LICHEN TAXONOMY FOR THE 21ST CENTURY: A REVISION OF THE GENUS *LEPRARIA* S.L. IN NORTH AMERICA NORTH OF MEXICO

by

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VOLUME 1

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Abstract

LICHEN TAXONOMY FOR THE 21ST CENTURY: A REVISION OF THE GENUS *LEPRARIA* S.L. IN NORTH AMERICA NORTH OF MEXICO

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James C. Lendemer

Advisor: Dr. William R. Buck

The results of a series of studies of lichens are presented. These studies are hoped to codify a forward for taxonomic progress in the group. Chapter one presents a standardized morphological terminology and descriptive scheme for the sterile crustose genus *Lepraria*. Chapters two through seven present a series of studies where molecular phylogenetic analyses of nrITS sequence were used to resolve species boundaries in *Cladonia* (III)*, Lepraria* (IV-VII), and *Punctelia* (II). Chapter eight reveals not all sterile crustose lichens resemble their congeners. Chapter nine presents a study in the use of molecular data to place an unknown sterile crustose lichen in higher level classification of fungi. Chapter ten uses that method to revise the circumscriptions of *Lepraria* and the sterile fruticose genus *Leprocaulon*. Finally, chapter eleven comprises a taxonomic revision of the crustose species of *Lepraria* s. str. that occur in North America north of Mexico.

DEDICATION

The present work is dedicated to William Buck, Samuel Hammer, Richard Harris, Kerry Knudsen, Rick McCourt, Thomas Nash, Herman Pfefferkorn, Alfred Schuyler, Earle Spammer and Isabelle Tavares. As a high school student in Philadelphia, Spammer tutored me in the *International Code for Zoological Nomenclature* and its companion *International Code for Botanical Nomenclature* while Pfefferkorn introduced me to a world of fossil plants and the value of appropriate sampling schemes. A chance invitation from McCourt to attend the NSF PEET IV conference in Berkeley, California led to meeting Hammer and Tavares. During a week of fieldwork for the NSF-funded Cladoniaceae PEET, Hammer encouraged me to study lichens in the field and question preconceived notions of morphological development. The meeting with Tavares led to a rich collaboration through which I learned the value of detailed anatomical studies of micromorphology, and to always question everything and everyone (most of all oneself). During my decade in Philadelphia I worked closely with Schuyler, whom I consider a true friend, historian, and connoisseur of a good martini. Also at that time, Knudsen and I began studying lichens, he opened my eyes to *Lepraria* and to California, and our friendship has continued ever since. He is also the one who pushed me to undertake a revision of *Lepraria* on our continent for my dissertation. Nash facilitated a formative experience in Phoenix, Arizona where I replaced Bruce Ryan for a short time after his passing. For more than a decade William Buck and Richard Harris have been both colleagues and friends. Without them, there is no way this work, and many others, would have been possible. This work would also not have been possible without the generous support of The City University of New York and The New York Botanical Garden.

A special section is here reserved for Brendan Hodkinson, with whom several chapters of this work are co-authored. America needs more young native lichenologists with original ideas, strong opinions, and a fondness for debating them. The tripartite collaboration between us and Harris has been extremely rewarding.

Lastly, this work is dedicated to Andrei Moroz, my companion in the field and in life. We have wandered everywhere from the hills of Appalachia to the *Araucaria* forests of the Andes. It is only fitting that the Appalachian endemics *Lepraria moroziana* Lendemer and *Opegrapha moroziana* Lendemer bear his name.

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Figure 50. Maximum likelihood (ML) phylogeny of *Lepraria* s. str., Stereocaulaceae and Cladoniaceae (outgroup) inferred from mtSSU sequence data. The topology shown here is derived from ML analyses of the full dataset. Support values are based on ML analyses of the full dataset as well as maximum parsimony (MP), ML, and Bayesian Inference (BI) analyses of the reduced dataset: MP-BP[reduced]/ML-BP[reduced]/ML-BP[full]/BI-PP[reduced]. A single value indicates support from the ML analyses of the full dataset for a clade of identical sequences that was collapsed in the reduced datset. Numbers in parentheses refer to the list of support values in the upper left corner of the figure. Thickened branches indicate support of $\geq$ 70% MP-BP and ML-BP as well as $\geq$ 0.95 BI-PP. Morphological variation in the Stereocaulaceae is illustrated in A–D. A, crustose placodioid thallus of *Lepraria oxybapha* (scale = 0.5 mm; *Lendemer 27101*). B, crustose aggregate thallus of *L. gelida* (scale = 0.5 mm; *Tønsberg 31049*). C, fruticose thallus (right, scale = 1.0 mm) with ecorticate granules (left, scale = 0.5 mm) in *L. subalbicans* (*McCune 30040*). D, fruticose thallus (right, scale = 2.0 mm) with corticated phyllocladia (left, scale = 0.5 mm) in *Stereocaulon glaucescens* (*Lendemer 27320*).

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Figure 51. Maximum likelihood (ML) phylogeny of the "*Halecania*-clade" and other members of the Teloschistales inferred from mtSSU sequence data. Members of the Teloschistiinae were selected as the outgroup based on prior studies that have shown the *Halecania*-clade to be sister to the Physciaceae (Nelsen et al. 2008). Support values are displayed as: MP-BP/ML-BP/BI-PP. Numbers in parentheses refer to the list of support values in the upper left corner of the figure. Thickened branches indicate support of $\geq$ 70% MP-BP and ML-BP as well as $\geq$ 0.95 BI-PP. Accepted family level names are indicated on the left. Note that *Speerschneidera euploca* is included in quotes because the sequence may represent a contaminant or chimera.

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Figure 52. Maximum likelihood (ML) phylogeny of the "*Halecania*-clade" (i.e., Leprocaulaceae) inferred from nrITS sequence data. The tree is displayed with midpoint rooting. Support values are displayed as: MP-BP/ML-BP/BI-PP. Thickened branches indicate support of ≥ 70% MP-BP and ML-BP as well as ≥ 0.95 BI-PP. Note that *Halecania alpivaga* and *Speerschneidera euploca* are not included because nrITS sequence data were not available in GenBank at the time of this study. Growth form and major secondary metabolites of the species within this group are indicated as thickened lines to the right (a = argopsin, p = pannarin, u = usnic acid, z = zeorin; black(c) = crustose, gray(f) = fruticose).

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Figure 53, morphological variation sterile asexually reproducing members of the newly established family Leprocaulaceae (order from A–H as in the phylogeny presented in figure 4). A, *Leprocaulon santamonicae* (scale = 0.25 mm; *Kocourková s.n.*). B, *L. terricola* (scale = 0.5 mm; *Knudsen 9608*). C–D, *L. quisquiliare* (scale = 1.0 & 0.25 mm respectively; *Buck 55960*). E, *L. adhaerens* (scale = 0.25 mm; *Beeching 11071*). F, *L. knudsenii* (scale = 0.25 mm; *Knudsen 9256*). G, *L. americanum* (scale = 1.0 mm; *Knudsen 9715*). H, *L. textum* (scale = 0.5 mm; *Lendemer 11500*).

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Figure 54. Maximum likelihood (ML) phylogeny of the Pilocarpaceae inferred from mtSSU sequence data and rooted with *Psilolechia* following Schmull et al. (2011). Support values are displayed as: MP-BP/ML-BP/BI-PP. Thickened branches indicate support of ≥ 70% MP-BP and ML-BP as well as ≥ 0.95 BI-PP. Genera are indicated with thickened bars to the right. *Nelsenium* is here described as a new genus to accommodate *Lepraria usnica* Sipman.

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Figure 55. Maximum likelihood (ML) phylogeny of sequences in the "*Lecanoria*-1" and "*Lecanora*-2" groups, with other reference sequences of members of the Lecanoraceae inferred from mtSSU sequence data. The tree is displayed with midpoint rooting. Support values are displayed as: MP-BP/ML-BP/BI-PP. Thickened branches indicate support of ≥ 70% MP-BP and ML-BP as well as ≥ 0.95 BI-PP.

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Figure 56, morphological variation of leprarioid Lecanoraceae included in figure 7. A, *Lecanora thysanophora* (*Lendemer 13179*). B, *Lepraria* sp. (*Lendemer 14816*). C, *Lepraria* sp. (*Lendemer 16832*). D, *Lepraria* sp. (*Lendemer 19499*). All scales = 0.5 mm.

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Chapter XI

Figure 57, North American collection localities visited by the author as part of this study.

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Figure 58, *Lepraria aurescens*. A, thallus surface, and edge with dark rhizohyphae (*Lendemer 15843A*, scale = 0.5 mm). B, thallus surface and edge with pale rhizohyphae (*Harris 39672*, scale = 0.5 mm). C, detail of thallus surface and edge (*Lendemer 15843A*, scale = 0.25 mm). D, detail of thallus with upturned margins and pale rhizohyphae (*Harris 39672*, scale = 0.25 mm).

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Figure 59, geographic distribution of *Lepraria aurescens* based on the specimens examined for this study.

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Figure 60, *Lepraria caesiella*, comparison of saxicolous population (all from *Lendemer 27454*) and corticolous population (all from *Lendemer 26684*). A–D, gross morphology of the thallus (scales = 0.5 mm). E–F, detail of the granules (scales = 0.25 mm).

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Figure 61, geographic distribution of *Lepraria caesiella* based on the specimens examined for this study.

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Figure 62, typical population of *Lepraria cryophila* from the Appalachian Mountains (all from *Lendemer 22721*). A, gross morphology of the thallus (scale = 1.0 mm). B, detail of the granules and thallus surface (scale = 0.25 mm). C–D, ascending thallus margins (scale = 0.5 mm). E–F, detail of ascending thallus margins, note the presence of pale rhizohyphae that begin to turn dirty brownish towards the tips (scales = 0.5 mm).

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Figure 63, typical population of *Lepraria cryophila* from the Ozark Ecoregion (all from *Lendemer 25898*). A, gross morphology of thallus (scale = 0.5 mm). B, detail of the thallus surface (scale = 0.25 mm). C, detail of thallus margin (scale = 0.25 mm). D, aspect of ascending thallus margins (scale = 0.5 mm). E, young thallus with ascending margins (scale = 0.5 mm). F, detail of thallus margin, not the poorly developed pale rhizohyphae (scale = 0.25 mm).

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Figure 64, geographic distribution of *Lepraria cryophila* based on the specimens examined for this study.

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Figure 65, comparison of type specimens of *Lepraria diffusa* (left, from holotype) and *L. diffusa* var. *chrysodetoides* (right, from isotype in BM). A–B, gross morphology of the thallus (scales = 1.0 mm). C–D, thallus surface (scales = 0.5 mm). E–F, detail of the granules (scales = 0.25 mm).

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Figure 66, comparison of North American populations of *Lepraria diffusa* (left, from *Lendemer 22344*) and *L. diffusa* "var. *chrysodetoides*" (right, from *McCune 30488*). A–B, gross morphology of the thallus (scales = 1.0 mm). C–D, thallus surface (scales = 0.5 mm). E–F, detail of the granules (scales = 0.25 mm).

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Figure 67, geographic distribution of *Lepraria diffusa* based on the specimens examined for this study.

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Figure 68, *Lepraria eburnea* (A–C and E from *Lendemer 22734*; D and F from *Lendemer 23046*). A, gross morphology of the thallus (scale = 1.0 mm). B, detail of the thallus margin (scale = 0.25 mm). C–D, aspect of the thallus margin (scales = 0.5 mm). E–F, detail of the granules (scales = 0.25 mm).

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Figure 69, geographic distribution of *Lepraria eburnea* based on the specimens examined for this study.

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Figure 70, comparison of the holotypes of *Lepraria elobata* (*Tønsberg 17404*, left) and *L. salazinica* (*Tønsberg 30941*, right). A–B, gross morphology of the thallus (scales = 0.25 mm and 0.5 mm respectively). C–D, detail of the granules (scales = 0.25 mm).

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Figure 71, North American populations of *Lepraria elobata* (*Lendemer 28116* is left; *Lendemer 28349A* is right). A–B, gross morphology of the thallus (scales = 1.0 mm). C–D, detail of thallus morphology (scales = 0.5 mm). E–F, detail of the granules (scales = 0.25 mm).

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Figure 74, geographic distribution of *Lepraria finkii* based on the specimens examined for this study.

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Figure 75, comparison of isotype of *Lepraria santosii* in GZU (A and B) with the lectotype of *L. lobificans* in H-NYL (C–F). A, gross morphology of the thallus in *L. santosii* (scale = 1.0 mm). B, detail of the thallus margin illustrating the crisped edge in *L. santosii* (scale = 0.5 mm). C, gross morphology of the thallus in *L. lobificans* (scale = 1.0 mm). D, detail of the thallus margin illustrating the crisped edge in *L. lobificans* (scale = 0.5 mm). E, thallus surface in *L. lobificans* (scale = 0.5 mm). F, lower surface in *L. lobificans* (scale = 0.5 mm).

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Figure 76, *Lepraria friabilis* (*Lendemer 21079A* is left; *Lendemer 21344* is right). A–B, gross morphology of the thallus (scales = 1.0 mm). C–D, detail of the thallus (scales = 0.5 mm). E–F, detail of the granules (scales = 0.25 mm).

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Figure 77, geographic distribution of *Lepraria friabilis* based on the specimens examined for this study.

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Figure 78, *Lepraria harrisiana* (all from *Lendemer 55730*). A, gross morphology of the thallus (scale = 1.0 mm). B–D, detail of the thallus, and thallus surface (scales = 0.5 mm). E–F, detail of the thallus edge, illustrating rhizohyphae and ascending margins (scales = 0.25 mm).

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Figure 79, geographic distribution of *Lepraria harrisiana* based on the specimens examined for this study.

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Figure 81, geographic distribution of *Lepraria hodkinsoniana* based on the specimens examined for this study.

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Figure 82, *Lepraria humida* (*Lendemer 12651* is left; *Lendemer 22865* is right). A–B, gross morphology of the thallus, note the tendency to form dispersed aggregations (scales = 1.0 mm). C–D, detail of the thallus (scales = 0.5 mm). E–F, detail of the granules (scales = 0.25 mm).

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Figure 83, geographic distribution of *Lepraria humida* based on the specimens examined for this study.

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Figure 84, comparison of holotype of *Lepraria jackii* (*Tønsberg 6176*, right) to North American population (*LaGreca 1980*, left). A–B, gross morphology of the thallus (scales = 0.5 mm and 1.0 respectively). C–D, detail of the thallus (scales = 0.5 mm). E–F, detail of the granules (scales = 0.25 mm).

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Figure 85, geographic distribution of *Lepraria jackii* based on the specimens examined for this study.

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Figure 86, *Lepraria lanata* (all from *Lendemer 23306*). A, gross morphology of the thallus (scale = 1.0 mm). B–C, detail of the thallus (scales = 0.5 mm). D–F, detail of the granules, note the tendency for some hyphae to become darkly pigmented (scales = 0.25 mm).

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Figure 87, geographic distribution of *Lepraria lanata* based on the specimens examined for this study.

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Figure 88. *Lepraria lecanorica* (all from the holotype). A–B, macromorphology of the thallus (scales = 2.0 mm and 1.0 mm respectively). C, detail of the granules (scale = 0.5 mm). D, detail of the thallus margin (scale = 0.5 mm).

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Figure 89, SEMs of *Lepraria lecanorica* (all from the holotype, scales as indicated). A, macromorphology of the thallus. B, thallus edge with prothalline hyphae extending outward from the granules. C, young ecorticate granule. D, mature granules with pseudocortex. E–F, detail of prothalline hyphae extending outward from the granules.

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Figure 90, geographic distribution of *Lepraria lecanorica* based on the specimens examined for this study (red dot = specimens examined for this study; yellow stars = vouchers from CNALH and protologue note seen for this study).

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Figure 91, SEMs of *Lepraria leprolomopsis* (all from *Harris 28456* unless otherwise indicated). A, detail of thallus edge. B, gross morphology of thallus. C, detail of thallus edge from below (*Guccion 1663*). D, granules encased in network of thalline hyphae. E, young granule developing on thallus surface. F, detail of hyphae forming the hypothallus (*Guccion 1663*).

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Figure 92, SEMs of *Crocynia pyxinoides* (all from *Lendemer 9067*, NY). A, gross morphology of thallus. B, detail of "lobe". C, detail of thallus margin illustrating both the upper surface and hypothallus extending outward from the underside of the "lobe" tips. D, detail of upper surface. E, thallus margin from below. F, detail of hyphae forming the hypothallus.

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Figure 93, color illustrations of *Crocynia pyxinoides* (A) and *Lepraria leprolomopsis* (B–F). A, gross morphology of *C. pyxinoides*, note well developed black hypothallus surrounding the "lobes" (*Lendemer 9067*, scale = 2 mm). B, gross morphology of thallus of *L. leprolomopsis* (*Harris 28456* , scale = 2 mm). C–D, detail of supper surface and aggregations of granules (*Harris 28456*, scales = 0.25 mm). E–F, underside of thallus illustrating unpigmented margins

(E) and pigmented portions near center of thallus (F) (both from *Guccion 1663*, scales = 1.0 and 0.5 mm respectively).

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Figure 94, geographic distribution of *Lepraria leprolomopsis* (red dots) based on the specimens examined for this study compared to the distribution of *Crocynia pyxinoides* (yellow stars) based on herbarium specimens at NY.

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Figure 95, *Lepraria membranacea* (all from *Lewis 450*). A–B, macromorphology of the thallus (scales = 1.0 mm). C–D, detail of the thallus (scales = 0.5 mm). C, detail of the granules and thallus edge (scale = 0.25 mm). F, section of the thallus illustrating thick white hypothallus (scale = 0.25 mm).

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Figure 96, geographic distribution of *Lepraria membranacea* based on the specimens examined for this study.

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Figure 97, *Lepraria neglecta* s.l., comparison of a saxicolous population (A–B, *Lendemer 24995*), a corticolous population (C–D, *Lendemer 23717*), and a population with an *alpina*-type thallus (E–F, *Buck 55113*). A, C and E, macromorphology of the thallus, note the tendency to form radiating rosettes (scales = 1.0 mm). B, D and F, detail of the granules (scales = 0.25 mm).

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Figure 98, geographic distributions of the chemotypes of *Lepraria neglecta* s.l. in North America based on specimens examined for this study. A, alectorialic acid chemotype (*L. neglecta* s.str.). B, fumarprotocetraric acid chemotype (*L. caesioalba*). C, psoromic acid chemotype (unnamed). D, stictic acid (stars, *L. svalbardensis*) and norstictic acid (circles, unnamed) chemotypes. E, porphyrilic acid chemotype (*L. alpina*). F, fatty acid chemotypes (populations with roccellic/angardianic acid were unnamed, those with rangiformic/jackinic acid were referred to *L. borealis*).

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Figure 99, *Lepraria nivalis* (all from *Lendemer 22375*). A–C, macromorphology of the thallus (scales = 1.0 mm). D–E, detail of the thallus (scales = 0.5 mm). F, detail of the granules and thallus margin (scale = 0.25 mm).

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Figure 100, geographic distribution of *Lepraria nivalis* based on the specimens examined for this study.

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Figure 101, *Lepraria normandinoides* (all from *Hollinger 2229* unless otherwise indicated). A–B, gross morphology of the thallus (scales = 1.0 mm). C, detail of the thallus margin (scale = 0.5 mm). D, detail of the thallus surface (scale = 0.25 mm). E, aggregation of granules becoming separated from the hypothallus (*Lendemer 25913*, scale = 0.25 mm). F, dark pigmented hyphae extending from the lower surface of the thallus (*Lendemer 26222*, scale = 0.25 mm).

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Figure 102, geographic distribution of *Lepraria normandinoides* based on the specimens examined for this study. A, protocetraric acid chemotype. B, acid deficient chemotype.

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Figure 103, *Lepraria oxybapha* (all from *Lendemer 29526*). A–B, gross morphology of the thallus (scales = 1.0 mm). C, detail of the thallus margin (scale = 0.5 mm). D, detail of the

thallus surface (scale = 0.25 mm). E, aggregation of granules becoming separated from the hypothallus (scale = 0.25 mm). F, dark pigmented rhizohyphae extending from the lower surface of the thallus (scale = 0.25 mm).

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Figure 104, geographic distribution of *Lepraria oxybapha* based on the specimens examined for this study.

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Figure 105, *Lepraria pacifica* (A–B from *Buck 53923*, C–D from *Lendemer 19532*, E–F from *Lendemer 19562*). A,C and E, gross morphology of the thallus (scales = 1.0 mm). B, D and F, detail of the granules (scales = 0.25 mm).

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Figure 106, geographic distribution of *Lepraria pacifica* based on the specimens examined for this study.

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Figure 107, *Lepraria rigidula* (A–D from *Lendemer 22691*, E from *McCune 30967*, F from *Lendemer 14831*). A–B, gross morphology of the thallus (scales = 1.0 mm). C, detail of the thallus (scale = 0.5 mm). D–F, detail of the granules (scales = 0.25 mm).

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Figure 108, geographic distribution of *Lepraria rigidula* based on the specimens examined for this study.

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Figure 109, comparison of populations of *Lepraria squamatica* from North America (left, *Buck 54362*) and Australia (right, *Elix 37755*). A–B, gross morphology of the thallus (scales = 1.0 mm). C–D, detail of the thallus (scales = 0.5 mm). E–F, detail of the granules and thallus margins (scales = 0.25 mm).

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Figure 110, geographic distribution of *Lepraria squamatica* based on the specimens examined for this study.

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Figure 111, comparison of populations of *Lepraria torii* from western North America (left, *Lendemer 22423*) and eastern North America (right, *Lendemer 27536*). A–B, gross morphology of the thallus (scales = 1.0 mm). C–D, detail of the thallus (scales = 0.5 mm). E–F, detail of the granules and thallus margins (scales = 0.25 mm).

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Figure 112, geographic distribution of *Lepraria torii* based on the specimens examined for this study.

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Figure 113, comparison of typical *Lepraria vouauxii* (right, *Lendemer 18816*) and its unnamed "pigmented" variant (left, *Buck 49831*). A–B, gross morphology of the thallus (scales = 1.0 mm). C–D, detail of the thallus margin (scales = 0.5 mm). E–F, detail of the granules (scales = 0.25 mm).

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Figure 114, geographic distribution of *Lepraria vouauxii* based on the specimens examined for this study.

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Figure 115. Comparison of the typical chemotype of *Lepraria xerophila* (left, *Bratt 5636*) and the chemotype that produces norascomatic acid (right, *Knudsen 7624*). A–B, gross morphology of the thallus (scales = 1.0 mm). C–E, detail of the thallus margins (scales = 0.5 mm). F, young thallus and large aggregations (scale = 0.5 mm).

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Figure 116, geographic distribution of *Lepraria xerophila* based on the specimens examined for this study.

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Figure 117, graphical representation of variability in hyphal width between species of *Lepraria* treated here. Numbers on the x-axis correspond to the species accounts herein, the y-axis refers to µm. Numerical ranges are illustrated as the average (large dot) +/- 1 standard deviation (small dots) and bounded by the extreme values observed (bars).

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Figure 118, graphical representation of variability in granule size between species of *Lepraria* treated here. Numbers on the x-axis correspond to the species accounts herein, the y-axis refers to µm. Numerical ranges are illustrated as the average (large dot) +/- 1 standard deviation (small dots) and bounded by the extreme values observed (bars).

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Figure 119, graphical representation of variability in photobiont cell diameter between species of *Lepraria* treated here. Numbers on the x-axis correspond to the species accounts herein, the y-axis refers to µm. Numerical ranges are illustrated as the average (large dot) +/- 1 standard deviation (small dots) and bounded by the extreme values observed (bars).

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INTRODUCTORY SUMMARY

Some may consider the title of the present work to be a bold one. Indeed if more than a decade of the 21st century has elapsed are we, as lichenologists, not by virtue of this, conducting "21st century" lichen research? In my view the answer to question is largely a resounding "no." As a lichenologist who began studying these organisms prior to the molecular revolution, I believe that during the last decade lichenology has become increasingly disconnected from its historical underpinnings and real-world applications. Further, it seems clear that in the absence of a major shift in paradigms, these changes will have long lasting negative impacts on the intellectual viability of our field as well as on the maintenance of the biodiversity of the organisms we study.

There was a nearly two century time span between when Erick Acharius began publishing his comprehensive works, and when I became interested in lichens while exploring dust laden packets in an herbarium in Philadelphia. During that time our field was revolutionized by manifold advances in microscopy that allowed ever more detailed examination of anatomical structures. These advances brought about new taxonomic schemes that better reflected the true evolutionary relationships of the organisms. Though the simplified system introduced by Zahlbruckner may be viewed as somewhat regressive in this respect, it was clearly a necessary step that was needed to synthesize all prior work and bring lichenology into the 20th century. Zahlbruckner's pragmatic approach effectively and efficiently cleared the way for a century of immense taxonomic progress.

Radical advances in our understanding of lichen chemistry, and chemical analytical techniques, also occurred throughout 20th century. These changes, which were largely used in concert with non-chemical characters, led to unparalleled revisions of species concepts in

lichens. As was the case for Zahlbruckner's system, some may view the chemotaxonomic approach championed during the latter half of the 20th century as having resulted in a misrepresentation of biological diversity. However, I would contend that this pragmatic approach set the stage for the modern era by providing myriad hypotheses to test with the new types of molecular data that were beginning to become available at that time.

The interface between the chemical age and the molecular age presented a crossroads for lichenology. We were given the option of adding a new tool to the toolbox that had served us well for more than two centuries, and using the contents of that toolbox selectively to complete a job. As is always the case with new things, we were also faced with the temptation to use it exclusively, even for jobs that it could not possibly do alone. When I began my dissertation in 2007 the first steps were being taken away from the crossroads. At that time the use of molecular data to evaluate species boundaries was not widespread and neither was the concept of the existence of "cryptic species" within lichen-forming fungi. The few studies that had been published used analyses of these data in concert with non-molecular characters (e.g., biogeography, chemistry, and morphology) to circumscribe monophyletic taxonomic entities that could be recognized by suites of non-molecular characters. It seemed that lichenologists had added a new tool to their toolbox, and had set about building a modern taxonomic system comparable to that of other groups of macroscopic organisms. This seemed particularly timely since well-characterized lucid taxonomic concepts are a critical component of protecting and conserving biodiversity. Such an improved understanding of lichen biodiversity would thus be pivotal to mitigating the effects of the then impending biodiversity crisis on these organisms.

Recognizing that I had an interest in the poorly understood groups of lichens that reproduce asexually through the dispersal of lichenized diaspores, I proposed to use molecular

data in concert with non-molecular datasets to overcome a serious taxonomic impediment in this group. Specifically I chose to study the genus *Lepraria* Ach. because it was widely accepted to consist of a polyphyletic assemblage of sterile lichen-forming fungi that had somehow managed to continue to evolve and speciate despite an apparent absence of sexual reproduction. Once modern procedures to elucidate the alpha- and beta- level diversity in this group were established, I thought they would then be able to be widely applied across the diverse lineages of sterile lichen-forming fungi. Additionally I hypothesized that an improved knowledge of *Lepraria* specifically would likely shed new light on the evolution of asexual reproduction and potentially result in the creation of a model system to examine the long term effects of sterility. I selected North America north of Mexico as the study area for my dissertation because the genus *Lepraria* is most diverse in temperate regions of the world, and the continent hosts representatives of virtually all the known lineages within *Lepraria* s.l. Indeed, it seems North America represents a center of diversity for *Lepraria*. Although after five years of study I suspect this may simply be due to our limited knowledge of the group in other temperate regions. When I began my dissertation studies I could not have predicted how the field of lichenology would develop during the next five years. Nor could I have predicted just how relevant the products of my dissertation would be to our field, specifically with regard to documenting biodiversity for the purposes of conservation and management.

Development and implementation of a methodology for species delineation in lichens

The first step towards completing a taxonomic revision of *Lepraria* in North America, north of Mexico involved elucidating the boundaries of the taxa present in the study area. In order to accomplish this task, it was necessary to develop a methodology that reconciled the results of molecular phylogenetic analyses with observable phenotypic reality. It was also

necessary to develop a morphological scheme and descriptive nomenclature to facilitate inter-investigator and inter-disciplinary dialogue in this group. Unfortunately, the vast majority of *Lepraria* species reported from North America were known from only a handful of voucher specimens, most of which were too old to use to generate molecular data or conduct detailed morphological investigations. As such, during the course of my dissertation I spent a total of more than 1.5 years of person time collecting fresh voucher specimens of *Lepraria* throughout North America, as well as in Australia (Tasmania) and Chile. These efforts not only generated the raw material needed to accomplish the aforementioned tasks, but they also allowed me to observe the ecology of nearly all the species in the field and understand the lichen communities in which they occurred. This was why I collected not only vouchers of *Lepraria* (a total of >1,000 collections) but of all the lichens at all the sites I visited (a total of >21,000 collections). It should be noted that the identification of these collections resulted in the description of many species that were new to science and numerous publications not cited here. Further, the sampling scheme for this procedure was not taxonomically biased, and an emphasis was placed on collecting replicates of all sterile crustose lichens. Thus, this collection represents a core resource for future studies of sterile crustose lichens in North America and abroad.

The process of developing a simplified morphological scheme and descriptive nomenclature for *Lepraria* involved intensive large scale studies of thallus ultrastructure with both light microscopy (LM) and scanning electron microscopy (SEM). Using the resources at The New York Botanical Garden for the former, and Lehman College of the City University of New York for the latter, I conducted detailed morphological investigations of hundreds of specimens representing every species known from North America. Additionally, the type specimens and representative specimens of most other described species of *Lepraria* s.l. were

borrowed from other herbaria and analyzed. These studies generated thousands of micrographs and allowed me to infer hypothetical processes of thalline development and ontogeny in *Lepraria*. The culmination of this aspect of my dissertation was the publication of a fully illustrated standardized descriptive scheme and morphological nomenclature for *Lepraria* s.l. (**chapter one herein**). The significance of this publication cannot be understated because it represents the first comprehensive attempt in modern times to understand and classify the morphology of a group of sterile crustose lichens on a large scale. With this scheme it instantly became possible for different workers to communicate the results of their research in *Lepraria* in a manner that is, at least theoretically, universally understandable.

Once it was possible to describe *Lepraria* species in the manner that reflected developmental reality, and could be understood by others, I set out to establish a methodology that would integrate molecular phylogenetic analyses into routine attempts to delineate species. This research was conducted in collaboration with Brendan Hodkinson, who was then a PhD student at Duke University. Recognizing the value of our complementary, and largely non-overlapping, skill-sets we began the development of a methodology that could be applied broadly to all lichen groups and easily carried out in a time- and cost- effective manner. After reviewing the available literature, and the data then available in GenBank, we decided to attempt to use nuITS sequence data to resolve species boundaries in unrelated groups of lichens. If these attempts were successful we would then implement the same procedure in *Lepraria* s.l. It is important to note that in 2007 there had been only a few studies to use nuITS sequence data to examine species boundaries in lichen-forming fungi. Although those few published studies concluded that nuITS sequence data were robust enough to resolve the taxa involved, many authors contrarily concluded that these data were unreliable or insufficient to resolve species

boundaries in this group of organisms. Since nuITS had been proposed as the region for use in barcoding fungal species, we wanted to establish a body of work that would effectively affirm or dispute its hypothetical viability for this purpose.

Our first study examined the delineation of the widespread foliose lichen *Punctelia subrudecta* (Nyl.) Krog. The concept of this species had been changed radically, several times, in contradictory ways, by authors in the early 2000s. As such when we began our study it was unclear whether three, two, or one species were present in North America. Further at least one of these entities had been considered to be rare and narrowly distributed in the central North America. After searching for correlations between non-molecular characters and the results of our molecular phylogenetic analyses we concluded that three easily recognizable species were present in North America. The publication of this work (**chapter two herein**) not only produced new insights into an old question, but encouraged us to move forward. We then used nuITS sequence data to examine the status of the apodetiate species of *Cladonia* in eastern North America. These species have long been neglected and my prior studies had uncovered a potentially undescribed species that was narrowly restricted to the southern Appalachian Mountains. Morphological analyses of this potential species led to questions about the relationships of the federally listed lichen *Cetradonia linearis* (A. Evans) Ahti & J.C. Wei. After searching for correlations between non-molecular characters and the results of molecular phylogenetic analyses of nuITS sequence data we again concluded that this methodology was effective in resolving species boundaries in the vast majority of taxa we studied (**chapter three herein**).

Upon completion of the work on *Cladonia* I began a series of four additional studies that were aimed at examining the delineations of the specific *Lepraria* taxa in the study area. The

first of these studies involved the divaricatic acid-producing populations which included the type species of the genus, *L. incana* (L.) Ach. Resolution of the status and delineation of the type species of *Lepraria* was the first important step toward a revision of the genus. The divaricatic acid-producing populations of *Lepraria* in North America appeared to correspond to three distinct taxonomic entities; however it was unclear how to resolve their boundaries and their relationships to European species (*L. incana* and *L. crassissima* (Hue) Lettau). The results of my study of this group (**chapter four herein**) revealed that the type species of *Lepraria*, as presently circumscribed, was polyophyletic and that in fact the species in a strict sense was absent from North America. It also affirmed the circumscriptions of the three hypothetical entities that I defined based on correlated non-molecular characters of chemistry, morphology, ecology, and biogeography. Interestingly, the study also revealed that nuITS sequences of eastern North American populations which were morphologically and chemically identical to the European populations of *L. incana*, were actually highly genetically divergent from sequences of European *L. incana*. These sets of populations, whose nuITS sequences were as divergent as any two species of sexually reproducing lichenized fungi thus represent an ideal case study in semi-cryptic speciation. This phenomenon was discussed in detail in that study and the North American populations were formally recognized as a distinct species, *L. hodkinsoniana* Lendemer.

Following study of the divaricatic acid species, I decided to examine the chemotypes of the common eastern North American species *Lepraria normandinoides* which I had described several years earlier (**chapter five herein**). Richard Harris and I separated the species from a broadly circumscribed *L. nivalis* J.R. Laundon using correlated suits of ecological, chemical, and morphological characters. However, within *L. normandinoides* we recognized the existence of

three sympatric chemotypes that each differed only in the presence/absence of a single secondary metabolite. Two of the chemotypes produce either protocetraric acid or fumarprotocetraric acid, which are biochemically closely related and differ only the presence/absence of a single chemical bond. The third chemotype lacked either of these substances. We hypothesized that these chemotypes would not represent discrete phylogenetic entities because they were sympatric and the compounds involved were closely related chemically. Surprisingly, molecular phylogenetic analyses of nuITS sequence data recovered the protocetraric acid and fumarprotocetraric acid chemotypes as strongly supported monophyletic entities. The acid deficient chemotype was recovered as embedded within the protocetraric acid chemotype, supporting the hypothesis that it merely represented a loss of the production of the depside protocetraric acid. These results are significant because they illustrate a case where morphologically identical sympatric chemotypes were recovered as distinct monophyletic entities. This contrasts with molecular phylogenetic studies of other groups of lichens that have reached entirely opposing conclusions. Not only were these results unexpected, but they were significant because the secondary metabolites involved are so closely related biosequentially. It was also noteworthy that this study was the first to lend support to the occurrence of "deficient" chemotypes in lichens, a phenomenon accepted by those who initially championed chemical studies of lichens, but subsequently rejected by later generations.

Since the studies detailed above supported the use of chemical characters in circumscribing monophyletic species of *Lepraria* I then conducted a study (**chapter six herein**) of the common eastern North American species, *L. caesiella*, to examine the robustness of the morphological scheme I had introduced previously. Both my and Richard Harris' studies of *L. caesiella* recognized the existence of two morphotypes within the chemically defined species.

