

***Chaenotheca subroscida* and *C. phaeocephala* in the Pacific Northwest of North America**

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Abstract. Morphological identification of *Chaenotheca phaeocephala* and *Chaenotheca subroscida* in the Pacific Northwest is difficult. Both have stalked mazaediate ascomata with yellow pruina and ascospores that are relatively large for the genus. In Scandinavia, their thalli are distinguishable and they occur in different habitats. We used thin-layer chromatography to examine the secondary metabolites in thalli of 36 specimens from Oregon and Washington identified as *C. phaeocephala* or *C. subroscida*. Nine specimens contained pseudoplacodiolic acid, which places them in *C. subroscida*. The thalli of six of these had dense whitish granules and no verrucae; other specimens had a mixture of verrucae and white granules. The rest of the specimens did not have pseudoplacodiolic acid and were therefore *C. phaeocephala*; their thalli had varying amounts of verrucae and granules. Our results suggest that identification of these two species in the Pacific Northwest is not possible without TLC.

Key words. Lichens, calicioids, TLC, pseudoplacodiolic acid.

INTRODUCTION

We investigated two calicioid lichens, *Chaenotheca phaeocephala* (Turner) Th. Fr. and *Chaenotheca subroscida* (Eitner) Zahlbr. in the Pacific Northwest, where they are difficult to distinguish from each other. The apothecia of the two species are morphologically very similar; they are stalked with mazaedia that have yellow pruina colored by vulpinic acid. They both have relatively large ascospores for the genus, 6–7 µm diameter. Tibell (1980) suggested that the difference between *C. phaeocephala* and *C. subroscida* could be due to phenotypic plasticity within a single species but Tibell and Beck (2002) found that the ITS sequences of the mycobionts were monophyletic with strong bootstrap support. Thus, although the species can be difficult to distinguish from each other, support for the two taxa is robust.

In Scandinavia, their thalli are quite different from each other and they occur in different habitats (Tibell and Beck 2002). On lignum, Scandinavian *Chaenotheca phaeocephala* have large thick brownish squamules, while on bark they are thinner, verrucose to granular and dull olive brown to greenish (Tibell 1999). *Chaenotheca subroscida* has a superficial pale gray thallus consisting of a thin layer of minute granules 0.05–0.1 mm diameter that look like soredia (Tibell 1999). In older parts of the thallus, smooth, flat, brownish or olivaceous verrucae develop and are covered by scattered minute, pale gray granules (Tibell and Beck 2002). In Scandinavia, *C. phaeocephala* grows on old *Quercus* in moderately shaded situations, and rarely on *Alnus*, *Fagus*, *Fraxinus*, *Tilia* and *Ulmus*; *C. subroscida* grows almost exclusively on the bark of old *Picea* in humid situations.

In the Pacific Northwest, these species not only appear to share habitats and substrates, but their thalli are far less distinct. Their thalli range from having distinct, shiny brownish or olive verrucae to having smaller verrucae, some becoming so small that they are only visible at 20x magnification. The amount and density of white granules also varies from dense and continuous to scattered or not visible at all.

While *Chaenotheca phaeocephala* is thought to be common in the Pacific Northwest, *Chaenotheca subroscida* is considered rare (USDI Bureau of Land Management 2007; USDA Forest Service, Pacific Northwest Region, 2008). The objective of this study was to determine if

both species are present in the Pacific Northwest, how abundant they are, and if they are being identified correctly. Further, we would like to better describe the thalli of the two species in the Pacific Northwest to aid in identification.

MATERIALS AND METHODS

We studied all Pacific Northwest specimens of the two species in the Oregon State Herbarium (OSC). Most of the specimens were collected by surveyors hired by the Forest Service and the Bureau of Land Management to search designated areas for rare species. The surveyors were not necessarily lichenologists but had been trained to recognize habitat and the appearance of rare species. Since *Chaenotheca subroscida* was considered rare, only small portions of the thalli were collected so as not to impact populations. Collections from this program were identified by Steven Selva, Jeanne Ponzetti and Daphne Stone.

