R2W Sec1</td>
<td>3000-3200</td>
<td>Y</td>
<td>Jackson</td>
<td>0509305 4721890</td>
</tr>
<tr>
<td>Emigrant Creek area</td>
<td>T40S-R2E-Sec25</td>
<td>3872</td>
<td>N</td>
<td>Jackson</td>
<td>0538265 4657049</td>
</tr>
<tr>
<td>Grizzly Peak</td>
<td>T38S R2E Sec7</td>
<td>4400-5800</td>
<td>N *</td>
<td>Jackson</td>
<td>0530044 4680600</td>
</tr>
<tr>
<td>Chimney Rock area</td>
<td>T37S R1E Sec25</td>
<td>3900</td>
<td>N *</td>
<td>Jackson</td>
<td>0529565 4686662</td>
</tr>
<tr>
<td>Lower Table Rock</td>
<td>T36S R2W Sec9</td>
<td>1870</td>
<td>N</td>
<td>Jackson</td>
<td>0504300 4699580</td>
</tr>
<tr>
<td>Galls Creek Road</td>
<td>T37S R3W Sec9</td>
<td>2890</td>
<td>N</td>
<td>Jackson</td>
<td>0494489 4691053</td>
</tr>
</tbody>
</table>

* both of these sites were large and seemed correct habitat. Further searches may turn up *L. pringlei*
**Rattlesnake Rocks** is a long, curving set of cliffs on a long promontory with east, south and west faces. The rock is igneous, but ancient and broken down in places. Large lamina of rock are peeling off in some areas, taking saxicolous lichens with them. The cliffs are cut into several separate chunks with steep talus slopes cutting down through them. The area has apparently been used by rock climbers who have built paths down the talus slopes, thus helping to preserve the area. Elevation is approximately 3000' to 3200'.

![Fig. 1. Rattlesnake Rocks. Upper left: Scot Loring and Tom Carlberg in an archway. Upper right: rock face with *L. pringlei* in small hole at arrow, rock delamination area in upper right. Lower left: *Miriquidica* sp. Lower right: *Lecanora pringlei* (presumed) in bubble hole in volcanic rock.](image)

Lichenologists Scot Loring, Tom Carlberg and Daphne Stone visited the area in spring 2011. Loring remembered seeing the *Lecanora pringlei* here several years before and we tried to relocate the area that he remembered. After spending about 4 hours scouring the cliffs, we were able to find only one bit of what appears to be *Lecanora pringlei*. Unfortunately, it is growing in a small air bubble hole in the rock. We were not able to collect it without destroying it, so it remains in place (Fig. 1). Other places along the cliff face seemed like the spot Loring remembered, but we did not find any *Lecanora pringlei* here. One such area has recently lost several square meters of surface rock by
delamination. We also found one lichen species that resembles young *L. pringlei*; this was later identified as *Miriquidica* sp. (Fig. 1).

**Near Joe's Rock, Emigrant Creek drainage.** This site is at 3872′, on a steep *Quercus garryana* dominated hillside (Fig. 2). The only appropriate habitat found in the described area were two low rock outcrops. The specimen collected here in 2007 (OSC#134693) looks like typical *Lecanora pringlei*, but is in fact *Toninia ruginosa*. We collected what looked like *L. pringlei* but it was growing in moss clumps which seemed unusual. It, too, is *Toninia ruginosa*.

Fig. 2. Emigrant Creek area. Upper left: Scot Loring and south rock. Upper right: north rock. Lower left: *Toninia ruginosa* with apothecia on mosses. Lower right: *Toninia ruginosa* forming small mound.
**Grizzly Peak area.** This site is at 4400' to 5800', and includes many cliffs and outcrops in open meadows (Fig. 3). It has been reported as a huge population with many subpopulations in several acres and two sections. Previous surveyors did not collect vouchers. What we found looked like typical *Lecanora pringlei* but was a bit shorter and less cushion-like than typical healthy thalli. We collected some and identified it as *Toninia ruginosa*. This site should be revisited to confirm the presence of *Lecanora pringlei*. Since the habitat is typical and the elevation is high enough, it is probable that *L. pringlei* is at this site and we overlooked it.

Fig. 3. Grizzly Peak area. A series of low outcrops and cliffs in open shrubby grassland.
Cliffs to the northwest of Chimney Rock. This site is at approximately 3900', and is a series of tall outcrops on a steep southwest-facing, grassy hillside (Fig. 4). At this site we found what could have been mistaken for a brown, young *L. pringlei*, but is actually *Acarospora thamnina*. The most common look-alike, *Toninia ruginosa*, was also collected here. No *L. pringlei* was found at this site.