However, after developing the morphological scheme for *Lepraria* it became clear that these two morphotypes corresponded to the two fundamentally different primary thallus types I had defined. Interestingly, molecular phylogenetic analyses of nuITS sequence data generated from both morphotypes of *L. caesiella* recovered them as strongly supported monophyletic entities. It also affirmed the separation of *L. caesiella* s. str. from *L. friabilis*, a morphologically similar species that I had described from the Coastal Plain of southeastern North America.

After having examined three sets of species of *Lepraria*, it was clear that nuITS sequence data was effective in resolving the boundaries of species in this group. Thus, I set out to examine the *L. neglecta* group which I had recognized as taxonomically problematical since the early days of my research on the genus (**chapter seven herein)**. Members of the *L. neglecta* group are unique within *Lepraria* s.l. because they occur in exposed habitats and have granules which develop a pseudocortex with age. In other words, the group is morphologically and ecologically distinct. Within the group however, two primary thallus types were recognized that differed in the size of the granules. Additionally the group displayed a remarkable degree of chemical variability that included the production of depsides, fatty acids, and dibenzofurans in various overlapping combinations. Many of these morphotypes and chemotypes had been recognized taxonomically, however they often occurred together as mixed populations. Considering the chemical overlap between chemotypes that had been described as different taxa, and the fact that the various forms often occurred together, I hypothesized that these entities would not likely form monophyletic groups. I also found it curious that this problem had never been studied previously with molecular data, despite that fact that the broadly defined *L. neglecta* group had been the subject of one previous dissertation. Extensive sampling of the chemotypes and morphotypes of *L. neglecta* throughout their geographic ranges in North America led to the

creation of a previously unparalleled dataset for the group. Molecular phylogenetic analyses of these data recovered a well-supported *L. neglecta* group, as had been done in previous studies, however there was no support for the recognition of any taxonomic entities within the group.

While constructing the *Lepraria neglecta* group dataset I decided to include representatives of the rare Appalachian species *L. salazinica* because of its resemblance to *L. elobata*. (*Lepraria elobata* was part of the outgroup sampling for *L. neglecta*.) The same analyses that failed to recover the variants of *L. neglecta* as monophyletic entities also recovered the sequences of *L. salazinica* as embedded within *L. elobata* with strong support. The results of this study clearly showed that while some species of *Lepraria* are chemically uniform, this is not always the case. It also reiterated the value of morphological and ecological characters in circumscribing monophyletic entities within the group.

The results of the studies of species boundaries within *Lepraria* discussed above are revolutionary, despite the fact that they may seem intuitive. Never before have so many representatives of a single sterile lichen group been studied in such great detail. And this diversity of study, which is mirrored by a diversity of outcomes, illustrates the need for radical change when delineating species of lichenized fungi. Indeed, these studies highlight that the use of a single character *a priori* is likely to result in the delineation of non-monophyletic entities. Clearly this presents significant problems when we attempt to apply our taxonomic concepts in other realms, such as conservation and management. It should be noted that the results for *Lepraria* parallel those that have been obtained by other research groups working with the lichen genus *Caloplaca*, and largely reinforce their conceptual methodology.

The application of single characters *a priori* has been a fixture of lichen taxonomy for the last two centuries. Unfortunately such “pigeon hole” taxonomy has not only continued to be used

in the 21st century, but has been expanded and imposed on ever larger groups with absolutely no analyses of independent molecular data. The potential of these untested schemes to misrepresent biodiversity is frightening when one considers the havoc it is likely to wreak on conservation measures. This is particularly true in cases were biogeographically discrete taxa circumscribed by suites of correlated characters have been broken up into entities with no discernable geographic or ecological integrity. The study of *Lepraria neglecta* is particularly salient to this point.

The study of *Lepraria incana* illustrates a problem that is paradoxically the converse of the above. Specifically it highlights the dangers associated with circumscribing taxa broadly using coarse characters while concurrently overlooking comparatively minor differences. In the case of *L. incana* all the divaricatic acid-producing populations in North America had previously been referred to *L. incana*, thus effectively obscuring three morphologically and chemically distinct species with discrete biogeographic affinities and potential conservation needs. Many would likely view the refinement of taxonomic concepts based on subtle characters to be part of normal taxonomic progress, no different from the discovery of new characters with improved microscopic techniques. However, in lichenology during the last five years there has been a trend by some research groups to introduce new taxonomic schemes for large, highly speciose groups, in which histogram-type character binning has been used to force continuous characters into discrete groups. The result of this has been that biogeographically distinct species with subtle, but statistically significant, character differences (e.g., ascospore size, or diahyphal morphology) are lumped together and thus excluded from the scientific dialogue. Unfortunately, unraveling and resolving the problems created by this practice is often prohibitively time-consuming and difficult, especially if rare, poorly sampled species with inadequate type specimens are involved.

The subject of so called "cryptic species" was also addressed extensively in the four chapters detailing the delineation of species. The concept of "cryptic" or "hidden" diversity in lichens has been widely discussed in the last five years. This has been coupled with a remarkable proliferation of studies purporting to describe such diversity. When dealing with "cryptic species" authors have taken two divergent approaches. Some research groups have taken a pragmatic approach and introduced a formalized scheme in which the vast majority of taxa uncovered with molecular data are not considered truly cryptic because they have diagnosable phenotypic characters. Although these groups have not encountered any taxa they would consider truly cryptic (i.e., diagnosable only using molecular characters) the implication of their scheme is that such taxa should not be formally described. Conversely, other research groups have taken an approach in which the term "cryptic" has been loosely defined to apply to any genetically distinct taxa they uncover regardless of whether they can be defined in the absence of molecular characters. The studies that Hodkinson and I conducted in conjunction with my dissertation have generated a large body of work that clearly supports the former approach. Unfortunately, the published dialogue on this topic has been dominated by the latter. This has culminated in a series of recent publications in which taxa diagnosable only by molecular characters have been formally described. As is outlined in the study of *L. neglecta* I consider this unproductive and a detriment to lichen taxonomy. It also resulted in the publication of a review paper questioning whether morphological (and other) characters can adequately describe lichen biodiversity. The conclusion of this review was that, in fact, only molecular data can achieve this goal. Clearly the results of my dissertation strongly refute this notion.

Resolving the impediment preventing the formal description of sterile crustose lichens

As has been discussed above, the genus *Lepraria* is a member of a diverse group of lichen taxa that reproduce asexually through the dispersal of lichenized diaspores. These taxa present a unique problem for taxonomists because although they are readily recognizable based on phenotypic characters, they cannot be placed within a higher level taxonomic framework because they lack sexual reproductive structures. Further, as my colleague Kerry Knudsen and I have discovered these taxa are not necessarily morphologically comparable to their congeners (**chapter eight herein**). Clearly our inability to adequately document these taxa is problematical, particularly in light of the fact that they can comprise as much as 50% of the lichen biodiversity in a given biota. In order to resolve this problem, Hodkinson and I collaborated to develop a methodology that would use molecular data to place these taxa in a higher level taxonomic framework and evaluate their putative circumscriptions. Such a methodology can be applied to all sterile crustose lichens and is particularly salient to *Lepraria* which has long been recognized as polyphyletic.

For this methodology we employed sequence data from the mtSSU gene to infer family and genus level placement for each species. We then used nuITS sequence data to examine the hypothetical circumscriptions of the taxa based on non-molecular characters. The mtSSU and nuITS genes were selected because their utility for each function has been affirmed in numerous publications, including our own, and because of the large amount of available data in GenBank. It is critical to have wide taxonomic coverage for each gene used in this methodology because the candidate species could potentially belong to any of the diverse lineages that comprise lichen-forming fungi.

Before embarking on a large scale revision of *Lepraria* s.l., we first tested this methodology using a common sterile crustose lichen from the Appalachian Mountains (**chapter nine herein**). Richard Harris and I have collected this taxon for more than five years and amassed well over one hundred vouchers spanning the geographic area between Alabama and New York. We also hypothesized based on non-molecular characters that this species would belong to the genus *Rinodina*, which is included in the Teloschistales. Using the approach we developed, Hodkinson and I inferred placement of the species in *Caloplaca*, which is another genus in the same order as *Rinodina*. We then used a diverse array of methods of phylogenetic inference to examine this placement and the potential species-level relationships of the taxon. Not only were we able to formally describe the species, but we uncovered potential problems with the methodologies and data sets currently used to resolve evolutionary relationships within the Teloschistales.

Upon completion of the above trial we began a revision of *Lepraria* s.l. the goal of which was to circumscribe the genus in a strict sense. Concurrently we hoped to infer higher level placement of the numerous taxa that were unrelated to the core of *Lepraria* s.l. This study (**chapter ten herein**) produced unexpected results that provide new data on the complex history of the evolution of macrothalline growth forms in lichens and on the evolution of asexual reproduction via lichenized diaspores. Using mtSSU sequence we were able to show that *Lepraria* s.l. includes representatives of at least five lineages belonging to four families in three orders. We were able to circumscribe a core group of *Lepraria* s.str. taxa that are related to the type species, *L. incana*, and as a group are sister to the genus *Stereocaulon*. Remarkably, we recovered some members of the sterile fruticose genus *Leprocaulon* within the *Lepraria* s. str. clade. Though this genus was suspected to belong to the family Stereocaulaceae, it was quite

unexpected that some of its members would be recovered within *Lepraria* s. str. Subsequent morphological studies supported this relationship and thus we concluded that some of the fruticose members of *Leprocaulon* actually represent an evolution of the fruticose growth form from the sterile crustose growth form. This is particularly remarkable because the same phenomenon appears to have occurred in the sister genus *Stereocaulon*. Our analyses of *Lepraria* s. str. also revealed a remarkably low degree of divergence between mtSSU sequences throughout the genus. This stands in stark contrast to both the divergence seen in analyses of nuITS and that observed in mtSSU sequences of lichen genera which reproduce sexually. Based on these data we have concluded that *Lepraria* s. str. may represent a model system in which to study the long term effects of sterility.

With regard to the members of *Lepraria* s.l. that did not correspond to *Lepraria* s.str. we were able to suggest placement in three families in two orders. One of these represented a previously unrecognized lineage within the Teloschistales that was sister to the family Physciaceae. This previously unrecognized lineage is notable in that it is comprised mostly of sterile asexually reproducing species with sterile crustose *or* sterile fruticose growth forms. In other words, the same morphological variability found within *Lepraria* s. str. was found to occur in another genus, belonging to a new family, in another order. In addition to this lineage, we also described a new genus, *Nelsenium*, to accommodate a leprarioid species that belongs to the Pilocarpaceae.

Taxonomic revision of *Lepraria* s. str.

After having established a monophyletic *Lepraria* s. str. well characterized by non-molecular characters, and after having studied the delineation of the morphologically or chemically variable species of that group, I was able to produce the intended product of my

dissertation (**chapter eleven herein**). This revision implements the new morphological and descriptive scheme on a large scale, and includes full descriptions and taxonomic accounts of twenty-six species. All the species are fully illustrated with color light micrographs and complemented by maps of their geographic distribution based on the specimens I examined during the last five years. This chapter is the culmination of more than five years of work, one and a half years in the field, and the review of thousands of voucher specimens. It is also the synthesis of the results outlined in the previous ten chapters. Hopefully this revision, coupled with the publication of the other chapters will provide a model for the direction lichen taxonomy should take in the rest of the 21st century.

CHAPTER I – A MORPHOLOGICAL SCHEME AND DESCRIPTIVE NOMENCLATURE FOR *LEPRARIA* S.L.[1]

James C. Lendemer

Abstract. – A standardized morphological terminology and descriptive scheme for *Lepraria* s.l. is presented. The contribution includes observations on development and ontogeny of morphological structures and is extensively illustrated with scanning electron and light micrographs.

Introduction

"*Lepraria* is an important lichen genus because its species are usually dominant on shaded rock, bark, and, to a lesser extent, earth in the temperate regions of the world. Unlike most lichen genera it never produces ascomata or conidiomata and its specialized unorganized thallus is deficient in morphological characters. On the other hand, most specimens contain lichen substances, and a study of their chemistry is therefore essential in providing an understanding of the species. The genus has been little studied by modern taxonomic methods."

Laundon (1992: 315)

The repeated and independent evolution of asexual reproduction is one of the most interesting aspects of lichen biology. Although asexual reproduction via lichenized diaspores occurs throughout the diverse lineages of lichens, the genus *Lepraria* represents a unique group (albeit presently polyphyletic) that has lost the ability to reproduce sexually yet continues to speciate (Myllys et al. 2005; Fehrer et al. 2008). The genus currently includes more than 70

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species representing an evolutionarily heterogeneous assemblage of asexually reproducing lichenized fungi (Nelsen et al. 2008; Saag et al. 2009; Lendemer 2010).

Historically, *Lepraria* has been used in an extremely broad sense as a catchall for all normally sterile lichens with leprose thalli (Laundon 1992), a circumscription clearly based on superficial similarity rather than shared evolutionary history. The first monograph of the group was published by Hue (1924), who described more than 100 new taxa based on specimens in the herbarium of Bouly de Lesdain. The destruction of de Lesdain's herbarium during World War II coupled with the cryptic nature of *Lepraria* species created an impasse in taxonomic progress that was only overcome when Laundon (1992) reconstructed Hue's work using duplicates of cited specimens that survived in other herbaria. Laundon's revision of *Lepraria* was the first to utilize chemotaxonomy in concert with morphological characters and thus made *Lepraria* accessible to present-day lichenologists. My current studies of the genus draw considerable inspiration from this seminal work.

Recently, *Lepraria* has become the subject of considerable attention (see summary in Saag et al. 2009), mostly falling into one of two categories. The first being attempts to accurately circumscribe and place *Lepraria* s. str. within the larger framework of the Lecanoromycetes (Ekman & Tønsberg 2002, Myllys et al. 2005, Nelsen et al. 2008). The second being descriptions of new taxa and documentation of range extensions (e.g., Knudsen & Elix 2007, Flakus & Kukwa 2007, Lendemer & Harris 2007, Saag et al. 2007, Tønsberg 2007, Lendemer et al. 2008, Lendemer 2010).

Unfortunately progress in *Lepraria* taxonomy following Laundon's revision has occurred in a highly haphazard and uneven fashion resulting in a systematic framework plagued by a fundamental lack of consistency in taxonomic concepts, as well as a lack of standardized

descriptive morphological terminology (Lendemer 2010). The chaotic state of present-day *Lepraria* taxonomy can be attributed to a diverse array of factors. Foremost among these is that due to the lack of apothecia and pycnidia, the genus is regarded as having few, if any, reliable morphological characters that can be used to differentiate taxa. With this preconception in mind, taxonomic workers in *Lepraria* have almost universally relied upon the diversity of its secondary metabolites, above morphology and ecology, when delineating taxa, despite ample evidence that chemotaxonomy does not necessarily result in the circumscription of monophyletic entities (e.g., Ekman & Tønsberg 2002, Tretiach et al. 2009). There is also mounting evidence that contrary to popular belief, morphology, ecology, and biogeography are salient taxonomic characters in this group (e.g., Lendemer & Harris 2007, Lendemer et al. 2008; Lendemer 2010).

After several years of initial study I began work on a revision of *Lepraria* s.l. in North America as the subject of my dissertation. In addition to the discovery of numerous apparently undescribed and overlooked taxa (e.g., Lendemer 2010) these studies revealed that it was almost impossible to reconcile accounts of species in the published literature. The primary reason for this was the lack of a standardized morphological terminology and descriptive scheme. This led me to develop such a system through extensive morphological studies conducted in conjunction with my revision. Though the modern roots of this system lie in the descriptions of Laundon (1992), in reality they draw from the excellent historical studies of *Lepraria* and other sterile lichens produced by a number of lichenologists (e.g., Hue 1924; Ozenda 1963). This system was first presented by Lendemer et al. (2008) in the context of an extended discussion of why the term "soredia" should be rejected from use in *Lepraria* descriptions. An improved version was then published by Lendemer (2010). Subsequent to that publication substantive additional morphological studies have been carried out to arrive at the treatment presented here. Many

aspects of this system however remain theoretical because they are based on observations of character states and discrete stages of developmental processes rather than continuous laboratory growth studies. That said my goal is to provide a conceptual framework through which the non-lichenized secondary structures in any given species can be described and placed in the broader context of tertiary thallus structure. It is hoped that the present contribution provides a simple system to facilitate dialogue in the *Lepraria* community, and renders this difficult genus more accessible to non-specialists.

Materials and methods

This study is based primarily on material of *Lepraria* s.l. in the herbarium of The New York Botanical Garden (NY), supplemented by material received on loan from the following herbaria: BG, CANL, COLO, CONN, DUKE, FH, GZU, H, KANU, MIN, MSC, SBBG, and UCR. Included in the holdings of this group at NY are the >1000 specimens collected by author during his extensive field studies of *Lepraria* throughout North America. To date these total 1.5 years in the field and span the period August, 2007 to December, 2010. Through these field studies the author has been able to gather firsthand observations of ecology and morphology for all the species known to occur in North America. The chemistry and morphology of all specimens was studied using the methodology outlined in detail by Lendemer et al. (2008) with the SEM methods as corrected by Lendemer and Elix (2010).

Results: a standardized morphological terminology

This section aims to elucidate a standardized morphological terminology for *Lepraria* by building the thallus from the ground up. It is divided into subsections that correspond to different scales of structural complexity similar to those used to describe biomolecular structure (i.e.,

primary, secondary, and tertiary structures; see figure 1). Employing this system of classification is advantageous because it forces anyone attempting to describe *Lepraria* thalli to contemplate ontogeny in addition to the morphological character states observed in any given specimen.

The discussion begins with the description of the smallest identifiable unit comprising the thallus, the hyphae. It continues with the description of the micromorphological structures (e.g., granules) formed by these units, and their known character states. Finally the section concludes with a survey of the thallus types displayed across *Lepraria* s.l. and how these macromorphologies are derived from differing combinations of character states of a set of shared micromorphological structures.

Primary structure

Fungal hyphae (figure 2) are the smallest identifiable unit of the *Lepraria* thallus. These are the primary structural units whose differing arrangements form all the component parts of the thallus and are integral to its ultimate macromorphology. In all the species that have been studied to date the morphology of the hyphae is uniform. To some degree this is remarkable considering the wildly polyphyletic nature of *Lepraria* as presently defined. There are no significant differences between the hyphae of different species within *Lepraria* s. str., or for that matter within *Lepraria* s.l. However, differing hyphal characteristics, particularly differences in thickness, have been noted between *Lepraria* and members of the Roccellaceae with byssoid or leprarioid thalli (Jørgensen & Tønsberg 1988; Lendemer & Knudsen 2010).

Hyphae in *Lepraria* species are 3–5 µm in diameter, hyaline, septate, and have a secund branching pattern. New branches form at the septa, consistently diverging in the same direction away from the primary branch (figure 2). Within any given *Lepraria* thallus, the hyphae can be

subdivided into two groups based on whether they are involved in the formation of lichenized or non-lichenized secondary structures. These subdivisions are artificial and are not derived from differences in the hyphae themselves but rather from the fundamental differences between the structures they eventually form. Those that form lichenized secondary structures (i.e., granules) are hereafter referred to as thalline hyphae. The hyphae that form non-lichenized secondary structures (i.e., the prothallus, hypothallus, and rhizohyphae) are referred to as prothalline hyphae.

The terms prothalline and thalline hyphae are introduced here to distinguish between hyphae that comprise non-lichenized and lichenized secondary structures, respectively. Although both groups of hyphae are morphologically identical, they form two fundamentally different sets of secondary structures. Thalline hyphae form lichenized secondary structures, which are those that incorporate photobiont cells. In all the species of *Lepraria*, granules represent the only lichenized secondary structures that have been observed. All other structures (i.e., the prothallus, hypothallus, and rhizohyphae) are formed from prothalline hyphae and do not incorporate photobiont cells.

Whether new *Lepraria* thalli are established through prothalline or thalline hyphae is not clear at present. It is also unknown whether prothalline hyphae are always derived from thalline hyphae, or vice-versa. Throughout this study prothalline hyphae were routinely observed extending from the thalline hyphae of granules (figure 6). Conversely prothalline hyphae were neither observed anastomosing photobiont cells nor forming granules. The possibility that new *Lepraria* thalli can form from prothalline hyphae rather than from a lichenized diaspore cannot, however, be excluded. Each secondary structure is described below.

Secondary structure

Secondary structures represent the next level of morphological complexity in the thallus in that their form is derived directly from the patterns of organization of the primary hyphal structure. These secondary structures are the basic parts of the thallus (e.g., granules, rhizohyphae, etc.). It is important to recognize that these structures are not the smallest divisible unit of the thallus, but rather are derived from different arrangements of the primary units (i.e., hyphae).

Lichenized secondary structures

I - The granules

Granules are the only secondary structures in *Lepraria* thalli that are lichenized. These structures are always spherical or globose in shape and are composed of one or more layers of fungal hyphae that form a layer encapsulating a core of photobiont cells. In the vast majority of species studied the granules vary from 20–50 μm in diameter. *Lepraria alpina* (de Lesd.) Tretiach & Barffuo, *L. gelida* Tønsberg & Zhurb., and *L. lanata* Tønsberg are notable exceptions in having enormous granules ~1.0 mm in diameter. It is important to describe the character states of the granules observed in a single thallus as well as across different populations. In most taxa the granules can be classified either as fluffy or compact, visual features that correlate to the thickness and degree of formation of a gelatinous layer around the outer layer of hyphae (figure 4C vs. 5D). These characters vary between species and can vary depending on the age of the granule (figure 4–5). It is tempting to refer to the outer layer of hyphae in the granules of some taxa (e.g., the *L. caesioalba-neglecta* group) as a cortex because it is thick and gelatinized, causing the granule to appear compact and corticate (figure 4). The use of the term "cortex" is

inappropriate however, because the granules in all species are initially ecorticate (i.e., the photobiont core is incompletely surrounded by a network of hyphae). Instead the term pseudocortex should be employed to describe cases in which the outer layer of hyphae appears to form a cortex-like layer (figure 4C).

Rather than growing exponentially in size the granules undergo a process of regular vegetative division (figure 3). It appears this process is initiated when the algal core reaches a critical size that can no longer be contained within a single continuous layer of hyphae. As the photobiont cells divide and the alga increases pressure on the wall of fungal hyphae, the hyphae begin to grow into the core and surround individual photobiont cells. Through this process the granule is cleaved apart to form a variable number of granules that begin the process anew.

Many previous workers have used differences in granule morphology to justify the circumscription of taxa. Unfortunately this has taken place while concurrently overlooking the fact that granules undergo a developmental series during which the granules of one taxon may resemble those of another. That this has led to considerable confusion in the descriptive literature is perhaps best illustrated by the use of the term "projecting hyphae." This term was first introduced to describe the conspicuous hyphae that extend outward from the hyphal layer in the mature granules of *Lepraria rigidula* (de Lesd.) Tønsberg (Tønsberg 1992; figure 5 herein). As subsequent workers studied other species the term began to be employed for any hyphae extending outward from the hyphal layer of a granule, and thus was included in the descriptions of more and more taxa. Eventually the taxonomic value of the character was obscured as its usage, and concept, expanded through the literature. Through careful study of granule development across different members of *Lepraria* s.l. it eventually became clear that the granules in all species produce some form of projecting hyphae at some stage of their

development (figure 3E). It also became clear that few species retain these hyphae in the final stages of development in the manner of *L. rigidula*. Clearly an understanding of the developmental stages of the granules, not just the final state, in a given species should be undertaken when allowed by available material and resources. Similarly, this type of problem will only be avoided when descriptions include this information, regardless of its hypothetical nature, rather than simply describing the granules in an idealized state.

Previous studies have referred to the granule as the basic structural unit of *Lepraria* thalli (Lendemer et al. 2008; Lendemer 2010). To some degree this is misleading because, as has been discussed above, fungal hyphae form the primary structure of the thallus. Regardless, the importance of the granule in *Lepraria* thalli cannot be underestimated because, in fact, it represents the only portion of the thallus that is actually involved in the process of lichenization. Assuming that *Lepraria* species reproduce through the dispersal of lichenized diaspores, then granules also represent the only means through which new thalli can be established. Thus granules have an unusual dual nature as both lichenized thallus and lichenized diaspore. Granules have also frequently been referred to as "soredia" by some workers. Detailed critiques of why this is both conceptually dubious and historically divergent from descriptive morphology in lichenized fungi have been published elsewhere (Lendemer *at al.* 2008; Lendemer 2010) and will not be repeated here.

Non-lichenized secondary structures

Most early morphological work in *Lepraria* focused on the lichenized portion of the thallus which is comprised of the granules. Focusing on these structures overlooks the fact that the gross morphology, or the aspect, of the thallus is owed entirely to development of the non-

lichenized portions of the thallus. The non-lichenized secondary structures in *Lepraria* collectively form the support structure for the granules. These structures unite the granules into a single thallus through a network of interconnecting hyphae (i.e., a prothallus or hypothallus) and serve to anchor/buffer them from the substrate through specialized attachment hyphae (i.e., rhizohyphae).

I - The prothallus

The first non-lichenized secondary structure to form during the establishment of a new thallus from a lichenized diaspore (i.e., granule) is the prothallus (figure 6). All species of *Lepraria* initially produce a prothallus. The prothallus is here defined as a network comprising one or more hyphae that extend outward from the granule, attaching it directly to the substrate and to the prothalli produced by other granules. In species with an aggregate thallus (see tertiary structure below) the hyphae eventually coalesce as the granules aggregate to form piles, at which time the prothallus is only visible as a carpet of hyphae extending outward from the edge of the piles (figure 7). The prothallus in such species remains distinct (figure 7E) and can be observed throughout all stages of development of the thallus. In species of *Lepraria* with thick placodioid thalli (see tertiary structure below) the prothallus either develops into, or is replaced by, a thick layer of interconnecting hyphae (i.e., hypothallus) in which the granules are embedded (figure 8).

II - The hypothallus

Here the term hypothallus is used to refer to the network of fungal hyphae in which the granules of species that have thick thalli are embedded (figure 8). As the thallus ages the thickness of the hypothallus increases as older granules senesce and are overgrown by newly

formed granules. Descriptions of the hypothallus should refer to the degree of development (thin to thick) and to the hyphal arrangement (compact or loose). The development of a hypothallus follows that of the prothallus in species of *Lepraria* with placodioid thalli (see tertiary structure below). Species of *Lepraria* with aggregate thalli (see tertiary structure below) do not produce a hypothallus.

The term "medulla" has incorrectly been applied to this structure by some workers presumably because it appears as a whitish layer just below the granules. Although it is unclear how and when the term was first used in the genus, it should be abandoned. This usage is in direct conflict with the traditional definition of the medulla as the layer of fungal hyphae below the cortex and algal layer (Kirk et al. 2001). *Lepraria* thalli are neither corticate nor produce an algal layer, therefore they cannot have a medulla. The usage of the term hypothallus for this structure is clearly more appropriate as it linguistically and conceptually links it to the prothallus while concurrently preserving the meaning as applied in the Pannariaceae.

III - Rhizohyphae

The term rhizohyphae is here applied to the fungal hyphae that extend vertically downward from the hypothallus to anchor the thallus to the substrate (figure 9). These structures are present only in the species that produce thick thalli with a well-developed hypothallus. They should not be confused with the hyphae of the prothallus that extend from the granules in the early stages of thallus formation (figure 6). In some species (e.g., *Lepraria normandinoides* Lendemer & R.C. Harris) the walls of the cells in the rhizohyphae are melanized giving them a distinctive dark color. The presence or absence of pigmentation in the rhizohyphae has been used as a character in delineating species when correlated with other non-molecular characters.

Tertiary structure

Tertiary structure represents the largest morphological scale in the descriptive terminology outlined here and is best understood as the gross morphology, or aspect, of the thallus. It is defined by the arrangement, combination, and character states expressed in the secondary structures of the thallus. Based on the species of *Lepraria* studied to date there are seven basic types of tertiary structure, each of which is outlined below. These types can be classified into two broad categories based on whether a hypothallus is present or absent. Species that lack a hypothallus have discontinuous thalli comprised of granules that are dispersed on a prothallus and aggregate into piles. Conversely species that produce a hypothallus have continuous thalli comprised of granules embedded in a well-developed network of prothalline hyphae. The former group is referred to as having "aggregate" thalli while the latter group is referred to as having "placodioid" thalli.

Any given specimen or species of *Lepraria* should be able to be classified using the scheme presented here, however it should be recognized that this scheme is preliminary. It should also be noted that shared tertiary structure does not *a priori* indicate a close evolutionary relationship. Such theoretical relationships can only be established using a combination of biogeographical, chemical, ecological, and morphological characters that are correlated with molecular characters.

Type 1 - Aggregate thalli

Species of *Lepraria* with aggregate thalli are characterized by a thin thallus that begins as isolated dispersed granules that form piles, or aggregations, as they divide (figures 10B, 11).

Aggregate thalli are best recognized by examining the thallus margins where the dispersion of the young granules is most readily observed (figures 11E–F). In this thallus type the piles of granules eventually overlap and become confluent, giving the false appearance of a continuous thallus in areas of older growth (figure 11A). Such areas can become thick and then misinterpreted as a hypothallus. This feature is best described as a necrotic layer that develops through the buildup of dead granules underlying the uppermost layer of lichenized granules. So far three subtypes of aggregate thalli have been observed. These subtypes are distinguished primarily by the degree of development of a pseudocortex and the size of granules.

Subtype 1 – *Caesioalba*-type (figure 11A–B)

Typified by *Lepraria caesioalba* (de Lesd.) J.R. Laundon

This type of aggregate thallus is found in members of the *Lepraria caesioalba*/*L. neglecta* group and is characterized by the dense, compact granules with a well-developed pseudocortex. When growing in exposed habitats these species produce rosette-forming thalli that grow in a radial fashion and appear placodioid. The absence of a conspicuous hypothallus in such cases indicates that the thallus is not truly placodioid (see Type 2 below).

Subtype 1a – *Alpina*-type (figure 11C–D)

Typified by *Lepraria alpina* (de Lesd.) Tretiach & Baruffo

Lepraria alpina and *L. gelida* are two species with *caesioalba*-type thalli that differ from other members of the *L. caesioalba/neglecta* group in having larger granules (≥100 μm vs. 30–50 μm diameter).

Subtype 2 – *Caesiella*-type (figure 11E–F)

Typified by *Lepraria caesiella* R.C. Harris

This type of aggregate thallus is found in many members of *Lepraria* s. str. and is characterized by loose, ± fluffy granules that do not develop a distinct pseudocortex with age. The type species of *Lepraria*, *L. incana* (L.) Ach., displays this thallus type, as does the common North American species *L. caesiella*.

Type 2 - Placodioid thalli

Species of *Lepraria* with placodioid thalli are characterized by a thick thallus with discrete margins and a well-developed hypothallus in which the lichenized granules are embedded (figure 10A). Placodioid thalli are best recognized by examining the thallus margins to determine whether they are discrete and distinctly separated from the substrate indicating the presence of a hypothallus (figures 12F, 13A–6). In many species with placodioid thalli a distinct fuzz of hyphae projects outward from the growing edge (figures 12F, 13D). The majority of species with placodioid thalli also produce rhizohyphae, which are best observed by overturning a portion of the thallus with a razor blade (figures 13E–F) or by making a section through the thallus with a fresh razor blade (figure 10A). So far four subtypes of placodioid thalli have been observed (figures 12–14). These subtypes are distinguished primarily by characteristics of the hypothallus (loose vs. compact) and the development of a pseudocortex on the granules.

Subtype 1 – *Cryophila*-type

Typified by *Lepraria cryophila* Lendemer (figures 12A–D)

This type of placodioid thallus is characterized by a hypothallus composed of loosely

intertwined hyphae, granules that do not develop a pseudocortex, the production of a weft of rhizohyphae on the lower surface, and thallus margins that are not distinctly "crisped" with a lip.

Subtype 2 – *Finkii*-type (figure 13)

Typified by *Lepraria finkii* (Hue) R.C. Harris (= *L. lobificans* auct.)

This type of placodioid thallus is characterized by a hypothallus composed of closely intertwined hyphae, granules that do not develop a pseudocortex, the sparse production of rhizohyphae on the lower surface, and thallus margins that are not distinctly "crisped" with a lip.

Subtype 3 – *Normandinoides*-type (figures 12E–F)

Typified by *Lepraria normandinoides* Lendemer & R.C. Harris

This thallus type is characterized by a compact hypothallus composed of closely intertwined hyphae, granules that develop a poorly defined pseudocortex, the production of abundant rhizohyphae on the lower surface, and thallus margins are distinctly "crisped" with a lip.

Subtype 4 – *Xerophila*-type (figure 14)

Typified by *Lepraria xerophila* Tønsberg

This thallus type is characterized by a compact hypothallus composed of tightly intertwined hyphae, granules that form a well-developed pseudocortex, the absence of rhizohyphae on the lower surface, and thallus margins that are distinctly "crisped" with a lip. Notably this thallus type has only been found in species that occur on stabilized soil in regions with a Mediterranean climate. It is represented by *L. xerophila* in North America, *L. isidiata*

(Llimona) Llimona & A. Crespo and *L. santosii* Argüello & A. Crespo (= *L. lobificans* Nyl. s.str. ?) in Europe, and *L. larrainiana* Lendemer in South America. Species with a *Xerophila*-type thallus appear to have a unique ontogeny in which the thallus initially forms through the apical unfolding of a granule (figures 14D–F; Tretiach et al. 2009, Lendemer 2010b). Molecular studies are currently underway to determine whether these taxa form a monophyletic group.

Key to the known thallus types in *Lepraria* s.l.