For preliminary identifications, we used the descriptions in Tibell (1999) and Tibell and Beck (2002) as well as criteria we learned from two calicioid experts who have identified most of the OSC specimens from the Pacific Northwest, Eric B. Peterson (pers. comm. 2012) and Steven Selva (pers. comm. 2020). We learned from Dr. Peterson that both species tend to have poorly developed thalli on *Pseudotsuga menziesii* (Mirbel) Franco, the most common substrate for both species in the Pacific Northwest. Tibell and Beck (2002) found poorly developed thalli on some substrates in Scandinavia; on *Betula* L. and decorticated *Picea* A. Dietr. twigs *Chaenotheca subroscida* has flattened olivaceous brown verrucae similar to those of *Chaenotheca phaeocephala*, and the minute whitish granules do not develop or develop only sparsely.

We selected 36 specimens that were large enough to provide material for thin-layer chromatography (TLC). They included specimens identified as both species and included thalli of brown verrucae, white granules and mixed verrucae and granules. Most of these were found on the bark of live *Pseudotsuga menziesii* (Mirbel) Franco (22), two specimens were on lignum (one of these on an old building) and the rest were on bark of live *Abies* Mill. spp. (7), *Callitropsis nootkatensis* (D. Don) D.P. Little (1), *Chrysolepis chrysophylla* (Douglas ex Hook.) Hjelmq. (1), *Picea engelmannii* Parry (1) and *Tsuga heterophylla* (Raf.) Sarg. (2) trees. We removed small thallus fragments from the 36 specimens and analyzed their secondary compounds using TLC. Solvents A and C were used on aluminum-backed silica gel plates, employing methods described by Culberson (1972). For controls we used *Rhizoplaca chrysoleuca* (Sm.) Zopf (McCune 6096) and *R. chrysoleuca* (McCune 12625) for pseudoplacodiolic acid; and *R. chrysoleuca* (McCune 12878c) for placodiolic acid. Geographic coordinates for the species were compiled and a distribution map was constructed using SimpleMappr (Shorthouse 2010).

All of the specimens used for TLC were small and not all had apothecia that were completely developed. Some specimens had very few apothecia, so none were harvested. We estimated the height of the stalk plus apothecium and mazaedium width with an accuracy of approximately 0.1 mm. We felt that the measurements, especially of the width of mature apothecia, overlapped so much they were not useful for determining the species.

DNA sequencing would have been a helpful tool for determining the identity of these specimens. However, since most of them are small and more than 5 years old, we did not attempt to generate sequences.

RESULTS

Of the 36 specimens that we examined, we determined that nine were *Chaenotheca subroscida* (pseudoplacodiolic acid was detected in solvent C) and 27 were *Chaenotheca phaeocephala* (pseudoplacodiolic acid was not detected in either solvent). None contained placodiolic acid. With these TLC results, we changed the determinations for 24 out of 33 specimens of previously determined as *C. subroscida* to *C. phaeocephala*. All three specimens previously determined as *C. phaeocephala* appeared to be correctly identified.

We used these specimens to refine our descriptions of the thalli of the two species to some degree. All specimens of *Chaenotheca subroscida* had a thallus of fairly dense white granules, and a few had visible brown verrucae (Fig. 1). The stalks of the apothecia were thin and delicate, averaging 0.97 mm tall. The width of the largest capitulum averaged 0.30 mm.

