Leptogium cyanescens - a catchall name for gray isidiate *Leptogium* species in the Pacific Northwest?

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Introduction

Leptogium is a lichen genus with worldwide distribution, great diversity, and sometimes subtle taxonomic distinctions. The Pacific Northwest hosts many species of *Leptogium*, with a number of species having been described from the area only recently (Jørgensen & Goward 1994; Jørgensen & Tønsberg 1999). Some of these species are considered rare and are subject to management on federal lands. Among these managed species is *Leptogium cyanescens*, an epiphytic foliose lichen that has a worldwide distribution; the species is considered common across much of the eastern United States (Brodo et al. 2001; McCune & Geiser 1994, Sierk 1964). In the Pacific Northwest, the species has been considered rare, and when found, the area of occupancy and population size usually appears to be small, with few thalli occurring within habitat (USDA & USDI 1994, 2000, 2003, ISMS 2004, McCune pers. comm. 2004).

During research conducted in 2004 and 2005, Lichenologists Dr. Daphne Stone (researcher and contractor) and Andrea Ruchty (botanist, Gifford Pinchot National Forest) began to suspect that the species that had been commonly identified as *L. cyanescens* in the Pacific Northwest is in fact a species complex that comprises at least two species. This complex comprises the 'unit' that has been managed on federal lands, under the Northwest Forest Plan (USDA & USDI 1994) as, first, a survey and manage category 4 lichen species, and, beginning in 2000 (USDA & USDI 2001) a survey and manage category A species (survey and manage all sites), and beginning in 2004 (USDA & USDI 2004), as a Forest Service Sensitive species across Region 6, and a Bureau of Land Management Tracking species in Washington and Oregon. Since federal land managers within the area of the Northwest Forest Plan are required to manage this species for persistence and viability (USDA & USDI 1994; USDA & USDI 2004; FSM 2670, BLM 6840), it is important that field botanists and staff specialists have the information to allow them to accurately identify and effectively manage *L. cyanescens*. To address these issues, this project was designed to address fundamental taxonomic and ecological questions about *L. cyanescens* by: (1) testing the hypothesis, using genetic sequencing, that a) *L. cyanescens* (as currently recognized within the northwestern United States), comprises at least 2 separate species; (2) identifying whether either member of the species complex is *L. cyanescens*; (3) identifying whether either member of the hypothesized species complex is a morphological variation of another potentially phenotypically plastic species of *Leptogium* (i.e. *L. tacomae*, *L. subaridum* or an undescribed species*)*; (4) compiling information on distribution, habitat and substrate for both members of the species complex within the Pacific Northwest, through examination of specimens at regional herbaria, including University of British Columbia, Victoria, Canada (UBC); University of Washington, Seattle (WTU); Washington State University, Pullman (WS); Oregon State University, Corvallis (OSC); University of California at Berkeley (UC); University of Idaho Herbarium, Boise (ID); University of Bergen, Norway (BG); personal collections of Dr. Bruce McCune, Oregon State University, Oregon; and Dr. Daphne Stone, Eugene, Oregon.

Results include verification that true *L. cyanescens* does indeed inhabit the Pacific Northwest, but the species is rare, with only five specimens identified from Northwest North America, with three of these from the Northwest Forest Plan area. The other member of the species complex is identified as *Leptogium tacomae*, a species recently described based on the single type collection (Jørgensen & Tønsberg 1999). Preliminary results from genetic testing support these morphological groupings, and are discussed in this report. An incidental result of this study has been the discovery of a new, apparently endemic species of *Leptogium*, with twenty-nine sites identified from southern Oregon and northern California. The full description of this species was published in the peer reviewed journal North American Fungi as "*Leptogium siskiyouensis*" (Stone & Ruchty 2008). This report will be used as the basis for a publication to be submitted to the peer reviewed journal The Bryologist.

As a straightforward and useful way to present the information resulting from this study, thorough species descriptions, with distributional maps, have been prepared for *Leptogium cyanescens*, *L. tacomae*, and *L. siskiyouensis*, while shorter reviews have been prepared for additional species with overlapping ranges and habitats that may be considered look-alikes. Species information pages are accompanied by photo collages designed to exhibit important morphological distinctions among species.

Leptogium cyanescens (Rabenh.)Korber

Systematics and Synonymy
Leptogium cyanescens was first described by Erik Acharius ("the father of lichenology") in 1810 under the name *Collema tremelloides* β. *caesium.* Since that time the species has been variously known as *Collema tremelloides* β. *cyanescens*, *L. tremelloides*, *Parmelia cyanescens* and *Collema cyanescens.* The currently recognized name, *Leptogium cyanescens* (Rabenh.) Korber, reached common use in the 1930's. Lichen nomenclature follows Esslinger and Egan (1995).

Technical Description
In his 1964 monograph of the genus *Leptogium* in North America north of Mexico, Herbert A. Sierk describes *L. cyanescens* as follows:

> **Thallus** 1-5 cm broad, flat, spreading, lead gray; lobes orbicular, 2-4 mm broad, margins entire to dentate to isidiate; surface of thallus smooth to occasionally slightly roughened but not wrinkled,; thallus attached to the substrate by scattered tufts of hairs. Lobes 35-110 μ thick; hyphae 2-3 μ in diameter, irregularly interwoven, rather compact and often with a horizontal orientation in the center, appearing paraplectenchymatous except in thin sections;
> **Cortex** of a single layer of irregularly idodiametric cells 5-8 μ in diameter, up to 10 rows developed below apothecia, the cells here up to 20 μ in diameter, thinning to 2-3 layers in exciple.
> **Lower cortex** with hairs here and there, 3-5 μ in diameter;
> **Isidia** commonly abundant on the upper surface, cylindrical to clavate to lobulate, commonly branching, concolorous with the thallus;
> **Apothecia** not common, sessile to short stipitate on the upper surface of thallus, 0.5-2.0 mm. broad; disc slightly concave to pane to convex, light brown to red-brown; thalloid exciple entire to isidiate, light gray to cream colored; 70-95 μ thick; proper exciple euparaplectenchymatous, poorly developed and difficult to discern in the center, 7-15 μ thick at the margins; subhymenium 45 μ thick, yellowish; hymenium 95-130 μ thick, hyaline with a thin yellow to brown epithecium; paraphyses unbranched, about 1 micron thick, little thickened at the apices; asci cylindrico-clavate, 80-105 X 12-14 μ;
> **Spores** 8, monostichous to irregular, ellipsoid with apices rounded to pointed, 18-23 X 6-10 μ, 3-septate transversely, 0-1 septate longitudinally;
> **Algal cells** 2-3 μ in diameter, spherical, in chains often tightly coiled and appearing in clusters.

Further, Sierk states:

> "This is the most common species of *Leptogium* in North America north of Mexico. Its range extends from the Gulf Coast north to Newfoundland, Quebec, and Ontario. It extends westward along the Gulf to eastern Texas and Oklahoma. In the Midwest it reaches into Iowa and Minnesota. To the west it has been collected in the Black Hills of South Dakota and in northern Colorado. Collections have been seen from British

Columbia and southern Alaska. This species is also abundant in Europe where Degelius (1935) has described it as an oceanic species. It is known also from Asia and the American tropics. It grows most commonly on the bark of deciduous trees, but also occurs on *Juniperus* and *Thuja* as well as on decaying logs and on rocks. In North America this species has generally been mistaken for *L. tremelloides*, a non-isidiate, Old World species".

Jorgensen & James (1983) report that *L. cyanescens* is never wrinkled, and may develop coralloid to lobulate isidia.

Study Results

We found that macroscopically, *L. cyanescens* has a consistently large-lobed (1 - 12 mm wide) smooth thallus (may have delicate texturing in places, but no definite wrinkling) with a recumbent growth habit, and gray-blue coloring with a distinctive matte tone, which brings to mind the look of a gray-blue eggshell finish paint chip. The color of this species is consistently blue-gray without brownish lobe tips or isidia. The isidia of *L. cyanescens* are fine, not subopuntioid, and may become flat-lobulate. The isidia emerge from the thallus as small light rounded bumps becoming round-tipped cylinders. These characters are somewhat subjectively interpreted, however, which may make "keying" specimens difficult in some cases. In addition to these hand-lens scale morphological characters that distinguish this species from other Pacific Northwest *Leptogium* species, microscopic characters including comparative spore size and thallus thickness may also be used to help distinguish species, though ranges for these characters do overlap for *L. cyanescens* and *L. tacomae*, the most easily confused pair (see Table 2).

In addition, we discovered differences in medullary structure that clearly and consistently differentiate *L. cyanescens* from *L. tacomae*, as well as from other Pacific Northwestern foliose *Leptogium* species. *Leptogium cyanescens* has a relatively thin thallus (generally 50-70 μ near edge of thallus), with a hyphal arrangement within its medulla consisting of tightly packed hyphal strands oriented parallel and perpendicular to the cortices, leaving few interhyphal spaces, with Nostoc chains scattered throughout (Figure 2). The impression within the medulla of *L. cyanescens* is of woven hyphal strands. To see this distinctive hyphal orientation, a very thin thallus cross section must be made and viewed at 400X (must be very thin – 1 or 2 cells thick).