1. Thallus aggregate-type [hypothallus absent; granules dispersed on a prothallus, eventually aggregating to form piles]....2

2. Granules ecorticate, appearing fluffy…*caesiella*-type

2. Granules with a well-developed pseudocortex, appearing compact…3

3. Granules <50 µm diameter…*caesioalba*-type

3. Granules >100 µm diameter…*alpina*-type

1. Thallus placodioid-type [hypothallus present; granules embedded in a hypothallus, always connected and forming a continuous thallus]…4

4. Thallus margins with a discrete "crisped" lip…5

5. Rhizohyphae present; granules ecorticate, appearing fluffy…*normandinoides*-type

5. Rhizohyphae absent; granules with a well-developed pseudocortex, appearing compact…*xerophila*-type

4. Thallus margins without a discrete "crisped" lip…6

6. Hypothallus composed of loosely arranged hyphae; rhizohyphae forming a well-developed waft...*cryophila*-type

6. Hypothallus composed of tightly arranged hyphae; rhizohyphae not forming a well-developed waft…*finkii*-type

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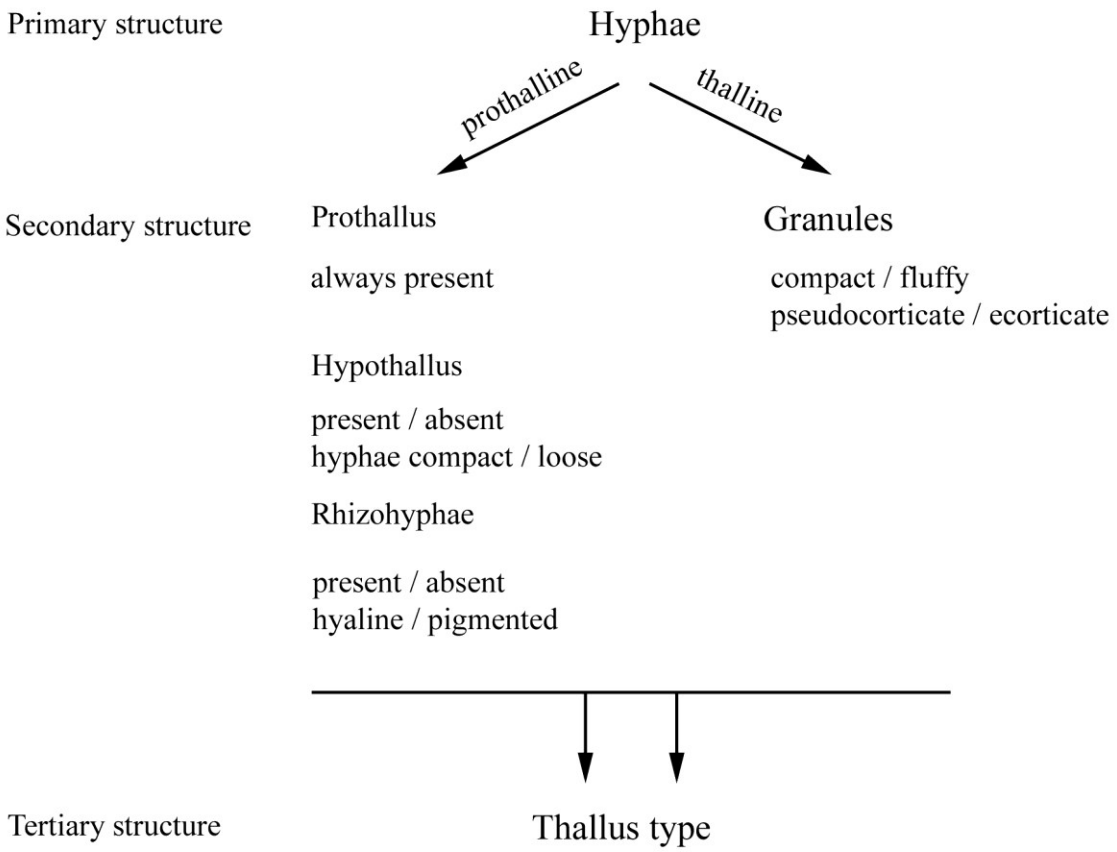

Figure 1. Schematic diagram of hierarchy of morphological terminology employed here.

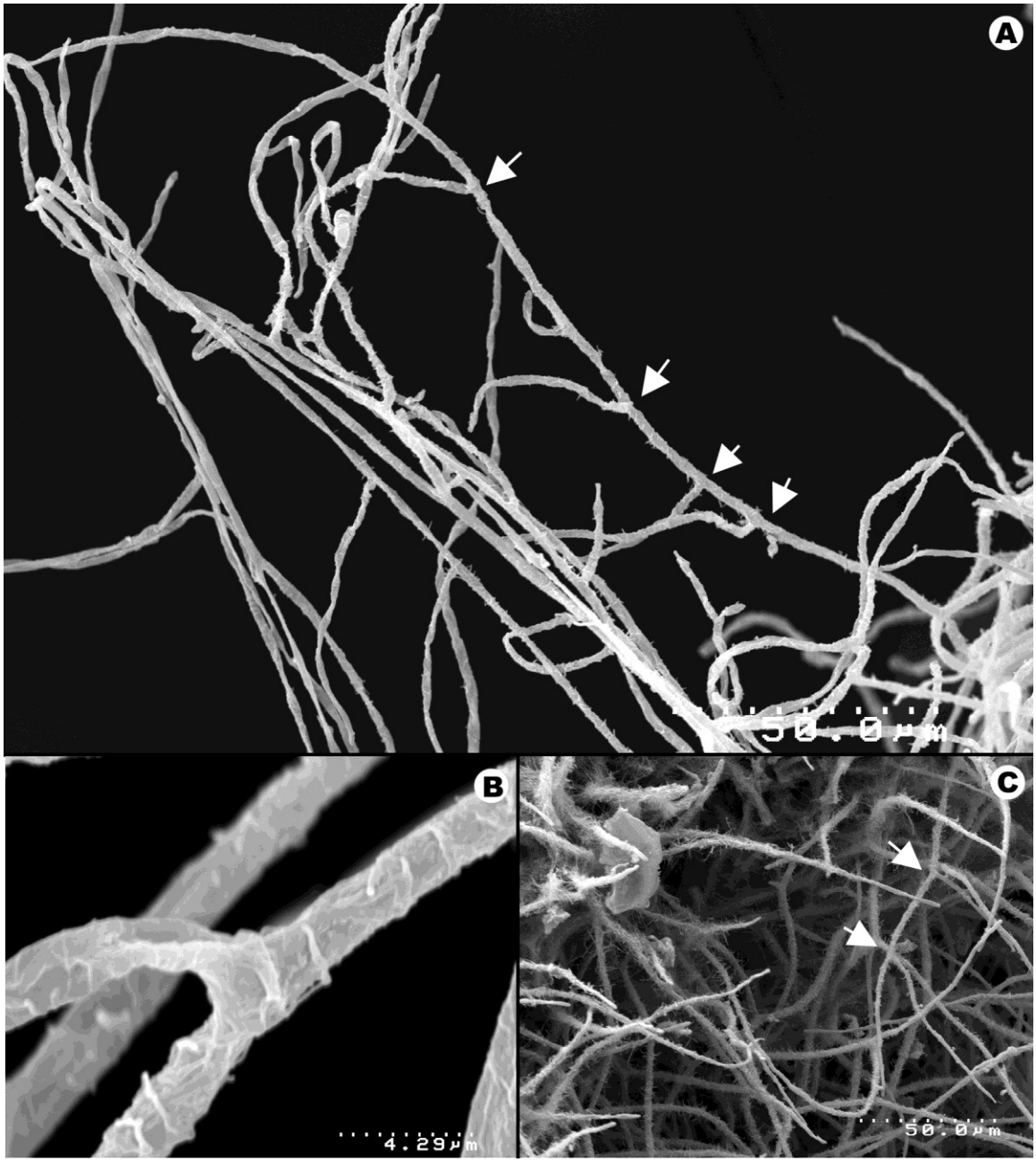

Figure 2. Hyphal branching. A, typical secund branching as seen when isolated from the hypothallus of *L. cryophila* (arrows point to branches). B, detail of branch attachment from A. C, typical secund branching in the context of the hypothallus in *L. lobificans* auct. (arrows point to secund branching in one hypha). (A and B, *Lendemer 18702*; C, *Lendemer s.n.*).

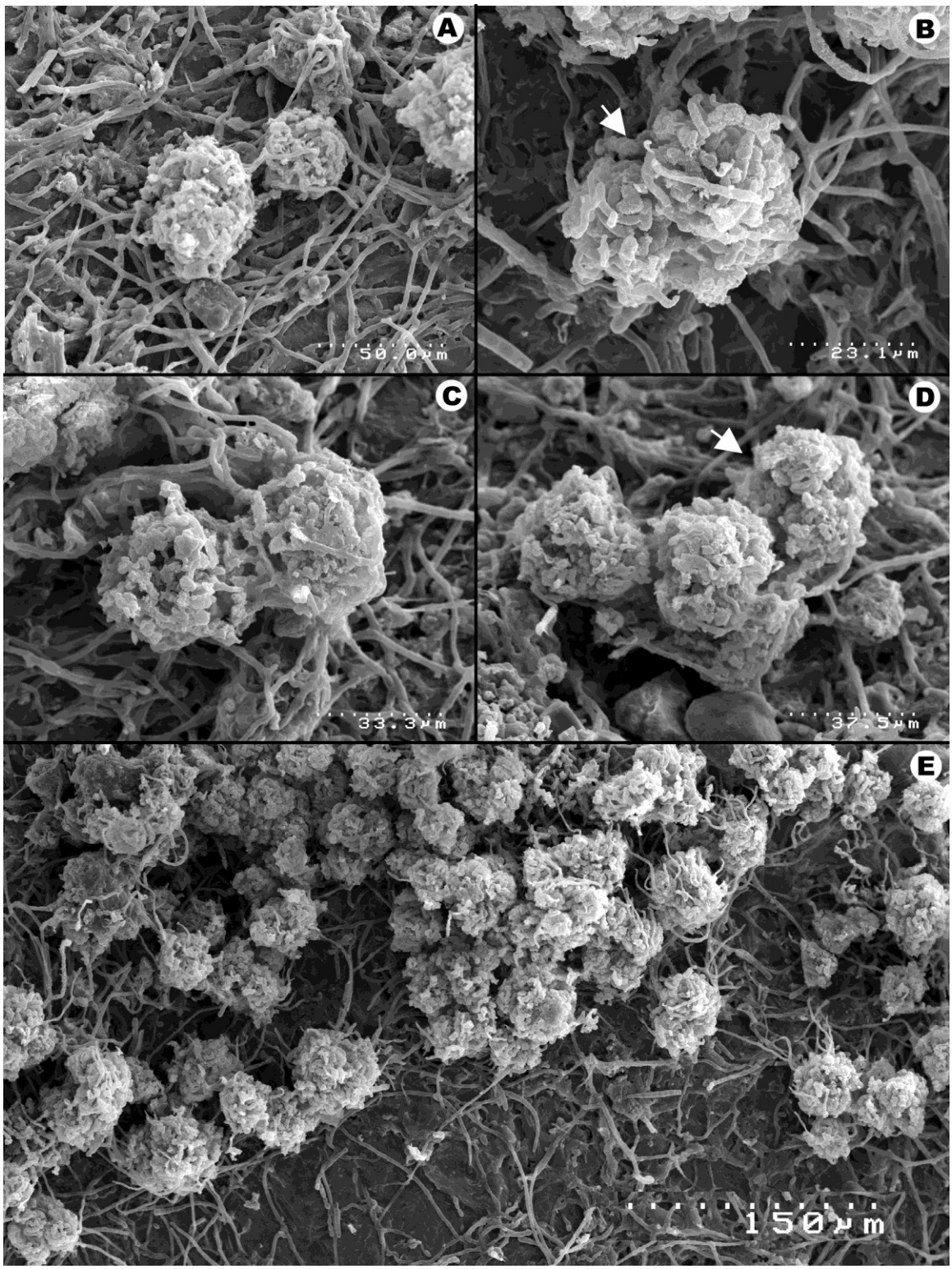

Figure 3. Granule division in *Lepraria friabilis* Lendemer. A, typical spherical granule and associated prothalline hyphae. B, initiation of vegetation division (arrow points to cleavage point). C, later stage of division. D, aggregation of divided granules (arrow points to granule undergoing further division). E, resultant tertiary structure of the thallus. (A, C, and D, from *Lendemer 9063*; B and E, from *Lendemer 8308*).

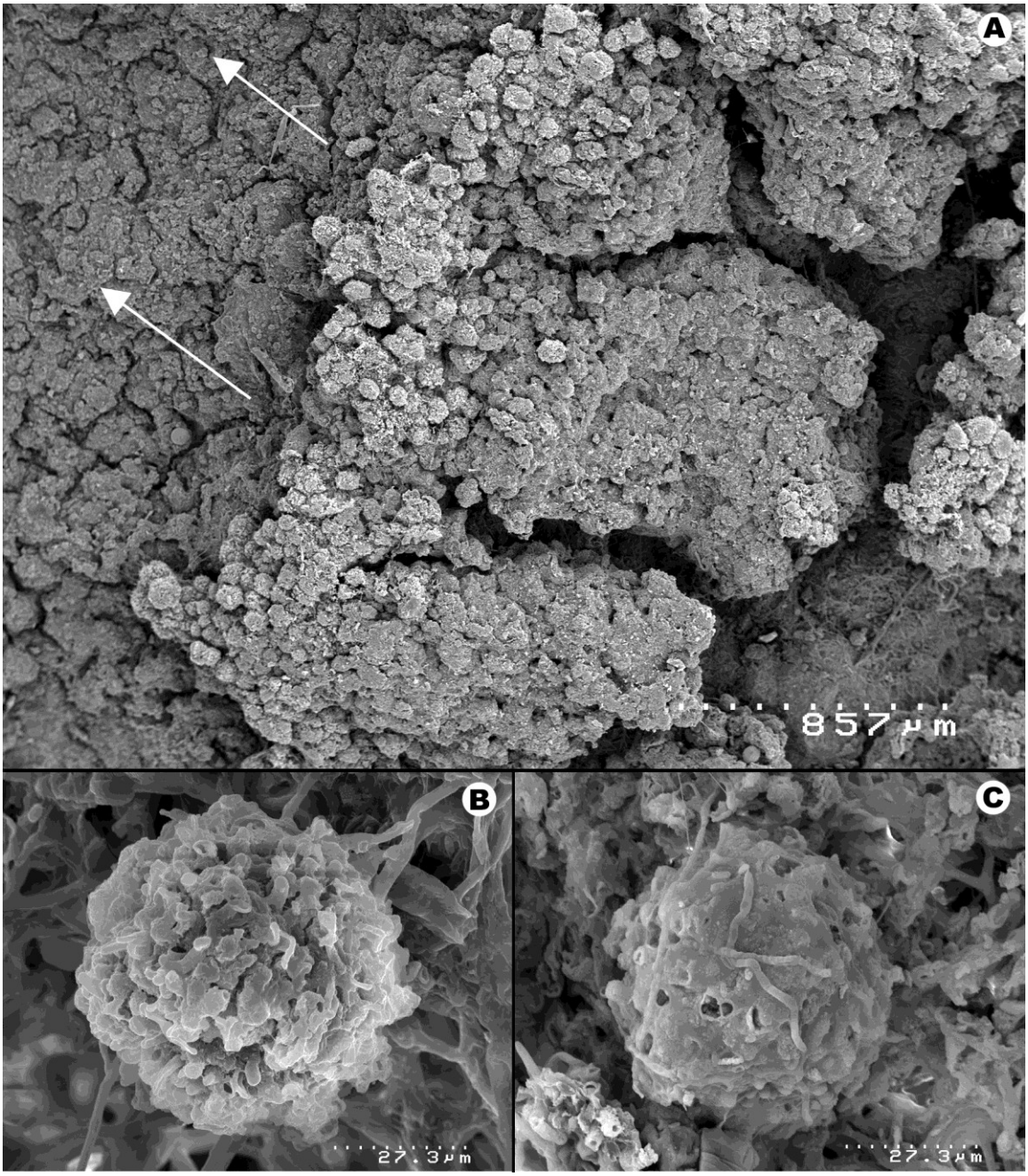

Figure 4. Developmental progression of the granules in *Lepraria caesioalba/neglecta*. A, macroscopic view of thallus illustrating progressive development of the pseudocortex and gelatinization with age (arrows indicate direction of new growth; *Lendemer 17098*, *L. caesioalba*). B, young granule with a poorly developed pseudocortex (*Lendemer 14020*, *L. neglecta*). C, senescent granule with fully developed strongly gelatinized pseudocortex (*Harris 55753*, *L. caesioalba*).

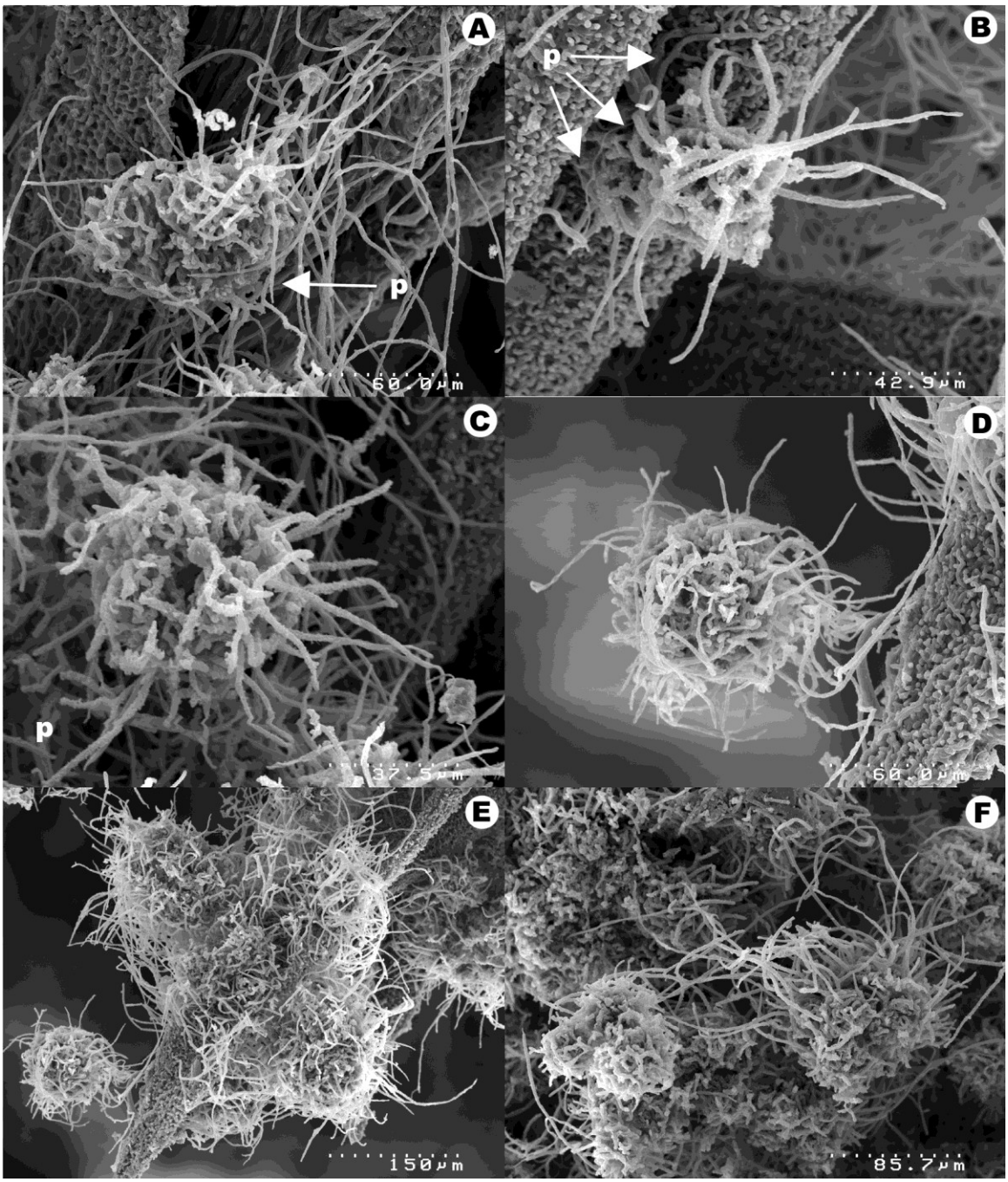

Figure 5. Development of granules in *Lepraria rigidula*. A, young granule establishing prothallus ("p") on substrate, note absence of projecting hyphae. B–C, further development of prothallus (p) including some projecting hyphae not attached to the substrate. D, mature granule nearly covered in projecting hyphae. E, projecting hyphae anchor granules together to form an aggregation. F, mature granules with (right) and without (left) projecting hyphae.

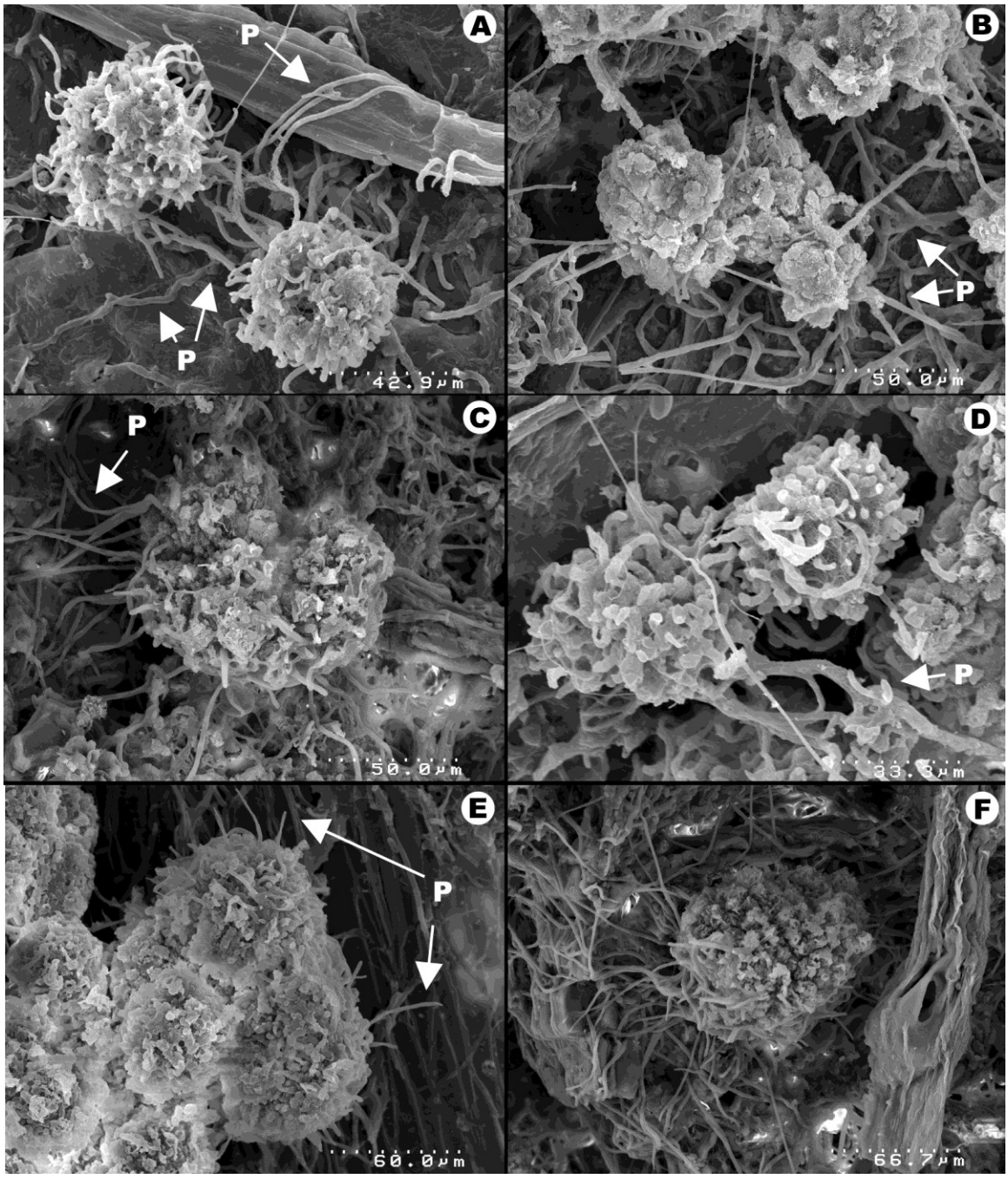

Figure 6. Development of the prothallus in different species of *Lepraria* ("p" points to the prothallus). A and D, *L. pacifica* (*Lendemer 19325*). B, *L. caesiella* (*Lendemer 18773*). C and F, *L. caesioalba* (*Harris 55753*). E, *L. neglecta* (*Lendemer 14020*).

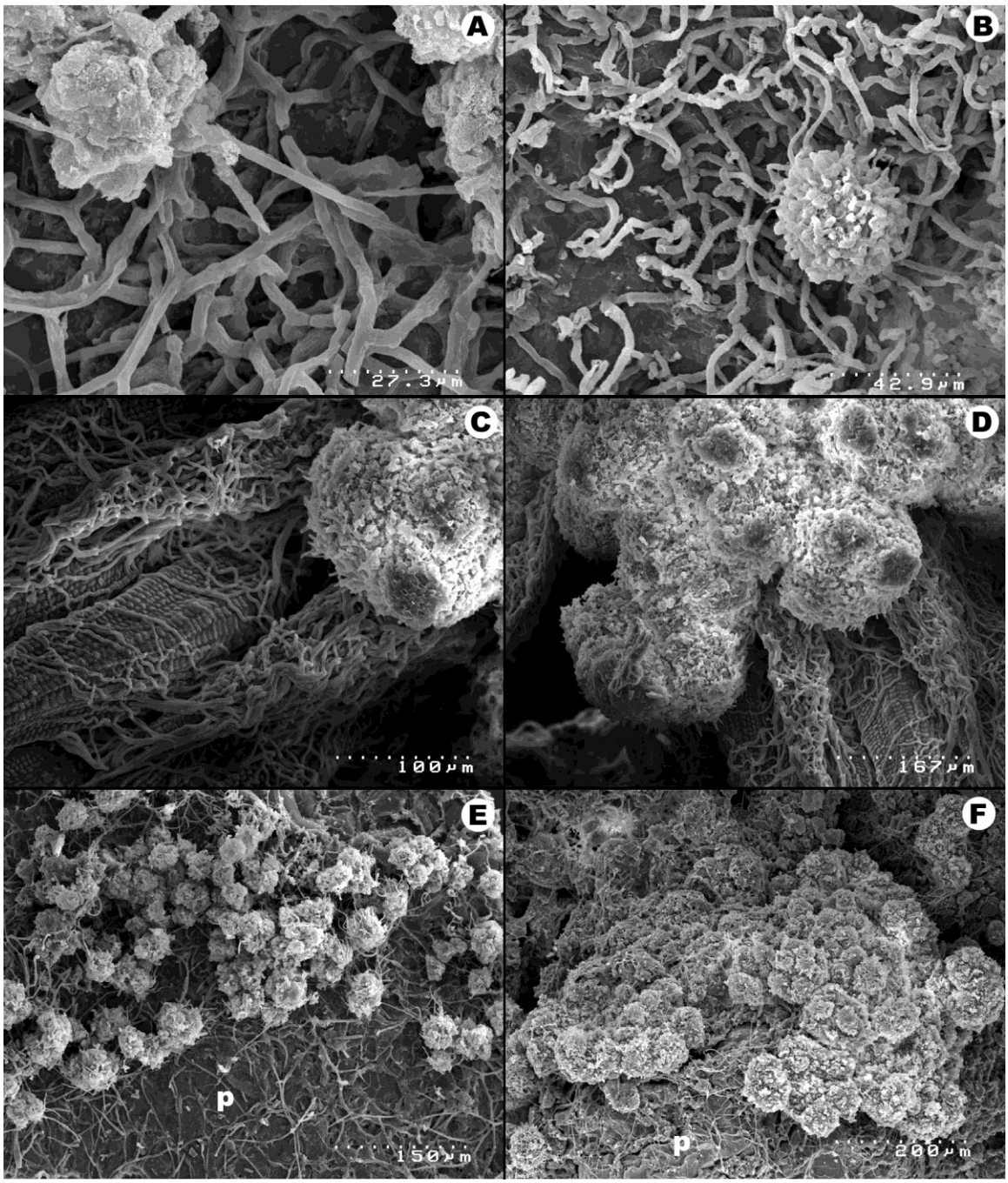

Figure 7. Prothalli of different *Lepraria* species ("p" indicates location of prothallus). A, *L. caesiella* (*Lendemer 18773*). B, *L. pacifica* (*Lendemer 19325*). C–D, *L. borealis* (*Hassellrot s.n.*). E, *L. frabilis* (*Lendemer 8308*). F, *L. neglecta* (*Harris 52596*).

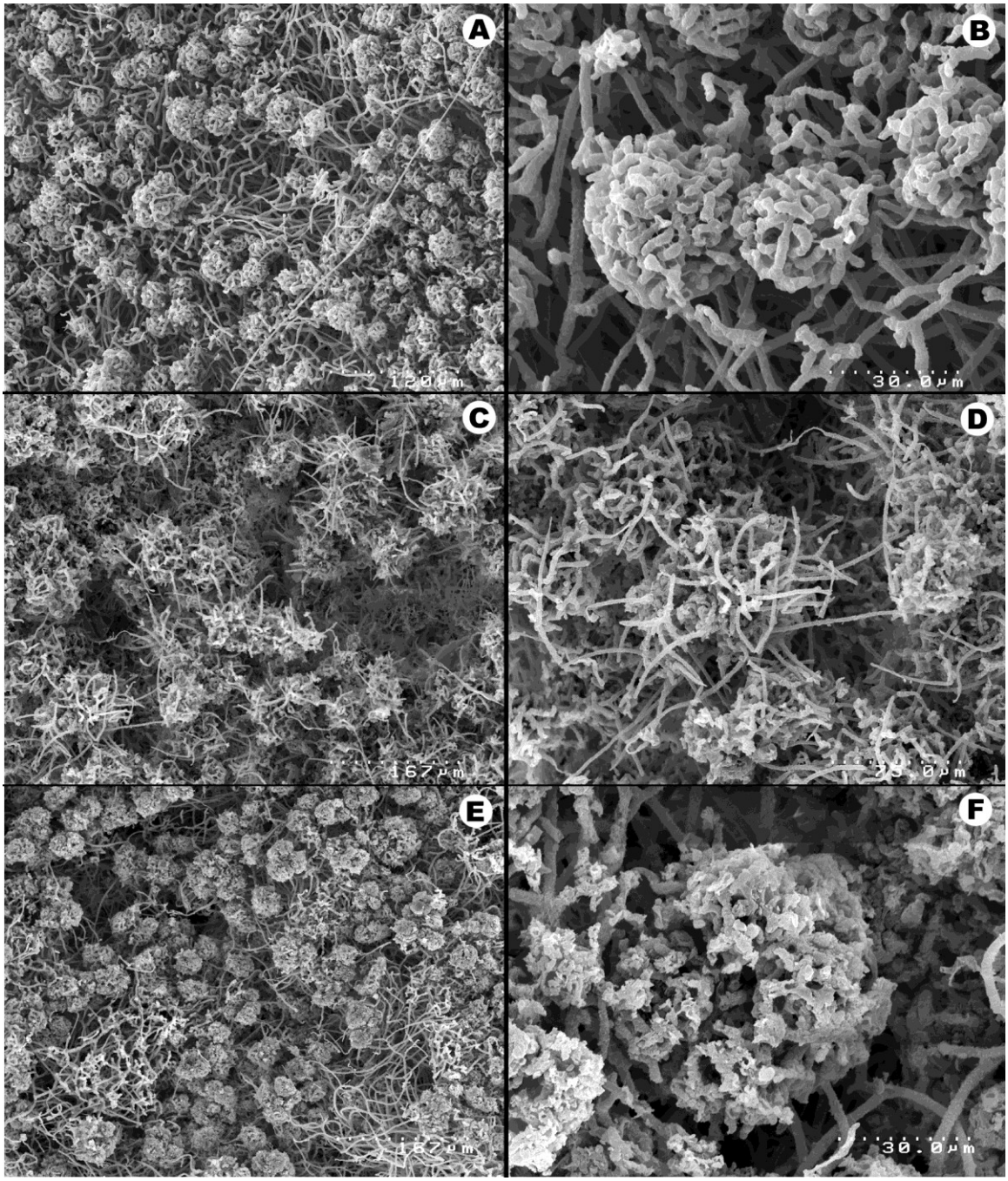

Figure 8. Hypothallus (left) and granules in the context of the hypothallus (right). A–B, *Lepraria cryophila* (*Lendemer 18702*). C–D, *L. rigidula* (*Lendemer 14812*). E–F, *L. squamatica* (*Elix 37755*).

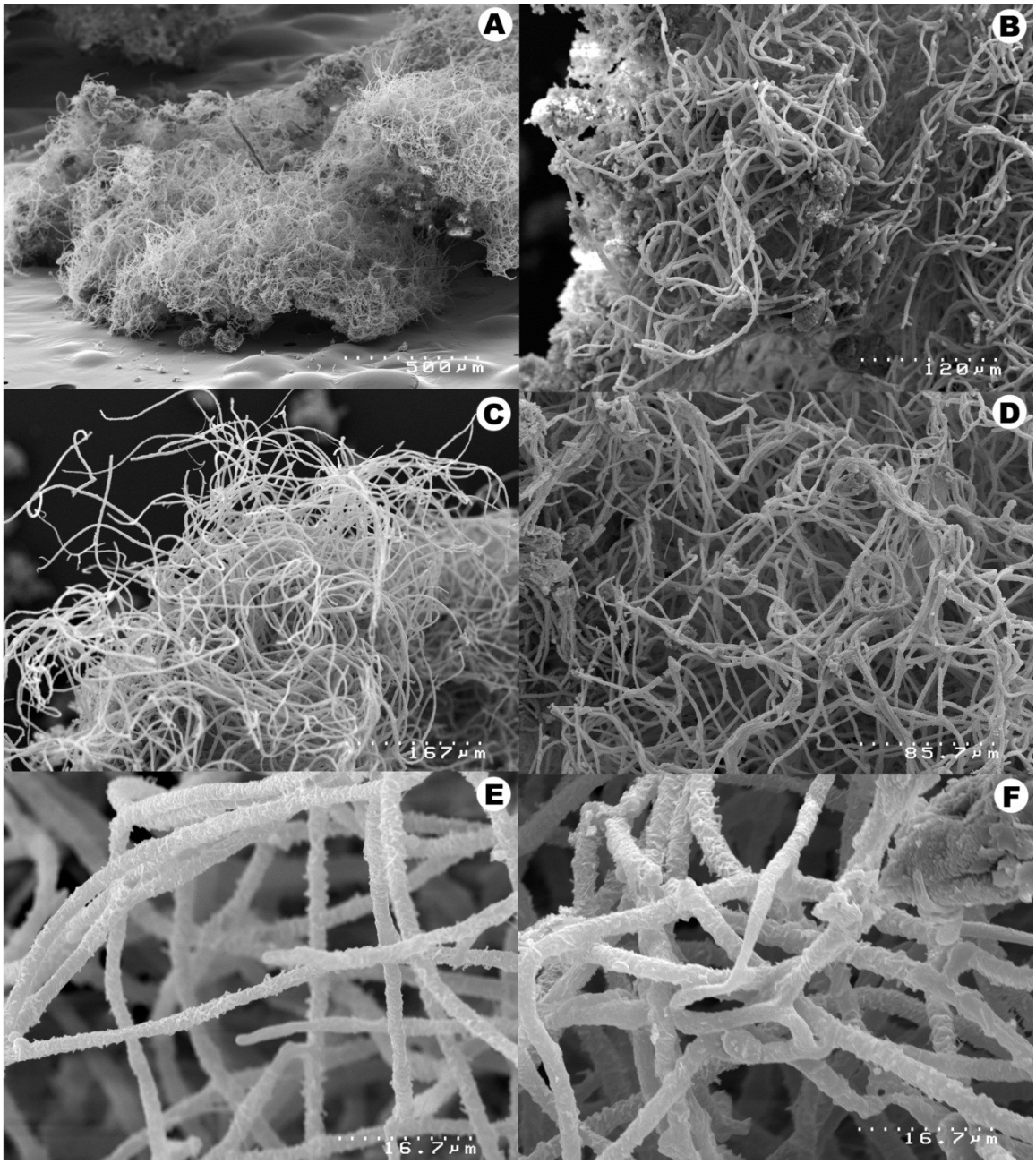

Figure 9. Rhizohyphae, gross aspect on overturned thallus (A), macromorphology (B–D), detail (E–F). A, B, D, E, *Lepraria cryophila* (A–D, *Lendemer 11961* and E, *Lendemer 18702*). C, F, *L. normandinoides* (*Lendemer 16746*).