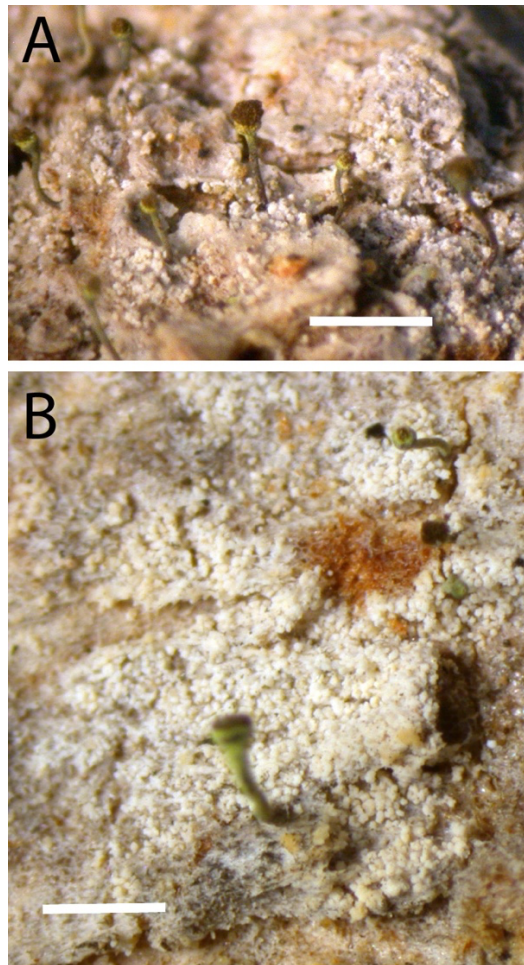


Figure 1. Thalli of *Chaenotheca subroscida*. A: sparse scattered thallus with some verrucae, see one at base of largest stalk (*Callagan 926-15*); B: dense discrete thallus of white granules, about 3 mm wide (*Stock SS121A*). Scale bars are 1 mm.

The most distinctive thallus of *Chaenotheca phaeocephala* was one collected on lignum of an old farm silo (Fig. 2). This specimen had large, nearly squamulose verrucae and relatively short ascomata with wider capitula (0.60 mm) and shorter (0.6 mm) stalks than most of the other specimens. The other *C. phaeocephala* specimens had taller (ave. 0.95 mm) stalks and narrower capitula (ave. width 0.37 mm), closer to those of the *Chaenotheca subroscida* specimens.

The 26 remaining *Chaenotheca phaeocephala* specimens exhibited several types of thalli. The color of thallus verrucae varied from green-brown to brown to pale yellow-brown. Some had verrucae without any white granules; some of these were definite, raised verrucae while others were low with indefinite edges giving the appearance of melting into the substrate. The only specimen on *Callitropsis nootkatensis* had small yellow-brown verrucae that were almost immersed among fibrous strands of bark. Other specimens had white granules growing densely or sparsely over low verrucae. One specimen had white granules with no brown lumps visible.