After examining hundreds of herbarium specimens identified as *L. cyanescens* from throughout the Pacific Northwest, only five specimens were found to be *L. cyanescens* (Table 1); the majority of specimens examined were found to be *L. tacomae*. The five *L. cyanescens* specimens were all found in humid localities, including near the Pacific coast and within riparian zones. Specimens were found growing epiphytically on both hardwoods and *Picea sitchensis* (a conifer), and on rock (Table 1). Based on our study results, we conclude that *L. cyanescens* is rarer than previously thought within the Pacific Northwest (see Figure 1 for distribution of *L. cyanescens* within the Pacific Northwest). Goward et al., in The Lichens of British Columbia (1994), describe *L. cyanescens* as a rare species restricted to humid localities. Our findings are consistent with this assessment.

Table 1. *Leptogium cyanescens* within the Pacific Northwest of North America

Herbarium	Collector/ collection date	Collection Number	Substrate	Location
University of British Columbia	T. Goward 8/2/1979	79-1108 (accession number L28397)	Muscicolous in crevice of siliceous rock	British Columbia, Canada: Wells Gray Provincial Park, Osprey Point Campground, S. shore of Azure Lake.
University of Washington	M. Arnot 8/23/1998	No collection number; accession number L1326	*Acer circinatum*	Washington State, USA: Snohomish County, Troublesome Creek Trail, ca. 10 miles NE of Index, Washington State. 1550 ft. elevation.
University of Washington	Scott Sundberg 2/20/1980	726	*Pyrus fusca* branches	Oregon State, USA: Lane County, 9 km. North of Florence, SE shore of Sutton Lake. 30 ft. elevation.
Bruce McCune	Bruce McCune 4/14/1996	23061	on crabapple trunk, among scattered shrubs and *Picea sitchensis*	Oregon State, USA: Lincoln County, along edge of water at Beaver Creek Marsh, just east of Ona Beach State Park, 1-3 km. inland from ocean.15-30 ft. elevation.
Bruce McCune	Linda Geiser 7/27/1989	TNFS # L-330	On *Picea sitchensis* twig.	Alaska, USA: Tongass National Forest, Stikine-LeConte Wilderness Mainland, Stikine R., Andrews Slough, between forks and moose hunting trees on clear water. <90 ft. elevation.

Figure 1:

Leptogium cyanescens
distribution in the Pacific Northwest of North America

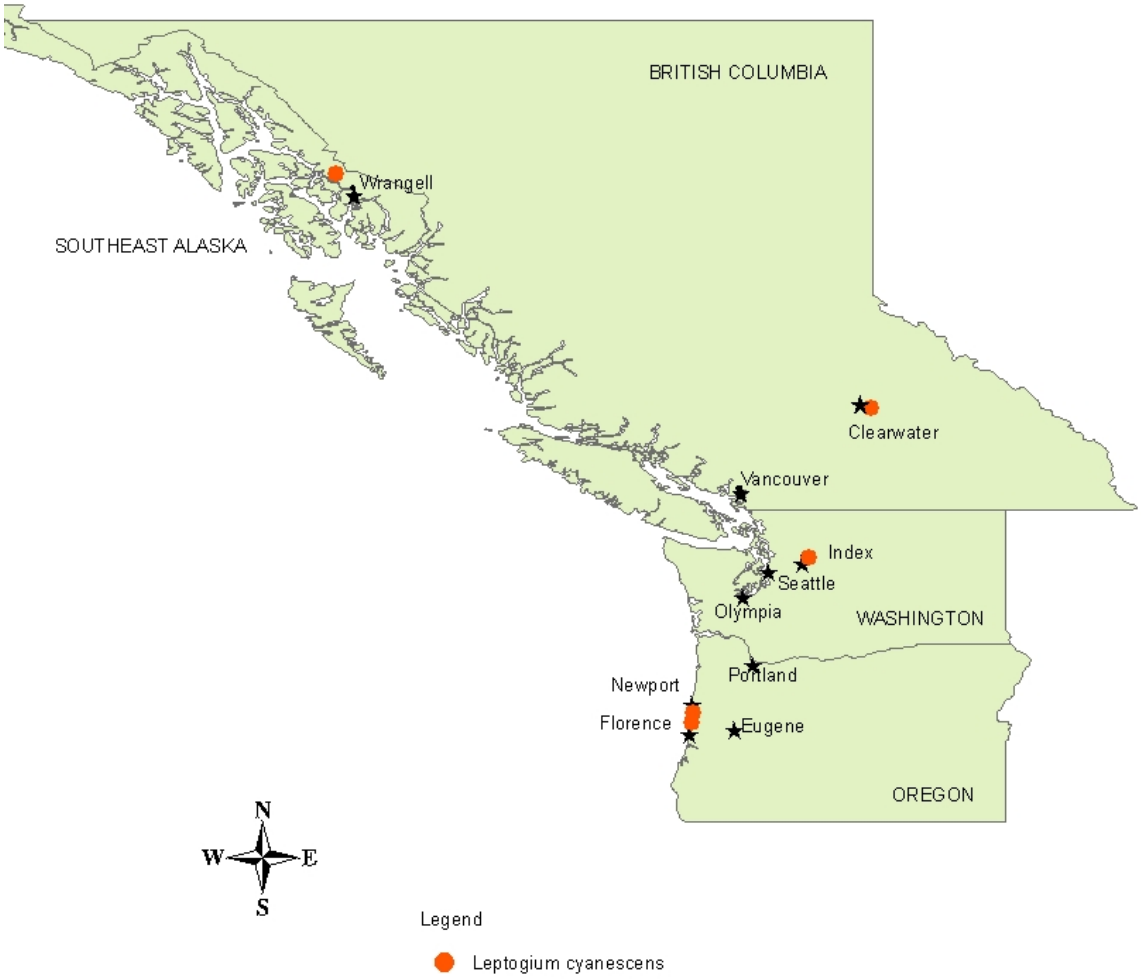
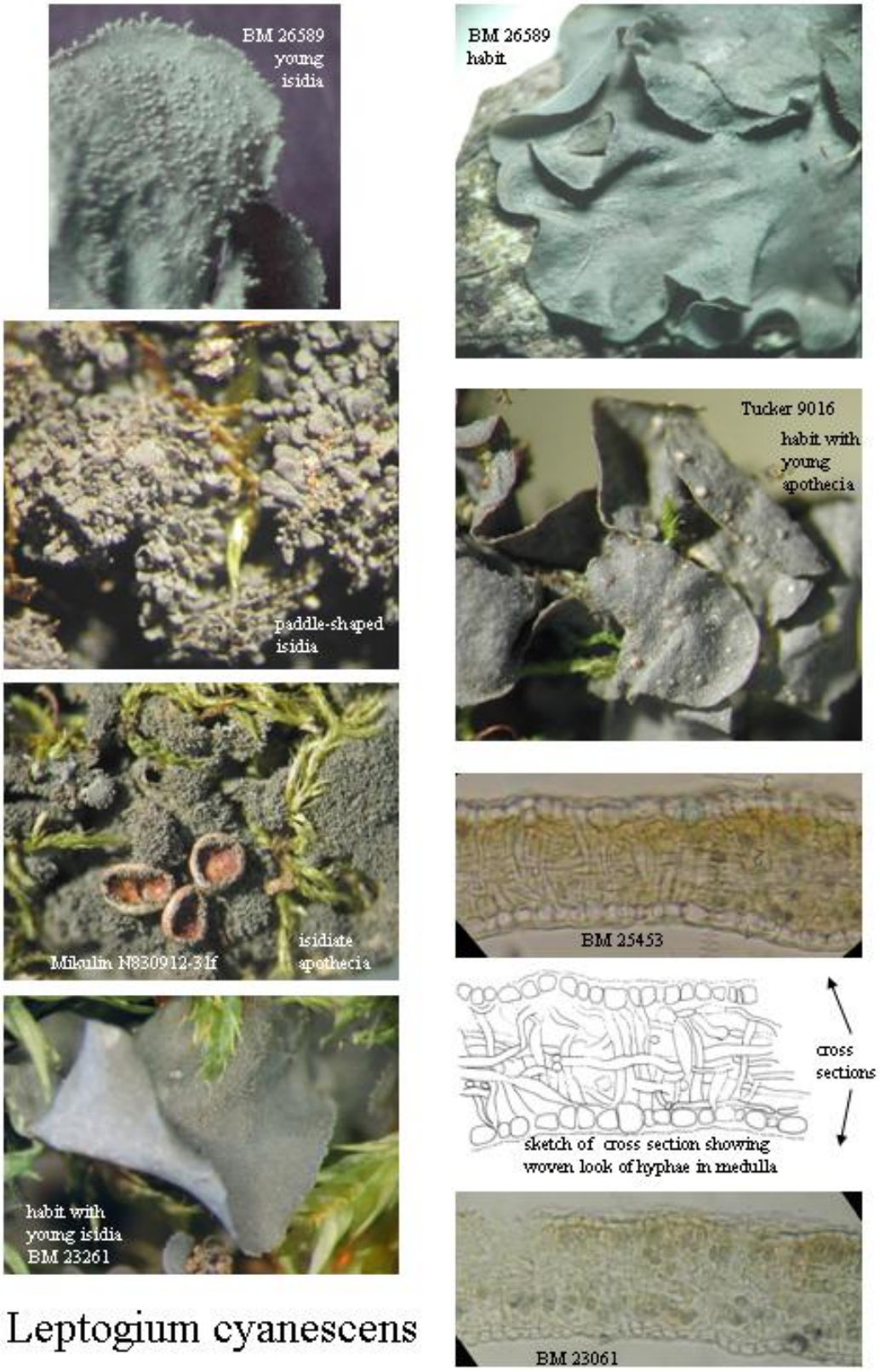


Figure 2: Photos of *Leptogium cyanescens*



Leptogium tacomae P.M. Jørg. & Tønsberg

Systematics and Synonymy: *Leptogium tacomae* was first described in 1999 by P. M. Jørgensen and T. Tønsberg, based on the single type collection taken from an *Alnus rubra* trunk, in Mt. Rainier National Park, Washington State.