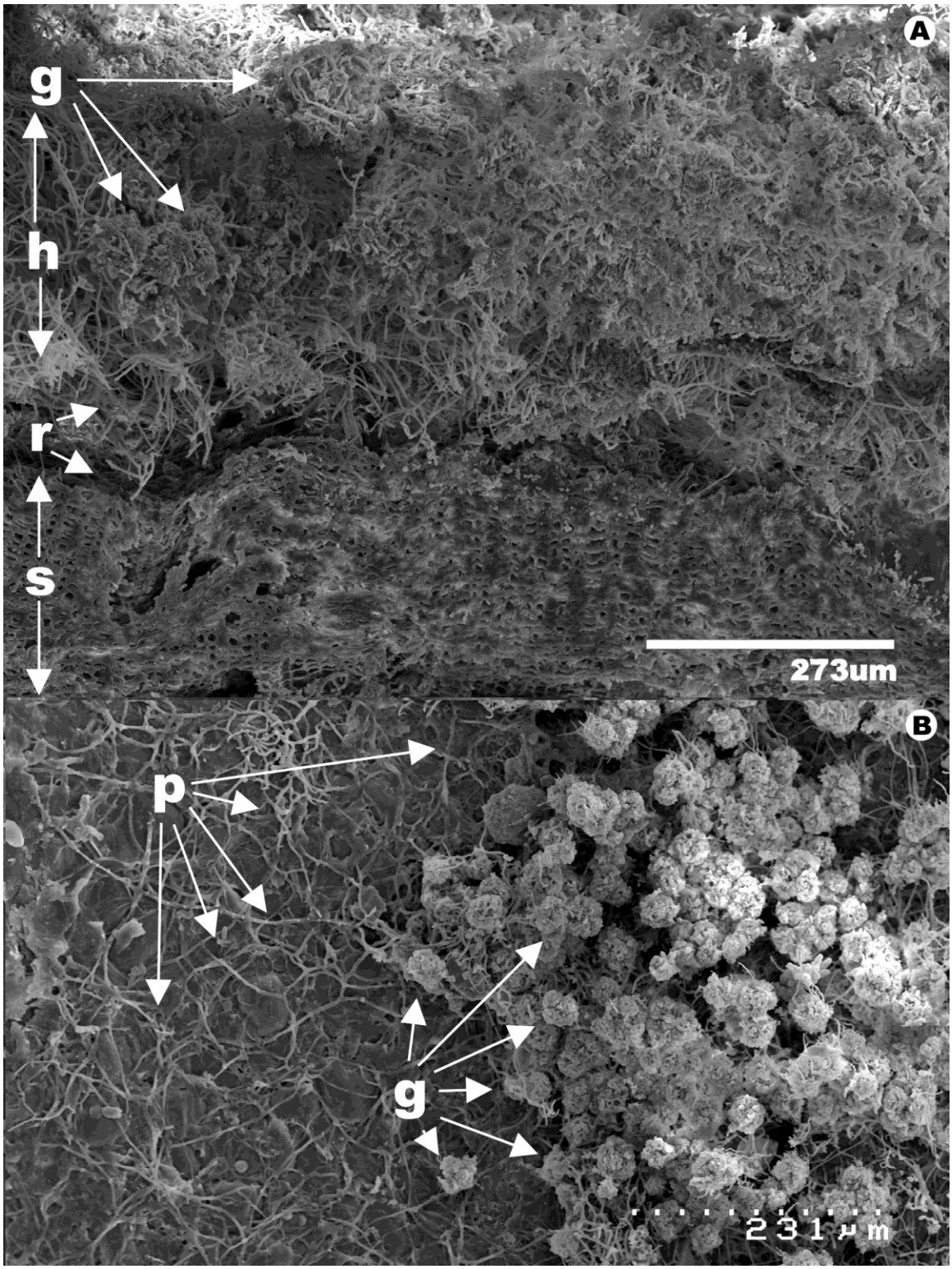

Figure 10. Tertiary structure (g= granules, h= hypothallus, p= prothallus, r= rhizohyphae, s= substrate). A, section of thallus of *Lepraria lobificans* auct. (*Lendemer 22897*). B, thallus of *L. friabilis* (*Lendemer 8308*).

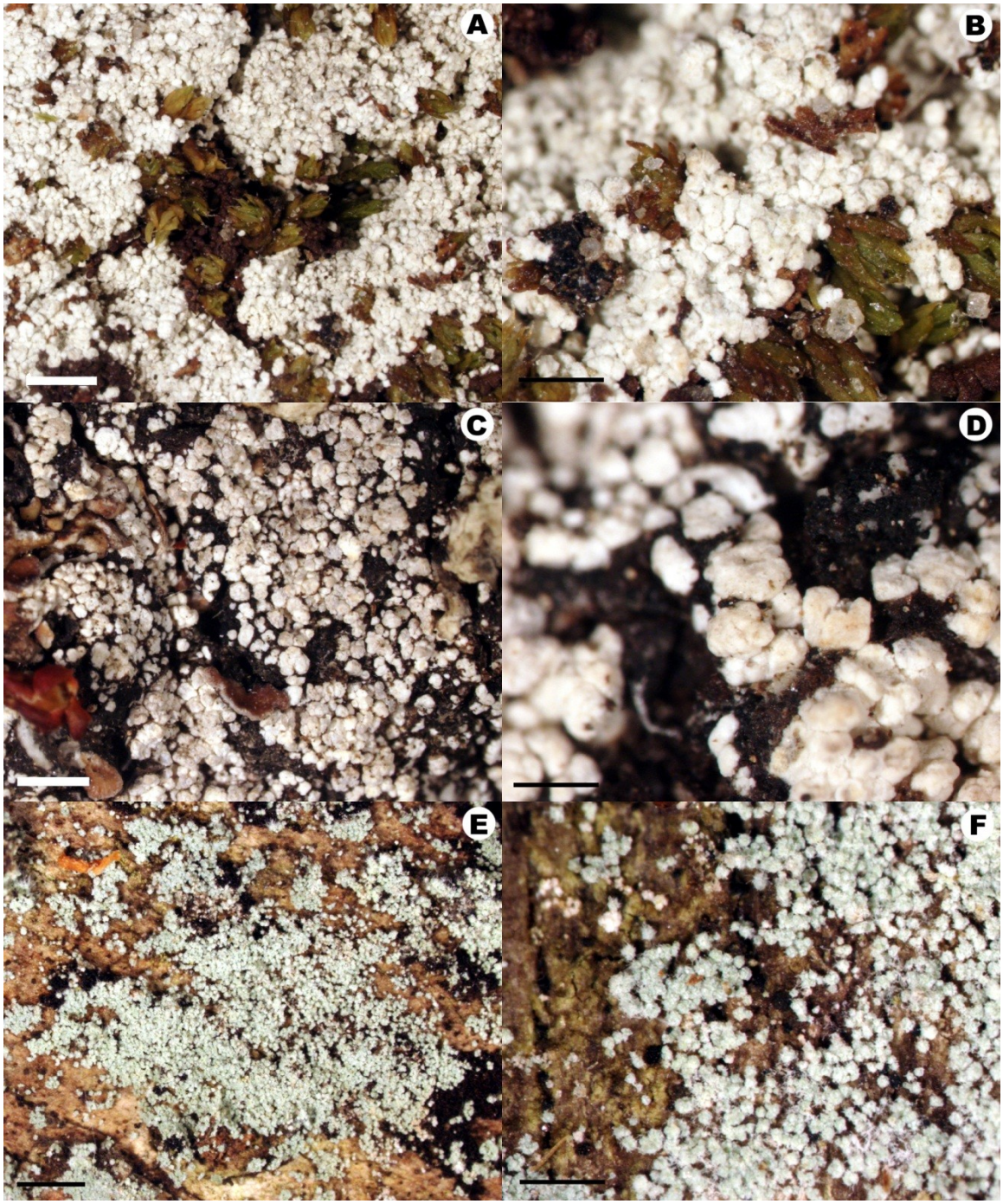

Figure 11. Subtypes of aggregate thalli. A–B, *caesioalba*-type exemplified by *Lepraria borealis* (holotype, *Tønsberg 1519*, BG). C–D, *alpina*-type exemplified by *L. alpina* (isoneotype, *Willard s.n.*, NY). E–F, *caesiella*-type exemplified by *L. caesiella* (*Lendemer 18767*, NY). Scales = 1.0 mm (A, C, E) and 0.5 mm (B, D, F).

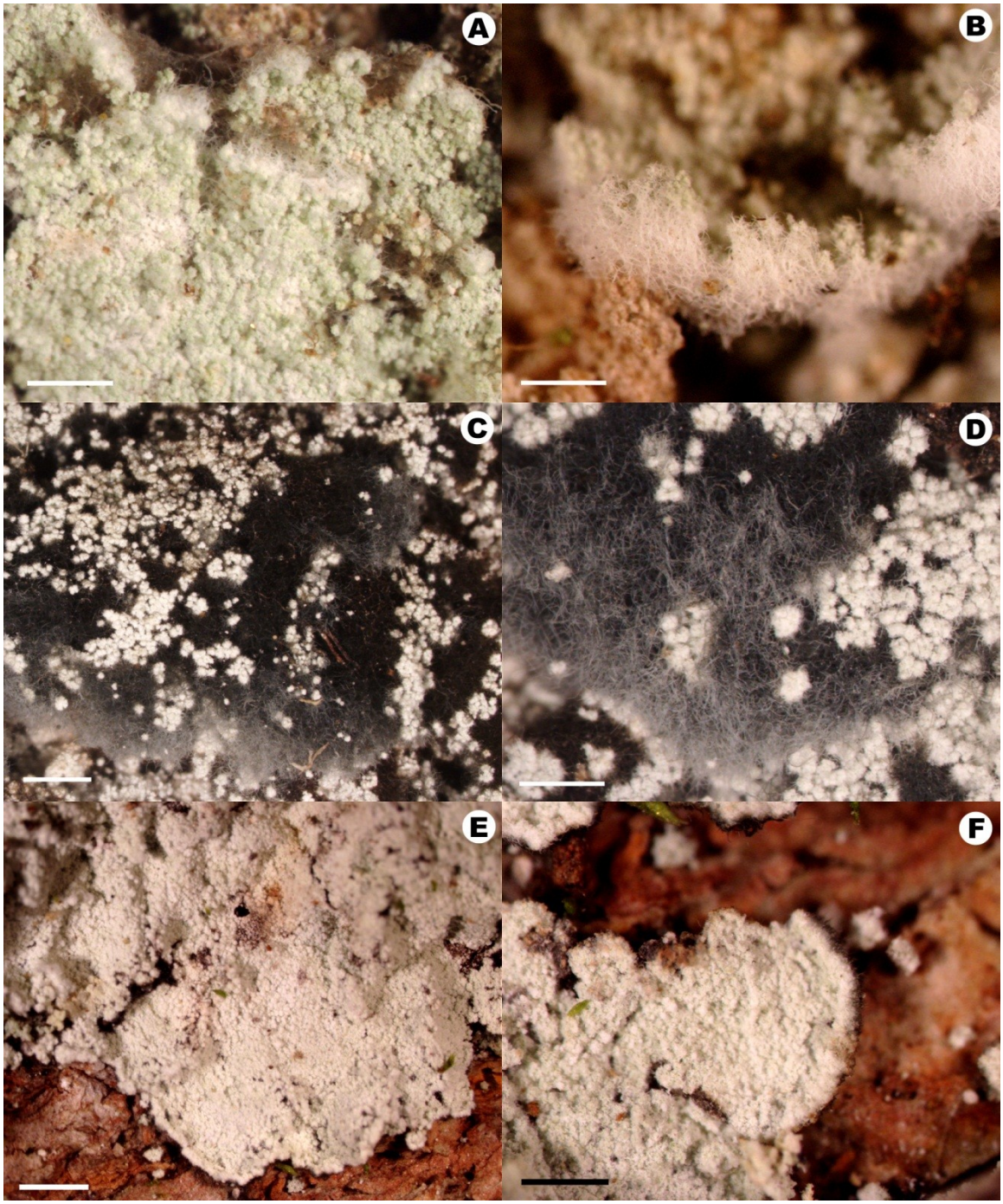

Figure 12. Subtypes of placodioid thalli. A–B, *cryophila*-type exemplified by *L. cryophila* (*Lendemer 11961*, NY). C–D, *cryophila*-type exemplified by *L. yunnaniana* (*Goffinet 10077*, NY). E–F, *normandinoides*-type exemplified by *L. normandinoides* (*Lendemer 18674*, NY). Scales = 1.0 mm (A, C, E,) and 0.5 mm (B,D, F).

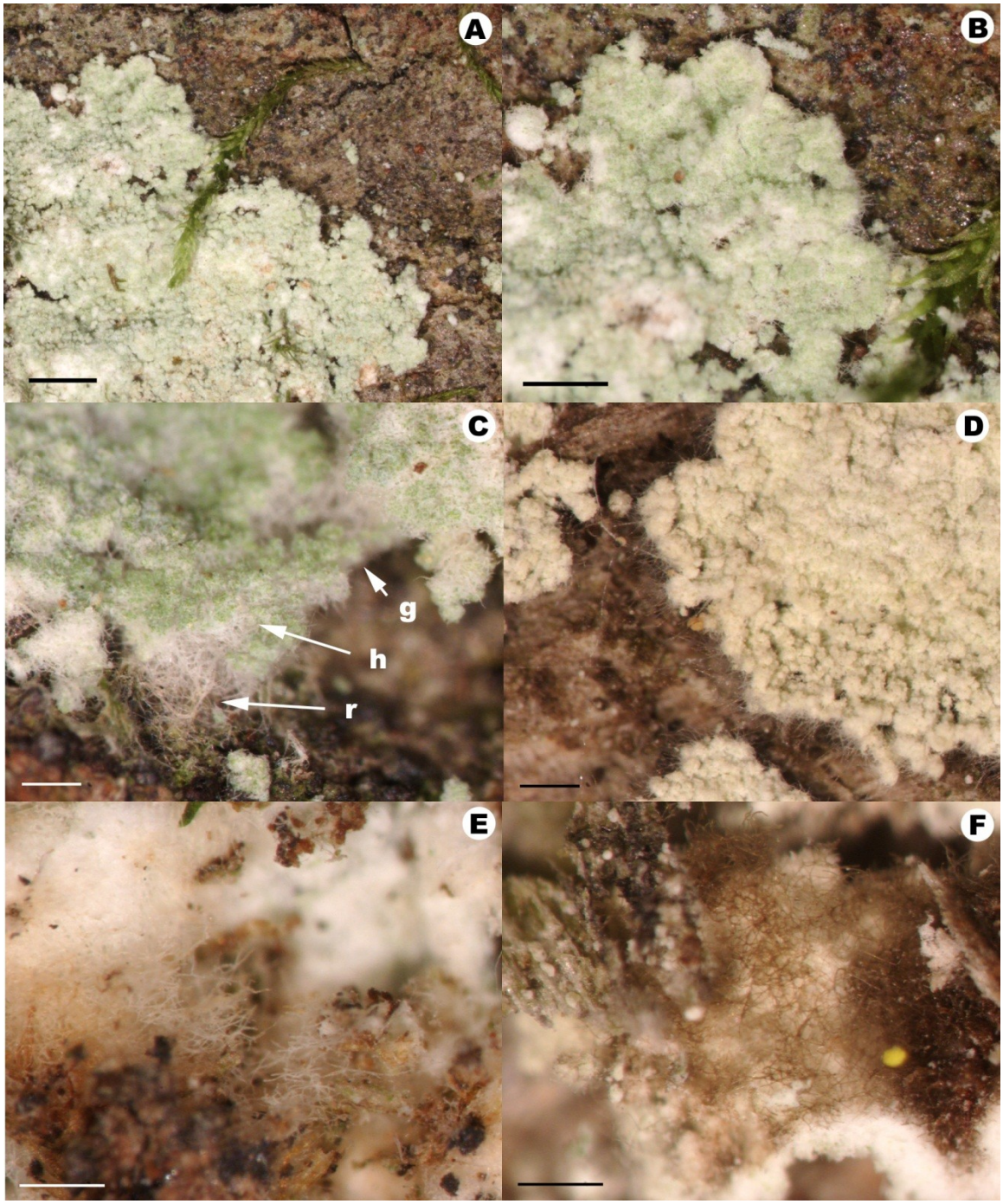

Figure 13. Subtypes of placodioid thalli, *finkii*-type (g = granules, h = hypothallus, r= rhizohyphae). A–C, *finkii*-type exemplified by *L. finkii* (*Lendemer 25433*, NY; scales = 1.0, 0.5, & 0.25 mm respectively). D, *finkii*-type exemplified by *L. membranacea* (*Lewis 450*, CANL; scale = 0.25 mm). E, pale rhizohyphae of *L. finkii* (as for A–C, scale = 0.25 mm). F, dark rhizohyphae of *L. membranacea* (as for D, scale = 0.25 mm).

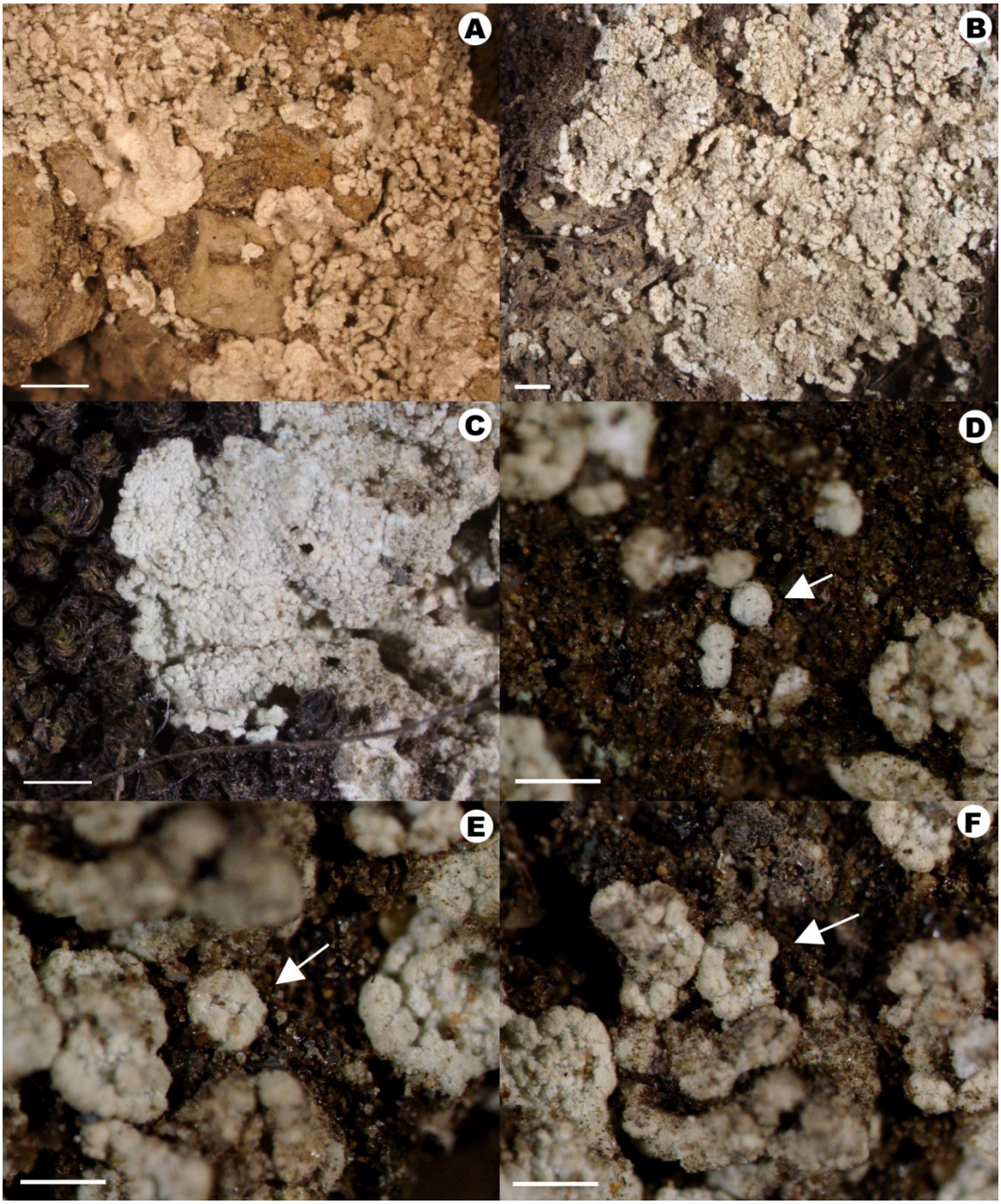

Figure 14. Subtypes of placodioid thalli, *xerophila*-type. A, *xerophila*-type exemplified by *Lepraria xerophila* (*Knudsen 7624*, NY). B–C, *xerophila*-type exemplified by *L. larrainiana* (*Lendemer 15936*, NY). D–F, development of thallus through the marginal expansion of the granules (as for B–C). Scales = 1.0 mm (A–C) and 0.5 mm (D–F).

CHAPTER II – IMPLEMENTATION OF A UNIFIED TAXONOMY INTEGRATING MOLECULAR AND NON-MOLECULAR DATA IN *PUNCTELIA SUBRUDECTA* S.L.[2]

James C. Lendemer & Brendan P. Hodkinson

Abstract. – In North America the names *Punctelia subrudecta* and *P. perreticulata* have variously been applied to corticolous sorediate *Punctelia* specimens with lecanoric acid and a pale lower surface. ITS1, 5.8S, and ITS2 sequence data were generated from a geographically and morphologically broad sampling from within these populations, and a molecular phylogeny was inferred. From the results of this analysis, we generated species concepts informed by morphology, geography, and phylogeny. This study revealed three distinct species in North America, one of which is described as new to science (*P. caseana*), one of which is finally confirmed for the continent (*P. jeckeri*), and one whose original circumscription is validated (*P. perreticulata*). The phylogeny inferred from ITS sequence data supports the taxonomic value of the following morphological characters in distinguishing species in this group: presence/absence of pruina; conidium type and length (although see discussion of *P. jeckeri*), and presence/absence of scrobiculae on the upper surface.

Introduction

The status of the sorediate, corticolous species *Punctelia subrudecta* in North America has been the subject of considerable study and debate (Adler & Ahti 1996; Aptroot 2003). While *P. subrudecta* has generally been considered to be distinct from *P. perreticulata* (e.g., Wilhelm & Ladd 1987), Adler and Ahti (1996) amended the definition of the latter species to exclude the

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scrobiculation of the upper surface as a diagnostic character, and added that the species could have either longer or shorter filiform conidia[3]. This change created a single, morphologically polymorphic species under the name *P. perreticulata* (the name *P. subrudecta* was correctly reserved for specimens with unciform conidia). Aptroot (2003), following these changes, assigned the name *P. perreticulata* to all corticolous, sorediate *Punctelia* specimens from North America with a pale lower surface and C+ red medulla (lecanoric acid). Through this process, *P. perreticulata* became the accepted name for all specimens from North America that were previously identified as *P. subrudecta* (since the conidia, when present, are always filiform, and not unciform). For some time, we have been uncomfortable with this treatment (Lendemer 2004), but lacked the ability to generate an alternate hypothesis using chemical, ecological, morphological, or phytogeographic data.

Recognizing the potential value of molecular data in addressing this problem, we undertook a study of the North American populations referred to *Punctelia perreticulata*. We first sampled broadly within the geographically widespread populations of *P. perreticulata* treated by Aptroot (2003), inferring a phylogeny of these organisms and some of their closest known relatives using ITS1, 5.8S, and ITS2 sequence data. Utilizing a phylogenetic species concept, we generated taxonomic hypotheses from this molecular analysis and attempted to correlate them to taxonomic hypotheses based on non-molecular characters (e.g., chemistry, ecology, morphology, phytogeography). Using a holistic taxonomic approach we integrated

[3]The reader should note that throughout this paper for the sake of consistency we follow the terms used by Krog (1982) to describe the conidia of *Punctelia* species (specifically the terms "unciform," "short filiform," and "long filiform"). We recognize that the term "filiform" does not seem to properly describe the straight rod-shaped conidia found in some *Punctelia* species (including those treated here) since these conidia are very different from the long threadlike conidia found in species of other genera such as *Bacidina* Vězda and *Lecanora* Ach.

these phylogenetic and "traditional" species concepts, accepting as species those that correlated and rejecting those that did not.

The results demonstrate the taxonomic utility of certain characters that have been rejected as uninformative by previous authors (e.g., conidium length and morphology of the upper surface; Adler and Ahti 1996), and reveal the presence of three distinct species within *Punctelia perreticulata sensu* Aptroot (2003) in North America.

Materials and Methods

Fieldwork and herbarium materials. – During our fieldwork throughout North America over the last five years, we have made an effort to collect sorediate *Punctelia* specimens with the goal of one day better understanding the circumscription of the taxa purported to be present in our region. We have collected hundreds of specimens of *P. perreticulata/subrudecta* across the continent including regions where both taxa had been reported to occur. Additionally, we examined the holdings of the Canadian Museum of Nature (CANL), The New York Botanical Garden (NY), and Duke University (DUKE). The ecological and distributional data used in this study have been gathered largely as part of our field studies, and molecular analyses have been carried out on samples collected by the first author and his collaborators (all materials have been deposited in the herbarium at NY).

Molecular data collection. – DNA was extracted from North American specimens of *Punctelia perreticulata* s.l. (e.g., including *P. subrudecta* auct. Amer. and *P. perreticulata sensu* Wihelm & Ladd) to generate a hypothetical circumscription of the species that could be tested with morphological characters as an independent dataset. Additionally, a sample of *P. missouriensis*, a species with weakly corticate squamiform isidiose-soredia, had its DNA

extracted in order to determine its relationship, if any, to specimens that were the main focus of the study. DNA extractions were performed at NY using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) with the instructions modified to include a prolonged (~12 hour) incubation period in the lysis buffer. Isolated DNA was resuspended in 100 µl of sterile water and stored at -20°C. PCR amplification of the ITS1, 5.8s, and ITS2 regions was performed at NY using the primers ITS4 and ITS5 (White et al. 1990). Amplification reactions of 25 µl contained each of the following: 2.5 µl 10× PCR Buffer (Qiagen), 2.5 µl dNTP solution (mixed to a concentration of 2 mM of each dNTP), 2.5 µl BSA solution (mixed to a concentration of 2.5 mg μl^{-1} of bovine serum albumin; Hillis et al. 1996), 0.2 µl Taq DNA Polymerase (= 1 U; Qiagen), 1 µl of each primer (in solution at a concentration of 10 µM), 9.3 µl of sterile water, 1 µl of extracted DNA, and 5 µl of Q-solution (Qiagen). PCR protocol followed Nelsen et al. (2007) and consisted of an initial denaturation of 95°C for 5 min, followed by 10 cycles of 95°C for 1 min, 62°C for 1 min, and 72°C for 1 min, then 35 cycles of 95°C for 1 min, 53°C for 1 min and 72°C for 1 min with a final extension for 7 minutes at 72°C. PCR products were visualized prior to sequencing by UV examination of a 1% agarose gel on which 1 µl of amplified PCR product had been subjected to electrophoresis and stained with ethidium bromide. Unpurified amplified PCR products were sent to the University of Washington Biochemistry DNA Sequencing Facility (BDSF) for sequencing. Sequences were assembled and manually edited using the software package Sequencher™ 4.9 (Gene Codes Corporation, Ann Arbor, MI, USA). In order to identify contaminants, nearest sequence matches were found by searching the nucleotide collection in GenBank using BLASTn (Altschul et al. 1997)

Phylogenetic analyses. – Molecular datasets were assembled using reference ITS

sequences representing our species of interest (e.g., all *Punctelia jeckeri/ulophylla, P. perreticulata,* and *P. subrudecta* sequences from GenBank) in addition to the sixteen sequences generated as part of this study. Sequences from the outgroup taxa *P. borreri* and *P. rudecta* were added because of their affinity to the species in question and the number of sequences (more than two) available from GenBank. Sequences were initially aligned using ClustalX (Thompson et al. 1997); those that contained numerous clear inconsistencies with all other aligned sequences were assumed to have resulted from the sequencing of heterogeneous amplicon pools, and were excluded from phylogenetic analyses (for a discussion of the benefits and drawbacks of using sequences generated from heterogenous amplicon pools in phylogenetic analyses, see Hodkinson & Lutzoni (2009)). Alignments were subsequently adjusted manually using Mesquite ver. 2.0 (Maddison & Maddison 2007), and the nucleotides in ambiguously-aligned regions were excluded from analyses (by defining them as members of an exclusion set). Ambiguous bases (e.g., N's, R's, Y's, etc.) and terminal gaps were changed to missing ('?') and the NEXUS file was saved with the taxa and characters compressed into a data block.

Using standard text editing software, the NEXUS alignment file was then prepared for a weighted maximum parsimony analysis with recoded ambiguously-aligned regions. First, constant sites were excluded from analyses by typing "constant" into the exset line of the assumptions block. Each of the ambiguously aligned regions was then re-coded using INAASE 3.0 (Lutzoni et al. 2000) and specific step-matrices were generated (through the same program) using a transition:transversion:gap ratio of 1:2:3. Step matrices produced by INAASE were subsequently altered based on manual adjustments to the provided pair-wise alignments of ambiguous regions. For those regions that could not be reasonably realigned in a pair-wise manner, INAASE characters were rejected and the regions were recoded using ARC

(Miadlikowska et al. 2003). ARC characters and accepted INAASE characters were then pasted immediately below the nucleotide matrix (as the final characters in an interleaved matrix). The data block was altered to accommodate the newly-inserted recoded characters. First, the number of characters (nchar in the dimensions line of the data block) was changed to the value equivalent to the number of characters in the nucleotide alignment + the number of INAASE regions + 23 times the number of ARC regions (since each ARC region is recoded as a series of 23 characters). Since the NEXUS file was originally saved in a non-interleaved format, the format was manually changed to interleaved (by typing "interleave" in the format line of the data block) to accommodate the insertion of ARC and INAASE characters into the character matrix. In the same line, "datatype=DNA" and "gap=-" were removed and replaced by “symbols = "A C G T - 0 1 2 ..."' listing each of the characters used in the nucleotide+INAASE+ARC character matrix.

Those ambiguously-aligned regions recoded using INAASE were subjected to the aforementioned manually-adjusted symmetric step-matrices initially produced by the same program. Likewise, unambiguously-aligned portions were subjected to their own step-matrices, which were computed separately for each of the three regions (ITS1, 5.8S, and ITS2) using STMatrix 3.0 (written by S. Zoller; *Miadlikowska* et al. 2002) as outlined by Gaya et al. (2003). The step-matrices produced from both INAASE and STMatrix were pasted directly into the assumptions block and renamed (name inserted between "usertype" and "stepmatrix") according to the region on which each would be employed. In the typeset line of the assumptions block, each of the regions subjected to a step-matrix was defined by typing the name of the region, followed by a colon and the identifier numbers of the character(s) contained within it. So as not to over-inflate the influence of certain characters that are inextricably linked, all but one of the 23 ARC characters was down-weighted for each ARC region as follows: characters 2–5 were

each given a weight of 0.25; characters 6–15 each had a weight of 0.10, and characters 16–23 were each assigned a weight of 0.5. Weights were entered manually into the wtset line of the assumptions block. The resulting NEXUS file is available for download at the second author's website, accessible at http://www.duke.edu/~bph8/. This filen was also submitted to the Dryad data repository (doi:10.5061/dryad.qv42j11b).

Phylogenetic analyses were run using weighted maximum parsimony (MP) in PAUP* 4.0b.8a (Swofford 2001). A first round of searches was performed with 1000 random-addition-sequence (RAS) replicates and TBR (tree bisection reconnection) branch swapping. The MULTREES option was in effect and zero-length branches were collapsed. All equally most parsimonious trees were saved with branch lengths, and a strict consensus tree was computed for reference. Branch support for MP trees was estimated through bootstrap analyses (Felsenstein 1985) by performing 1000 bootstrap replicates with 10 RAS per bootstrap replicate, with all other settings as above. Clades with high support (>70% MP-BP) on branches that had a length of ten or more changes in all most parsimonious reconstructions were designated as 'phylogenetic species' to be tested using an independent morphological dataset.

Chemical and morphological analyses. – All specimens were studied dry using a Bausch & Lomb StereoZoom 7 dissecting microscope. Characters of size, broadness, aspect, and basal color were noted. All specimens were also subjected to chemical analysis using standard spot tests (reagents are abbreviated following Brodo et al. (2001)) or Thin Layer Chromatography (TLC). Thin Layer Chromatography was carried out using solvent systems A or C following the standardized methods of Culberson & Kristinsson (1970).

Results and Discussion

The results of our morphological and phylogenetic analyses of *Punctelia subrudecta* s.l. in North America are presented below, followed by a summary taxonomic treatment including additional discussion, distribution maps, and illustrations. As the proceeding discussion should make clear, we do not consider the present study to be the final word on the status of the sorediate lecanoric acid-producing species of *Punctelia* with a pale lower surface in North America. Rather, our study is intended to be a jumping off point for more detailed research that is needed to understand the characters used to circumscribe species of *Punctelia*, and to understand the relationships between populations of '*P. subrudecta*' in North America and abroad.

In total, we generated fifteen new ITS sequences from North American specimens of corticolous sorediate *Punctelia* species with lecanoric acid and a pale underside, in addition to one sequence from the isidiose-sorediate *P. missouriensis*. Phylogenetic analyses resulted in four equally most parsimonious trees, one of which was topologically equivalent to the strict consensus of all four trees; this tree is displayed in figure 15. The new sequences from sorediate *Punctelia* specimens with lecanoric acid sorted into three distinct clades, two of which already had representatives in GenBank (*P. jeckeri* and *P. perreticulata*; figure 15). Certain combinations of morphological characters were found to correlate with each of these monophyletic entities (table 3), leading us to accept the phylogenetic species suggested by our molecular analyses.

Considering the differences in conidia reported by Adler and Ahti (1996) for European and North American populations of *Punctelia perreticulata*, we expected that phylogenetic analyses of a diverse sampling of populations from within *P. perreticulata sensu* Aptroot (2003)

would likely reveal the existence of more than one morphologically recognizable species (especially since comparable differences in conidium length were used to distinguish *P. semansiana* C. Culb. & W.L. Culb. (= *P. graminicola* (de Lesd.) R.S. Egan) from *P. hypoleucites* (Nyl.) Krog by Culberson and Culberson (1980)). We were, however, quite surprised that these data revealed the existence of three distinct species (none of which is *P. subrudecta*) that can be distinguished both morphologically and phylogenetically (see figure 15). Each of these species is discussed more fully below.

The outgroup taxa *Punctelia borreri* (which has a black underside, gyrophoric acid, and soredia) and *P. rudecta* (which has a pale underside, lecanoric acid, and isidia) formed distinct, monophyletic entities (Figure 1), confirming the utility of underside color, medullary chemistry, and diaspore type for separating these species from the species at issue in the present study. The sequence generated from *P. missouriensis* produced a rather unexpected result. Strangely, this sequence formed a strongly-supported clade with a single GenBank sequence generated from Chinese material (tentatively identified as "*P. aff. rudecta*"; figure 15), suggesting that this species, previously known only from North and South America, may actually also exist in Asia.

The status of *Punctelia perreticulata* sensu Aptroot (2003)

A - *Punctelia perreticulata* s. str.

One of the primary goals of our study was to determine the status of *Punctelia perreticulata* s. str. in North America. The type specimen of *P. perreticulata* is from Italy (van Herk & Aptroot 2000) and the species has traditionally been separated from others with a pale underside and lecanoric acid by its scrobiculate upper surface with few pseudocyphellae and

medium sized (6–8 µm long) filiform conidia. The species, in the strict sense, was considered to occur rarely in south-central North America (Hale 1979, Wetmore 1976, Wilhelm & Ladd 1987). We examined material at NY identified as *P. perreticulata* and found several specimens that corresponded to the strict concept *P. perreticulata*. We successfully extracted DNA from one of these specimens. In our analysis the sequence generated from this specimen formed a well supported clade along with another specimen from North America and one from Spain. The presence of *P. perreticulata* s. str. in North America is thus confirmed and the original circumscription of *P. perreticulata* strongly supported by molecular data.