![Fig. 4. Chimney Rock area. Upper left: *Acarospora thamnina* (in circle). Lower left: *Toninia ruginosa* (in circle). Right: Scot Loring at base of cliffs.](image)

Lower Table Rock. This site is located at 1870' at the base of the east side of Lower Table Rock. Access is from the northeast side of Lower Table Rock, walking south along the base of the slope under the powerline. The forest below the cliffs is a young mixed conifer-hardwood forest with a few old conifer trees and snags. The cliffs are in direct sun only part of the day, partly because of the east aspect and partly because of a set of small tors beside the cliff at this spot. This interesting site is easily located because it lies on the main cliff directly behind these tors that are separate from the main Table Rock cliffs. Lining up Mt. McLaughlin and the edge of the south-most tor, the line points directly at the site.

The location examined is in an interesting spot with several unusual lichens including
*Peltula euploca*. Loring mentioned that the *L. pringlei* here were brown and a bit farther along the cliff from a marked *Peltula euploca* site. I found the *P. euploca* marked with a small piece of bumblebee flagging stuck in a crack in the rock. Moving south a bit along the cliff base I found what I believe Loring mistook for Clay’s *L. pringlei* but is actually *Acarospora thamnina*. *Toninia ruginosa* also grows in the area. No photos were taken at this site.

**Galls Creek Road.** This site is on a gentle southeast-facing ridge in a thinned, mixed forest at 2890’. The site as far as I could tell is two meter-long boulders on the ridge. This would be a really strange place for *L. pringlei* to grow, and none was found here, and there were no other species that would be confused with *L. pringlei*.

![Fig. 5. Gall's Creek Rd. Two boulders, no Lecanora pringlei.](image)

**Habitat of Lecanora pringlei**

By compiling known sites of *Lecanora pringlei* in southwestern Oregon, we are able to get a good idea of the range in elevation in which this species occurs. Among the verified specimens from southwest Oregon, two are from elevations just over 3600’ (OSC# 134702, 140422). No sites lower than this were found except for the minute, unverifiable population at Rattlesnake Rock, at approximately 3000’. The highest elevation site is above 6000’ and *L. pringlei* probably can grow at higher elevations that have not been surveyed as intensively as lower elevations.

All the sites with *L. pringlei* that I visited are on igneous rock. Three sites with verified specimens are reported as on serpentine rock (OSC# 140421, 140422, 140423). One verified site is reported as on metamorphic rock (OSC#134702)

**Look-alikes**

*Lecanora pringlei* thalli range from ivory colored where protected from the sun to black where exposed, and with a variety of greenish to yellowish tan colors in areas that have intermediate exposure. The surface of the thallus is shiny, with a transparent, waxy look to the cortex. The lobes grow as inflated, hollow, irregularly dented stipitate areoles
rising vertically from the rock substrate in tight groups forming small cushions (Fig. 6). Apothecia form immersed in the top of these areoles, beginning as small circular, slightly matte circles and with age covering the top of the areole. Occasionally the areole pinches in below the apothecium so that a thalline rim is formed (Fig. 7).

Fig. 6. *Lecanora pringlei* thalli showing variation in thallus color and shape. Scale bars are 1 mm except as noted.
Fig. 7. *Lecanora pringlei* apothecia. Apothecia begin as small area on top of thallus (center), then grow to cover top of lobe, and when mature can be wider than original lobe (top). Scale bars are 1 mm.
Four look-alike species were identified. The first, and most commonly misidentified as *L. pringlei*, is *Toninia ruginosa*. This species has a similarly shiny cortex and greenish-black color on the upper side of its thallus, and has a lighter color in parts not as exposed to sunlight. It grows in patches over mosses (often *Grimmiaceae* spp.) and has a lumpy, uneven thallus that is easily mistaken for the tops of *L. pringlei* areoles. The thallus often wraps over the tips of a moss clump forming a hollow envelope, much like the cross section of a *L. pringlei* areole. The apothecia of *T. ruginosa* are lecideine, with a black fruiting surface and a black rim without photosymbiotic algae. The epithecium is dark reddish brown and the hypothecium is pale reddish brown. Spores are narrowly ellipsoid to acicular, 1-7 septate, 12-42 X 3-4.5 µm (Nash et al 2002). Although the apothecia are not immersed, they look very much like well-developed apothecia of *L. pringlei* that have covered the entire areole, so that there is only a small, blackened rim left around the apothecial margin (Fig. 8).

![Fig. 8. *Toninia ruginosa* thalli. Upper: thallus growing over mosses. Lower left: thallus growing over moss debris so it looks stalked. Lower right: typical thallus with apothecia. Scale bars are 1 mm.](image-url)
The second look-alike is *Miriquidica* sp. Although it fits into the genus well, the small specimen does not appear to be any previously described species. It has small, brown, 3-dimensional areoles that look much like young *L. pringlei*, however the thallus is not shiny and is a uniform brown without lightening on lower parts of the areoles (Fig. 9). The thallus contains norstictic acid and crystals that shine in polarized light. The black, lecideine apothecia are not immersed and are microscopically rough on the fruiting surface. The hymenium is red-brown above and blue-green below. The spores are approximately 7.5 X 6-6.5 µm.