Technical Description: Jørgensen and Tønsberg describe the species as follows:

Thallus foliose, appearing subfruticose when densely isidiate or when the isidia develop directly from the substratum to form new thalli, in ± pulvinate colonies to 5 cm diam. Squamules rounded to irregular, to 1.5 (rarely 2.5) mm wide, brown or in shaded parts (and below) grayish blue, smooth or weakly wrinkled with laminal and more frequently marginal isidia; thallus (60-) 80-120 µ thick, wrinkled parts up to 215 µ; upper and lower cortices present, mainly with one cell layer, the cells occasionally dividing periclinally in two; cells best developed in lower cortex, particularly underneath apothecia where they form a palisade of standing, rectangular cells 7-12 X 15-22 µ.
Upper cortex partly brownish
Lower cortex partly with groups of simple, long-celled anchoring hyphae.
Isidia coralloid branched to 0.6 mm long, forming dense aggregations, though sparse on richly fruiting specimens; subopuntioid with brown-pigmented cortex, 50 µ wide in subapical parts, individual segments easily detached.
Apothecia mostly laminal, rarely formed on isidia, up to 0.5 mm wide, brownish with distinct thalline margin, often with corona of isidia; thalline margine up to 100 µ thick; proper margin narrow, in lateral parts 12-25 µ widening above to 35 µ, composed in lateral parts of cells with narrow lumina, below subhymenium with isodiametric lumina. Subhymenium 20-25 µ. Hymenium 130-170 µ, colorless, except in upper part, that is pale brown, I+ blue; paraphyses simple to 2 µ wide, apically enlarged to 4 µ. Asci clavate, 90-120 X 12-15 µ, 8-spored.
Spores ellipsoid, (sub)muriform with 5-6 transverse septa, 28-36(-40) X 10-15 µ, often with attenuated ends.
Pycnidia not observed.
Cyanobiont Nostoc, singly or in chains, individual cells oval to globose, 7.0-7.5 µ diam., mostly concentrated in two layers near cortices, or occasionally evenly dispersed in loose medulla of irregular hyphae.

Jørgensen and Tønsberg also state:

> *"Leptogium tacomae* in its typical form would key out close to *L. subaridum* P.M. Jørg. & Goward as it is an isidiate, small-lobed, brownish species, although the two species may not be closely related. *Leptogium tacomae* is easily separated by its characteristic, clustered, laminal isidia as well as by paler color, fertility, and corticolous habitat in more humid environments. *Leptogium tacomae* appears to be a quite variable species. The isidia are sometimes poorly developed, particularly in richly fertile specimens, which then may approach *L. polycarpum* P.M. Jørg. & Goward in appearance, but do not have 4-spored asci and have a browner, less wrinkled thallus than that species. Some thalli are predominantly

isidiate and then easily recognizable by characteristically branched, subopuntioid isidia that are different from those of the similar, much smaller, *L. teretiusculum* (Wallr.) Arn. and *L. cellulosum* P.M. Jørg. & Tønsberg, both of which have different anatomy, being paraplectenchymatous throughout".

Study Results

We found *L. tacomae* to be a highly variable species. We realized that the morphology of *L. tacomae* ranges from:

1) gray-blue thalli, with lobes that are longer than wide, few simple, cylindrical "finger like" isidia and no apothecia, with a sprawling, recumbent growth habit (usually among moss), to

2) rich brown thalli composed of many overlapping, rounded lobes densely covered with coralloid, sometimes "subopuntioid" isidia (in places so dense as to obscure the thallus) and copious apothecia.

The first morphology causes specimens to be "keyed" to *L. cyanescens* in McCune and Geiser 1997 and in Goward et al.1994, the two most commonly used dichotomous keys for foliose lichen species within the maritime Pacific Northwest. Close comparison of attributes of these specimens to Sierk's 1964 technical description of *L. cyanescens* establishes, however, that these specimens do not closely conform to the description of that species.

The second morphology does not readily "key out" in any of the published dichotomous keys currently available.

Most *L. tacomae* we examined fell somewhere between the two morphological extremes described above, with many thalli partially gray and partially brown; with moderate distribution of isidia (often more marginal than laminal); with varying levels of branching (ranging from simple cylindrical to subopuntioid on a single thallus); and variable numbers of apothecia. Although the isidia are described by Jørgensen and Tønsberg as subopuntioid, in many cases the form is longer (at least 0.5 mm) and less pinched, having instead the look of long fingers with prominent knuckles ("arthritic fingers"). Isidia begin as small, brown, shiny flat-tipped or indented-tipped cylinders on the thallus.

We hypothesize that the great morphological variation seen in *L. tacomae* may, to some extent, depend on thalli age and sun/shade exposure, with recumbent, strap shaped lobed specimens with consistent gray-blue coloration being younger, and possibly developing in more shaded microhabitats, while rich brown, specimens with highly branching subopuntioid isidia may be older specimens exposed to more sun. This hypothesis is partially based on the idea that the constrictions seen in "arthritic fingers" and subopuntioid isidia may indicate yearly growth spurts (T. Goward pers. comm. 2006), indicating that thalli with well-developed coralloid isidia may be older than those with simple isidia. In addition, intermediate specimens suggested that the strap shaped lobes of the gray-blue specimens may be growing and filling out to eventually form rosettes with rounded lobes.

Importantly, we discovered that all *L. tacomae* specimens do share certain characteristics that clearly distinguish this taxon from *L. cyanescens.* First, we found that the structure of the medulla in these two species consistently differs. *Leptogium tacomae* has a generally thicker thallus ((70) 80-120 (200) μ), with a medulla made up of segments of hyphae (to10:1 length to width ratio) with hyphae joined at 45 to 60 degree angles, the spaces between these hyphae containing chains and single cells of *Nostoc*, but with much of the inter-hyphal space not filled. Overall, the impression is of hyphal segments forming polygons, sparsely interspersed with *Nostoc* chains (chicken wire and green pearl necklaces) (Figure 4). *L. cyanescens*, in contrast, generally has a thinner thallus (usually 50-70 μ near edge of thallus), with a hyphal arrangement within its medulla consisting of hyphal strands oriented parallel and perpendicular to the cortices, packed tightly leaving few inter-hyphal spaces, with Nostoc chains scattered throughout. The impression within the medulla of *L. cyanescens* is of basket-woven hyphal strands, which is clearly distinguishable from *L. tacomae* with a thin cross section (must be very thin – 1 or 2 cells thick).

At the time of the publication of the species' description (1999) Jørgensen and Tønsberg expressed their belief that *L. tacomae* may have been previously overlooked, and may have a wider distribution than reflected in their type collection. This prediction has proved to be accurate.

During the course of this study, it became clear that most specimens commonly identified as *L. cyanescens* within the Pacific Northwest are actually *L. tacomae*. Among collections housed in 7 herbaria, and the personal collections of Dr. Bruce McCune and Dr. Daphne Stone, we discovered only five specimens from the Pacific Northwest that are actually *L. cyanescens.* In contrast, we found *L. tacomae* distributed widely in Washington, Oregon and northern California (Figure 3).

Our study establishes that *L. tacomae* is a widely distributed species with specimens identified from Mt. Baker Snoqualmie and Gifford Pinchot National Forests in Washington State; Mt. Hood, Willamette, Rogue River and Siskiyou National Forests and Medford Bureau of Land Management in Oregon; and Six-Rivers and Lassen National Forests in California.

Leptogium tacomae has been found growing in abundance on hardwoods in moist forested locations; many collections viewed from the Willamette and Gifford Pinchot National Forests were reported growing along the horizontal stems of *Acer circinatum*, among moss. The species also grows on mossy rocks and boulders, *Acer macrophyllum*, *Sambucus cerulea*, *Alnus sinuata*, *Arbutus menziesii* and *Quercus* spp. In southern Oregon and northern California, *Quercus kelloggii* is also a substrate, demonstrating that this species may be found in drier habitats that originally reported by Jørgensen and Tønsberg. Clearly, *L. tacomae* has a wide ecological amplitude. This species is likely under-collected and under-reported, and has often been misidentified as *L. cyanescens*.