Adler and Ahti (1996) expanded the concept of *Punctelia perreticulata* to include specimens from Australasia and North America with longer conidia (9–12 µm) and smooth to moderately ridged upper surfaces. Our results do not support this expanded concept since North American specimens with the above characters form two distinct highly supported clades quite distant from *P. perreticulata* s. str.; these are discussed in the subsequent sections.

It should be noted that we are not the first to reject Adler and Ahti's (1996) broad circumscription of *Punctelia perreticulata*. Thell et al. (2005) also arrived at this conclusion in conjunction with their larger phylogenetic studies of *Punctelia*. Their conclusion was based on the fact that in their analysis sequences of two specimens identified as *P. perreticulata* from China grouped with sequences of *P. borreri*, rather than with a sequence of *P. perreticulata* from North America (identified by D. Ladd and presumably thus congruent with the concept of the taxon outlined by Wilhelm & Ladd (1987)). Thell et al. (2005) also reported *P. perreticulata* as new to China despite the fact that their own data indicated *P. perreticulata* was polyphyletic (and that the Chinese samples did not represent *P. perreticulata* s. str.).

Since the publication by Thell et al. (2005) a number of additional sequences of *Punctelia borreri* have accumulated in GenBank and we included these in our analysis. In our analysis the Chinese sequences of *P. perreticulata* generated by Thell et al. (2005) were nested within a well-supported clade otherwise comprised of sequences of *P. borreri* from Africa, Europe, and Asia. We examined the voucher (*Aptroot 56094*, hb. Aptroot) which is the basis of AY773124 and found it to be a mixture of *P. borreri* (gyrophoric acid detected by GE) and a sorediate species with a pale underside. Almost certainly the fragment used for molecular analysis represented *P. borreri*. Unfortunately we were unable to examine the other Chinese specimen (*Aptroot 56005*) cited as *P. perreticulata* by Thell et al. (2005); however, in light of the above it is very likely to be *P. borreri*, and at the very least, not *P. perreticulata*.

B - *Punctelia subrudecta* sensu Lendemer (2004)

As has been mentioned above, the sequences derived from North American samples that correspond to the expanded concept of *Punctelia perreticulata* proposed by Adler and Ahti (1996) actually belong to two well-supported clades distinct from *P. perreticulata* s. str. One of these (identified as *P. caseana* in Figure 1) corresponds to *P. subrudecta* sensu Lendemer (2004) and is sister to a clade comprised of sequences from GenBank identified as *P. jeckeri* and those from our study generated from specimens of *P. subrudecta s.l.* from western North America.

The clade recognized here as *Punctelia caseana* is comprised entirely of sequences generated from specimens collected in eastern North America. We were initially surprised that our study supported these populations as distinct from those in western North America because both sets of populations have long filiform conidia (8–12 µm) and a smooth to moderately ridged thallus surface. Further study in light of the results of our molecular analysis led to the

recognition that the *P. caseana* and *P. jeckeri* clades were distinguished respectively by the absence and presence of pruina on the lobe tips. Since there is no available name for the eastern North American populations previously referred to *P. subrudecta*, we describe them herein as a new species, *P. caseana*.

In our analysis, *Punctelia caseana* contains within it a single, well-supported, monophyletic entity that seems to have experienced a higher rate of ITS nucleotide substitution than the rest of the members of the species (hence the long branch on which it sits). The remainder of the sequences form a group that cannot be definitively characterized as either monophyletic or paraphyletic based on ITS sequence data. These two sets of sequences correspond to two morphotypes that are reasonably well defined on morphological and biogeographic grounds. While each morphotype dominates a given geographic region, both often occur at the same locality in northern Pennsylvania/southern New York.

The morphotype that cannot be definitively characterized as monophyletic in our analysis ("Morph-I" in Figure 1) is most common in northern North America and also frequently occurs at high elevations throughout the central and southern Appalachian Mountains. This morphotype is often extremely distinctive because its large size, sparse pseudocyphellae, and primarily marginal soralia with fine soredia give it the appearance of a species of the genus *Cetrelia* C. Culb. & W.L. Culb. in the field (all *Cetrelia* species have a black rather than pale underside and different medullary chemistry). It is illustrated in Hinds and Hinds (2007) and has clearly been a source of confusion for lichenologists as many specimens had been identified as *Punctelia perreticulata* or *P. subrudecta* with some indication of hesitation (e.g., "?" or "s.l.").

The second morphotype ("Morph-II" in figure 15) is exemplified by the type collection of *Punctelia caseana* and is most common in the Coastal Plain and Piedmont of North America.

It also occurs frequently at middle to low elevations (e.g., ~2000 ft. and below) in the Appalachian Mountains and in the Ozark Ecoregion. Populations of this morphotype can be recognized by their smaller thalli with narrower lobes (compared to the above morphotype), and primarily laminal soralia with coarse granular soredia that often aggregate and give the appearance of squamules or lobules (this causes confusion with *P. missouriensis*).

C - *Punctelia perreticulata* sensu Egan & Aptroot (2004)

Though Egan (2002) reported *Punctelia jeckeri* (syn. *P. ulophylla*) for the western North American state of New Mexico in 2002 (after Bouly de *Lesdain* 1942), Egan and Aptroot (2004) later applied the name *P. perreticulata* to all populations of *P. subrudecta* s.l. in western North America, essentially following the evaluation of these populations by Aptroot (2003). Despite this treatment by Aptroot, *P. ulophylla* was once again reported for the continent by Tucker et al. (2006) as part of a lichen survey of Pinnacles National Monument in California. We sampled broadly within the populations occurring in California; however, we were unable to obtain fresh material from populations in the arid southwest and Rocky Mountains. Like the populations from eastern North America, the western populations that we sampled are characterized by long filiform conidia (8–12 µm long) and a smooth to moderately ridged upper surface.

In our molecular analysis the sequences derived from the western populations of *Punctelia subrudecta* s.l. are indistinguishable from those sequences generated from European specimens identified as *P. jeckeri* and thus form a well-supported clade sister to *P. caseana* which is characterized by the presence of pruina on the tips of the lobes. Our results do not support the separation of *P. perreticulata* sensu Egan and Aptroot (2004) from *P. jeckeri* and thus we report these populations under the latter name here.

The use of the name *Punctelia jeckeri* for these populations is problematic because *P. jeckeri* has unciform to short-filiform conidia, while the western North American populations uniformly have long-filiform conidia. Since differences in the type and length of the conidia are supported as species-level characters elsewhere in our study, it is curious that they would not be useful in the case of *P. jeckeri*. We have not revised all the vouchers upon which the European sequences of *P. jeckeri* are based[4] and thus are unable to state with certainty what type of conidia they have (if conidia are present). If these vouchers do have the typical conidia reported for European *P. jeckeri*, this phenomenon could potentially be explained by a founder effect, and a single anomalous individual or set of individuals could have given rise to the representatives of this species on one of the two continents. However, it is also possible that there is another species present in Europe that is morphologically similar to *P. jeckeri* but differs in conidium type. Clearly, further study of this taxon is needed.

Taxonomic Section

Below we provide an account of the three North American species of sorediate *Punctelia* with a pale lower surface and lecanoric acid in the medulla. The new species *P. caseana* is described and treated in full, while *P. jeckeri* and *P. perreticulata* are treated in a shortened form primarily to detail their separation from *P. caseana* and each other. A key to the genus *Punctelia* in North America (north of Mexico) is included below.

[4]We have examined *Aptroot 57873* (hb. Aptroot) which is the voucher for AY773121 identified as *P. ulophylla* by Thell et al. (2005) and found that it lacks pycnidia.

Key to genus *Punctelia* in North America (north of Mexico)

1. Thallus **lacking lichenized diaspores**; apothecia often present…2

    2. Lower surface black; medulla C+ pink (gyrophoric acid); Texas…*P. subpraesignis*

    2. Lower surface pale; medulla C+ red (lecanoric acid) or C-; distribution various…3

        3. Medulla C- (fatty acids present)…*P. bolliana*

        3. Medulla C+ red (lecanoric acid)…4

            4. Conidia (3–)5–6(–9) µm long…*P. graminicola*

            4. Conidia (7–)10–13(–16) µm long…*P. hypoleucites*

1. Thallus **with isidia**, **lobules**, or **soredia**…5

    5. Thallus **sorediate**…6

        6. Lower surface pale-brown…7

            7. Saxicolous; coastal southern California; thallus with false soredia developing from the breakdown of papilliform isidia…*P. punctilla*

            7. Corticolous (rarely saxicolous); distribution and thallus not as above…8

                8. Soredia coarse and isidioid, squamiform and partially corticate, few per soralium; central and eastern North America…*P. missouriensis*

                8. Soredia fine, not isidioid, not squamiform, and fully ecorticate, many per soralium; widespread…9

                    9. Upper surface smooth to somewhat ridged, not scrobiculate; lobe tips pruinose or not…10

                        10. Lobe tips epruinose; central and eastern North America…*P. caseana*

10. Lobe tips pruinose; western and southwestern North America…*P. jeckeri*

9. Upper surface scrobiculate; lobe tips pruinose…*P. perreticulata*

6. Lower surface black…11

11. Saxicolous; rare, medulla C+ pink (gyrophoric acid)…*P. stictica*

11. Corticolous (rarely saxicolous); medulla C+ pink or C-…12

12. Medulla C+ pink (gyrophoric acid)…*P. borreri*

12. Medulla C- (fatty acids)…*P. reddenda*

5. Thallus **isidiate** or **lobulate**….13

13. Thallus **isidiate**…14

14. Saxicolous; coastal southern California; isidia short and papilliform, breaking down into coarse soredia…*P. punctilla*

14. Corticolous (rarely saxicolous); absent from southern California; isidia otherwise…15

15. Very common; isidia tall, cylindrical to branched…*P. rudecta*

15. Less common (except in central North America); isidia short and squamiform, restricted to pseudocyphellae and resembling heaps of corticate soredia…*P. missouriensis*

13. Thallus **lobulate**…16

16. Lower surface black…17

17. Medulla C+ pink (gyrophoric acid); Texas…*P. subpraesignis*

17. Medulla C- (fatty acids); Appalachian…*P. appalachensis*

16. Lower surface pale…*P. bolliana*

Account of the Species

1. *Punctelia caseana* Lendemer & Hodkinson sp. nov.

Mycobank #514021.

Figure 16.

Thallus ut in *Punctelia jeckeri* sed epruinosus et conidiis longioribus differt.

TYPE: **U.S.A. NEW JERSEY.** ATLANTIC CO.: Nature Conservancy Reserve, SE of Egg Harbor City, NE of Germania, 1/3 mi S of NJ 561, 21.x.2003, on branches of *Vaccinium*, *J.C. Lendemer et al. 1431* (NY!, holotype; isotypes distributed as *Lich. East. N. Amer. Exs. III: 149*).

Description. – Thallus corticolous, closely attached to the substrate, highly variable in size depending on microhabitat conditions, sorediate. Lobes flat to ridged, often upturned at the margins. Upper surface gray-blue, glabrous, with a narrow brown zone near the lobe tips, epruinose. Lower surface pale to off white, rarely light brown but never black. Soralia frequent, primarily marginal along the secondary lobes with laminal soralia arising from the pseudocyphellae in the older portions of the thallus. Soredia large, coarse. Apothecia rare (seen only in *Lendemer 17592*, DUKE, NY), lecanorine, with sorediate margins. Ascospores 8-ascus, hyaline, 14–17 × 10–12 µm. Pycnidia infrequent, immersed, black. Conidia filiform, hyaline, (8–)9–10(–12) µm long.

Published color illustrations. – Flenniken (1999, as *Punctelia subrudecta*), Hinds and Hinds (2007, as *P. perreticulata*).

Etymology. – The epithet "caseana" honors the undergraduate major professor of the second author, Dr. Martha Case, who supported his interest in lichenology and encouraged him to study the lichens of the state of Virginia, U.S.A., where the new species commonly occurs.

Chemistry. – Atranorin (cortex), lecanoric acid (medulla). Spot tests: cortex, K+ yellow, C-, KC+ yellowish, P-, UV-; medulla, K-, C+ red, KC+ red, P-, UV-.

Ecology and distribution. – The new species essentially follows the distribution of *Punctelia subrudecta* in eastern North America as figured and described by Brodo et al. (2001). It is common on the bark of conifers (*Pinus, Tsuga*) and, in some regions, is equally common on the bark of hardwoods (*Acer, Quercus*).

The reader should note that the distribution map presented here is heavily biased against portions of central and northern North America because these regions are not well represented in the NY herbarium. The available literature indicates the taxon to be common in the region highlighted by Brodo et al. (2001) west of the Appalachian Mountains (see e.g., the distribution map of *Punctelia subrudecta* for Ohio provided by Showman and Flenniken (2004)). The apparent rarity of *P. caseana* in the coastal plain and piedmont of southeastern North America south of Virginia is likely "real" because the macrolichens in this region were intensively collected by Clyde F. Reed whose herbarium has been incorporated into NY.

All specimens identified as *Punctelia subrudecta* from southwestern North America that we have examined have proved to be *P. jeckeri*. However Egan (2002) noted both *P. subrudecta* and *P. ulophylla* (= *P. jeckeri*) to be present in New Mexico, USA and it is thus possible that both *P. caseana* and *P. jeckeri* occur in that region.

Exsiccati specimens examined. – *Lich. East N. Amer. Exs. 149* (NY, type collection), *355* (NY, exemplar of morphotype II), *356* (NY, exemplar of morphotype I).

Selected specimens examined. – **CANADA. ONTARIO.** HASTINGS CO.: 3.5 mi E of Marmora, 1976, *P.Y. Wong 2251 & P.A. Bowler* (CANL). PEEL CO.: NW of Palgrave, 1957, *R.F. Cain 26398* (CANL, NY). YORK CO.: NW of Nobleton, 1958, *R.F. Cain 27122* (CANL, NY). **U.S.A. CONNECTICUT.** FAIRFIELD CO.: Highstead Arboretum, 2005, *R.C. Harris 51579* (NY). LITCHIELD CO.: Holleran Swamp Preserve, 2003, *R.C. Harris 48071* (NY). **DELAWARE.** SUSSEX CO.: Delaware Wild Lands Cypress Swamp Conservation Area, 1989, *R.C. Harris 24962* (NY). **GEORGIA.** RABUN CO.: Chattahoochee National Forest, Rabun Bald, 1997, *R.C. Harris 41270* (NY). WALKER CO.: 1992, *R.C. Harris 28194* (NY). **KENTUCKY.** BATH CO.: Daniel Boone National Forest, Stoney Cove Recreation Area, 1995, *R.C. Harris 36956* (NY). BELL CO.: Pineville, 1981, *C.F. Reed 111115* (NY). BULLITT CO.: Bernheim Arboretum, 2002, *D. Ladd 23522* (NY). CARTER CO.: Carter Caves State Park, 1981, *P.G. Reed 1981-15* (NY). ESTILL CO.: Daniel Boone National Forest, ~1.5 mi NW of Cottage Furnace Campground, 1995, *R.C. Harris 36976* (NY). FLEMING CO.: Grange City, 1981, *C.F. Reed 112890* (NY). JESSAMINE CO.: near Wilmore, 1962, *C.F. Reed 57279* (NY). LAUREL CO.: Cumberland National Forest, Morgan Ridge, 1962, *C.F. Reed 58214* (NY).

LETCHER CO.: Bad Branch Nature Preserve, 1991, *R.C. Harris 27076* (NY). MONTGOMERY CO.: Reid Village, 1980, *C.F. Reed 111133* (NY). OWEN CO.: New Liberty, 1980, *C.F. Reed 120850* (NY). ROWAN CO.: Daniel Boone National Forest, SE end of Cave Run Lake, 1995, *R.C. Harris 36803* (NY). SPENCER CO.: 1962, S of Elk Creek, 1962, *C.F. Reed 56780-B* (NY). WAYNE CO.: Mill Springs, 1962, *C.F. Reed 58399* (NY). **MAINE.** LINCOLN CO.: Spruce Point, 1994, *B. Allen 15785* (NY). YORK CO.: Ferry Beach State Park, 2002, *R.C. Harris 46352* (NY). **MARYLAND.** ALLEGANY CO.: near Midlands, 1963, *C.F. Reed 64615B* (NY). BALTIMORE CO.: Ward's Chapel Road, 1980, *E.G. Worthley s.n.* (NY). CECIL CO.: Elk Neck, 2.xii.1987, *C.F. Reed 126459* (NY). FREDERICK CO.: Thurmont, 1962, *C.F. Reed 59360A* (NY). GARRETT CO.: High Bog, 1989, *R.C. Harris 24583* (NY). SOMERSET CO.: near Mt. Vernon, 1963, *C.F. Reed 64576* (NY). WASHINGTON CO.: near Big Pool, 1963, *C.F. Reed 64966* (NY). WICOMICO CO.: 5 mi SW of Sharptown, 1980, *E.G. Worthley s.n.* (NY). **MICHIGAN.** EMMET CO.: S of Wycamp Lake, 1974, *W.R. Buck s.n.* (NY). HOUGHTON CO.: near Calumet, 1957, *H.A. Imshaug 20849* (CANL). **MINNESOTA.** PINE CO.: Nemadji State Forest, 1983, *J.P. Schuster 1655* (CANL). **MISSOURI.** CARTER CO.: MOFEP site 9, 1007, *D. Ladd 20501* (NY). IRON CO.: St. Francois Mountain, Clark National Forest, 1993, *R.C. Harris 31116* (NY). MADISON CO.: Mark Twain National Forest, Rock Pile Mountain Wilderness, 2003, *R.C. Harris 48267* (NY). SHANNON CO.: MOFEP site 4, 1996, *D. Ladd 19494* (NY). ST. GENEVIEVE CO.: Pickle Springs Natural Area, 1990, *R.C. Harris 25975* (NY). WASHINGTON CO.: Hughes Mountain Conservation Area, 2002, *R.C. Harris 46452* (NY). **NEW HAMPSHIRE.** GRAFTON CO.: Benton Tr. N Moosilauke, 1935, *G.P. Anderson s.n.* (NY). **NEW JERSEY.** ALTANTIC CO.: Wharton State Forest, Batsto Natural Area, 2003, *J.C. Lendemer et al. 985* (NY). BURLINGTON CO.: Wharton State Forest, Skit Branch, 1995,

R.C. Harris 36618 (NY). CAMDEN CO.: Winslow Wildlife Management Area, 2009, *J.C. Lendemer 15327* (NY). CAPE MAY CO.: Peaslee Wildlife Management Area, 2009, *J.C. Lendemer 15172* (NY). CUMBERLAND CO.: Peaslee Wildlife Management Area, 2009, *J.C. Lendemer 15140* (NY). HUNTERDON CO.: Mitchell Property, 1992, *R.C. Harris 29034* (NY). MONMOUTH CO.: Allaire State Park, 2009, *J.C. Lendemer 15411* (NY). OCEAN CO.: 3 mi SW Warren Grove, 1982, *L. Brako 4947* (NY). SALEM CO.: Parvin State Park, 2008, *J.C. Lendemer et al. 15041* (NY). SUSSEX CO.: Stokes State Forest, 2008, *J.C. Lendemer et al. 11592* (NY). **NEW YORK.** ALBANY CO.: Limestone Rise Preserve, 2005, *R.C. Harris 51878* (NY). DUTCHESS CO.: Roger Perry Memorial Preserve, 2008, *R.C. Harris 54826* (NY). GREENE CO.: Catskill Park, Blackhead Range Trail, 1996, *R.C. Harris 38573* (NY). PUTNAM CO.: Peach Lake Natural Area, 2006, *W.R. Buck 51201 & R.C. Harris* (NY). ROCKLAND CO.: Harriman State Park, 2008, *J.C. Lendemer et al.* 11541 (NY). SUFFOLK CO.: Long Island, 1991, *R.C. Harris 26839* (NY). ULSTER CO.: summit area of Giant Ledge, 1993, *R.C. Harris 30509* (NY). **NORTH CAROLINA.** ASHE CO.: Horse Gap, 1982, *C.F. Reed 143278-B* (NY). CALDWELL CO.: Moses G. Cone Memorial Park, 1981, *P.G. Reed 1981-105 & L.L. Reed* (NY). GRAHAM CO.:Co., Nantahala National Forest, along Santeelah Creek, 1994, *R.C. Harris 32923* (NY). HAYWOOD CO.: Great Smoky Mountains National Park, vicinity of Baxter Creek, 2008, *J.C. Lendemer et al. 11917* (NY). HENDERSON CO.: Pisgah National Forest, North Mills River Recreation Area, 2006, *J.C. Lendemer et al. 7084* (NY). MACON CO.: Nantahala National Forest, Southern Nantahala Wilderness Area, 1997, *R.C. Harris 41109* (NY). MITCHELL CO.: Pisgah National Forest, trail to Roan High Bluff, 1993, *R.C. Harris 30918* (NY). STOKES CO.: Hanging Rock State Park, 1993, *R.C. Harris 30699* (NY). **OHIO.** ADAMS CO.: Adams Co., Chaparral Prairie State Nature Preserve, 2006, *R.C. Harris 52804*

(NY). HOCKING CO.: Crane Hollow, 1968, *I.M. Brodo 14502* (CANL). SCIOTO CO.: Shawnee State Forest, 2006, *J.C. Lendemer et al. 7231* (NY). **PENNSYLVANIA.** CARBON CO.: Hickory Run State Park, 2008, *J.C. Lendemer 12594* (NY). CHESTER CO.: Nottingham County Park, 2003, *J.C. Lendemer 1678 & E.W. McCardell* (NY). COLUMBIA CO.: State Game Lands No. 58, 2008, *R.C. Harris 54332* (NY). Huntingdon Co: Rothrock State Forest, 2008, *J.C. Lendemer et al. 11702* (NY). LANCASTER CO.: Susquehannock State Park, 2008, *J.C. Lendemer 11349 & E. Tripp* (NY). LACKAWANNA CO.: Lackawanna State Forest, Phelps Road (loop), 2008, *J.C. Lendemer 13089* (NY). LUZERNE CO.: State Game Lands No. 91, 2008, *J.C. Lendemer 13123* (NY). MONROE CO.: Tobyhanna State Park, 2008, *J.C. Lendemer 13766* (NY). PIKE CO.: Delaware State Forest, Bruce Lake, 2008, *J.C. Lendemer 12087* (NY). SULLIVAN CO.: Wyoming State Forest, 2004, *J.C. Lendemer 2255 & J.A. Macklin* (NY). WAYNE CO.: Lacawac Sanctuary, 2008, *J.C. Lendemer 12205* (NY). WYOMING CO.: State Game Lands No. 57, 2008, *J.C. Lendemer 13604* (NY). **TENNESSEE.** CARTER CO.: Ripshin Bog on Tiger Creek Road, 1993, *R.C. Harris 30955* (NY). GREENE CO.: Horsehitch Gap, 1991, *R.C. Harris 27212* (NY). POLK CO.: Cherokee National Forest, Cohutta Wilderness Area, 1998, *R.C. Harris 42467* (NY). SULLIVAN CO.: 3 mi S of Bluff City, 1964, *C.F. Reed 66918* (NY). WASHINGTON CO.: 3 mi N of Johnson City, 1964, *C.F. Reed 66735* (NY). **VERMONT.** ESSEX CO.: Victory State Forest, 2008, *R.C. Harris 54394* (NY). GRAND ISLE CO.: North Hero State Park, 1996, *W.R. Buck 30736* (NY). LAMOILLE CO.: Babcock Nature Preserve, 2005, *R.C. Harris 51402* (NY). WINDSOR CO.: Hartland Bog, 1986, *I.M. Brodo 25473* (CANL). **VIRGINIA.** ACCOMACK CO.: N of Pungoteague, 1957, *C.F. Reed 38567-A* (NY). AMHERST CO.: Long Mt., 1963, *C.F. Reed 62199* (NY). BLAND CO.: E of Ceres, 1964, *C.F. Reed 66951* (NY). FAIRFAX CO.: Pohick Church, 1962, *C.F. Reed 60340* (NY).

Franklin Co., near Snydersville, 1947, *C.F. Reed 10056* (NY). GILES CO.: Moonshine Dell, 1995, *R.C. Harris 36688* (NY). GRAYSON CO.: Grayson Highlands State Park, 1991, *R.C. Harris 26924* (NY). MADISON CO.: Big Meadows, 1966, *I.M. Brodo 9509* (CANL). NORTHAMPTON CO.: 3 mi NE of Eastville, 1963, *C.F. Reed 64166* (NY). PITTSYLVANIA CO.: 3 mi W of Chatham, 1963, *C.F. Reed 138253* (NY). ROCKINGHAM CO.: Endless Caverns, 1964, *P.G. Reed 67529* (NY). Rappahannock Co., just N of Thornton Gap, 1964, *C.F. Reed 66966* (NY). SMYTH CO.: Jefferson National Forest, Whitetop Mountain, 2008, *R.C. Harris 54122* (NY). WARREN CO.: Smithsonian CRC, 2005, *J.C. Lendemer 3839* (NY). **WEST VIRGINIA.** HARDY CO.: South Branch Mountain, 1964, *C.F. Reed 66510* (NY). TUCKER CO.: Monongahela National Forest, along FS 13, 2001, *R.C. Harris 44941* (NY). PENDLETON CO.: Spruce Knob, 1976, *R.C. Harris 10626* (CANL). POCAHONTAS CO.: Monongahela National Forest, upper slopes of Spruce Mountain, 2007, *J.C. Lendemer 9896 & A. Moroz* (NY). PRESTON CO.: Cranesville Swamp, 1963, *C.F. Reed 64440* (NY). **WISCONSIN.** BAYFIELD CO.: Point Detour, 1965, *I.M. Brodo 5776* (CANL). IOWA CO.: 6 mi W of Arena, 1965, *I.M. Brodo 5643* (CANL). ONEIDA CO.: Patterson Hemlocks State Natural Area, 29.iv.2002, *R.C. Harris 46046* (NY).

2. *Punctelia jeckeri* (Roum.) Kalb, Biblioth. Lichenol. 95: 312. 2007.

Sticta jeckeri Roum., Revue Mycol. 3: 33. 1881. TYPE: "sur les roches de la cascade Crévent (Deux-Sévres)", *M. Jecker s.n. = Lichenes Gallici Exs. 245* (UPS [n.v.], lectotype).

Parmelia caperata var. *ulophylla* Ach., Lichenogr. Univ., p. 458. 1810. TYPE: **SWITZERLAND:** sine loc, *J. Schleicher s.n.* (H-ACH-1338[n.v.], lectotype).

Parmelia rudecta var. *ulophylla* (Ach.) Ach., Synops. Lich., p. 197. 1814.

Parmelia borreri var. *ulophylla* (Ach.) Nyl., Flora, 55: 547. 1872.

Parmelia ulophylla (Ach.) F. Wilson, Pap. Proc. R. Soc. Tasm., p. 172. 1893.

Parmelia dubia var. *ulophylla* (Ach.) Harm., Bull. Soc. Sci. Nancy, ser. 2, 31: 224. 1897.

Imbricaria borreri var. *ulophylla* (Ach.) Jatta, Nov. Giorn. Bot. Ital., new series, 9: 470. 1902.

Punctelia ulophylla (Ach.) van Herk & Aptroot, Lichenologist, 32: 239. 2000.

Figure 17.

Published color illustrations. – Brodo et al. (2001, as *Punctelia subrudecta*), St. Clair (1999, as *P. subrudecta*).

Description. – See van Herk & Aptroot (2000) under the name *Punctelia ulophylla* (Ach.) van Herk & Aptroot.

Discussion. – In North America, this taxon is restricted to the western portion of the continent, and its distribution centers around two geographic regions: the Pacific Coast of California, Oregon, and Washington; and the arid mountains of Arizona, Colorado, and New Mexico north to Alberta. The species typically occurs on the bark of hardwoods in coastal regions, and occurs on conifers in the arid interior. Further study of populations from southwestern North America is needed to determine the eastern edge of the range of this taxon, and whether it is sympatric with *Punctelia caseana* in this region, as has been suggested by Egan (2002).

Tucker et al. (2006) reported this taxon for the first time from North America under the name *Punctelia ulophylla* based on a specimen from San Benito County, California. We have examined the collection and confirmed its identity as *P. jeckeri*.

Exsiccati specimens examined. – *Lich. Exs. ASU 132* (NY).

Selected specimens examined. – **CANADA. ALBERTA:** Elk Island National Park, 1961, *G.W. Scotter 657* (CANL). **BRITISH COLUMBIA:** Gulf of Georgia, Galiano Island, 1977, *T. Ahti 34085 & W.J. Noble* (CANL). **MANITOBA:** 40 mi SW of Winnipeg, 1969, *I.M. Brodo et al. 15987* (CANL). **U.S.A. ARIZONA.** GRAHAM CO.: Coronado National Forest, Mount Graham, 1995, *S.D. Sharnoff & S. Sharnoff 1644.24* CANL, photo voucher for Brodo et al. (2001)). PIMA CO.: Santa Catalina Mtns, Mount Lemmon, 1972, *T.H. Nash 4019* (CANL). **CALIFORNIA.** CONTRA COSTA CO.: Briones Regional Park, 1991, *S.D. Sharnoff & S. Sharnoff 833.1* (CANL). LAKE CO.: end of Hell's Peak Rd., 1973, *D. Toren 978* (CANL, fertile). MONTEREY CO.: UC Hastings Reservation, 1992, *S.D. Sharnoff & S. Sharnoff 844.03* (CANL). RIVERSIDE CO.: Santa Ana Mountains, Santa Rosa Plateau, 2008, *J.C. Lendemer 14695 & K. Knudsen* (NY), *J.C. Lendemer 14701 & K. Knudsen* (NY), *J.C. Lendemer 14739 & K. Knudsen* (NY). SAN BENITO CO.: Pinnacles National Monument, 2005, *S.C. Tucker et al. 38694* (SBBG). SAN LUIS OBISPO CO.: Green Valley and W of Black Mtn., 1999, *R.E. Riefner 20-290-A* (NY); Los Padres National Forest, Hi Mtn., 2000, *C. Bratt 11619* (NY); Cuesta Ridge, 1974, *McLeod et al. 11912-B* (CANL). SANTA BARBARA CO.: Channel Islands, Santa Rosa Island, 2006, *K. Knudsen et al. 7687-A* (NY, mixed with *P. borreri*); Santa Cruz Island, 2007, *I.M. Brodo et al. 31925* (CANL). **COLORADO.** BOULDER CO.: Boulder Canyon above

the “Narrows”, 1994, *W.A. Weber L-89611 & J. Corbridge* (NY). JEFFERSON CO.: Coal Creek Canyon, 8.iii.1962, *S. Shushan 1129* (CANL, NY). **NEW MEXICO.** OTERO CO.: Cloudcroft, 2001, *I.M. Brodo 30444 & F. Brodo* (CANL). **OREGON.** BENTON CO.: Oregon State University Campus, 2008, *B. McCune 29576* (NY, OSC). **WASHINGTON.** SAN JUAN CO.: central and E part of San Juan Island, 1969, *I.M. Brodo et al. 11569B* (CANL).

Comparative specimen examined. – **AUSTRIA:** Steiermark, Steirisches Randgebirge, 2005, *W. Obermeyer 10786* (NY, = *Dupla Graecensia Lichenum 467*).

3. *Punctelia perreticulata* (Räsänen) G. Wilh. & Ladd, Mycotaxon 28: 249. 1987.

Parmelia dubosquii var. *perreticulata* Räsänen *in* Sbarbaro, Ann. Mus. Civic. Storia Nat. Genova 41: 40. 1941. TYPE: Italy, Liguria, Spotorno, 1936, *C. Sbarbaro* (= *Lichenotheca parva 72*) (H[n.v.], epitype).

Parmelia perreticulata (Räsänen) Hale, Southwest. Naturalist 3: 212. 1959.

Figure 18.

Description. – See Wilhelm & Ladd (1987).

Discussion. – While Wilhelm and Ladd (1987) correctly separated *Punctelia caseana* from *P. perreticulata*, they used the name *P. subrudecta* for the former taxon because, at that time, conidia from the type of *P. subrudecta* had not been studied. It was only when Adler and Ahti (1996) reported on the separation of *P. perreticulata* and *P. subrudecta* that the difference in conidium-type between the North American material referred to *P. subrudecta* (hereafter referred to as “*P. subrudecta* auct Amer.”) and that of the type of *P. subrudecta* was actually

realized. These authors included *P. subrudecta* auct. Amer. (= *P. caseana*) in their concept of *P. perreticulata* because both taxa have rod-shaped to filiform conidia, and they considering the statistically significant differences in the length of the conidia (see table 3) to have no taxonomic value.

We believe that Adler and Ahti (1996) included *Punctelia subrudecta* auct. Amer. in their concept of *P. perreticulata* because they did not actually examine North American material of *P. perreticulata* s. str., and that their report of long conidia actually refers to a mixture of *P. caseana* and *P. jeckeri*. This supposition is based on the fact that in North America *P. perreticulata* s. str. is known only from the Ozark Ecoregion and a handful of scattered populations in rare Ozark-like habitats (e.g., glades), and all the specimens cited by Adler and Ahti (1996) were from outside of this region and from other habitats. In fact, specimens of *P. perreticulata sensu* Wilhelm and Ladd (= *P. perreticulata* s. str.) have the short conidia (Harris & Ladd 2005) reported as characteristic of European populations of this species. In light of our results, we reject the emendation of *P. perreticulata* proposed by Adler and Ahti (1996), and recognize this species as originally conceived.

While the confusion discussed above is understandable in light of the lack of available material, we find the subsequent treatment by Aptroot (2003) considerably more puzzling because that author was presumably well acquainted with *Punctelia jeckeri* (syn. *P. ulophylla*), *P. perreticulata*, and *P. subrudecta* as a result of his studies of these taxa in Europe (van Herk & Aptroot 2000). Despite having correctly separated these species in the past, the North American material was all lumped into a broad concept of *P. perreticulata*, following the arguments and implications of Adler and Ahti (1996).

Specimens examined. – **ITALY:** Liguria, Spotorno (Savona), 1952, *C. Sbarbaro s.n.* (NY ×2); Alassio, 1950, *C. Sbarbaro s.n.* (NY). **U.S.A. ARKANSAS.** CARROLL CO.: along US 62 NE of White River, 2000, *R.C. Harris 44601, 44610, 44622* (all NY). GARLAND CO.: Ouachita National Forest, 1993, *S.D. Sharnoff & S. Sharnoff 1058.13* (CANL). **MISSOURI.** CARTER CO.: Peck Ranch Conservation Area, 1997, *R.C. Harris 40429* (NY). OZARK CO.: Mark Twain National Forest, Smoke Tree Scenic Lookout, 1997, *R.C. Harris 41393* (NY). SHANNON CO.: Ozark National Scenic Riverways along CR NN, 1990, *R.C. Harris 25723* (NY). STONE CO.: Ashe Juniper Natural Area, 2002, *R.C. Harris 44685, 46702* (both NY). WASHINGTON CO.: Hughes Mountain Conservation Area, 2002, *A. Amtoft 326* (NY). **ILLINOIS.** GALLATIN CO.: Gallatin Co., Shawnee National Forest, 1999, *I.M. Brodo 29732* (CANL). **OHIO.** SCIOTO CO.: Shawnee State Forest, Copperhead Lookout, 2006, *J.C. Lendemer et al. 7230* (NY).