Fig. 9. *Miriquidica* sp. Apothecia not visible in photo, but they are raised, matte and black. Scale bar is 0.5 mm.
A third look-alike is *Acarospora thamnina*. This species has shiny, red-brown, three-dimensional areoles. It looks like young, reddish brown *L. pringlei* just beginning to form 3-dimensional cushions. It has one to several small apothecia immersed in the tops of the areoles (Fig. 10). The apothecia produce asci filled with hundreds of tiny spores, 4-5 X 1 - 1.8 μm (Nash et al. 2007). The ephymenium is dark reddish-brown.

Fig. 10. *Acarospora thamnina*. Apothecia show as small dots. Scale bar 1 mm.
A fourth look-alike was only collected once (Fig. 11). It appeared to be an old, weathered thallus of *Toninia ruginosa* but was identified by Bruce McCune as an unnamed species of *Lecidea*. We were only able to get a single stranded sequence from this collection, and it did not have any close affiliates in GenBank.

![Figure 11](image)

**Fig. 11.** *Lecidea* sp. looks like an old, weathered thallus of *Lecanora pringlei*. Note black apothecium at top. Scale bar 1 mm.

**Sequencing Results**  
As often happens, we were unsuccessful sequencing many of our specimens. However, we did get ten double-stranded ITS sequences; these are shown in the tree in Figure 12. The three *Lecanora pringlei* specimens grouped together closely. Unfortunately no other authors' sequences of *L. pringlei* are currently in GenBank, and we do not have material from the type or type location, so we are presuming that what we are calling *L. pringlei* is indeed that species. These three collections are from widely distributed sites in Oregon: McCune 29823 from South Sister, Deschutes Co.; McCune 30116 from Grayback Mountain, Josephine Co.; and Copeland SC70, from Pilot Rock, Jackson Co.
Three other species that were collected because they look similar to *L. pringlei* are spread widely in the rest of the tree, showing that they are not closely related. None of these species are currently represented by sequences in GenBank, however our *Acarospora thamnina* appears to be a sister species to *A. sinopica*. Three specimens of *Toninia ruginosa* (Stone 8343.1 and 8342.1, Grizzly Peak, Jackson Co., OR and Stone 8349, Chimney Rock area, Jackson Co., OR) group together in the same species cluster. Stone 8341, Jackson Co., OR occurs on a separate branch and may represent an undescribed sister species. All sequenced specimens are deposited at OSC (Oregon State University Herbarium) or in the personal collection of Dr. Bruce McCune, that will be given to OSC in the future.
Fig. 12. Tree of all successful double-stranded ITS sequences of Lecanora pringlei and its look-alikes.
Discussion

Identity of "Clayton's Variety"
No *Lecanora pringlei* that is brownish and growing at low elevations was found in this study. However, much was learned about this species, and should help clarify its identity in the future. (Note that one low-elevation thallus presumed to be *L. pringlei* that is brown was found at Rattlesnake Rocks, but could not be collected.)

Status of *Lecanora pringlei*
What we currently call *Lecanora pringlei* is not rare, although it is seldom found at elevations below 4000'. It thrives on basalt rock at elevations up to 5500' and most likely higher, on Cascade ridges and mountains. Because of less intensive use of forests at high elevations, these areas are also surveyed less than lower elevation areas, leading to few high elevation known sites of *L. pringlei*. Because of the abundance of the habitat and the wide distribution of the species in the Pacific Northwest and nearby states (Herbarium of Bruce McCune, CPNWH), I recommend that this species be removed from its status as a strategic species and from the ORNHIC list as an S3 species.

Note that the type specimen for this species was not examined during this study. Also, as found in many recent studies of widespread lichens (Lumbsch & Leavitt 2011), what we now consider one species may, with genetic examination, be found to be several species in the future.

Ryan states that *L. pringlei*, together with *L. cavicola* and possibly some additional species should be segregated from *Lecanora* (Nash et al. 2004). Our *L. pringlei* does not fit the description of the subspecies *brandegei* which Ryan describes (Nash et al. 2004).

Acknowledgements
My thanks to the Interagency Special Status/Sensitive Species Program for funding this research. Thanks also to Scot Loring for remembering the history of Clay's variety and the sites, and to Tom Carlberg for field help. Dr. Bruce McCune identified the look-alikes and lent many specimens for perusal and sequencing. Marcia Wineteer initiated this study and requested funding.

References