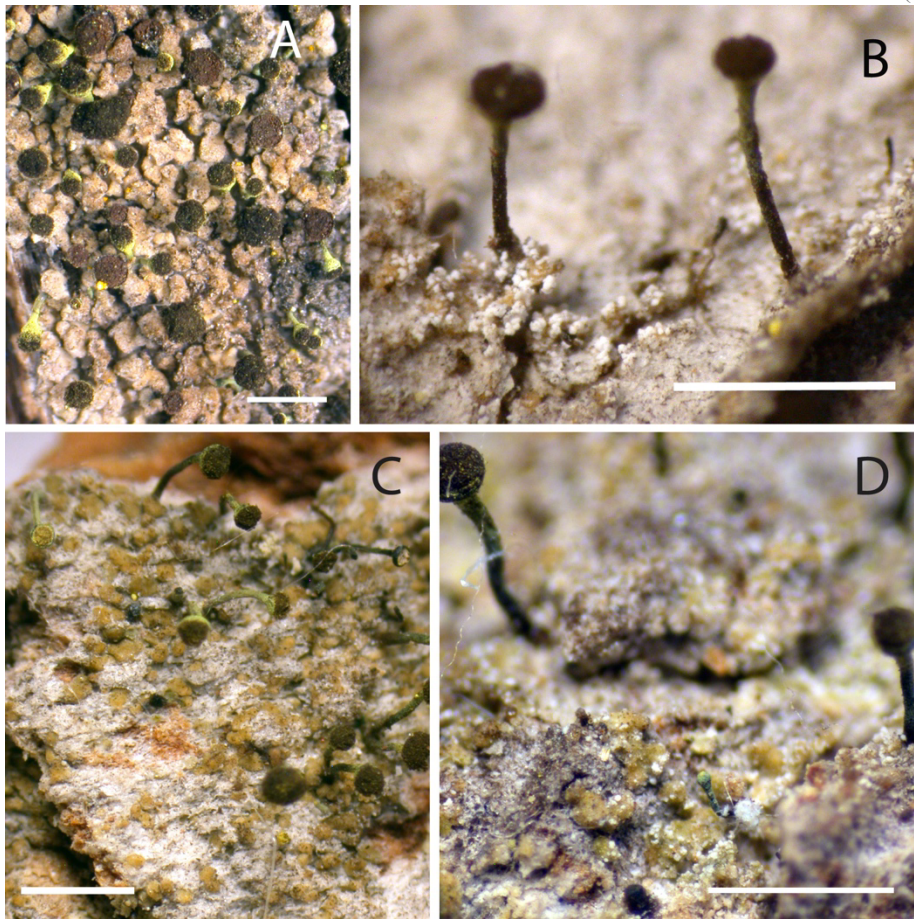


Figure 2. Thalli of *Chaenotheca phaeocephala*. A: thick brown verrucae on old barn silo (Stone 6217); B: brown verrucae with white granules (Clinch JC-LICH-264); C: low brown verrucae, some appearing to melt into the substrate (Brock 10-131RB); D: low brown verrucae with sparse white granules (Clark JC15-184). Scale bars are 1 mm.

The *Chaenotheca subroscida* specimens in our study were from west of the Cascade crest, from 646 to 1433 m elevation (Fig. 3). They were found in moist to dry forest types, often in old-growth stands, and sometimes near openings or wet meadows. Rikkinen (2003) reported it as frequent in humid montane and subalpine forests in his survey of a wider area that included higher elevations than that of our specimens. The *Chaenotheca phaeocephala* specimens in our study came from elevations between 144 and 1433 m, with even distribution along that elevation range in the Coast Range, Willamette Valley and into the Cascade Range, in dense or open, late-successional or old-growth forests. Six were from east of the Cascade crest in riparian areas. Rikkinen (2003) similarly reported that *C. phaeocephala* was frequent in montane forests and the Willamette Valley. The minimal label details and small sample size made it difficult to make more inferences about their respective habitat preferences. We had very few collections of either species from east of the Cascade crest, so we are not able to describe habitat of *C. subroscida* or infer abundance of either species from that area.

Note that the abundance of each species in OSC is not representative of their true abundances, because surveyors were required to submit specimens of *Chaenotheca subroscida* since it was considered to be a Special Status Species at the time (USDI Bureau of Land Management 2007; USDA Forest Service, Pacific Northwest Region. 2008), while vouchers of *Chaenotheca phaeocephala* were not required.

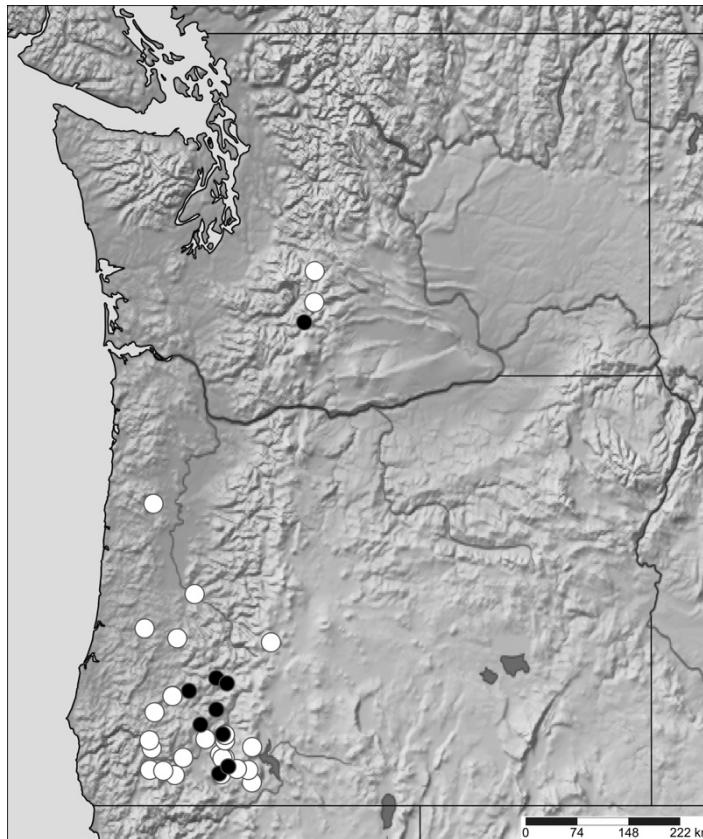


Figure 3. Map of Oregon and Washington with locations of TLC-verified *Chaenotheca phaeocephala* (white dots) and *C. subroscida* (black dots).