Look-alike species:
The newly described species "*Leptogium siskiyouensis*," may closely resemble *L. tacomae*, as may *L. pseudofurfuraceum*, all species that are frequently found on *Quercus kelloggii*. *Leptogium tacomae* may also resemble *L. subaridum*; both of these species are found on moss covered rock outcrops. Although *L. subaridum* has not been found growing as an epiphyte in our area, it has been reported as an epiphyte from Europe, and we feel that it may be found growing on tree bases in dry habitats, where it may also be found growing with *L. tacomae.* See species' descriptions for characters to distinguish these look-alikes.

Figure 3:

Leptogium tacomae
distribution in the Pacific Northwest of North America
Seattle
Olympia
WASHINGTON
Portland
Florence
Eugene
Bend
OREGON
Medford
Arcata
Redding
San Franciso
CALIFORNIA
N
W
E
S
Legend
Leptogium tacomae

Figure 4: Photos of *Leptogium tacomae*

Leptogium siskiyouensis Daphne Stone & Andrea Ruchty

Systematics and Synonymy: *Leptogium siskiyouensis* was first described in 2008 by the authors of this report as a result of examining specimens previously identified as *L. cyanescens*. The holotype specimen is from a *Quercus chrysolepis* tree trunk, northeast of Cave Junction in Josephine County, Oregon (Stone & Ruchty 2008).

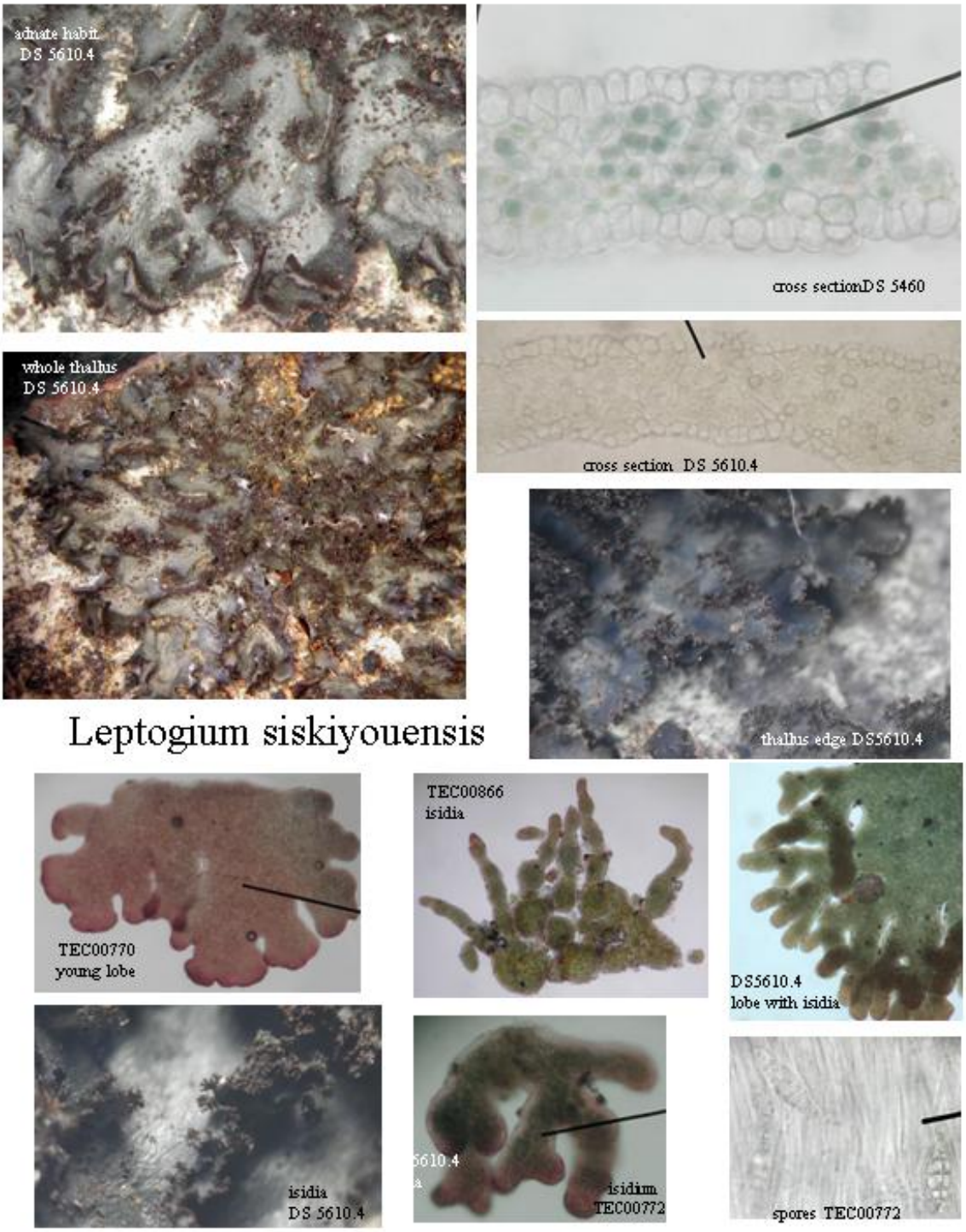
Thallus lobes grow in a regular, radial pattern, forming a delicate, closely adnate circular thallus of up to about 3 cm. The general shape of lobe edges is rounded, and sinuses between lobes are rounded, leaving a small hole through which the bark is visible. As each lobe matures it is closely attached to the bark down the center but raised almost to 90º from the bark along the edges. Lobes are about 0.3 to 4 mm wide, flat to slightly wavy on the outer edge. The thallus is thin, 44 - 87 (125) μ, and usually smooth and shiny, matte in a few specimens. It is not wrinkled, although in some specimens there is some irregular texture. The thallus appears gray with lobe tips and isidia becoming brown.
Medulla with short hyphae usually no longer than 3:1 (length : width) but on occasion as long as 5:1. These cellular hyphae are packed closely; most spaces between hyphae are filled with the photobiont.
Photobiont clumped or single cyanobacterial cells.
Cortices one cell thick, made of isodiametric cells about 5-8 μ across. Upper and lower cortices are similar in cross section, with exterior surfaces rounded and the upper cortex sometimes thickened slightly. Tufts of white hairs are found on the lower cortex at least 1 mm from the edge of the thallus.
Isidia form a dense fringe along the upturned lobe edges, sometimes starting as simple, narrow, knobby cylinders, becoming isodiametrically forked and then coralloid, and sometimes starting as finely dissected lobe edges approaching lobules in appearance, that develop simple isidia that become knobby, forked, and then coralloid (these sometimes give the impression of raised hands). Isidia are corticate and matte to shiny, and gray becoming brown to blackish at the tips. There is no dimpling on the isidia; instead they are convex to opuntioid (having several constricted points along their length). Isidia also arise laminally, and these laminal isidia tend to remain smaller and less coralloid than the marginal isidia.
Apothecia are not common; we have seen only one fertile specimen. Young apothecia are spherical, later becoming flattened or semispherical. Apothecia are not immersed; some appear almost stalked. One apothecium appears to have rhizines connecting its lower surface to the upper cortex of the thallus.
Habitat is epiphytic, frequently on the smooth bark of young oak trunks or oak branches (particularly *Quercus kelloggii*), in the Siskiyou Mountains of southern Oregon, and the Trinity Alps of northern California, at elevations between 2000 and 4800 ft. In addition, it has been collected in Monterey, Shasta, and Humboldt Counties in California, at elevations of 4200 ft., 1300 ft., and 3800 ft., respectively. The species has also been found growing on *Pseudotsuga menziesii* (single specimen from Trinity County, California), and on *Fraxinus latifolia* (single specimen from Shasta County, California).

Look-alike species: *Leptogium siskiyouensis* can be confused superficially with *Leptogium pseudofurfuraceum*. Several characters distinguish the two. *L. pseudofurfuraceum* has a brown, finely wrinkled thallus that has lobes to 1 cm wide and grows to a much larger diameter than *L. siskiyouensis*. In *L. pseudofurfuraceum*, lobe edges are downturned and it has a mallotium-type hairy lower surface. The medulla of *L. pseudofurfuraceum* has hyphae at least 5:1 and usually much longer, with space between the photobiont and the hyphae in cross-section. The isidia of *L. pseudofurfuraceum* are dimpled and often somewhat flattened. *L. siskiyouensis* differs from both *L. tacomae* and *L. subaridum* in having a tightly packed, cellular interior. In addition, highly isidiate *L. tacomae* specimens generally form less adnate rosettes than *L. siskiyouensis*, with lobes arising to form a lettuce-like to cushion-like thallus. In contrast, *L. siskiyouensis* is usually tightly adnate, with steeply rising lobe edges with upright, branched to coralloid isidia that are more consistently shiny at the tips. *L. subaridum* never has coralloid isidia, but has simple to branched clavate isidia that are often deeply dimpled, appearing like deflated balloons. In addition, *L. subaridum* has dentate lobes and a distinctive blackish brown color.