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Conservancy. The molecular data obtained during this study was generated using the facilities of the Lewis B. and Dorothy Cullman Progam for Molecular Systematics.

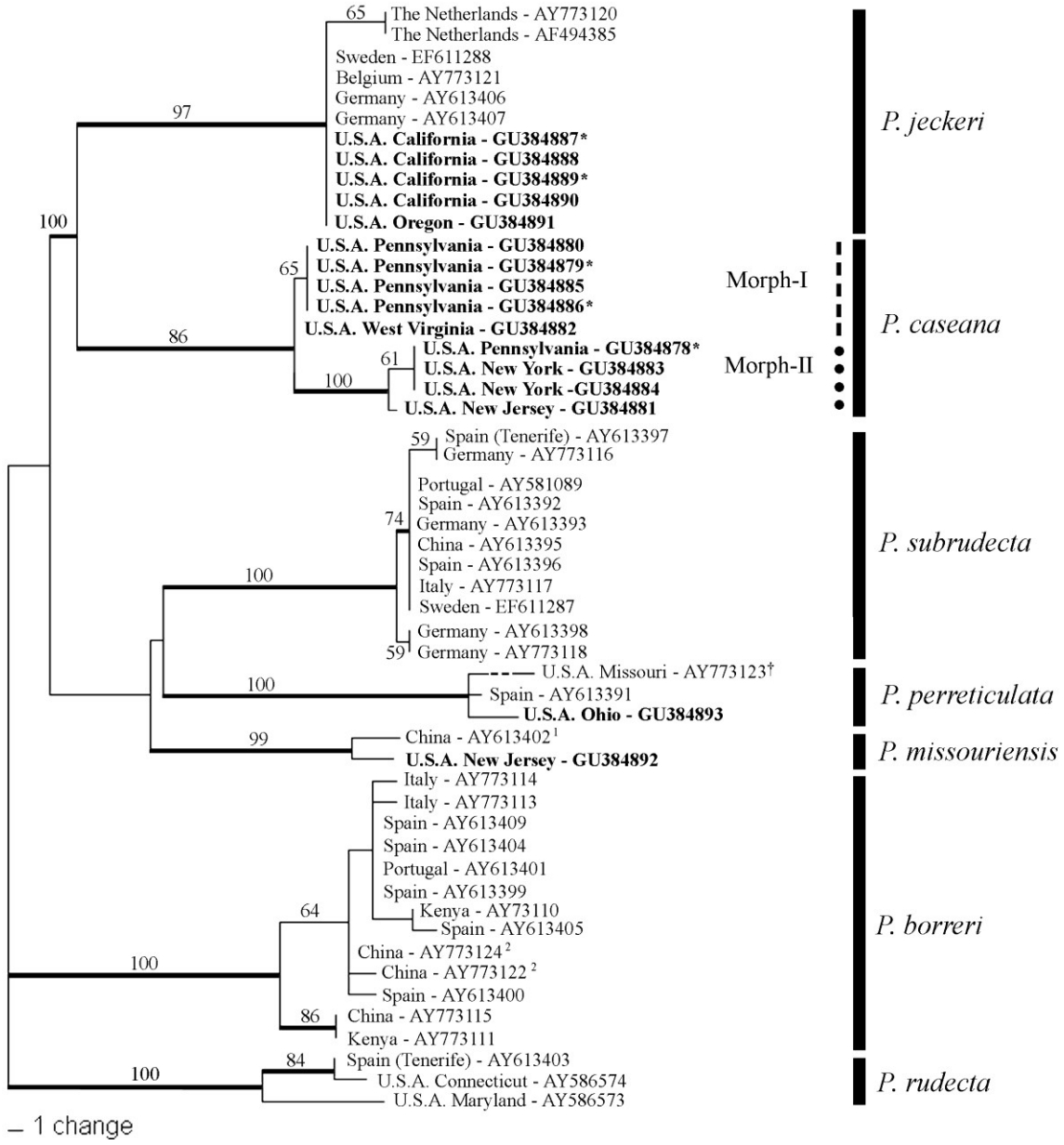

Figure 15. Phylogeny of *Punctelia subrudecta* s.l. and its relatives based on ITS1, 5.8S, and ITS2 sequence data, inferred using weighted maximum parsimony (MP). MP-bootstrap proportions (BP) from 1000 resamplings are shown at each node that has a support value >50%; branches with MP-BP support >70% are thickened. For sequences generated as part of this study, the locality and accession number are emboldened. An asterisk (*) following a sequence indicates that we have found and examined conidia in the voucher specimen. A dagger (†) is used to denote a sequence on a long terminal branch (12.77 changes in length) that has been shortened for space (as indicated by the hashed branch). Based on our analysis, it is likely that the following sequences in GenBank represent misidentifications: [1] – AY613402 as "aff. rudecta" is likely *P. missouriensis*; [2] – AY773122 and AY773124 as "perreticulata" are likely *P. borreri* (see text for discussion).

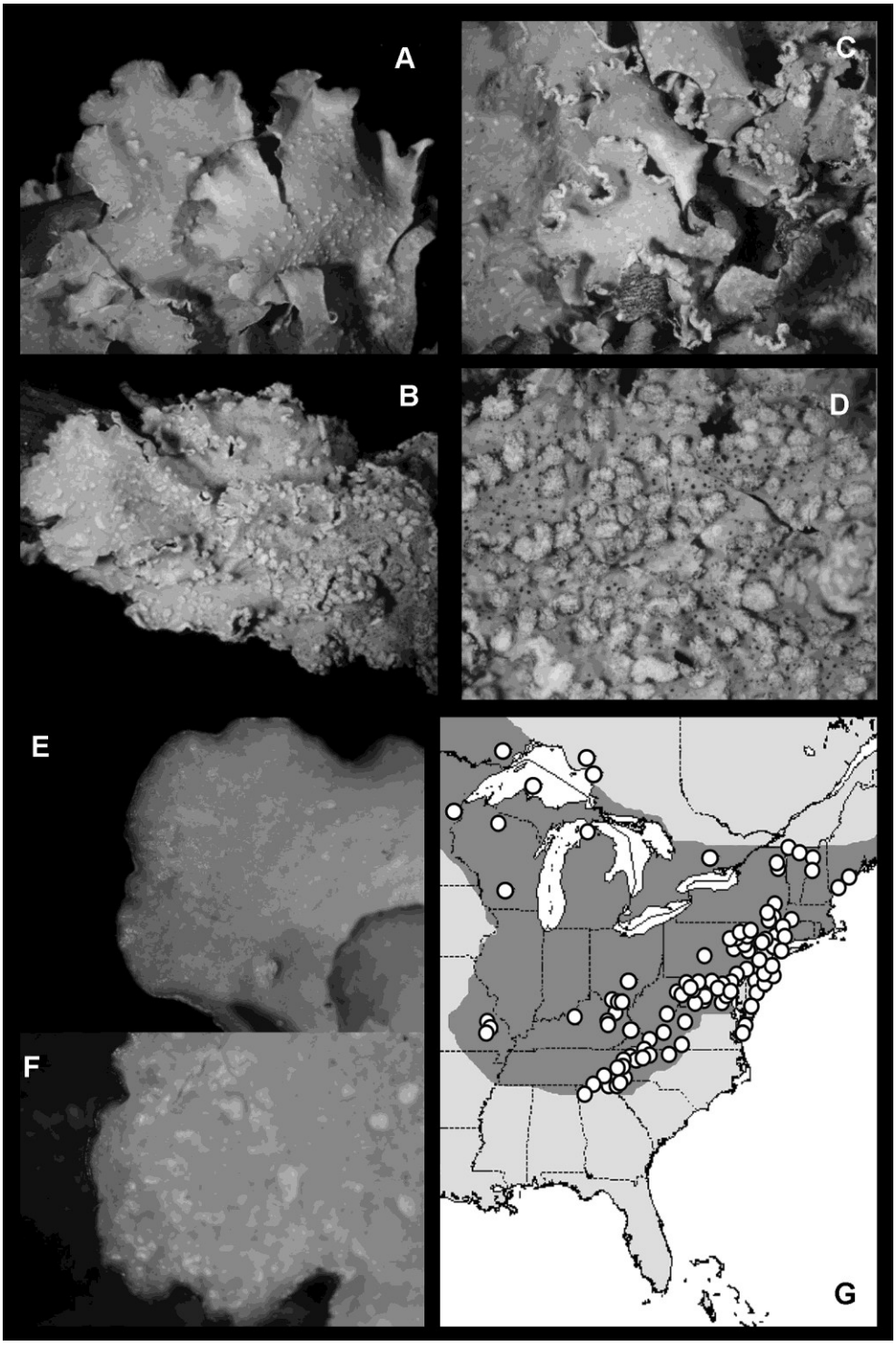

Figure 16. *Punctelia caseana*. Figures A–B, thallus margin, ×0.5 (A = morphotype 1; B= morphotype 2). Figures C–D, soralia, ×1 (C = morphotype 1; D = morphotype 2). Figures E–F, epruinose lobe tips, ×3 (E = morphotype 1; F = morphotype 2). Figure G, geographic distribution based on selected herbarium material at CANL and NY; shaded region approximates the eastern range mapped by Brodo et al. (2001)). Images of morphotype 1 are from *Lendemer 12205* (NY). Images of morphotype 2 are from *Harris 54826* (NY).

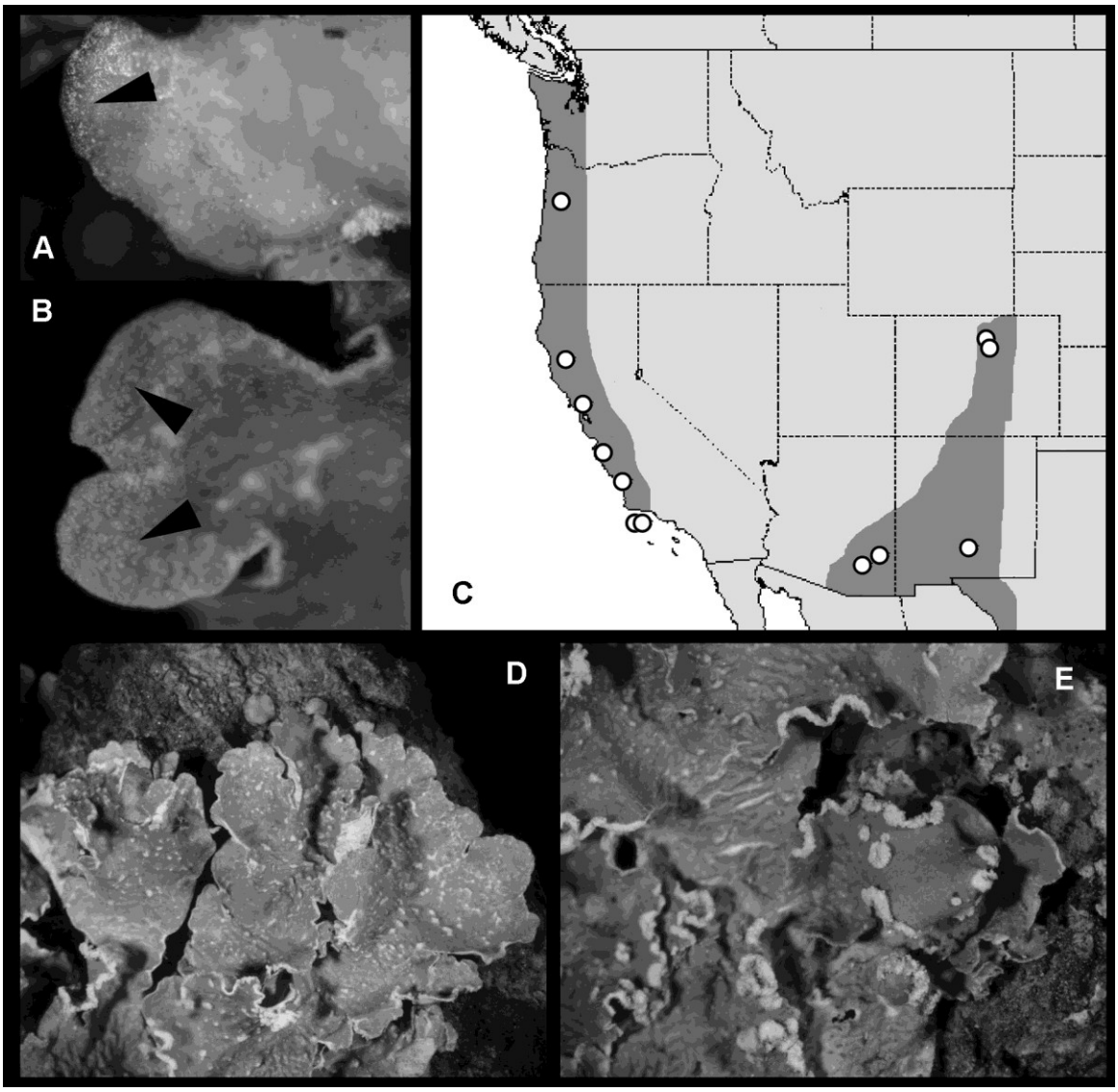

Figure 17. *Punctelia jeckeri* (all from *Lendemer 14695*, NY). Figures A–B, pruinose lobe tips with arrows highlighting position of the pruina, ×5. Figure C, geographic distribution based on herbarium material at NY, shaded regions correspond to western populations mapped by Brodo et al. (2001); note that several populations in Canada are not mapped due to space constraints. Figure D, thallus margin, ×0.5. Figure E, soralia, x1.

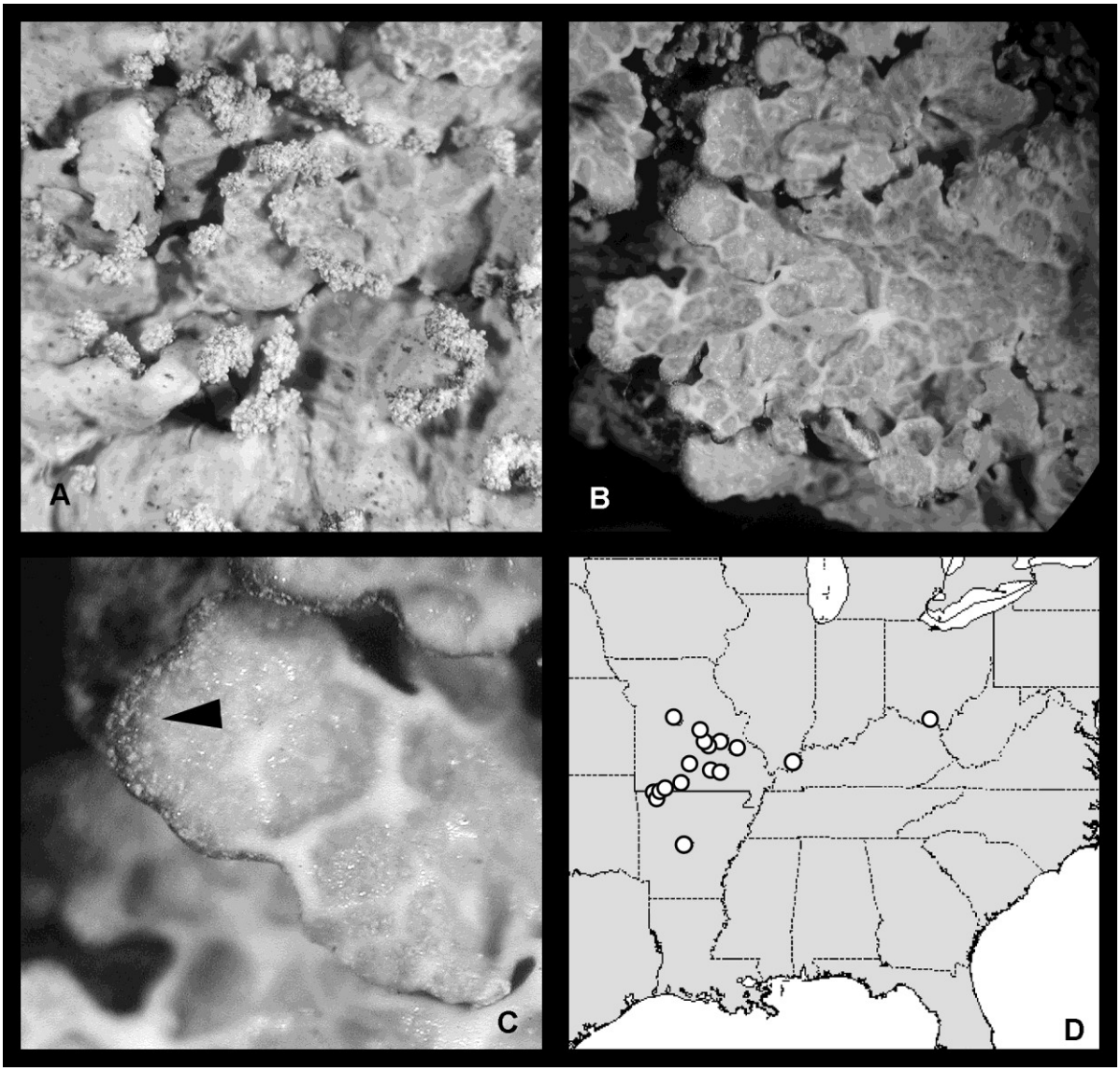

Figure 18. *Punctelia perreticulata* (all from *Lendemer 7230*, NY). Figure A, soralia, ×1. Figure B, thallus margin, ×0.5. Figure C, pruinose lobe margin, ×4. Figure D, geographic distribution based on herbarium material at CANL and NY.

	P. caseana	*P. jeckeri s. str.*	*P. jeckeri s.l.*	*P. perreticulata*	*P. subrudecta*
conidia					
type	filiform	unciform/short-filiform	filiform	filiform	unciform
length	9-12 µm	3-5 µm	9-12 µm	6.5-8 µm	4-6 µm
upper surface	smooth	smooth	smooth	scrobiculate	smooth
pruinose lobe tips	no	yes	yes	yes	no

Table 1. Tabular comparison of the species discussed in this study.

Taxon	Isolate No.	Genbank No.	Collection	Locality
P. caseana	NY47	GU384878	*J.C. Lendemer 12563*	U.S.A., Pennsylvania, Carbon Co.
	NY48	GU384879	*J.C. Lendemer 12733*	U.S.A., Pennsylvania, Lackawanna Co.
	NY81	GU384880	*J.C. Lendemer 13604*	U.S.A., Pennsylvania, Wyoming Co.
	NY160	GU384881	*J.C. Lendemer 15041*	U.S.A., New Jersey, Salem Co.
	NY277	GU384882	*J.C. Lendemer 9896*	U.S.A, West Virginia, Pocahontas Co.
	NY280	GU384883	*J.C. Lendemer 11541*	U.S.A., New York, Rockland Co.
	NY282	GU384884	*R.C. Harris 54826*	U.S.A., New York, Dutchess Co.
	NY283	GU384885	*J.C. Lendemer 13805*	U.S.A., Pennsylvana, Wyoming Co.
	NY284	GU384886	*J.C. Lendemer 13428*	U.S.A, Pennsylvania, Wyoming Co.
P. jeckeri	NY273	GU384887	*K. Knudsen 7687*	U.S.A., California, Santa Barbara Co.
	NY274	GU384888	*J.C. Lendemer 14695*	U.S.A., California, Riverside Co.
	NY275	GU384889	*J.C. Lendemer 14701*	U.S.A., California, Riverside Co.
	NY276	GU384890	*J.C. Lendemer 14739*	U.S.A., California, Riverside Co.
	NY323	GU384891	*B. McCune 29576*	U.S.A., Oregon, Benton Co.
P. missouriensis	NY164	GU384892	*J.C. Lendemer 15006*	U.S.A., New Jersey, Salem Co.
P. perreticulata	NY272	GU384893	*J.C. Lendemer 7230*	U.S.A., Ohio, Scioto Co.

Table 2. Data for voucher specimens from which sequences were generated for this study.

CHAPTER III – IMPLEMENTATION OF A UNIFIED TAXONOMY INTEGRATING MOLECULAR AND NON-MOLECULAR DATA IN APODETIATE *CLADONIA* SPECIES IN NORTH AMERICA[5]

James C. Lendemer & Brendan P. Hodkinson

Abstract. – The taxonomy of the North American apodetiate and superficially apodetiate species of *Cladonia* with persistent, primarily squamulose thalli (viz., *C. apodocarpa*, *C. caespiticia*, and *C. petrophila*) is revised using morphological, chemical, and ecological data in combination with molecular phylogenetic analyses of ITS1, 5.8S, and ITS2 sequence data. The results of this approach confirm some established taxonomic concepts in the group and raise doubts about others. The taxon known as *C. apodocarpa* is well supported by likelihood- and parsimony-based molecular phylogenetic inferences as well as morphology, chemistry, and ecology. However, molecular evidence gives an equivocal answer to the question of whether *C. petrophila* is a distinct monophyletic entity or if *C. apodocarpa* is nested within it. In addition, no phylogenetic structure is detected for the chemical races of *C. petrophila* with and without fumarprotocetraric acid, based on the markers used in these analyses. However, *C. petrophila* does remain well supported as a taxon by chemistry (i.e., the presence of sphaerophorin), morphology, and ecology. A morphologically and ecologically distinct new taxon is described as *C. stipitata*. We confirm a distant relationship of these taxa (*C. apodocarpa*, *C. petrophila*, and *C. stipitata*) to *C. caespiticia*, a species with stipitate or pseudopodetiate (vs. sessile) apothecia. A treatment of the North American apodetiate species of *Cladonia* including distribution maps,

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cited specimens, and illustrations is presented. A key to the taxa that includes unrelated, frequently sterile species of *Cladonia* found in eastern North America is also provided.

Introduction

Species of the genus *Cladonia* P. Browne are generally characterized by their distinctive fruticose podetia, which often bear terminal apothecia. There is, however, a small group of species currently classified in *Cladonia* sect. *Helopodium* (Ach.) S. Stenroos in which the podetia are extremely reduced or absent and whose thalli consist of conspicuous, persistent, primary squamules (Ahti 2000, Stenroos et al. 2002, Jahns & Beltman 1973). In North America, this group is represented by three described species, *C. apodocarpa* A. Evans, *C. caespiticia* (Pers.) Flörke, and *C. petrophila* R.C. Harris, all of which are restricted to the eastern portion of the continent. Thalli of these species are commonly encountered and frequently sterile, or at least appear so in the field. Thus, it is not surprising that these taxa are perceived as taxonomically difficult and as such often ignored by collectors because their collection and identification is seen as a futile pursuit (Harris 1992). However, as is evidenced by the description of new species by Harris (1992) and Ahti (2000), collection and study of this group is not without reward.

With the above in mind, we have made a distinct effort to collect primarily squamulose thalli of *Cladonia* during our field studies in eastern North America in hopes of finding a sufficient way of dealing with these problematic lichens and potentially uncovering additional, overlooked taxa. As specimens accumulated, we became aware of several entities from the Appalachian Mountains whose taxonomic status and relationships were obscured by their cryptic habit and morphology that is reduced compared to other species of *Cladonia*. Because we could not rely solely on so-called "traditional" characters to fully evaluate these entities, we decided to undertake the present study and utilize molecular data to provide an additional dataset against

which we could test our taxonomic hypotheses. Toward this end, we generated ITS sequences from a geographically broad sampling of the collections that we had gathered and aligned these with sequences that we generated from an equally broad sampling of *C. apodocarpa* and *C. petrophila*.

The results of our study clearly illustrate the utility of an approach to alpha-taxonomy that involves integrating molecular data with so-called "traditional" characters of biogeography, chemistry, ecology, and morphology. A unified approach such as we have undertaken here often reveals the taxonomic value of characters that have previously been overlooked or underestimated (Amtoft et al. 2008, Argüello et al. 2007, Arup 2006, Hodkinson & Lendemer 2011, Lendemer & Hodkinson 2010). In the present study ecological characters and vegetative morphology were found to be of unexpected value and, when combined with molecular data, led us to recognize four apodetiate species of *Cladonia* in eastern North America: *C. apodocarpa, C. caespiticia, C. petrophila*, and the newly described *C. stipitata* Lendemer & Hodkinson.

Materials and Methods

Fieldwork and herbarium materials. – Since learning of the existence of *Cladonia petrophila*, we have made a special effort to observe and collect primarily squamulose *Cladonia* thalli and their associated ecological data in conjunction with our fieldwork. The result of this effort has been the accumulation of hundreds of freshly-collected herbarium specimens from throughout eastern North America that represent an unbiased sample of the apodetiate *Cladonia* species that occur in the region. These specimens served as the primary resource for this study and have been deposited in the herbarium of The New York Botanical Garden (NY). Due to the unassuming nature of these taxa and the assumption that they are often sterile and thus unidentifiable, they are often poorly represented in herbaria. As such, we limited this study to our

own collections and the holdings of undetermined sterile *Cladonia* specimens, *C. apodocarpa, C. caespiticia,* and *C. petrophila* at DUKE and NY.

Molecular methods. – DNA extractions were performed at NY using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) with the instructions modified to include a prolonged (~12 hour) incubation period in the lysis buffer. Isolated DNA was resuspended in 100 µl of sterile water and stored at -20°C. PCR amplification was performed at NY using the primers ITS4 and ITS5 (White et al. 1990). Amplification reactions of 25 µl contained each of the following: 2.5 µl 10X PCR Buffer (Qiagen), 2.5 µl dNTP solution (mixed to a concentration of 2 mM of each dNTP), 2.5 µl BSA solution (mixed to a concentration of 2.5 mg μl^{-1} of bovine serum albumin; Hillis et al. 1996), 0.2 µl Taq DNA Polymerase (= 1 U; Qiagen), 1 µl of each primer (in solution at a concentration of 10 µM), 9.3 µl of sterile water, 1 µl of extracted DNA, and 5 µl of Q-solution (Qiagen). PCR protocol followed Nelsen et al. (2007) and consisted of an initial denaturation of 95°C for 5 min, followed by 10 cycles of 95°C for 1 min, 62°C for 1 min, and 72°C for 1 min, then 35 cycles of 95°C for 1 min, 53°C for 1 min and 72°C for 1 min with a final extension for 7 minutes at 72°C. PCR products were visualized prior to sequencing by UV examination of a 1% agarose gel on which 1 µl of amplified PCR product had been subjected to electrophoresis and stained with ethidium bromide. Unpurified amplified PCR products were sent to the University of Washington Biochemistry DNA Sequencing Facility (BDSF) for sequencing. Sequences were assembled and manually edited using the software package Sequencher™ 4.9 (Gene Codes Corporation, Ann Arbor, MI, USA). In order to identify contaminants, nearest sequence matches were found by searching the nucleotide collection in GenBank using BLASTn (Altschul et al. 1997).

Taxon Sampling. – The goal of the present study was to resolve the placement of several groups of apodetiate *Cladonia* populations from eastern North America that we considered to potentially represent distinct taxonomic entities. We conducted analyses of a dataset consisting of sequences from the nuclear ribosomal ITS region (ITS1, 5.8S, and ITS2). In addition to sequences (*n*=12) that we generated from populations of the putative taxa, we generated additional sequences from populations representing typical *C. apodocarpa* (*n*=5), *C. caespiticia* (*n*=1), and *C. petrophila* (*n*=7) to supplement those available in GenBank (one sequence for each of the aforementioned taxa). For outgroups, we used selected sequences from Stenroos et al. (2002) that (1) represented taxa for which more than one sequence of the full ITS region was available and (2) could be reasonably aligned with those from the members of section *Helopodium* that we studied without the need to exclude/recode large amounts of the analyzed nucleotide alignment (see "*Sequence Alignment*" and "*Phylogenetic Analyses*" sections below, and note that similar criteria are outlined by Hodkinson & Lendemer (2011) and Lendemer & Hodkinson (2010)).

Sequence Alignment. – Sequences were initially aligned using ClustalX 2.0 (Larkin et al. 2007), and were subsequently adjusted manually in Mesquite 2.6 (Maddison & Maddison 2009) taking into consideration rRNA secondary structure (Kjer 1995) based on models developed by Beiggi and Piercey-Normore (2007) for *Cladonia grayi* G. Merr. *ex* Sandst. During the process of sequence alignment, it became clear (by comparing our sequences to the reference sequences from section *Helopodium*) that, as part of the editing process, numerous insertions representing sites that were apparently unique to a given species had inadvertently been deleted from the

reference sequences. When an insertion was present in all sequences of a particular taxon in section *Helopodium*, except for the reference sequence, the 'gap' symbol ('-') in the reference sequence was replaced by the 'missing' symbol ('?') to offset the effect of this potentially erroneous sequence editing (since 'gap' was treated as a fifth character state in subsequent analyses). The alignment used for these analyses was deposited in the Dyrad data repositiory (doi:10.5061/dryad.nv1sv92n).

Phylogenetic Analyses. – Weighted maximum parsimony (MP) inferences were made using PAUP* 4.0b10 (Swofford 2001). Introns, nucSSU/nucLSU residues, constant sites, and ambiguously-aligned regions were excluded from analyses. Ambiguously aligned regions were re-coded using INAASE 3.0 (Lutzoni et al. 2000), reintegrated as new characters, and subjected to specific step-matrices (as outlined by Hodkinson & Lutzoni (2009)); for a review of logistical details regarding reintegrating characters and implementing step-matrices, see Hodkinson & Lendemer (2011)). Unambiguously-aligned portions were subjected to symmetric step matrices computed in STMatrix 3.0 (Miadlikowska et al. 2002; available at http://www.lutzonilab.net/pages/download.shtml) as outlined by Gaya et al. (2003, 2008). For each dataset, a first round of searches was performed with 1000 random-addition-sequence (RAS) replicates and TBR (tree bisection-reconnection) branch swapping. The MULTREES option was in effect and zero-length branches were collapsed. This analysis resulted in the best tree island being hit in 75.2% of the RAS replicates. Branch support was estimated through bootstrap analyses (Felsenstein 1985) by performing 1000 bootstrap replicates with 5 RAS per bootstrap replicate, with all other settings as above. Maximum likelihood (ML) topology and bootstrap searches were performed using RAxML 7.0.4 with GTRMIX and 1000 resamplings

(Stamatakis 2006).

Chemical and Morphological Analyses. – All specimens were studied dry using a Bausch & Lomb StereoZoom 7 dissecting microscope. Macromorphological characters of thallus size, squamule broadness, branching, aspect, and basal color were noted. All specimens were also subjected to chemical analysis using both standard spot tests (reagents abbreviated following Brodo et al. (2001)) and Thin Layer Chromatography (TLC). TLC was carried out using solvent systems A or C following the standardized methods of Culberson & Kristinsson (1970).

Results and Discussion

I. – General remarks

As has been discussed in the introduction above, the primary goal of the present study was to evaluate the taxonomic status of some unusual apodetiate *Cladonia* populations from the Appalachian Mountains. These populations seemed to be distinct from the three species known to occur in the region (*C. apodocarpa, C. caespiticia,* and *C. petrophila*) and could be grouped into three putative taxonomic entities. These entities have been little discussed in print (e.g., Lendemer & Tripp 2008) and are generally not represented in herbaria because they have either been overlooked or have gone uncollected as a result of their apparent sterility and lack of podetia. We only became aware of them after several years of concentrated fieldwork led us to suspect that more than just *C. apodocarpa*, *C. caespiticia,* and *C. petrophila* were present in the region (although *C. stipitata* had been recognized in NY as a chemotype of *C. petrophila*). In fact, while our study did resolve the status of these putative taxa (see sections IIa–c below), the increased sampling in our molecular dataset also afforded us the opportunity to examine in detail the circumscriptions and relationships of the taxa that had already been described in the group.

The results of our phylogenetic analyses (figure 19) confirmed the findings of Stenroos et al. (2002) that *Cladonia caespiticia* is distantly related to *C. apodocarpa* and *C. petrophila*, and thus that *Cladonia* sect. *Helopodium* is not monophyletic. This distant relationship is not surprising considering that *C. caespiticia* is morphologically dissimilar to other apodetiate species in both vegetative morphology and in the development of the apothecia (see section IIa below). The robust sampling within *C. apodocarpa* and *C. petrophila* s.l. revealed that the ITS region alone does not seem to be sufficient for resolving relationships within this species complex. In the phylogeny presented by Stenroos et al. (2002) the two species, each represented by a single terminal, were found to be sister to each other. However, increased sampling revealed that, while the sequences of *C. apodocarpa* form a well-supported monophyletic clade, it is not clear whether this clade is nested within *C. petrophila* or if the latter species is itself a monophyletic grouping that simply cannot be defined on the basis of ITS sequence data alone. Considering the equivocal nature of the molecular data, and the fact that *C. apodocarpa* and *C. petrophila* are well characterized by a suite of traditional characters (see taxonomic section below), we prefer to retain them as distinct here pending further study with molecular methods.

II. – Discussion and resolution of the putative taxonomic entites

IIa. – *Cladonia caespiticia* s.l.

The first putative taxon that we examined was seemingly restricted to sandstone boulders in boulder fields and talus slopes in Pennsylvania. Thalli from these populations were consistently sterile but distinctive because of their large primary squamules (often > 1 cm long), otherwise resembling those of *Cladonia caespiticia* in being thin, delicate, and finely incised.

While the morphological similarity of these populations to *C. caespiticia* was further correlated to chemistry (i.e., the presence of only fumarprotocetraric acid), their ecology and size were striking enough to warrant further study.

We generated sequences from two populations of this putative taxon and included them in our analysis with a sequence of *Cladonia caespiticia* from GenBank as well as one that we generated from a typical fertile population in southern New Jersey. In the results of our analysis (Plate 1), the sequences derived from the unusual saxicolous populations from Pennsylvania form a well-supported monophyletic group with those of typical *C. caespiticia*. *Cladonia caespiticia* is generally considered to be a terricolous species that can also occur on the bases of trees and on mossy shaded rocks. Our results indicate that the species has a wider ecological amplitude than previously thought. This conclusion was further supported when we examined the holdings of *C. caespiticia* at NY and found several saxicolous collections that were fertile and otherwise morphologically typical in having small squamules.

Interestingly, our results also strongly support the taxonomic value of vegetative morphology in circumscribing this taxon. The aberrant populations that we examined differed from typical *Cladonia caespiticia* only in their larger size and ecology. Our molecular analyses also confirmed the distant relationship of *C. caespiticia* to the rest of the known North American apodetiate taxa. This distant relationship is further supported by differences in the morphology of the apothecia; while the apothecia of *C. apodocarpa, C. petrophila,* and a new member of this group (described below) are sessile on the squamules at maturity, the apothecia of *C. caespiticia* are distinctly elevated because they form a stipe or short ecorticate pseudopodetium (Brodo et al. 2001, figure 217).

IIb. – *Cladonia petrophila* s.l.

The second putative taxon that we examined was restricted to shaded granitic rocks at low- to middle- elevations in the southern Appalachian Mountains. The populations were sympatric with, and morphologically identical to, *Cladonia petrophila* but differed chemically in the absence of fumarprotocetraric acid. The presence of this chemotype was not mentioned in the protologue of *C. petrophila* (Harris 1992) and it does not appear to have been mentioned otherwise in print. After collecting the chemotype several times during fieldwork in North Carolina we examined the holdings of *C. petrophila* at NY and discovered several additional specimens from other portions of the southern Appalachians.