We verified that *Chaenotheca subroscida* and *Chaenotheca phaeocephala* are present and sympatric in the Pacific Northwest. The morphology of the nine verified *C. subroscida* specimens suggest that this species does not have brown verrucae among the white granules of its thallus in the Pacific Northwest, and that the white granules usually form a fairly dense cover. However, two of the specimens we TLC'd did have brown verrucae among the white granules; we believe that these were examples of where the two species were growing together; we were unable to prove that because *C. phaeocephala* does not produce lichen acids that would show up in mixed thalli.

The difficulty in identification of *Chaenotheca subroscida* in the Pacific Northwest stems from the fact that presence of small verrucae among the granules is normal for that species in Scandinavia, and because the verrucae are small and cryptic. We have now found that both of these species, with identical-looking apothecia, grow adjacent to each other on the same substrates in the Pacific Northwest, typically with thalli as small as a few square millimeters. Although it is clear that thalli with only white granules are *C. subroscida*, the species determination of those thalli that have both brown verrucae and white granules will still be uncertain. In these cases, we recommend the use of chromatography for accurate determinations.

In conclusion, it appears that *Chaenotheca subroscida* is indeed rare, given that only 1/3 of the specimens collected as this species were correctly identified, and that these specimens are the result of intense searching by all lichen surveyors in Washington and Oregon under the NW Forest Plan.

Finally, this research presents the possibility that what we have been calling *Chaenotheca subroscida* in the Pacific Northwest may be an undescribed species. This could explain the

differences between morphology and habitat between the Pacific Northwest and Scandinavian specimens. Phylogenetic studies would help to solidify our species concepts.

Specimens Examined:

***Chaenotheca phaeocephala*—U.S.A. OREGON. DOUGLAS CO.:** Council Creek, 240 yr old late seral multi-canopied forest, 80% overstory, 20% understory, on trunk of *Pseudotsuga menziesii*, 683 m, 42.892302°N, -123.425361°W, 19 May 2015, *Brock RB15-44* (OSC 155435); Swiftwater Resource Area, 25% slope; 268° aspect, on *Pseudotsuga menziesii*, 433 m, 43.590179°N, -123.13208°W, 22 July 2009, *Vos 2009-03-GV* (OSC 140445); Umpqua National Forest, South Myrtle Creek, PSME/dry shrub plant association, on tree, 524 m, 43.198333°N, -122.330167°W, 30 July 2007, *Brock RB07-100* (OSC 137680); South River Resource Area, South Myrtle Creek, PSME/dry shrub plant association, on ridgeline, on *Pseudotsuga menziesii*, 463 m, 43.045457°N, -123.194037°W, 18 August 2007, *Brock RB07-128* (OSC 137683); **JACKSON CO.:** Medford District BLM, Upper Salt Creek, late successional *Pseudotsuga menziesii*, *Abies concolor* and *Cornus nuttallii*, occasional older legacy trees, on *Abies concolor* trunk, 1311 m, 42.4558°N, 122.5207°W, 29 October 2015, *Brock RB15-132* (OSC 155523); Medford District BLM, north of city of Gold Hill, partially shaded ridge, open forest, 50% slope, 290°, on live *Pseudotsuga menziesii* and snag, 622 m, 42.4599°N, 123.0561°W, *Scelza JDS16-144* (OSC 155524); Medford District BLM, Ashland Resource Area, moist 30% slope, 30°, on bark of *Abies concolor* trunk, 1472 m, 42.3014°N, 122.5663°W, 15 August 2009, *Brock 09-49* (OSC 140437); Medford District BLM, Upper Slagle Creek, fully shaded upper slope, late successional Douglas fir-madrone/poison oak forest, *Pseudotsuga menziesii*/dry shrub plant association, 80% slope, 330°, on *Pseudotsuga menziesii* trunk of 40 inch DBH, 884 m, 42.2941°N, 123.1641°W, 24 April 2015, *Brock RB15-19* (OSC 155683); Medford District BLM, Upper Salt Creek, dense forest, fully shaded, 40° aspect, 20% slope, on *Pseudotsuga menziesii*, 1082 m, 42.4530°N, 122.54917°W, 1 December 2015, *Brock RB15-145* (OSC 155527); Medford District BLM, Upper Salt Creek, filtered, dense forest, 45% slope, 340° aspect, on *Abies concolor* trunk, 1189 m, 42.4927°N, 122.5798°W, 30 October 2015, *Clark JC15-184* (OSC 155526); Friese Camp Area, near Jackass Creek, late seral stand of *Pseudotsuga menziesii* and *Abies grandis*, with fairly open upper canopy, on *Abies grandis* branches, 1263 m, 42.625321°N, -122.516389°W, 2 May 2012, *Stone 8625.1* (OSC 144142); Little Tom Folley Creek watershed, north of Elkton, 50-year-old mid-seral (with remnant 200-year-old) forest, 75% overstory, 10% understory, on *Pseudotsuga menziesii* within a younger stand, 229 m, 43.682684°N, -123.55341°W, 25 August 2012, *Vos GV12-188* (OSC 155437); Butte Falls Resource Area, ABCO-BENE2-LIBOL plant association, on *Chrysolepis chrysophylla*, 1356 m, 42.677208°N, -122.517794°W, 13 January 2015, *Callagan RC-922-15* (OSC 151043); southwest of Bear Mountain and south of Brush Creek, partially shaded, dense forest, 50% slope, 285° aspect, on *Pseudotsuga menziesii* trunk, 605 m, 42.638356°N, -122.768786°W, 4 March 2016, *Scelza JDS16-010* (OSC 155522); Rogue River-Siskiyou National Forest, High Cascades Ranger District, partial cut old growth with *Abies concolor* understory and large PSME overstory, on bark of *Pseudotsuga menziesii* and *Abies concolor*, 1433 m, 42.352353°N, -122.363327°W, 16 August 2010, *Brock RB10-138* (OSC 159634); **JOSEPHINE CO.:** Grants Pass Resource Area, dense forest of a late successional Douglas fir-madrone-black oak, on 38" DBH *Pseudotsuga menziesii*, 497 m, 42.548802°N, -123.460986°W, 13 May 2009, *Brock 09-04* (OSC 140408); mid to late seral stand of mixed conifer-hardwood forest, PSME the main overstory tree with QUCH2 and ARME occupying significant cover, on *Pseudotsuga menziesii*, 739 m, 42.626015°N, -123.491933°W, 29 September 2008, *Whitridge 269w* (OSC 155537); Siskiyou Mountains, Cheney Creek, in rocky draw in open overstory old growth, moist site with relatively open canopy, on *Pseudotsuga menziesii*, 421 m, 42.343549°N, -123.48482°W, 22 November 2007, *Brock 07-185* (OSC 139081); Grants Pass Resource Area, Siskiyou Mountains, wide swale on