Figure 5:

Leptogium siskiyouensis
distribution in the Pacific Northwest of North America

Figure 6: Photos of *Leptogium siskiyouensis*



Leptogium cellulosum P. M. Jørg. & Tønsberg

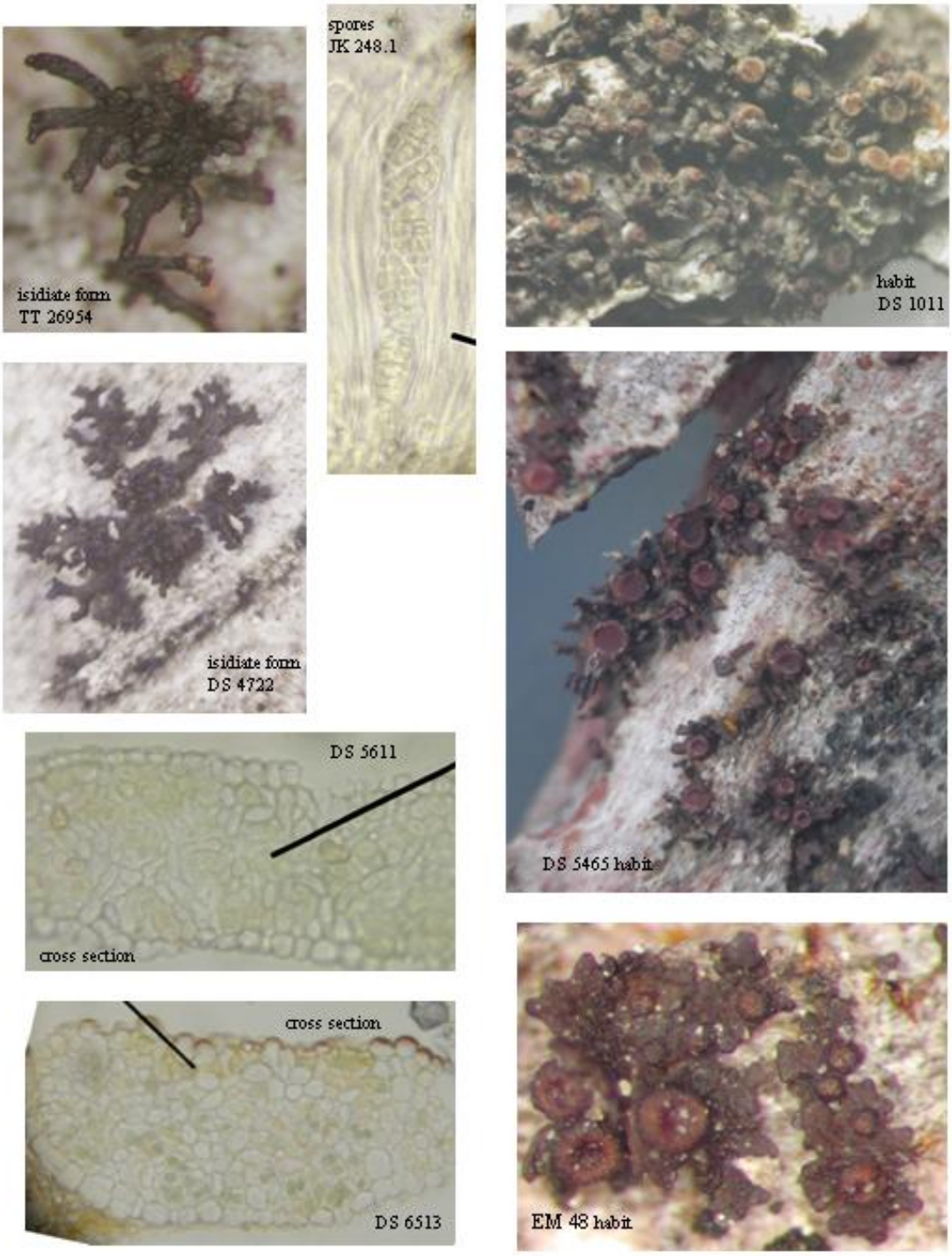
Leptogium cellulosum, first described by Jørgensen and Tønsberg in 1999, is one of the smaller species covered in this study. The thallus is composed of flat, gray to brown, squared to dentate lobes generally 0.1 - 0.6 mm. wide. There are two forms described in the literature. Fertile specimens have abundant apothecia grouped on the small lobes and grow in irregular colonies of up to about 2 cm (Fig. 7). Apothecia are globose when young, becoming flattened to a semi-spherical shape when mature. Apothecia are formed on the isidia, so they appear stalked.

Non-fertile thalli support granular to cylindrical to irregularly flattened narrow isidia on their margins and lamina, the isidia becoming so dense as to completely hide the lobes (Martin et al. 2002). Isidia are sometimes dimpled at the tip. Isidiate thalli tend to be dark brown to nearly black (Fig. 7).

Leptogium cellulosum grows on hardwood trees in the Pacific Northwest, east and west of the Cascade Mountains, Canada, and eastern North America, as well as in Europe, and seems to be particularly common on *Populus trichocarpa* boles in broad river valleys, where it may sometimes be found growing with the look-alike species *L. teretiusculum* (Martin et al. 2002). The isidiate form appears to be more common east of the Cascade Range, but since the thalli are so small, it is probably under-collected.

Look-alike species: The isidiate form of *L. cellulosum* and *L. teretiusculum* are sometimes nearly indistinguishable. *L. cellulosum* is a rich brown to tan color, in contrast to the brownish gray *L. teretiusculum*. In addition, the isidia of *L. cellulosum* tend to be cylindrical to flattened, while *L. teretiusculum* isidia tend to be pinched at the base, appearing swollen. Martin et al. (2002) describe the morphological differences between these species in detail.

Figure 7: Photos of *Leptogium cellulosum*



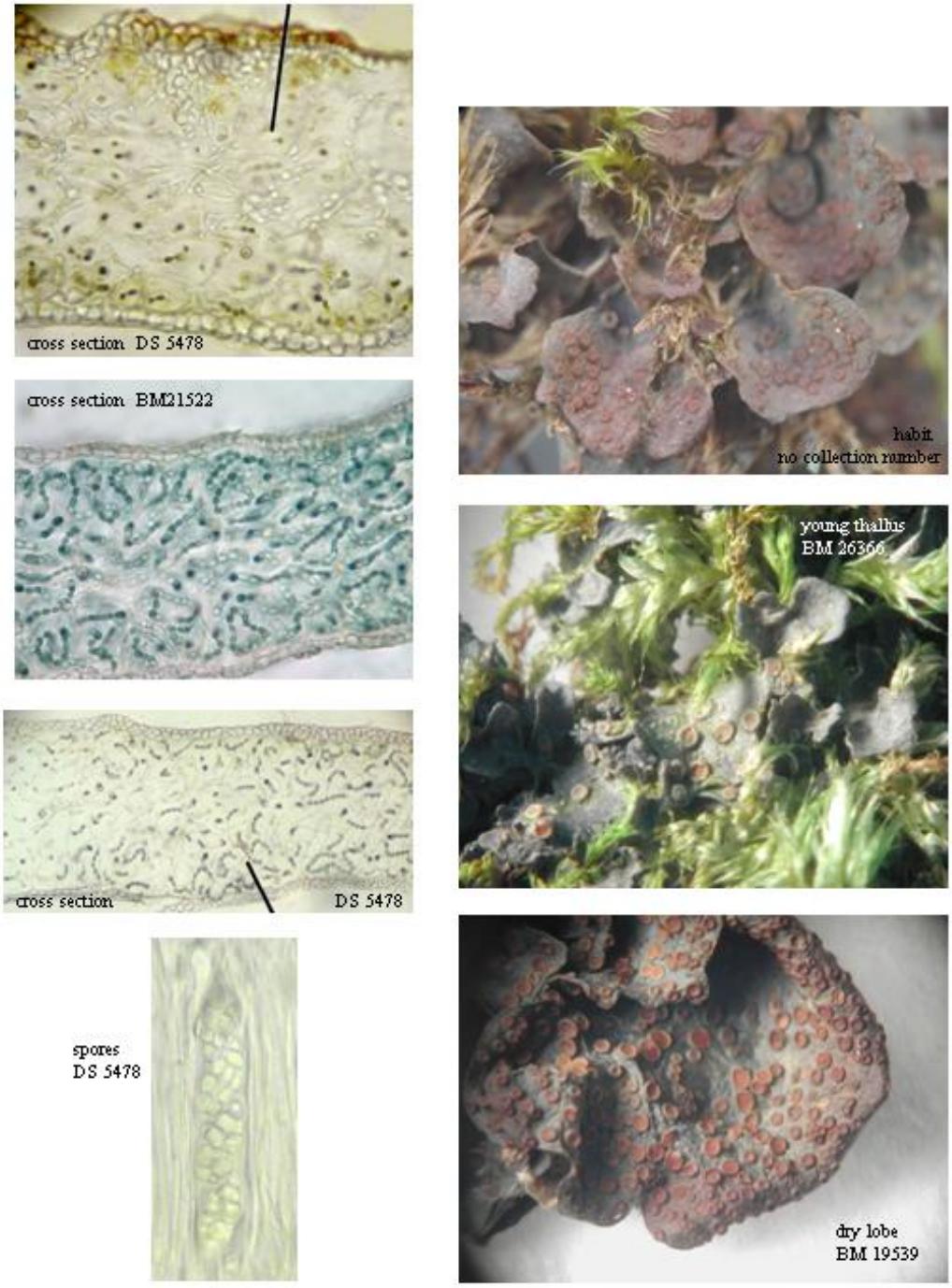
Leptogium cellulosum

Leptogium polycarpum P. M. Jørg. & Goward

Leptogium polycarpum was first described in 1994 by Jørgensen and Goward 1994. *L. polycarpum* is a non-isidiate species with rounded lobes. Edges of the lobes are flat or down-turned, and lobes range from gray in shaded habitats to brown in exposed habitats. The thallus is about 75 - 180 µ thick. Most thalli are abundantly fertile, with immersed apothecia containing 4 spores per ascus. It grows among mosses and leafy liverworts on tree boles and rocks throughout the Pacific Northwest, including British Columbia.