Because we could not detect any ecological or morphological differences between the two chemotypes, and both were found at the same locality, we decided to use molecular data to investigate whether a previously unrecognized geographically restricted cryptic taxon was involved. We generated five sequences from three populations of the chemotype that lacks fumarprotocetraric acid. We then generated an additional seven sequences of the typical chemotype of *Cladonia petrophila* from seven populations across its geographic range including one from a region where the chemotypes are sympatric. These additional sequences were generated both to increase the robustness of our sampling and to supplement the one sequence available in GenBank (AF455222[6]). Our analyses of these sequences (Plate 1) revealed that the chemotypes do not seem to represent distinct monophyletic groups and thus the hypothesis that the chemotype lacking fumarprotocetraric acid is a distinct taxon is not supported by molecular data from the ITS region. We propose that the circumscription of *C. petrophila* should be expanded to include populations that lack fumarprotocetraric acid.

[6] Unfortunately the chemical content of the voucher upon which AF455222 is based is neither noted in GenBank nor in print (Stenroos et al. 2002), and we have not reviewed the specimen.

While conducting background research for this study, we discovered that the chemical phenomenon observed in *Cladonia petrophila* appears to be widespread in the genus *Cladonia.* There are apparently a number of cases in which species that produce major secondary metabolites in addition to fumarprotocetraric are known to have chemotypes in which the latter substance is either present or absent while the primary compound is consistently present (e.g., the two chemotypes of *C. grayi*). The results of our study of *C. petrophila* would seem to support the stance that such chemotypes should not be treated as distinct taxa, even when they appear to be geographically distinct. We do not, however, believe that our results justify any taxonomic changes outside of the current taxon. Rather we believe that the status of morphologically identical chemotypes should be evaluated with molecular methods on an individual basis.

IIc. – *Cladonia stipitata*

The final putative taxon that we examined has an interesting history of study (see the proceeding taxonomic section) and is an excellent example of the value of a unified approach to alpha-taxonomy. Several years ago the first author (JCL) first encountered a population of this perplexing entity on an exposed low-elevation granitic bald in North Carolina. Although the thalli in this population were morphologically similar to *Cladonia petrophila*, they were chemically different in lacking sphaerophorin and producing atranorin as a major substance. The chemistry (atranorin and fumarprotocetraric acid) drew comparison to *C. apodocarpa*; however, that taxon has much larger squamules and occurs on soil or humus in disturbed habitats. Lendemer and Tripp (2008) recognized that the problem required further study and thus reported the population as *C. petrophila* s.l., noting the absence of sphaerophorin. During subsequent fieldwork the first author and his colleagues at NY found additional populations of this entity at

other granitic balds elsewhere in the southern Appalachians. Study of the undetermined *Cladonia* collections at NY and DUKE also revealed the existence of several additional collections from the region (most of which had been recognized as a chemotype of *C. petrophila* at NY), as well as from massive granite outcrops in the Piedmont of Georgia, and one from a similar habitat in Rhode Island.

Confronted with what seemed to be an ecologically and geographically distinct entity (with a unique morphology that could have been induced by its distinctive ecology), we generated five sequences from three populations. In our analyses (figure 19), these sequences emerged as a well-supported monophyletic group, supporting its status as a taxon distinct from *Cladonia apodocarpa* and *C. petrophila*. We thus describe these populations as a new species, *C. stipitata*, in the following section.

It is noteworthy that the sequences of *Cladonia stipitata* possess a ubiquitous (*n*=5) ~210 base-pair group I intron. The presence of this intron was confirmed both by gel electrophoresis and sequencing. The latter method revealed that the intron is actually found in the small portion of the nucSSU rDNA that is amplified by the ITS primers used in this study. Interestingly, the intron was entirely absent in the sequences of *C. apodocarpa* (*n*=5) and *C. petrophila* (*n*=12) examined. In *C. caespiticia* it was present in two of the three sequences that we generated. The situation found in *C. caespiticia* is apparently typical in *Cladonia* (Myllys et al. 2003); however, the ubiquity of this intron in one phylogenetic species (i.e., *C. stipitata*) and its complete absence in a closely related clade (i.e., *C. apodocarpa/petrophila*) suggests that there may be forces selecting for the presence and/or absence of introns in this group of species. It has been shown previously that introns can improve transcriptional and translational yield in fungi (Juneau et al. 2006), which can be especially beneficial under stressful conditions (Parenteau et al. 2008). The

trend seen here may be correlated with the fact that *C. stipitata* lives in an environment that is more barren and receives more ultraviolet radiation than the environments inhabited by *C. apodocarpa* and *C. petrophila*; however, further study is required before any conclusions of this nature can be drawn.

III. – Taxonomic Section

IIIa – Key to the frequently-sterile *Cladonia* species of eastern North America*

1. Squamules C+ green (strepsilin present)……..*C. strepsilis* (Ach.) Grognot
1. Squamules C- (strepsilin absent)…….2

2. Squamules extremely large (10–20 mm long) and branching; P+ red, K+ yellow (fumarprotocetraric acid and atranorin present); SE coastal plain……*C. prostrata* A. Evans
2. Squamules mostly smaller (<10 mm long) and not branching; chemistry various; distribution various……...3

3. On calcareous substrates; squamules thick and appressed to the substrate, appearing foliose; podetia cupped when present; northern…….…….....*C. pocillum* (Ach.) Grognot
3. On non-calcareous substrates; squamules not as above, usually ascending; podetia rare or unknown……4

4. Squamules yellowish-green, KC+ golden (usnic acid and barbatic acid present)...*C. robbinsii* A. Evans

4. Squamules gray or blue-gray, KC- (usnic acid and barbatic acid absent; atranorin present or absent) ……...……5

5. Medulla UV+ blue-white (sphaerophorin present), P± red (fumarprotocetraric acid present or absent); typical of rock outcrops in shaded forests…….*C. petrophila* R.C. Harris

5. Medulla UV- (sphaerophorin absent), P+ red (fumarprotocetraric acid always present); typical of other habitats……...6

6. Squamules K+ yellow (atranorin present)…….…7

7. Squamules long and erect, upper portions convex-reflexed to show the white underside, without a blackened narrow stipe; thallus not forming distinct cushions; typical of roadside banks and disturbed areas; widespread…....*C. apodocarpa* Robbins

7. Squamules shorter and not distinctly erect, upper portions concave ± obscuring the underside, with a distinct blackened narrowed stipe; thallus forming low dense cushions or mats; typical of granitic outcrops in sunny openings; generally known from the central-southern Appalachians and Piedmont…*C. stipitata* Lendemer & Hodkinson

6. Squamules K- (atranorin absent)…….....8

8. Squamules large and broad, margins not finely divided; podetia rare, when present forming tall irregular cup-like structures……..……..*C. mateocyatha* Robbins

8. Squamules smaller, narrow, margins finely divided; podetia absent; apothecia when present borne on a stipe/ecorticate pseudopodetium.....……*C. caespiticia* (Pers.) Flörke

*This key does not include a number of species that normally produce podetia but can also be found sterile. The excluded species fall into two main groups: 1) species with sorediate squamules or squamules that break down into granules or micro-squamules, and 2) members of the *Cladonia subcariosa* group with squamules that are generally short and not easily confused with the squamules of species in this key. Additionally, the most frequently sterile members of the *C. subcariosa* group have distinctive ecologies (they tend to occur on soil and sand in disturbed areas) and chemistries (e.g., *C. brevis* (Sandst.) Sandst. contains psoromic acid, *C. polycarpia* G. Merr. contains stictic acid, and *C. polycarpoides* Nyl. contains norstictic acid). *Cladonia sobolescens* Nyl. *ex* Vain., a member of the *C. subcariosa* group with fumarprotocetraric acid, is rarely sterile; however, sterile specimens of this species would be almost impossible to determine in light of the numerous other species with small squamules and similar chemistries.

IIIb. – The Species

Although we recognize that the group of taxa treated here is artificial, we believe that a treatment of the four species is useful in light of their superficial similarities. It is also useful because when Harris (1992) described *Cladonia petrophila* he drew comparisons to *C. apodocarpa* and *C. caespiticia*. Because the aforementioned species have been well illustrated and described in recent publications (Ahti 2000, Brodo et al. 2001, Harris 1992, Hinds & Hinds 2007), we present abbreviated treatments for these taxa below and describe only the new species *C. stipitata* in detail.

1. *Cladonia apodocarpa* Robbins, Rhodora, 27: 211. 1926. TYPE: **U.S.A. MASSACSHUETTS.** PLYMOUTH CO.: Wareham, 1924, *C.A. Robbins s.n.* (FH[n.v.], lectotype [selected by Ahti (1993)]).

Figure 20.

Descriptions. – Ahti (2000: 237), Thomson (1967: 87).

Published color illustration. – Brodo et al. (2001: 238 [fig. 210]).

Chemistry. – Atranorin (major), fumarprotocetraric acid (major). Spot tests: K+ yellow, C-, KC-, P+ red, UV-.

Ecology. – This species typically occurs on soil and humus in recently disturbed habitats or other areas such as forest openings where succession is arrested because of natural factors such as periodic fire. It typically occurs in edge habitats where it is not fully exposed to the sun

but rather protected by shade, at least periodically. Although we have not observed saxicolous populations of *Cladonia apodocarpa*, several specimens we examined listed the ecology as "vertical rock faces" or "boulders in light shade". Thalli in these specimens appeared to represent typical *C. apodocarpa* and almost certainly occurred on a layer of soil or humus rather than directly on rock.

Distribution. – *Cladonia apodocarpa* is known primarily from eastern North America, where it is common and widespread at low to middle elevations. Although the species is most common south of the boreal region, records from as far north as Quebec have been reported (Thomson 1967). Ahti (2000) also reported the species from a single locality in Uruguay and noted a questionable literature report by Thomson (1967) from Haiti.

Discussion. – Due to the large size of its squamules, *Cladonia apodocarpa* is not easily confused with any of its close relatives, namely *C. petrophila* and *C. stipitata*. Rather, it is likely to be confused with sterile thalli of members of the *C. subcariosa* group, with which it frequently occurs. Members of the *C. subcariosa* group that occur in eastern North America differ chemically from *C. apodocarpa* in lacking the combination of atranorin and fumarprotocetraric acid. The primary squamules of taxa in the *C. subcariosa* group also tend to be coarser, less fragile, and shorter than those of *C. apodocarpa* although these characters are variable and distinguishing among species in the field (i.e., without access to chemical data) can be difficult. In most cases, however, careful examination of a given, apparently sterile population will reveal the presence of some podetia. Several species of *Cladonia* with cushion-forming primary squamules that could be confused with *C. apodocarpa*, particularly *C. robbinsii* and *C. strepsilis*, are distinguished in the above key.

Although the apothecia of *Cladonia apodocarpa* have generally been described as being sessile on the primary squamules of the thallus (Brodo et al. 2001, Evans 1930, Hinds & Hinds 2007, Robbins 1925, Thomson 1967), they were recently noted to be borne on short podetia by Ahti (2000). Unfortunately, we cannot confirm either interpretation because mature apothecia were not present on any of the specimens that we examined.

Selected specimens examined. – **U.S.A. ALABAMA.** CLAY CO.: Talladega National Forest, Cheaha Wilderness, 24.i.1992, *R.C. Harris 28367* (NY**). JACKSON CO.:** Pisgah, 2.x.1999, *R.C. Harris 43354* (NY). **ARKANSAS.** BAXTER CO.: Ozark National Forest, Leatherwood Wilderness, 18.iv.2005, *R.C. Harris 51178* (NY). FRANKLIN CO.: Ozark National Forest, Spy Rock Hollow, 8.vi.2002, *W.R. Buck 37245* (NY). GARLAND CO.: Hot Springs National Park, 22.v.2001, *C.M. Wetmore 26025* (NY). IZARD CO.: NE corner of Devil's Knob-Devil's Backbone Natural Area, 24.x.2001, *R.C. Harris 45372* (NY). JEFFERSON CO.: Pine Bluff Arsenal, 1.xii.1999, *D. Ladd 21818 & M. Pederson* (NY). MADISON CO.: Madison County Wildlife Management Area, 2.xi.2000, *R.C. Harris 44674* (NY). NEWTON CO.: Ozark National Forest, Boston Mountains, 24.iv.1988, *R.C. Harris 21486* (NY). POPE CO.: Ozark National Forest, Kings Bluff, 7.xi.2002, *R.C. Harris 46874* (NY). SEARCY CO.: Buffalo National River, N of Woolum Campground, 17.iv.2005, *R.C. Harris 51020* (NY). SHARP CO.: Strawberry River Preserve, 25.x.2001, *R.C. Harris 45477* (NY). STONE CO.: Ozark National Forest, Gunner Pool Recreation Area, 25.iv.1988, *R.C. Harris 21594* (NY). **DELAWARE.** KENT CO.: Milford Crossroads, 6.xi.1886, *A. Commons 102* (NY). NEW CASTLE CO.: Wilmington, 3.i.1890, *A. Commons s.n.* (NY). SUSSEX CO.: Rt. 113 near Ellendale, xi.1936, *R.H. Torrey s.n.* (NY). **GEORGIA.** DADE CO.: Sitton's Gulf, W side of Lookout Mt.,

17.iii.1939, *R. McVaugh 4731* (NY). DAWSON CO.: Amicalola Falls State Park, 25.xi.2000, *W.R. Buck 38878* (NY). STEPHENS CO.: Chattahoochee National Forest, ~2 km above Toccoa Falls, 21.ix.1992, *R.C. Harris 28000* (NY). TOWNS CO.: Southern Nantahala Wilderness, Hightower Gap to Rich Knob, 11.xi.2007, *J.C. Lendemer et al. 10948* (NY). WALKER CO.: Chattahoochee National Forest, Keown Falls, 23.ix.1992, *R.C. Harris 28277* (NY). **ILLINOIS.** COLES CO.: Fox Ridge State Park, 19.ix.1986, *T. Motley 32* (NY). POPE CO.: Shawnee National Forest, Hayes Creek, 16.x.1993, *R.C. Harris 31405* (NY). UNION CO.: Berryville Shale Glade Nature Preserve, 6.xi.2004, *R.C. Harris 50518* (NY). **INDIANA.** HARRISON CO.: 2.5 mi N of Leavenworth, 28.viii.1945, *C.C. Deam 63646* (NY). **KENTUCKY.** BULLITT CO.: Bernheim Arboretum, 25.iii.2002, *D. Ladd 23331 & M. Ladd* (NY). ELLIOTT CO.: Mauk Ridge, 3.v.1981, *C.F. Reed 112325* (NY). KNOX CO.: Rt. 11, Greenroad, 30.iv.1981, *C.F. Reed 118004* (NY). MARTIN CO.: Wolf Creek, 26.ii.1962, *R.M. Reed 16* (NY). ROWAN CO.: Daniel Boone National Forest, 9.x.1995, *R.C. Harris 36793* (NY). **MARYLAND.** BALTIMORE CO.: Bens Run, 25.ii.1933, *C.C. Plitt 84* (NY). CALVERT CO.: 5 mi N of Solomons Island, 12.x.1962, *C.F. Reed 59078* (NY). CARROLL CO.: 2 mi N of Mt. Airy, 22.iv.1963, *C.F. Reed 61599* (NY). FREDERICK CO.: Sugar Loaf Mt., 19.iv.1962, *C.F. Reed 55765* (NY). GARRETT CO.: 2 mi S of Redhouse, 24.vi.1962, *C.F. Reed 54424* (NY). HARFORD CO.: 2.5 mi S of Dublin, 24.v.1965, *C.F. Reed 69768* (NY). HOWARD CO.: Savage, 17.i.1962, *C.F. Reed 53990* (NY). MONTGOMERY CO.: Plummer's Island, 7.xi.1937, *E.C. Leonard 2710* (NY). PRINCE GEORGES CO.: near Croom, 2.iii.1962, *C.F. Reed 55137* (NY). **MASSACHUSETTS.** BERKSHIRE CO.: Bartholomew's Cobble, 28.iii.1981, *R.C. Harris 13547* (NY). ESSEX CO.: S of Newburyport, 30.v.1973, *C.F. Reed 94215* (NY). FRANKLIN CO.: Town of Rowe, 19.ix.1998, *R.C. Harris 42277* (NY). WORCESTER CO.: Sturbridge, 22.x.1955, *B.N. Gates 112*

(NY). **MISSOURI.** BARRY CO.: Mark Twain National Forest, Piney Creek Wilderness, 27.iii.2006, *R.C. Harris 52186* (NY). CARTER CO.: S of Van Buren, 23.ix.1990, *R.C. Harris 25649A* (NY). CHRISTIAN CO.: Mark Twain National Forest, Devreaux Ridge, 21.v.2003, *R.C. Harris 47683* (NY). GASCONADE CO.: Canaan Conservation Area, 25.iii.2006, *R.C. Harris 52026* (NY). IRON CO.: E slope Royal Gorge, 27.iv.1988, *R.C. Harris 21771* (NY). JEFFERSON CO.: Don Robertson Property, 24.iii.2006, *J.C. Lendemer et al. 5989* (NY). LAWRENCE CO.: Fall Hollow Gorge, 27.iii.2006, *R.C. Harris 52144* (NY). MADISON CO.: Mark Twain National Forest, near N end of CR 408/FS 2604, 14.x.2003, *R.C. Harris 48350* (NY). McDONALD CO.: Huckleberry Ridge Conservation Area, 29.x.2000, *R.C. Harris 44211* (NY). MONTGOMERY CO.: Graham Cave State Park, 27.x.2001, *R.C. Harris 45735* (NY). MORGAN CO.: Frank E. Carpenter Memorial Conservation Area, 15.iv.2005, *W.R. Buck 48599* (NY). OZARK CO.: Mark Twain National Forest, Smoke Tree Scenic Lookout, 18.iv.1997, *R.C. Harris 40630* (NY). PHELPS CO.: Mark Twain National Forest, Bradford Branch, 14.vi.1995, *D. Ladd 18915* (NY). SHANNON CO.: Angeline Conservation Area, 19.iv.2005, *R.C. Harris 51276* (NY). ST. CLAIR CO.: Buzzard Bluff, 22.iv.1988, *R.C. Harris 21269* (NY). ST. FRANCOIS CO.: W of Knob Lick Fire Tower, 19.ix.1990, *R.C. Harris 25492* (NY). ST. LOUIS CO.: Rockwoods Reservation, 26.x.2001, *R.C. Harris 45663* (NY). TANEY CO.: Mark Twain National Forest, Hercules Glades Wilderness, 22.v.2003, *R.C. Harris 47749* (NY). TEXAS CO.: 16 km SSE of Yukon, 18.x.1984, *W.L. Culberson 19699* (NY). WRIGHT CO.: John Alva Fuson M.S. Conservation Area, 4.xi.2002, *R.C. Harris 46645* (NY). **NEW JERSEY.** BERGEN CO.: Closter, *C.F. Austin 513* (NY). PASSAIC CO.: Ringwood Mines, 1935, *R.H. Torrey s.n.* (NY). SALEM CO.: Rd. to Jericho, 6.iv.1991, *C.F. Reed 133921* (NY). SUSSEX CO.: Stokes State Forest, 29.iii.2008, *J.C. Lendemer et al. 11561* (NY). **NORTH CAROLINA.** CLAY CO.:

Nantahala National Forest, Buck Creek Barren, 10.xi.2007, *J.C. Lendemer et al. 10769* (NY). HENDERSON CO.: Chimney Rock Falls, 14.vi.1981, *C.F. Reed 1981-118* (NY). JACKSON CO.: Nantahala National Forest, Panthertown Valley, 29.iv.2006, *R.C. Harris 52497* (NY). MACON CO.: Nantahala National Forest, McDowell Mountain, 12.viii.1994, *R.C. Harris 33126* (NY). MCDOWELL CO.: 2 mi S of Deer Lick Gap Overlook, 6.v.1967, *S.B. Pravda s.n.* (NY). MONTGOMERY CO.: Uwharrie National Forest, 19.iv.2008, *G.B. Perlmutter et al. 1391* (NY). SWAIN CO.: Great Smoky Mountain National Park, Hughes Ridge Trail, 16.v.1976, *R.C. Harris 10977* (NY). WAKE CO.: William B. Umstead State Park, 14.i.2007, *J.C. Lendemer et al. 8362* (NY). WILKES CO.: Stone Mountain State Park, 23.ix.1993, *R.C. Harris 30802* (NY). YANCEY CO.: Newdale, 26.ii.1982, *C.F. Reed 114994* (NY). **OHIO.** GALLIA CO.: Wayne National Forest, Symmes Creek Natural Area, 19.v.2006, *R.C. Harris 52660* (NY). SCIOTO CO.: Shawnee State Forest, at S jct of FS 3 & FS 6, 21.v.2006, *R.C. Harris 52736* (NY). **OKLAHOMA.** CHEROKEE CO.: Cookson Wildlife Management Area, 14.iv.2004, *R.C. Harris 48926* (NY). **PENNSYLVANIA.** BRADFORD CO.: New Albany, 10.x.1987, *J.K. McGrath 511-6* (NY). CHESTER CO.: Street Rd. near West Chester Pike, 12.ii.1966, *C.F. Reed 73076* (NY). DELAWARE CO.: Tyler Arboretum, Pink Hill, 17.vii.2005, *J.C. Lendemer 4491 & A.E. Schuyler* (NY). HUNTINGDON CO.: Trough Creek State Park, 22.iv.2008, *J.C. Lendemer et al. 11803* (NY). LANCASTER CO.: New Texas Serpentine Barrens, 8.iv.2000, *R.C. Harris 43838* (NY). MONROE CO.: Delaware Water Gap, 27.iv.1986, *M.G. Peidl 7037-A* (NY). MONTGOMERY CO.: Fulshaw Craeg Preserve, 9.iv.2004, *J.C. Lendemer 2179 & A.F. Rhoads* (NY). PIKE CO.: Delaware Water Gap National Recreation Area, PEEC, *J.C. Lendemer 5051* (NY). WYOMING CO.: Bowmans Creek Ledges, 20.vii.2008, *J.C. Lendemer 13503* (NY). **YORK CO.:** Big Mount Rd., 18.iv.1988, *C.F. Reed 141194* (NY). **SOUTH CAROLINA.**

LANCASTER CO.: Forty Acre Rock Heritage Preserve, 5.x.1999, *R.C. Harris 43479* (NY). LAURENS CO.: N of Gray Court, 20.iv.1982, *C.F. Reed 15768* (NY). OCONEE CO.: Mountain Rest, *C.F. Reed 115651* (NY). PICKENS CO.: Rocky Bottom Church Rd., 27.ix.1989, *R.C. Harris 24794* (NY). **TENNESSEE.** MONROE CO.: Cherokee National Forest, Bald River Falls Quad., 6.viii.1994, *R.C. Harris 32898* (NY). POLK CO.: Cherokee National Forest, Cohutta Wilderness Area, 5.x.1998, *R.C. Harris 42471* (NY). **VIRGINIA.** WILLAMSBURG: College of William & Mary, 2.iv.2004, *B. Hodkinson 1946* (DUKE).

2. *Cladonia caespiticia* (Pers.) Flörke, De Cladon., p. 8. 1827.

Baeomyces caespiticia Pers., Ann. Bot. (Usteri), 7: 155. 1794. TYPE: "The Netherlands?", *C.H. Persoon s.n.* (H-ACH 1722A, lectotype n.v. [selected by Ahti (1993)]).

Figure 21(1–3).

Descriptions. – Ahti (2000: 237), Thomson (1967: 124).

Published color illustrations. – Brodo et al. (2001: 242 [fig. 217]), Hinds and Hinds (2007: 168 [fig. 50]).

Chemistry. – Fumarprotocetraric acid. – Spot tests: K+ dirty yellow, C-, KC-, P+ red, UV-.

Ecology. – *Cladonia caespiticia* is a common species that occurs on humus, bare soil, and the bases of trees (especially hardwoods). It typically occurs in shaded habitats with a history of

low-grade persistent disturbance such as the compacted soil or duff on the margins of trails actively used by hikers.

Distribution. – *Cladonia caespiticia* is widespread and common throughout eastern North America with scattered occurrences in the Great Plains. It has also been reported from every other continent except Australia and Antarctica (see distribution map in Litterski & Ahti (2004)).

Discussion. – Morphologically, *Cladonia caespiticia* can be recognized by its thallus composed of delicate, finely divided primary squamules. The two other species with small squamules discussed in the proceeding sections differ in having robust simple squamules that never become finely divided. *Cladonia caespiticia* is clearly unrelated to the others discussed here because it is only superficially apodetiate. In fact, the apothecia of *C. caespiticia* are not sessile but rather borne on very short ecorticate podetia that give the appearance of a stipe (the reader will note that we have referred to the apothecia as "stipitate" or "pseudopodetiate" throughout this paper). *Cladonia caespiticia* is generally collected only when it is fertile because, as noted by Harris (1992), "only fools collect sterile *Cladonia*." As part of our study, we generated ITS sequences from several sterile *Cladonia* specimens with incised squamules that were filed only to genus at NY because, although distinctive, they could not be identified with certainty. These sequences formed a monophyletic group with those generated from fertile specimens of *C. caespiticia.* Thus, we conclude that sterile *Cladonia* thalli on soil and humus from shaded habitats in eastern North America can be referred to this taxon on the basis of the presence of only fumarprotocetraric acid and delicate, incised, esorediate primary squamules.

Selected specimens examined. – **CANADA. NEWFOUNDLAND AND LABRADOR.** NEWFOUNDLAND: Bay of Islands, 9–10.viii.1901, *C.D. Howe & W.F. Lang 1162* (NY). **NOVA SCOTIA.** COLCHESTER CO.: Economy River Wilderness Area, 14.v.2001, *R.C. Harris 49763* (NY). **U.S.A. ALABAMA.** LEE CO.: Auburn, 12.ii.1897, *F.S. Earle & C.F. Baker s.n.* (NY). **ARKANSAS.** BAXTER CO.: Ozark National Forest, Leatherwood Wilderness, 18.iv.2005, *R.C. Harris 51164* (NY). JEFFERSON CO.: Pine Bluff Arsenal, 2.xii.1999, *D. Ladd 21965 & M. Pederson* (NY). **CONNECTICUT.** LITCHFIELD CO.: Town of Canaan, Robbins Swamp, 19.ix.2003, *R.C. Harris 47995* (NY). **DELAWARE.** SUSSEX CO.: near Piney Grove, 24.ii.1990, *C.F. Reed 13144* (NY). **GEORGIA.** ROCKDALE CO.: Panola Mountain State Park, 17.iv.2007, *J.C. Lendemer et al. 9001* (NY). RABUN CO.: 12.2 mi W of Clayon, 11.vi.1981, *R.C. Harris 13795* (NY). TOWNS CO.: Reed Branch Wet Meadow TNC Preserve, 12.xi.2007, *J.C. Lendemer et al. 10366* (NY). UNION CO.: Chattahoochee National Forest, along Duncan Ridge Trail, 6.x.1998, *R.C. Harris 42606* (NY). **ILLINOIS.** UNION CO.: Berryville Shale Glade Nature Preserve, 6.xi.2004, *R.C. Harris 50520* (NY). **INDIANA.** PUTNAM CO.: Fern, 1893, *L.M. Underwood 23* (NY). **KANSAS.** CHEROKEE CO.: 4 mi E of Baxter Springs, 13.vi.1950, *C.L. Kramer 180* (NY). WOODSON CO.: Lake Fegan, 9.ix.1950, *C.L. Kramer 574* (NY). **KENTUCKY.** BULLITT CO.: Bernheim Arboretum, 28.iii.2002, *D. Ladd 23624 & M. Ladd* (NY). LAUREL CO.: near Baldrock, 15.vi.1962, *C.F. Reed 58115* (NY). LETCHER CO.: Bad Branch Nature Preserve, 14.ix.1991, *R.C. Harris 27064* (NY). **MAINE.** HANCOCK CO.: T.10 SD, N of ME182, 27.vii.2006, *R.C. Harris 53086* (NY). KENNEBEC CO.: Mud Pond, 19.ix.1987, *R.C. Harris 20886* (NY). **MARYLAND.** ANNE ARUNDEL CO.: 1 mi E of Odenton, 23.iii.1962, *C.F. Reed 54279* (NY). BALTIMORE CO.: between Lutherville and Texas, 14.iii.1911, *C.C. Plitt s.n.* (NY). CAROLINE CO.: Rt. 404 near Ridgely, 20.iv.1962, *C.F. Reed*

55305 (NY). DORCHESTER CO.: N of Vienna, 12.iv.1963, *C.F. Reed 61697* (NY). GARRETT CO.: Savage River State Forest, 23.ix.1989, *W.R. Buck 17586* (NY). MONTGOMERY CO.: Travilah Rd. near Piney Meetinghouse Rd., 11.iii.1966, *C.F. Reed 73391* (NY). WICOMICO CO.: 1 mi S of Upper Ferry, 22.iv.1962, *C.F. Reed 56061* (NY). WORCESTER CO.: Pocomoke Swamp, 22.iv.1962, *C.F. Reed 56084* (NY). **MASSACHUSETTS.** BERKSHIRE CO.: Mt. Greylock State Reservation, 6.v.1995, *R.C. Harris 36501* (NY). WORCESTER CO.: Oxford, 25.viii.1955, *V. Ahmadjian s.n.* (NY). **MICHIGAN.** CHEBOYGAN CO.: Carp Creek Gorge, 6.viii.1958, *J.T. Mickel L144* (NY). WASHTENAW CO.: W of Whitmore Lake, 10.vii.1979, *R.C. Harris 13133* (NY). **MINNESOTA.** LAKE CO.: Caribou Falls State Park, 9.vii.1978, *L. Brako 411* (NY). **MISSOURI.** BULTER CO.: Mark Twain National Forest, Mud Creek Natural Area, 16.x.2003, *R.C. Harris 48499* (NY). CARTER CO.: Peck Ranch Conservation Area, 16.iv.1997, *R.C. Harris 40428* (NY). JEFFERSON CO.: E of Don Robinson Rd., 12.x.2003, *R.C. Harris 48096* (NY). LINCOLN CO.: Cuiver River State Park, 23.v.2003, *A. Amtoft 1274* (NY). MONTGOMERY CO.: Graham Cave State Park, 27.x.2001, *R.C. Harris 45745* (NY). TANEY CO.: Mark Twain National Forest, Hercules Glades Wilderness, 22.v.2003, *R.C. Harris 47790* (NY). WAYNE CO.: Sam A. Baker State Park, 15.x.2003, *C. Guedian 1083* (NY). **NEW HAMPSHIRE.** GRAFTON CO.: Benton Trail, Mount Moosilauke, 4.vii.1935, *G.P. Anderson s.n.* (NY). **NEW JERSEY.** ATLANTIC CO.: Makepeace Wildlife Mangement Area, 31.iii.2007, *J.C. Lendemer 8744 & A. Moroz* (NY = *Lich. East. N. Amer. Exs. VII: 313*). BERGEN CO.: Closter, 1871, *C.F. Austin 507* (NY). BURLINGTON CO.: Atsion, 7.xi.1986, *M.G. Peidl 5071* (NY). MONMOUTH CO.: Allaire State Park, 5.ii.2009, *J.C. Lendemer 15427* (NY). OCEAN CO.: Manchester Wildlife Management Area, 5.ii.2009, *J.C. Lendemer 15365* (NY). SUSSEX CO.: Stokes State Forest, Tillman Ravine, 12.ix.1992, *R.C. Harris 27959* (NY). **NEW YORK.**

ESSEX CO.: Town of Keene, Chapel Pond, 19.ix.2004, *R.C. Harris 50032* (NY). PUTNAM CO.: Town of Southeast, Peach Lake Natural Area, 21.xii.2006, *W.R. Buck 51194 & R.C. Harris* (NY). ROCKLAND CO.: Harriman State Park, 9.iii.2008, *J.C. Lendemer et al. 11550* (NY). ULSTER CO.: Town of Shandaken, W slope of Slide Mountain, 13.ix.2008, *J.C. Lendemer 14004* (NY). **NORTH CAROLINA.** CLAY CO.: Nantahala National Forest, 1–1.5 mi N of US64 on Buck Creek Road., 10.xi.2007, *J.C. Lendemer et al. 10439* (NY). GRAHAM CO.: Nantahala National Forest, along Santeetlah Creek, 6.viii.1994, *R.C. Harris 32944* (NY). JACKSON CO.: Cedar Cliff Mountain, 11.viii.1994, *R.C. Harris 33031-A* (NY). STOKES CO.: sine loc., 10.iv.1939, *P.O. Schallert s.n.* (NY). WAKE CO.: along Barton Creek, 27.xi.1969, *C.F. Reed 95824* (NY). **OHIO.** SCIOTO CO.: Shawnee State Forest, 21.v.2006, *R.C. Harris 52734-A* (NY). SUMMITT CO.: near Akron, 3.ci.1892, *E.E. Bogue s.n.* (NY). **PENNSYLVANIA.** BRADFORD CO.: State Game Lands No. 36, 18.v.2009, *R.C. Harris 55158* (NY). CUMBERLAND CO.: Michaux State Forest, Woodrow Rd., 2.vi.2009, *J.C. Lendemer 18331* (NY). JEFFERSON CO.: Brookville, 6.ix.1987, *J.K. McGrath 511-17* (NY). LACKAWANNA CO.: Merli-Sarnoski County Park, 17.vii.2008, *J.C. Lendemer 13221* (NY). LANCASTER CO.: Susquehannock State Park, 3.i.2008, *J.C. Lendemer 13360 & E. Tripp* (NY). LYCOMING CO.: State Game Lands No. 133, 12.v.2009, *J.C. Lendemer 16680 & D. Atha* (NY). MONROE CO.: Delaware Water Gap National Recreation Area, Community Drive Wetlands, 24.iv.2004, *R.C. Harris 49524* (NY). PIKE CO.: Delaware State Forest, Bruce Lake Natural Area, 17.v.2009, *R.C. Harris 55152* (NY). SULLIVAN CO.: Eagle's Mere, 1937, *R.H. Torrey s.n.* (NY). TIOGA CO.: Tioga State Forest, W-facing slopes of Callahan Hill, 14.v.2009, *J.C. Lendemer 16903* (NY). WAYNE CO.: State Game Lands No. 312, 27.vii.2009, *J.C. Lendemer 18586* (NY). WYOMING CO.: State Game Lands No. 57, 21.vii.2008, *J.C. Lendemer 13864* (NY). **SOUTH CAROLINA.**

GREENVILLE CO.: Bald Rock, 14.iii.1997, *R.C. Harris 40071* (NY). **TENNESSEE.** CARTER CO.: Doe River Gorge, 26.ix.1993, *R.C. Harris 30986* (NY). CHATHAM CO.: Sycamore, 30.iii.1934, *A.J. Sharp 3* (NY). **VERMONT.** ESSEX CO.: Town of Averill, Quimby Country Resort Property, 19.v.2008, *W.R. Buck 53753* (NY). **VIRGINIA.** BEDFORD CO.: 3 mi E Gillespe, 17.iv.1966, *C.F. Reed 138969* (NY). FAIRFAX CO.: small branch of Turkey Run, 29.xi.1970, *J.C. Guccion 1089* (NY). HIGHLAND CO.: ~9 mi S of Monterey on US220, 10.v.1976, *R.C. Harris 10660* (NY). KING GEORGE CO.: Rt. 301 near Dogue, 4.iv.1961, *C.F. Reed 49192* (NY). RICHMOND CO.: 2 mi S Farnham, 26.v.1962, *C.F. Reed 60552* (NY). SYMTH CO.: Jefferson National Forest, Whitetop Mountain, 5.iv.2008, *R.C. Harris 54087* (NY). WILLIAMSBURG: College of William & Mary, 7.viii.2004, *B. Hodkinson 1628* (NY). WISE CO.: Jefferson National Forest, High Knob, 13.ix.1991, *R.C. Harris 26935* (NY). **WEST VIRGINIA.** POCAHONTAS CO.: Monongahela National Forest, 0–1 mi S of intersection of FS and CR 23, 21.x.2007, *J.C. Lendemer 9867 & A. Moroz* (NY). TUCKER CO.: Monongahela National Forest, Olsen's Bog, 22.iv.2001, *R.C. Harris 44859* (NY). **WISCONSIN.** LINCOLN CO.: Town of Rock Falls, 27.iv.2002, *R.C. Harris 45880* (NY). ONEIDA CO.: Patterson Hemlocks State Natural Area, 29.iv.2002, *R.C. Harris 46016* (NY).