west aspect in madrone-black oak dominated forest, lower slope in draw, on large 36" DBH *Pseudotsuga menziesii* snag/stump, 396 m, 42.334791°N, -123.312398°W, 22 November 2007, *Brock 07-187* (OSC 139082); **KLAMATH CO.:** Lakeview District BLM, Surveyor Mtn, dense forest, fully shaded, mesic, 35% slope, 350 degrees, on *Abies concolor* trunk, 1692 m, 42.2233°N, 122.1707°W, 7 August 2010, *Brock 10-131RB* (OSC 151129); Klamath Ranger District, Rock Creek, rocky creek running through subalpine old-growth forest with several springs feeding it, some exposed volcanic rock/boulders on creek and up creek bank, on *Abies grandis*, 1615 m, 42.560982°N, -122.166599°W, 7 August 2003, *Clinch JC-LICH-186* (OSC 137297); Winema National Forest, Rainbow Creek, meadow with small perennial creek surrounded by old-growth forest, flat terrain, on trunk of *Pseudotsuga menziesii*, 1433 m, 42.344267°N, -122.214918°W, 4 August 2003, *Maertens FS0620/L/TM-206* (OSC 137304); Deschutes National Forest, Odell Creek, large wooded meadow with several openings without overstory, many seeps/springs with adjacent bogs eventually fed into ever-widening creek, on bark and decorticated branch of *Picea engelmannii*, 1413 m, 43.553853°N, -121.923652°W, 19 October 2003, *Clinch JC-LICH-264* (OSC 137306); **LANE CO.:** Mt. Pisgah Arboretum, SE of Eugene, on north side of old wooden silo, on lignum, 148 m, 44.0035°N, 122.9083°W, 9 February 2005, *Stone 6217* (OSC 173398); **POLK CO.:** Falls City, Little Sinks RNA, 1.5 miles S of Falls City, *Pseudotsuga menziesii/Acer macrophyllum* forest occurring on broken topography, characterized by slumps, ponds, and geologically recent natural disturbance, on firm wood of rotten stump, 213 m, 44.838167°N, -123.439833°W, 24 February 1996, *Ponzetti 637* (OSC 167728). **WASHINGTON. YAKIMA CO.:** Okanogan-Wenatchee National Forest, mixed conifer stand near creek, on bark of 33 inch *Abies grandis* snag, 1049 m, 46.93388°N, 121.35952°W, 15 August 2013, *Hardman 6120* (OSC 156833); Okanogan-Wenatchee National Forest, old growth *Tsuga heterophylla* and mix of other conifers, on bark of *Callitropsis nootkatensis* bole, 1311 m, 46.65729°N, 121.36518°W (NAD83), 16 August 2013, *Hamill 1002.2* (OSC 173399).