Look-alike species: Although *L. polycarpum* is not an isidiate species, we have included it in this study because it could be confused with young, non-isidiate thalli of normally isidiate species, particularly *L. tacomae*; these species are sometimes found growing together. The structure of the medulla in these two species are subtly different, with *L. polycarpum* generally thicker (Table 2), with a more loose organization of hyphae and nostoc chains than is generally seen in *L. tacomae* (Figures 4 and 8). *L. polycarpum* is generally more consistently wrinkled than *L. tacomae*, and sometimes shows cracking in the upper thallus surface, a character we have not seen in *L. tacomae*. A non-isidiate, fertile lobe of *Leptogium cyanescens* could also be confused with *L. polycarpum*, because both of these species have apothecia that are partially immersed when young. In this case, a section of a mature apothecium would reveal 4-spored asci in *L. polycarpum*, and 8-spored asci in other *Leptogium* species.

Figure 8: Photos of *Leptogium polycarpum*



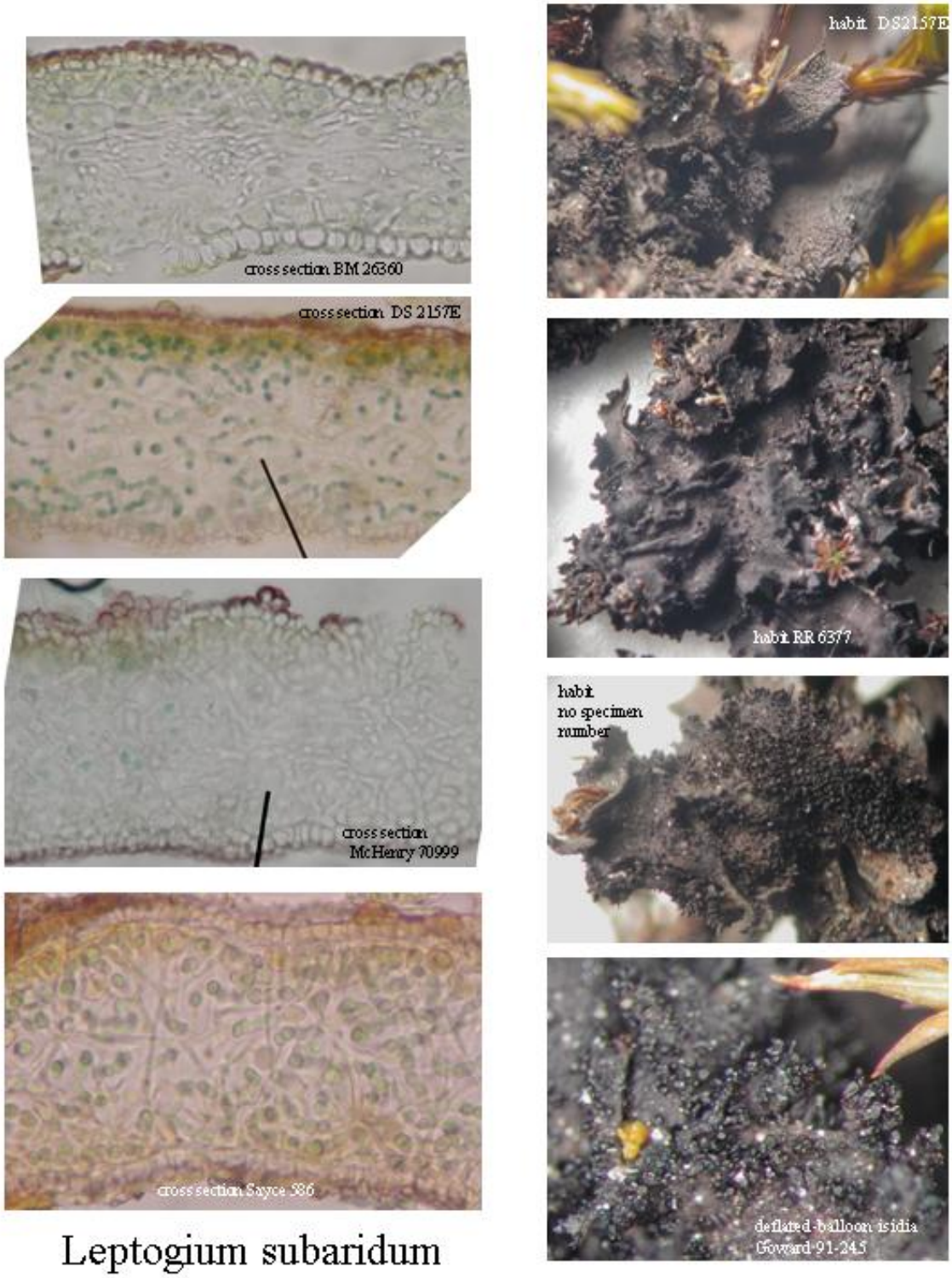
Leptogium polycarpum

Leptogium subaridum P. M. Jørg. & Goward

Leptogium subaridum was first described by Jørgensen and Gøward in 1994. It is an isidiate species found in North America, southern Europe and northern Africa (Aragon et al. 2004). In moist habitats the thallus grows in a non-regular manner with lobes growing up and around moss stems, some vertical and some horizontal, giving an overall untidy look (Figure 2 in Jorgensen & Goward 2004 shows this quite clearly). Lobes are rounded or squared, sometimes with the edges turned down, but usually dentate or dissected, about 75-125µ thick. In drier habitats east of the Cascade Mountains the thallus is typically composed of cupped, rounded, dissected-edged lobes standing on edge forming a cushion surrounded by mosses. Laminal and marginal isidia are clavate, with dimpling on the sides, giving them the appearance of partially deflated balloons. The lobes are matte and dark brown, while isidia are usually shiny and brown to black. In North America, *L. subaridum* is found on mossy rocks and occasionally on the bases of trees (D. Stone 6616), while in southern Europe and northern Africa it is found on tree trunks more commonly (Aragon et al. 2004).

Look-alikes: We included *L. subaridum* in this study because of its similarity to some thalli of *L. tacomae*. Although the typical thalli of *L. tacomae* and *L. subaridum* are clearly different morphologically, occasional thalli are found which are matte, gray with brown tips, and have clavate, dimpled, shiny, brown isidia, as opposed to typical *L. tacomae* isidia that are lumpy-cylindrical, thickened in places but not clavate, and not dimpled except occasionally at the tips. Several of these thalli came to our attention during the course of this study, and we are hopeful that DNA sequencing will clarify how they fit into this species complex.

Figure 9: Photos of *Leptogium subaridum*



Leptogium teretiusculum (Flörke *in* Wallr.) Arn.

Leptogium teretiusculum is the smallest of the species covered in this study, and is included in Sierk's The Genus *Leptogium* in North America North of Mexico (1964). The thallus is composed of many small isidia with a medulla composed of isodiametric cells to 2:1 (Figure 10), and is very similar to *L. cellulosum* (Figure 7). This species is reported by Martin et al. (2002) from British Columbia, Oregon, Idaho and Montana.

Look-alikes: There is difficulty in determining the difference between *L. teretiusculum* and isidiate forms of *L. cellulosum*. European specimens of *L. teretiusculum* have an overall gray color with some browning at the tips and have thin isidia that are knobby, or constricted in places, the basal parts of which become wider as the isidia mature. We have not seen this extreme widening of older parts of the isidia in North American specimens. However some North American specimens with delicate, knobby coralloid isidia appear to be the same taxon (Figure 10). Specimens with coarser isidia that are dimpled on the tips are more likely to be the isidiate form of *L cellulosum*. Martin et al. (2002) discuss morphological distinctions between *L. teretiusculum* and *L. cellulosum* at length.

Figure 10: Photos of *Leptogium teretiusculum*

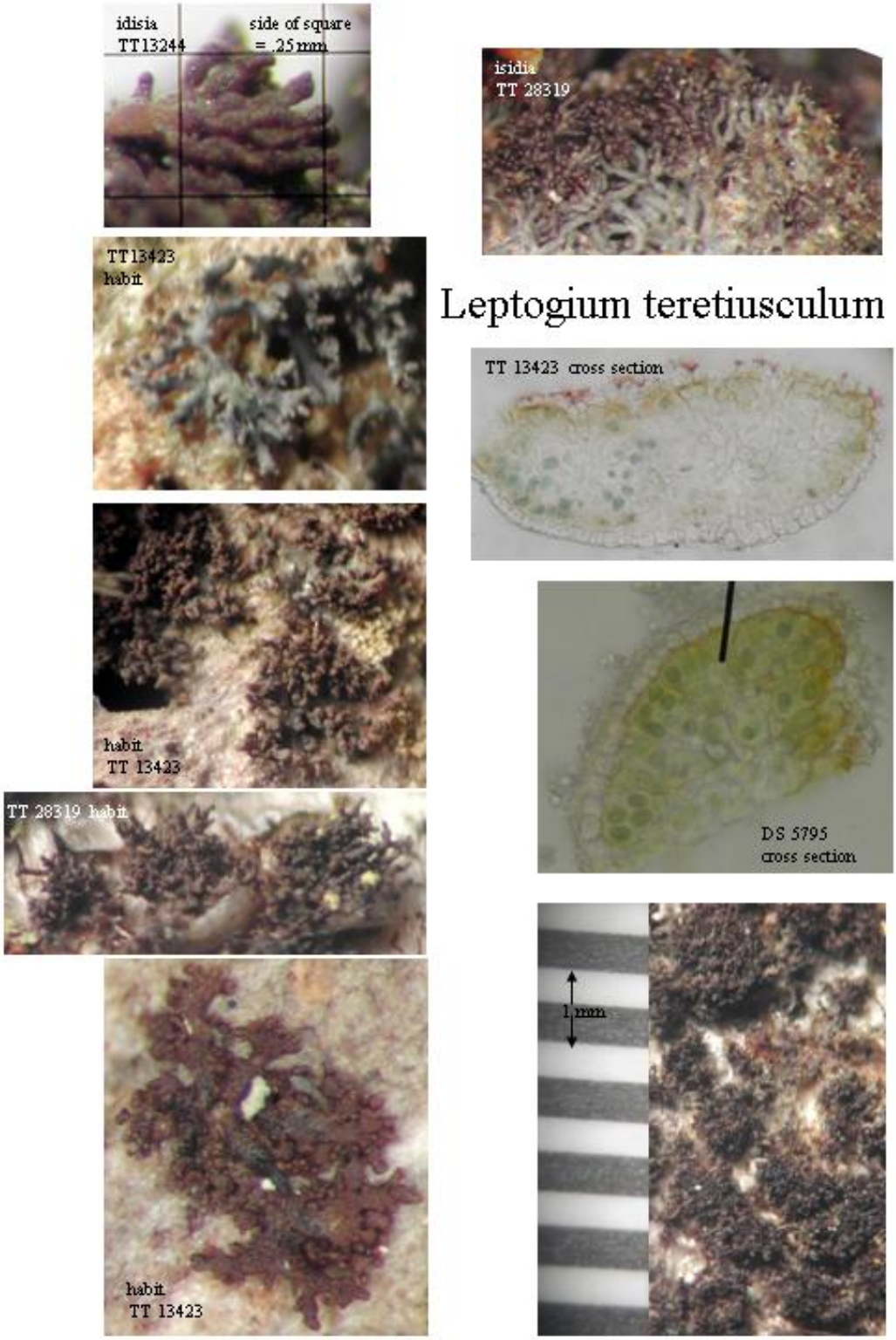


Table 2. Comparison of Small *Leptogium* Species in the Pacific Northwest

SPECIES	SPORES	APOTHECIA	INTERIOR	SUBSTRATE	ISIDIA	LOBES	THALLUS	REFERENCES Observations by Stone & Ruchty incorporated
cellulosum	4 - 6 (8)/ ascus 27-34 x 13-17µ (4) 5(-7) transv septa often w/ attenuated ends	abundant to .5 mm begin globose, become flattened	isodiametric cells some cells longer than 2:1 no space between hyphae and photobiont	hardwood bark	no laminal isidia, marginal isidia-like protruberances martin morph: granular to cylindric isidia, simple or branched	lobes (squamules) 0.1-0.6 (-3) mm wide 50-80 µ thick	brown matte grainy surface rosettiform to irreg often >2mm diam	Jorgensen & Tonsberg 1999 Martin et al 2002
teretiusculum	8/ ascus 20-25 x 10 3-5 transv septa	rare begin as spheres hymenium becomes concave	isodiametric cells some cells to 2:1 no space between hyphae and photobiont	hardwood bark	terete, knobby	lobes are isidia .01 - 0.3 mm broad pinched at bottom,	small cushions lead gray to brown	Sierk 1964
subaridum	8 / ascus 30-32.5µ x 12.5µ 5 transv septae 1 longi septum	rare	hyphae to 20:1 wide angles open spaces long chains photobiont some hyphal joints widened	soil or mosses on rock, tree trunks	clavate with dimpled tips and sides shiny, laminal and marginal	lobes 2-3 mm (1-10 mm) wide margins dentate (90) 100-125 (140) µ thick	brown to black 2-5 cm	Jorgensen & Goward 1994
tacomae	8 spores/ascus (19) 28-36 (38) x (7) 10-15 (17)µ 5-6 transv septae some attenuated ends	sparse to abundant To 0.5 mm. begin partially immersed or globose mature not globose can have isidiate margin	hyphae 3:1 - 10:1 wide angles, open spaces hyphal joints widened	hardwood bark mossy rocks	laminal and marginal knobby become coralloid	rounded to squared 0.5 -3 (.25-7) mm broad (60) 80-120 (150)µ thick wrinkled to 215 µ thick smooth to weakly wrinkled flat to cupped	~100 µ (44 – 144) µ thick blue gray in shade brown in sun cushion-forming foliose to 5 cm	Jorgensen & Tonsberg 1999
cyanescens	8 spores/ascus 18-23 X 6-10µ, 3 sept transv, 0-1 septa longi	Reported as abundant in general references; absent to sparse in specimens from Pacific Northwest 0.5-2.0 mm can have finely isidiate margins	hyphae to 20:1 with horizontal and vertical orientation compact,woven appearance hyphal joints not widened	tree trunks, rotten logs, rocks + trees	fine to paddle-shaped to lobular laminal and marginal	lobes up to 12 mm wide 35-110µ thick, older parts to 110µ. smooth or mildly wrinkled	blue gray to dark gray 1-5 cm, flat	Sierk 1964, Jorgensen & James 1983
polycarpum	4 sp/ascus 25-40 µ x 12-15 µ	abundant 0.2 - 0.5 mm partly sunken	hyphae to 20:1 wide angles open spaces long chains photobiont	mossy bark mossy rock	none	.5-1.0 (8) cm wide 125 to 190 µ thick (up to 400 µ when fully hydrated) margins entire, broadly rounded, surface wrinkled and sometimes cracked when dry	blue gray to brownish gray 0.5-2 (4) cm flat to cupped	Jorgensen &Goward 1994 Noble 1982
siskiyouensis	8 sp/ ascus 37.5 x 11 µ	rare begin as spheres, become flattened not immersed	isodiametric cells hyphae to 6:1 no space between hyphae and photobiont hyphal joints widened	smooth bark of oaks (particularly *Quercus kelloggii*), occ. on conifers and other hardwoods	laminal and marginal simple becoming coralloid shiny	lobes 0.3 - 4 mm wide 44 - 87µ (125) thick smooth, shiny to matte unwrinkled	gray to brown-tipped to 3 cm wide centers of lobes closely adnate edges of lobes erect, coralloid-isidiate	Stone & Ruchty 2008

Sequencing *Leptogium* DNA

Methods

Well-preserved thalli of *Leptogium* spp. 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 20 years old) were sampled for DNA extraction. Sampled specimens were an assortment of collections from different university herbaria as well as from the herbaria of the authors.

An assortment of specimens was used in order to include a range of locations. Since the genus *Leptogium* is poorly represented in GenBank, we also sampled more species than were encompassed by this study, in order to enter known, documented specimens into the sample pool for baselines and comparisons. Extractions were attempted from approximately 100 specimens. Of these, only 20 were successfully sequenced. Failure of the process could be due to age of the specimens, their treatment in Herbaria, or contamination by fungal parasites.

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% polyvinylpolypyrollidone, 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 (LSU) DNAwas amplified from the template DNA using primers LROR and LR7 (Vilgalys and Sun 1994). PCR reactions were carried out in a 1X buffer, 200 um dNTP, 0.4M forward and reverse primers, 0.05 U/uL RedTaq DNA polymerase (Sigma, St. Louis), 5% blocking powder in an H_2O solution - 0.5 ul per 50ul reaction, 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 Reserach, 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 80º 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). Sequences identified by BLAST searches as having high homology to the test specimens sequences, and those identified as Leptogium spp in the GenBank database were included in the analysis.