***Chaenotheca subroscida*—U.S.A. OREGON. DOUGLAS CO.:** Roseburg District BLM, dense, mesic, *Pseudotsuga menziesii* forest, on the bark of PSME trunk, 646 m, 43.2161°N - 122.629°W, 30 November 2009, *Clark 904* (OSC 140444); Umpqua National Forest, in partial shade on the bark in an open/cut, mesic forest, on *Pseudotsuga menziesii* base, 1159 m, 43.23361111°N, -122.3433333°W, 24 August 2000, *Delinks TD753* (OSC 140199); South River Resource Area, grove of remnant large diameter overstory trees, along a dry draw, on *Pseudotsuga menziesii*, 1018 m, 43.094436°N, -122.980598°W, 9 August 2011, *Vos GV-2011-81* (OSC 144123); Umpqua National Forest, partial shade, slope, open forest, on *Pseudotsuga menziesii* rotten log, 1433 m, 42.91805°N, -122.62883°W, 26 September 2000, *Stock SS121A* (OSC 124457); **JACKSON CO.:** Medford District BLM, Trail Creek, open mature mixed conifer forest, overstory *Pseudotsuga menziesii*, understory sparse *Pseudotsuga menziesii* with *Abies concolor*, *Chrysopsis chrysophylla* and *Arbutus menziesii* on a 3 foot diameter at breast height (DBH) *Pseudotsuga menziesii* trunk, 1031 m, 42.7749°N 122.8327°W, 15 May 2013, *Werling 05152013* (OSC 155530); Grizzly Peak, Lost Creek Road, north facing slope among large rock outcrops and dense shrubs, *Abies concolor* series plant association, on mature *Pseudotsuga menziesii*, 1366 m, 42.308148°N, -122.592232°W, 12 August 2015, *Carey GC15-243* (OSC 155684); Rogue River National Forest, old-growth Douglas fir forest with high amount of mistletoe, dense forest except for canopy gaps, on bark of 42" DBH *Pseudotsuga menziesii*, 1372 m, 42.375856°N, -122.474763°W, 30 June 2007, *Brock RB07-4* (OSC 137524); Butte Falls Resource Area, 15% slope, 250° aspect, partially shaded, *Abies concolor-Berberis nervosa* plant association, on *Pseudotsuga menziesii* trunk, 1268 m, 42.684315°N, -122.544678°W, 31 January 2015, *Callagan RC-926-15* (OSC 151042). **WASHINGTON. LEWIS CO.:** Gifford Pinchot National Forest, forest of *Tsuga heterophylla*, *Abies amabilis* and *Callitropsis nootkatensis*, on bark of *Tsuga heterophylla*, 1184 m, 46.48024°N, -121.49425°W, 14 August 2014, *Hardman 6065* (OSC 156834).

ACKNOWLEDGEMENTS

We thank Bruce McCune for his assistance with interpretation of the TLC plates. Eric B. Peterson and Steven Selva have been helpful over the years with their thoughts on identification of these two species. Darci Pankratz prepared and databased most of the specimens at the Interagency Special Status/Sensitive Species Program (ISSSSP) and helped us navigate the ISSSSP's agency lists. The suggestions by Troy McMullen, our reviewer, improved the paper immensely. Financial support was through the Oregon State University Lichen Herbarium Improvement Grant provided by Shirley Tucker.

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