Results and Discussion

Results from comparisons of DNA sequences of this group of *Leptogium* taxa is consistent with recognition of lichen species based on morphological characters, and indicates areas with remaining problems. The neighbor-joining tree resulting from the nLSR sequence analysis indicates strong support for recognizing the monophyletic species *L. cyanescens*, *L. tacomae*, *L. "siskiyouensis"* and *L. polycarpum*. The horizontal distances on the tree show relative differences (number of base pair changes) between the sequences of each species. The numbers given by certain nodes are bootstrap values. Bootstrap values, expressed as percents, are the proportion of times that the grouping occurred in the tree in 1000 iterations. Bootstrap values over 70% are generally considered significant, and only bootstrap values> 70% are shown on Figure 11. Only the horizontal distances on the tree are meaningful. The scale of 0.01 at the bottom of the figure is the number of bases per 100 changes.

The two specimens of *L. cyanescens* are grouped together with bootstrap support of 100% but are grouped somewhat distantly from the other species considered in this study. The two specimens representing *L. cyanescens* were collected from widely different locations. *Leptogium cyanescens* DS 6616 was collected in Vermont and *L. cyanescens* McCune 27346 was collected in New Zealand. Despite the presumed reproductive isolation of the two populations, the LSU analysis provides evidence that this species has a global distribution. Including more examples of *L. cyanescens* in the study, particularly from North America, would likely reveal variation within the species. The terminal branches suggest some divergence between the two specimens. *Leptogium brebissonii* was used in the study as an outgroup. Both *L. cyanescens* and *L. brebissonii* are genetically dissimilar to the other species in this study, and thus they are artificially grouped, an artifact of the tree-forming process.

The sequence analysis reveals variation within *Leptogium subaridum*, indicating that this taxon as currently understood comprises two distinct groups. Both groups have very strong bootstrap support, but their relationship to other *Leptogium* species is not resolved in the analysis. One group contains an isotype collected by Goward in British Columbia, Canada, and a specimen from Josephine Co., Oregon. The other "*L. subaridum*" is represented by two specimens from Wasco County, WA. Thalli of these specimens differ somewhat from the isotype in having tightly packed, erect lobes that form a cushion, as opposed to horizontal lobes that are often spread among mosses. Because of the morphological differences and LSU sequence divergence between this eastern Washington "*L. subaridum"* and the isotype, we suspect that the eastern Washington specimens comprise an undescribed species.

Two other species groupings indicate areas where further investigation is needed. First, there is low bootstrap support for linking the two representatives of *L. cellulosum*, one an isidiate thallus and one fertile specimen, despite very slight sequence divergence between the two. The reason for the low bootstrap support is not apparent.

The strong grouping (bootstrap 87.2%) of a specimen of *L. lichenoides* group from Douglas Co., Oregon with a specimen of *L. teretiusculum* from Norway is unexpected. These species are superficially extremely different. *Leptogium teretiusculum* is a minute thallus of tiny lobes covered with abundant isidia, growing on bark, in contrast to the larger, erect and dissected but not isidiate lobes of the *lichenoides* group. In addition, their medullar structure is different. The medulla of *L. teretiusculum* is composed of hyphae forming isodiametric cells with scattered cyanobacterial cells, giving it a parenchymatous look, while the *L. lichenoides* group has a medulla made of long, moderately branched hyphae with interspersed strings of cyanobacteria. The species of the *lichenoides* group differ in the amount of space between hyphae, with *L. lichenoides* having widely spaced hyphae, *L. intermedium* with less space between long hyphae, to *L. californicum* that has fairly tightly packed long hyphae with fewer strings of cyanobacteria visible. We refer to the "lichenoides group" because we feel that the species *L. californicum* and *L. intermedium* are morphologically similar but not well-defined, and there is an additional taxon, represented by collections from the Pacific Northwest, that possesses characteristics of both *L. californicum* and *L. lichenoides*. The species *L. quercicola*, described from Spain (Otalora et al., 2004) is in this group as well, and may represent this undetermined Pacific Northwest species.

The placement of these two species in a sister relationship in the analysis suggests that *L. lichenoides* and *L. teretiusculum* are closely related. More sampling is needed to determine whether North American *L. teretiusculum* is related to European *teretiusculum*, and whether North American *L. lichenoides* is a separate species from the European *L. lichenoides*. Unfortunately, although these results are very suggestive, it is not possible to draw firm conclusions on the relationships of these taxa from the limited scope of sampling in this study. These results, however, do point to some problems where further molecular phylogenetic studies may add to our understanding of this genus.

Finally, it is informative to compare the placement of species in the LSU neighbor-joining analysis with the corresponding medullar structure, which has been considered a useful diagnostic character for the genus. The placement of species in the molecular analysis indicates that medullar structure is not a stable phylogenetic character. Instead of being grouped by different medullar structures, species having very different looking medullae are scattered throughout the tree. For instance, *L. siskiyouensis*, with a cellular medulla, is placed near *L. lichenoides* with long, hyphal strands in the medulla. A similar grouping occurs with *L. cellulosum* being placed near *L. polycarpum*. The bootstrap values of these groupings are very low, indicating that they are not always grouped together. However the fact that the different medullar types are not significantly grouped indicates that medullar type is of little importance and has occurred several times during the evolution of the species in the genus *Leptogium*.

11. Phylogenetic Tree of some *Leptogium* species.

Table 3: Specimens used in DNA sequencing

Herbarium in parentheses, McCune=personal herbarium of Dr. Bruce McCune, DS=personal herbarium of Dr. Daphne Stone, others are official Herbarium abbreviations.

GenBank number follows successfully sequenced specimens

Leptogium californicum
Tør Tønsberg 15409 Kodiak Island E, AK 1991 (BG)

Leptogium cellulosum
Daphne Stone 5465 Josephine Co., OR 2002 (DS)
Bruce McCune 25342a Lane Co., OR 2000 (McCune) EU166330
Tør Tønsberg 26954 TOPOTYPE Kittitas Co., WA 1998 (BG) EU166344
Erin Martin 48 Benewah Co., ID 1999 (BG)

Leptogium cyanescens
Grenon no number, as DS 6616 Grafton Co., NH 2006 (DS) EU166339
Arnot L1326 Snohomish Co., WA 1998 (WTU)
Bruce McCune 23061 Lincoln Co., OR 1996 (McCune)
Bruce McCune 27346 Northland District, New Zealand 2003 (McCune) EU166335

Leptogium platynum
Bruce McCune 26095 Hood River Co., OR 2001 (McCune) EU166332

Leptogium intermedium
Roger Rosentreter 7034 Kootenai Co., ID 1991 (McCune)
JMP1341 Benton Co., WA 1999 (McCune)
Czarnota L-31744 South Poland, Poland 1994 (BG)

Leptogium lichenoides
Bruce McCune 21751 Wallowa Co., OR 1994 (McCune)
Tør Tønsberg 26094 Upper Austria, Austria 1998 (BG)
Bruce McCune 25720 Douglas Co., OR 2000 (McCune) EU166331
J. I. Johnsen L-71984 Rogaland, Norway 2001 (BG)

Leptogium polycarpum
Daphne Stone 5478 Jackson Co., OR 2002 (DS) EU166336

Leptogium tacomae
Eric McEwen EM1 Josephine Co., OR 2003 (OSC) EU166340
Tør Tønsberg 20292 HOLOTYPE Pierce Co., WA 1994 (BG)
Noel Bachellor NJB14JUN05-F Linn Co., OR 2005 (OSC) EU166341
Bruce McCune 26367 Marion Co., OR 2002 (McCune) EU166334
Shana Gross 080101SEG-10 Snohomish Co., WA 2001 (WTU)
Bruce McCune 11707 Idaho Co. ID 1982 (McCune)

Leptogium teretiusculum
Tør Tønsberg 28319 Hordaland, Norway 2000 (BG) EU166345
Tør Tønsberg 13423 Hordaland, Norway 1990 (BG)

Tør Tønsberg 13244 Østfold, Norway 1990 (BG)

Leptogium siskiyouensis
Bruce McCune 18354 Josephine Co., OR 1990 (McCune) EU166328
Bruce McCune 25896 Monterey County, CA 2001 (McCune)
Tom Carlberg 00866 Humboldt Co., CA 2003 (UC) EU166342
Daphne Stone 5610.4 TYPE Josephine Co., OR 2003 (OSC) EU166337

Leptogium subaridum
Yana Valacovic 269.1 Josephine Co., OR 1999 (DS) EU166346
Bruce McCune 26360 Wasco Co., WA 2002 (McCune) EU166333
Bruce McCune 21528 Mono Co., CA 1994 (McCune)
Trevor Goward 91-245 ISOTYPE Vaseux Lake area, BC, Canada 1991 (UBC) EU166343
Roger Rosentreter 6377 Lincoln Co., WA 1990 (McCune)
Arnot L590 Kittitas Co., WA (WTU)
Daphne Stone 5723 Butte Co., CA 2004 (DS)

Leptogium subtile
Botnen 2412 Hordaland, Norway 1992 (BG)

undetermined Leptogium spp.
Mark Boyle ARM143-10 Hood River Co., OR 1994 (McCune)
Daphne Stone 5725 Butte Co., CA 2003 (DS)
Bruce McCune 25823 Humboldt Co., CA 2001 (McCune)
Bruce McCune 25474 Jefferson Co., OR 2000 (McCune)
Daphne Stone 6115 Jackson Co., OR 2004 (DS)

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