3. *Cladonia petrophila* R.C. Harris, Brittonia, 44: 326. 1992. TYPE: **U.S.A. TENNESSEE.** CARTER CO.: Cherokee National Forest, Dennis Cove, 1976, *R.C. Harris 10730* (NY!, holotype).

Figures 21(4–6) & 22.

Description. – Harris (1992: 326).

Published color illustration. – Hinds and Hinds (2007: 195 [fig. 75]).

Chemistry. – Chemotype I: sphaerophorin (major), atranorin (minor), fumarprotocetraric acid and accessories (major or minor). Spot tests: K+ weak yellow, C-, KC+ weak purple, P+ orange red (especially near the growing edge of the squamule), UV+ blue white (medulla). Chemotype II: sphaerophorin (major), ± atranorin (minor). Spot tests: K-, C-, KC-, P-, UV+ blue-white (medulla).

Ecology. – As was discussed by Harris (1992), *Cladonia petrophila* is restricted to shaded non-calcareous boulders and rock outcrops in intact hardwood forests, often near streams, streambeds, or habitats with high humidity. In particularly humid localities, the species can also occur on the roots and bases of hardwood trees; however, typical saxicolous thalli are always present in the immediate vicinity.

Distribution. – *Cladonia petrophila* is known only from eastern North America where it is widely distributed in the Appalachian Mountains, Piedmont, and Ozark Ecoregion. A chemotype lacking fumarprotocetraric is restricted to the southern Appalachian Mountains (see specimens cited below) and outlying granite outcrops in the Piedmont of the southeastern United States.

Discussion. – *Cladonia petrophila* is a common species throughout eastern North America and the Ozarks. It cannot be confused in the lab with any other *Cladonia* in the region because of its diminutive usually imbricate primarily squamulose thallus and the presence of

sphaerophorin in the medulla. In the field, it could be confused with sterile thalli of species with esorediate primary squamules, particularly *C. ochrochlora* Flörke; however, it can be readily distinguished from these by its distinctive medullary chemistry. The distinction between *C. petrophila* and *C. stipitata* is addressed in the following section. It should be noted that examination of the holotype of *C. petrophila* revealed that the figure published as part of the protologue (Harris 1992: 327, figure 1) illustrates pycnidia rather than apothecia (see figure 21, herein).

Selected specimens examined [FUMARPROTOCETRARIC ACID PRESENT CHEMOTYPE]. – **U.S.A. ALABAMA.** CLAY CO.: Talladega National Forest, 24.ix.1992, *R.C. Harris 28387* (NY). FRANKLIN CO.: Dismals Wonder Garden, 26.ix.1992, *R.C. Harris 28455* (NY). JACKSON CO.: Buck's Pocket State Park, 3.x.1998, *R.C. Harris 42379* (NY). LEE CO.: Auburn, 20.ii.1897, *F.S. Earle 125 & C.F. Baker* (NY). TALLAPOOSA CO.: Double Bridges, 5.xi.1898, *F.S. Earle s.n.* (NY). WINSTON CO.: Natural Bridge, 26.ix.1992, *R.C. Harris 28488* (NY). **ARKANSAS.** CRAWFORD CO.: Ozark National Forest, along FS 1725 (CR257), 15.iv.2004, *R.C. Harris 49186* (NY). FRANKIN CO.: Ozark National Forest, Shores Lake, 16.iv.2004, *R.C. Harris 49201* (NY). IZARD CO.: NE corner of Devil's Knob-Devil's Backbone, 24.x.2001, *R.C. Harris 45379* (NY). NEWTON CO.: Ozark National Forest, Alum Cove Recreation Area, 24.ix.1988, *R.C. Harris 21472* (NY). STONE CO.: Ozark National Forest, City Rock Bluff Special Interest Area, 18.iv.2005, *R.C. Harris 51249* (NY). **CONNECTICUT.** FAIRFIELD CO.: Town of Redding, 9.vi.2005, *R.C. Harris 51547* (NY). WINDHAM CO.: Town of Ashford, 24.vii.2002, *R.C. Harris 46094* (NY). **GEORGIA.** COFFEE CO.: Broxton Rocks Ecological Preserve, 7.x.1999, *W.R. Buck 36609* (NY). GILMER

CO.: Chattahoochee National Forest, Barnes Creek Picnic Area, 22.ix.1992, *R.C. Harris 28192* (NY). JEFF DAVIS CO.: 0.4 mi E of Coffee Co. Line, 18.ix.1996, *R.C. Harris 38770* (NY). **ILLINOIS.** JACKSON CO.: Shawnee National Forest, Pomona Natural Bridge, 15.x.1993, *R.C. Harris 31285* (NY). POPE CO.: Shawnee National Forest, Hayes Creek, 16.x.1993, *R.C. Harris 31373* (NY). RANDOLPH CO.: Piney Creek Ravine Nature Preserve, 7.xi.2004, *R.C. Harris 50588* (NY). **KENTUCKY.** BATH CO.: Daniel Boone National Forest, Tater Knob, 10.x.1995, *R.C. Harris 36875* (NY). BULLITT CO.: Bernheim Arboretum and Research Forest, 25.iii.2002, *D. Ladd 23455 & M. Ladd* (NY). BULTER CO.: Union-Boston area, 17,.vi.1962, *C.F. Reed 58940* (NY). EDMONSON CO.: Area of Temple Hill Cemetery, ix.1979, *J.P. Dey et al. 10985* (NY). ESTILL CO.: Daniel Boone National Forest, ca. 1.5 mi NW of Cottage Furnace Campground, 11.x.1995, *R.C. Harris 36963* (NY). HARLAN CO.: Profile Rock, Kentenia State Forest, 16.ix.1991, *R.C. Harris 27193* (NY). LETCHER CO.: Bad Branch Nature Preserve, 14.ix.1991, *R.C. Harris 26997* (NY). PERRY CO.: Daniel Boone National Forest, Old Field Branch of Leatherwood Creek, 6.x.2001, *R.C. Harris 44946* (NY). WOLFE CO.: Red River Gorge, 1.v.1981, *C.F. Reed 113186* (NY). **MARYLAND.** BALTIMORE CO.: Bear Island, 12.ii.1982, *C.F. Reed 114782* (NY). CECIL CO.: mouth of Conowingo Creek, 17.iii.1962, *C.F. Reed 55209* (NY). HARFORD CO.: along Deer Creek at US 1, 24.iv.1988, *C.F. Reed 127060* (NY). MONTGOMERY CO.: Rt. 28, just S of Dawsonville, 19.iv.1962, *C.F. Reed 55780* (NY). WASHINGTON CO.: Pleasant Valley Rd. at Rt. 77, 28.iv.1962, *C.F. Reed 55595* (NY). **MASSACHUSETTS.** BERKSHIRE CO.: Bartholomew's Cobble, 1982, *R.C. Harris s.n.* (NY). NORFOLK CO.: Chickatawbut Hill, 18.iv.1992, *E. Lay et al. 93-020* (NY). **MISSISSIPPI.** TISHOMINGO CO.: Tishomingo State Park, 27.ix.1992, *R.C. Harris 28570* (NY). **MISSOURI.** DOUGLAS CO.: Mark Twain National Forest, Dimcher Hollow, 11.x.1997,

R.C. Harris 41429 (NY). IRON CO.: E slope of Royal Gorge, 27.iv.1988, *R.C. Harris 21784* (NY). JEFFERSON CO.: Don Robinson Property, 24.iii.2006, *R.C. Harris 51980* (NY). LAWRENCE CO.: Fall Hollow Gorge, 27.iii.2006, *R.C. Harris 51235* (NY). PHELPS CO.: Mark Twain National Forest, Roluf Spring Woodland Resoration Area, 6.ii.1994, *D. Ladd et al. 17842* (NY). ST. CLAIR CO.: Buzzard Bluff, 22 iv.1988, *R.C. Harris 21257* (NY). ST. FRANCOIS CO.: St. Francois State Park, 13.x.2003, *R.C. Harris 48181* (NY). ST. GENEVIEVE CO.: Pickle Springs Natural Area, 26.ix.1990, *R.C. Harris 25989* (NY). WAYNE CO.: Sam A. Baker State Park, 15.x.2003, *W.R. Buck 45379* (NY). **NEW JERSEY.** PASSAIC CO.: Ringwood, 7.xi.1935, *G.P. Anderson s.n.* (NY). SUSSEX CO.: Stokes State Forest, 12.ix.1992, *R.C. Harris 27956* (NY). **NEW YORK.** BRONX CO.: New York Botanical Garden, 10.v.1980, *R.C. Harris 13294* (NY). DUTCHESS CO.: Cary Aboretum, 14.x.1981, *R.C. Harris 14050* (NY). GREENE CO.: Long Path, 9.v.1993, *R.C. Harris 30472* (NY). ORANGE CO.: Harriman State Park, 19.iv.1998, *R.C. Harris 42138* (NY). PUTNAM CO.: Town of Putnam Valley, 27.x.1998, *R.C. Harris 42827* (NY). ROCKLAND CO.: Harriman State Park, 26.iii.1997, *R.C. Harris 40359* (NY). WESTCHESTER CO.: Mianus River Gorge, 16.v.1981, *R.C. Harris 13585* (NY). **NORTH CAROLINA.** CLAY CO.: Nantahala National Forest, Buck Creek Barren, 10.xi.2007, *J.C. Lendemer et al. 10867* (NY). HAYWOOD CO.: Great Smoky Mountains National Park, Big Creek, 25.viii.1977, *J.W. Thomson 19577* (NY). JACKSON CO.: Bad Creek Trail to Ellicott's Rock, 28.ix.1989, *R.C. Harris 24857* (NY). MACON CO.: Nantahala National Forest, Glen Falls, 17.vii.1996, *W.R. Buck 30274* (NY). **OHIO.** GALLIA CO.: Wayne National Forest, Symmes Creek Natural Area, 21.v.2006, *W.R. Buck 50325* (NY). SCIOTO CO.: Shawnee State Forest, along Pond Like Run, 21.v.2006, *R.C. Harris 52761* (NY). **PENNSYLVANIA.** BEDFORD CO.: Buchanan State Forest, Pleasant Valley, 17.v.2006, *R.C.*

Harris 52589 (NY). BERKS CO.: French Creek State Park, 27.xii.2006, *J.C. Lendemer 8272 & A. Moroz* (NY). BLAIR CO.: State Game Lands No. 73, 22.iv.2008, *R.C. Harris 54294* (NY). BUCKS CO.: Ringing Rocks County Park, 15.ix.2005, *J.C. Lendemer 4940 & A.E. Schuyler* (NY). HUNTINGDON CO.: Trough Creek State Park, 22.iv.2008, *R.C. Harris 25257* (NY). LACKAWANNA CO.: SGL No. 300, 3.vii.2008, *J.C. Lendemer 12417* (NY). LUZERNE CO.: SGL No. 91, 15.vii.2008, *J.C. Lendemer 13152* (NY). MONROE CO.: Delaware Water Gap National Recreation Area, Mount Minsi, 3.v.2006, *J.C. Lendemer 6818 & N. Howe* (NY). MONTGOMERY CO.: Fulshaw Craeg Preserve, 9.iv.2004, *J.C. Lendemer 2143 & A.F. Rhoads* (NY, PH). PIKE CO.: SGL No. 180, 11.vii.2008, *J.C. Lendemer 12975* (NY). **RHODE ISLAND.** PROVIDENCE CO.: Town of Lincoln, 17.ix.2006, *R.C. Harris 53204* (NY). WASHINGTON CO.: Town of Hopkinton, 16.ix.2006, *R.C. Harris 51379* (NY). **SOUTH CAROLINA.** LEXINGTON CO.: Peachtree Rock Nature Preserve, 13.iii.1997, *R.C. Harris 39914* (NY). PICKENS CO.: along Eastatoe Creek ~2.5 mi SW of Rocky Bottom, 27.ix.1989, *R.C. Harris 24785* (NY). SPARTANSBURG/LAURENS CO.: Rt. 101 at Enoree River, 20.iv.1982, *C.F. Reed 117896* (NY). **TENNESSEE.** BLOUNT CO.: Great Smoky Mountains National Park, Cades Cove, 23.vii.1977, *R.W. Becking 77071318* (NY). HAMILTON CO.: Lookout Mountain, *W.W. Calkins s.n.* (NY). MONROE CO.: Cherokee National Forest, Bald River Falls Quad., 6.viii.1994, *R.C. Harris 32897* (NY). SCOTT CO.: Big South Fork National River and Recreation Area, 7.viii.1994, *R.C. Harris 32978* (NY). SEVIER CO.: Greenbrier, 5.viii.1977, *R.W. Becking 7708061* (NY). **VERMONT.** ADDISON CO.: Bristol Cliffs Wilderness Area, 16.ix.2000, *E. Lay 0059* (NY). **VIRGINIA.** GILES CO.: Mountain Lake Biological Station, Moonshine Dell, 7.x.1995, *R.C. Harris 36723* (NY). WARREN CO.: Smithsonian Conservation & Research Center, 11.iii.2005, *J.C. Lendemer 3845* (NY). **WEST**

VIRGINIA. GRANT CO.: Greenland Gap, 21.iv.2001, *R.C. Harris 44841* (NY). **WISCONSIN.** RICHLAND CO.: Rockbridge Park, 28.vii.1974, *M. Nee 13150* (NY).

Selected specimens examined [FUMARPROTOCETRARIC ACID ABSENT CHEMOTYPE]. – **U.S.A. ALABAMA.** JACKSON CO.: Pisgah, Jones Cove, 2.x.1999, *R.C. Harris 43352* (NY). MARION CO.: North Fork Creek, 4.x.1999, *R.C. Harris 43425* (NY). **GEORGIA.** HEARD CO.: Camp Meeting Rock Preserve, 1.x.1999, *R.C. Harris 43310* (NY). PUTNAM CO.: Eatonon Granite Outcrop, along W shore of Oconee Lake, 8.x.1999, *R.C. Harris 43726* (NY). TOWNS CO.: Southern Nantahala Wilderness, Hightower Gap to Rich Knob, 11.xi.2007, *J.C. Lendemer et al. 10973* (NY). **NORTH CAROLINA.** HAYWOOD CO.: Great Smoky Mountains National Park, 3 mi SE of Waterville, 28.x.2006, *J.C. Lendemer 8208 & E. Tripp* (NY). JACKSON CO.: Cedar Cliff Mountain, 11.viii.1994, *R.C. Harris 33084* (NY). MACON CO.: Nantahala National Forest, Wayah Bald, 30.ix.1997, *R.C. Harris 40968* (NY). TRANSYLVANIA CO.: Gorges State Park, ~1/2 mi N of Ray Fisher Farm, 9.viii.2005, *J.C. Lendemer 4690 & E.A. Tripp* (NY). **TENNESSEE.** MONROE CO.: Cherokee National Forest, Bald River Falls Quad., 6.viii.1994, *W.R. Buck 25164* (NY).

4. *Cladonia stipitata* Lendemer & Hodkinson sp. nov.

Mycobank. #515515

Figure 23.

Similis *C. petrophilae* apotheciis sessilibus sed differt squamulis stipitatibus, acidum sphaerophoricum nullum et acidum atranoricum contintente.

TYPE: **U.S.A. NORTH CAROLINA.** TRANSYLVANIA CO.: Gorges State Park, SW face of Grassy Ridge, Snake Rock, 3150–3200 ft., exposed granitic bald with extensive bryophyte-*Selaginella* cushions, on steep slope marginally forested by pitch pine (*Pinus rigida*), oak (*Quercus*), and sourwood, 19.ix.2005, on granite in full sun, *J.C. Lendemer 7615 & A. Moroz* (NY!, holotype; DUKE!, isotype).

Description. – **Primary thallus** squamulose, forming distinct cushion-like colonies to several centimeters in diameter. **Squamules** digitate, dichotomously branching, crowded, erect, forming tight dense cushions, not distinctly recurved near the tips, 0.7–1.25 mm wide, (2.5)–5–8.75 mm long, with multiple central cartilaginous strands; upper surface blue-gray though often discolored to brownish near the tips; lower surface white; margins and tips crenate. **Podetia** absent. **Apothecia** frequently present, although, mature apothecia not observed, sessile, plane, reddish-brown darkening to blackish-brown, ca. 0.5 mm in diameter. **Pycnidia** sessile on the squamules, reddish-brown, darkening to blackish, subglobose to pyriform, ca. 0.5 mm diam, occasionally elongating apically to becoming ca. 1.0 mm tall. **Conidia** hyaline, short and rod-like, often slightly curved, (7.7)–8.9–(10.2) × 0.75–1 μm.

Etymology. – The epithet “stipitata” refers to the distinctive stipitate morphology of the primary squamules of the species.

Chemistry. – Atranorin (major), fumarprotocetraric acid (major). Spot tests: K+ yellow, C-, KC+ weak purple, P+ orange-red (especially near the growing edge of the squamules), UV-.

Ecology. – *Cladonia stipitata* is essentially restricted to fully exposed rock slabs and boulders on granitic balds at middle to low elevations (e.g., 2000–3000 ft.) of the southern Appalachian Mountains. To date, the species has not been found in the humid shaded hardwood forests that typically surround the granitic balds where it occurs. It has also not been found in similar habitats at the highest elevations of the southern Appalachians (e.g., >3000 ft.).

Distribution. – *Cladonia stipitata* is presently known only from a handful of localities in the southern Appalachian Mountains (in Georgia, Kentucky, North Carolina, and South Carolina) and from a single disjunct population on an exposed granite outcrop near sea level in Rhode Island. The occurrence of this disjunct population of *C. stipitata* suggests that the species may actually have a typical Appalachian-Great Lakes distribution and that exhaustive searching of regional herbaria or additional fieldwork would turn up additional records. It should be noted, however, that our attempts to locate the species in seemingly appropriate habitats outside of its currently documented range (e.g., in Maryland, Pennsylvania, and Virginia) were unsuccessful.

History of study. – We were first alerted to the existence of *Cladonia stipitata* when the first author (JCL) and Erin Tripp collected it during a survey of the lichens of Gorges State Park in North Carolina. Considerable study by JCL and Richard Harris failed to resolve its status, and despite its aberrant ecology, chemistry, and distinctly stipitate squamules it was reported by Lendemer & Tripp (2008) as "*Cladonia petrophila* s.l.". In an effort to better understand the species, a specimen that had been deposited at DUKE was studied with molecular methods as part of the AFTOL project and sequences generated from that specimen were included in the dataset used to produce the inferred phylogeny of the Lecanoromycetes by Miadlikowska et al.

(2006). As a result of that study, this species is now represented in GenBank by nucSSU, nucLSU, *RPB*1, *RPB*2, and mitSSU sequences. Unfortunately, Miadlikowska et al. (2006) included this sample under the name "*Cetradonia* sp." based on the specimen label and the initial communication from JCL suggesting that molecular data might resolve whether the taxon should be included in *Cetradonia* J.C. Wei & Ahti or *Cladonia*. It was only after additional collections of this taxon were discovered and we generated additional sequences for it and other apodetiate *Cladonia* species that we decided that formal recognition was required.

Discussion. – Among the apodetiate species of *Cladonia* with sessile apothecia borne on the squamules found in eastern North America, *C. stipitata* can be recognized by its unique ecology, restricted distribution, and stipitate squamules with a blackened base. Morphologically, the species resembles *C. petrophila*, which also occurs on non-calcareous rocks; however, *C. petrophila* is restricted to shaded habitats in intact forests, consistently produces sphaerophorin in the medulla, and produces atranorin only as a minor substance that is usually detectable as only a trace with TLC. Chemically, the species is identical to *C. apodocarpa* in producing high concentrations of atranorin and fumarprotocetraric acid; however, that species occupies an entirely different habitat (soil and humus in disturbed open areas) and has much longer squamules that are not distinctly stipitate or blackened at the base.

The discovery of a new species of *Cladonia* that is essentially restricted to exposed granitic balds in the southern Appalachian Mountains is not surprising when one considers how poorly collected the middle and low elevations of the mountain range are in that region (Lendemer & Tripp 2008). An endemic unispecific genus, *Cetradonia* J.C. Wei & Ahti, is already known from shaded granitic seepage faces in the same region and ongoing studies by the authors

and their colleagues continue to result in the discovery of previously unrecognized disjunct and endemic taxa (e.g., Harris & Lendemer 2009; Hodkinson 2010; Lendemer 2007, 2009, 2009a, 2009b; Lendemer et al. 2008; Lendemer & Tripp 2008; Sheard et al. 2008).

Additional specimens examined. – **U.S.A. GEORGIA.** RABUN CO.: Chattahoochee National Forest, Rabun Bald, 20.ix.1996, *R.C. Harris 38964* (NY). ROCKDALE CO.: Panola Mountain State Park, 17.iv.2007, *J.C. Lendemer 8994* (NY). **KENTUCKY.** HARLAN CO.: Profile Rock, Kentenia State Forest, 16.ix.1991, *R.C. Harris 27148* (NY). **NORTH CAROLINA.** JACKSON CO.: Nantahala National Forest, Panthertown Valley, 29.iv.2006, *R.C. Harris 52488* (NY), *J.C. Lendemer et al. 6905* (NY). TRANSYLVANIA CO.: Gorges State Park, 9.vii.2005, *J.C. Lendemer 4689 & E. Tripp* (DUKE), *J.C. Lendemer 4856 & E. Tripp* (NY, DUKE, AFToL voucher #1657). WILKES CO.: Stone Mountain State Park, 23.ix.1993, *R.C. Harris 30810* (NY). **RHODE ISLAND.** WASHINGTON CO.: Town of Hopkinton, Long & Ell Ponds Natural Area, 16.ix.2006, *R.C. Harris 53180* (NY). **SOUTH CAROLINA.** GREENVILLE CO.: Bald Rock, 14.iii.1997, *R.C. Harris 40063* (NY); vicinity of Bald Rock, 5.x.1999, *R. Yahr 1987* (DUKE). PICKENS CO.: 1.5 mi N of Liberty on Hwy 178, 21.vi.1973, *J.N. Knox 125* (DUKE).

Acknowledgments

We thank Richard Harris for his helpful discussions of *Cladonia petrophila* and other sterile *Cladonia*. Thanks to Teuvo Ahti and Erin Tripp for reviewing the manuscript. The second author thanks François Lutzoni and Jolanta Miadlikowska for training in phylogenetic methods. Special thanks to Sean Beeching, Bill Buck, Richard Harris, Malcolm Hodges, Andy Moroz, and Erin Tripp for their companionship and help with logistics associated with fieldwork. Fieldwork

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Taxon	Chemotype	Isolate No.	GenBank No.	Collection	Locality
C. apodocarpa		NY373	GU207223	J.C. Lendemer 11561	U.S.A., New Jersey, Sussex Co.
		NY374	GU207224	J.C. Lendemer 10769	U.S.A., North Carolina, Clay Co.
		NY377	GU207225	R.C. Harris 52660	U.S.A., Ohio, Gallia Co.
		NY378	GU207226	J.C. Lendemer 13503	U.S.A., Pennsylvania, Wyoming Co,
		NY379	GU207227	R.C. Harris 54374	U.S.A., Vermont, Essex Co.
C. caespiticia		NY228	GU207228	J.C. Lendemer 15185	U.S.A., New Jersey, Cape May Co.
		NY368	GU207229	J.C. Lendemer 13221	U.S.A., Pennsylvania, Lackawanna Co.
		NY96	GU207230	J.C. Lendemer 12579	U.S.A., Pennsylvania, Carbon Co.
C. petrophila	fumar-	NY358	GU207231	J.C. Lendemer 8208a	U.S.A., North Carolina, Haywood Co.
	fumar+	NY366	GU207232	R.C. Harris 51547	U.S.A., Connecticut, Fairfield Co.
	fumar+	NY369	GU207233	J.C. Lendemer 12975	U.S.A., Pennsylvania, Pike Co.
	fumar-	NY37	GU207234	J.C. Lendemer 8208b	U.S.A., North Carolina, Haywood Co.
	fumar+	NY370	GU207235	J.C. Lendemer 12145	U.S.A., Pennsylvania, Pike Co.
	fumar+	NY371	GU207236	R.C. Harris 52527	U.S.A., Pennsylvania, Huntingdon Co.
	fumar+	NY372	GU207237	J.C. Lendemer 11858	U.S.A., Pennsylvania, Blair Co.
	fumar+	NY38	GU207238	J.C. Lendemer 7371	U.S.A., Ohio, Gallia Co.
	fumar-	NY404	GU207239	J.C. Lendemer 10973	U.S.A., Georgia, Towns Co.
	fumar-	NY405	GU207240	J.C. Lendemer 4690	U.S.A., North Carolina, Transylvania Co.
	fumar+	NY406	GU207241	J.C. Lendemer 10867	U.S.A., North Carolina, Clay Co.
	fumar-	NY407	GU207242	J.C. Lendemer 8208c	U.S.A., North Carolina, Haywood Co.
C. stipitata		NY360	GU207243	J.C. Lendemer 4856	U.S.A., North Carolina, Transylvania Co.
		NY400	GU207244	J.C. Lendemer 7615a	U.S.A., North Carolina, Transylvania Co.
		NY401	GU207245	J.C. Lendemer 7615b	U.S.A., North Carolina, Transylvania Co.
		NY402	GU207246	J.C. Lendemer 6905	U.S.A., North Carolina, Jackson Co.
		NY403	GU207247	J.C. Lendemer 8994	U.S.A., Georgia, Rockdale Co.

Table 3. Data for molecular vouchers and GenBank accessions for ITS sequences generated as part of this study.

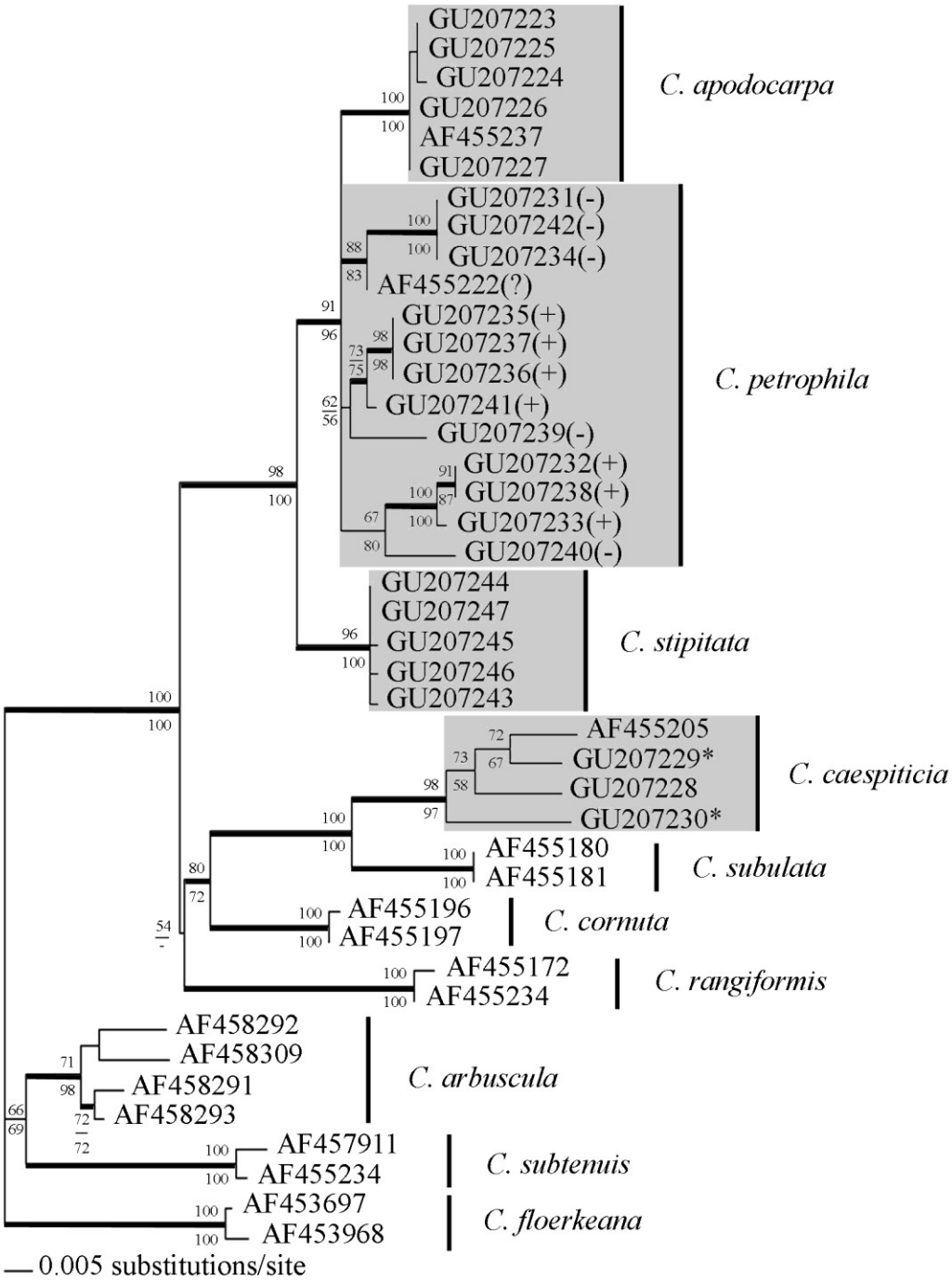

Figure 19. Maximum likelihood (ML) phylogenetic tree showing inferred relationships among selected members of the genus *Cladonia* based on ITS1, 5.8S, and ITS2 sequence data. Support is shown in the form of ML bootstrap proportions (BP) and maximum parsimony (MP) BP, each based on 1000 replicate resamplings. BP values ≥50% are shown above (ML-BP) and below (MP-BP) each internode, with branches supported by both ML-BP and MP-BP ≥70% thickened. Plus "+" and minus "-" symbols following the accession numbers of sequences of *C. petrophila* refer to the presence/absence of fumarprotocetraric acid. Asterisks "*" following sequences in the clade representing *C. caespiticia* indicate vouchers of the first putative taxon. Members of *Cladonia* sect. *Helopodium* are shaded in gray

Figure 20. *Cladonia apodocarpa*. 1, typical habitat (Lackawanna Co., Pennsylvania, USA). 2, thallus (*Harris 24794*, scale = 5 mm). 3, detail of primary squamules (*Harris 24794*, scale = 10 mm).

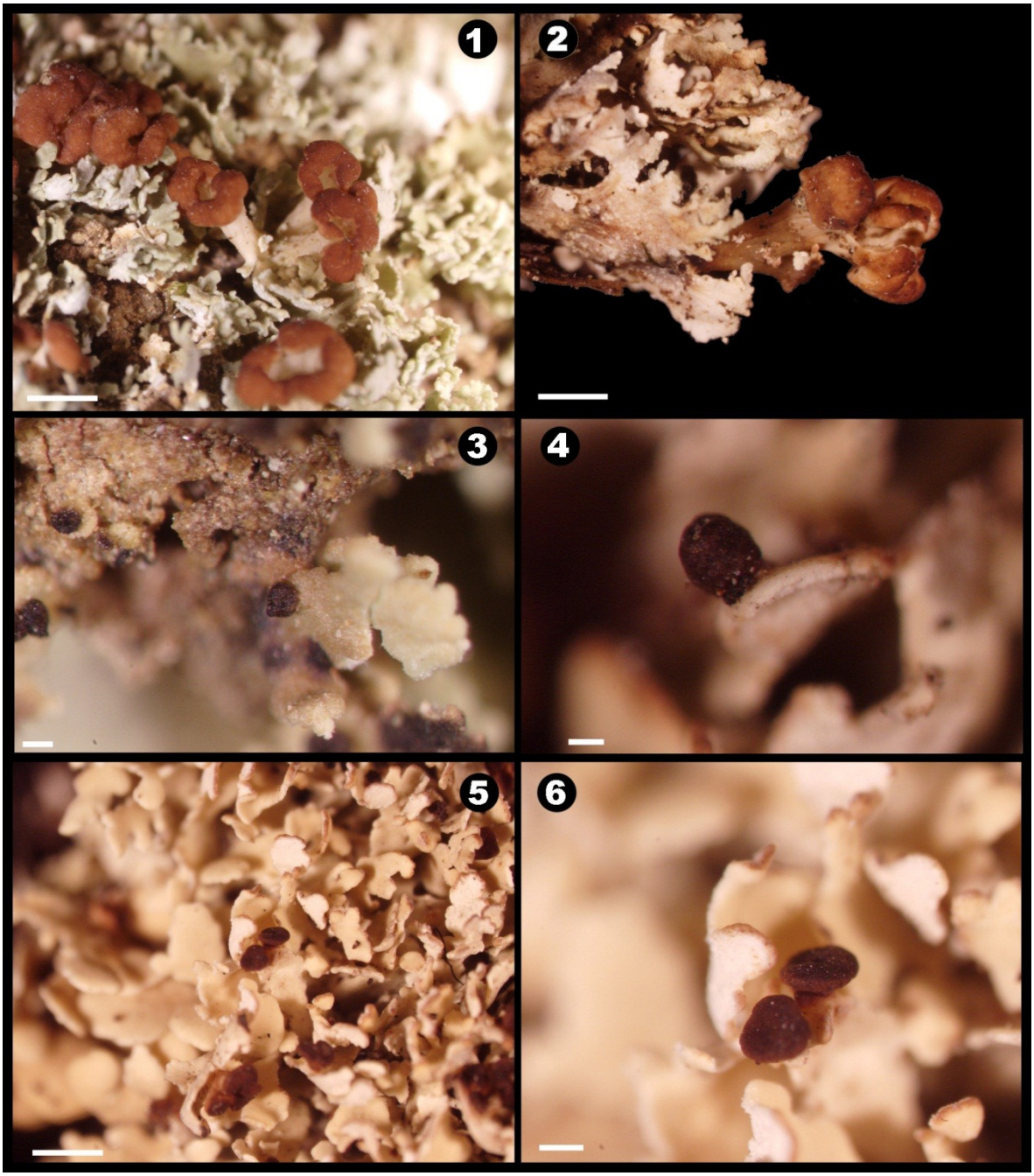

Figure 21. 1, mature apothecia of *Cladonia caespiticia* (*Harris 52734A*; scale = 1 mm). 2, mature apothecia and primary squamules of *C. caespiticia* (*Buck 53753*; scale = 1 mm). 3, pycnidia of *C. caespiticia* (*Harris 52734A*; scale = 0.5 mm). 4, pycnidium of *C. petrophila* (*Harris 10730*; scale = 0.1 mm). 5, mature apothecia and primary squamules of *C. petrophila* (*Harris 10730*; scale = 1 mm). 6, detail of mature apothecia of *C. petrophila* (*Harris 10730*; scale = 0.25 mm).

Figure 22. *Cladonia petrophila*. 1, typical habitat (Pike Co., Pennsylvania, USA). 2, detail of the primary squamules (*Buck 50325*, scale = 1 mm). 3, thallus (*Buck 53025*, scale = 5 mm).

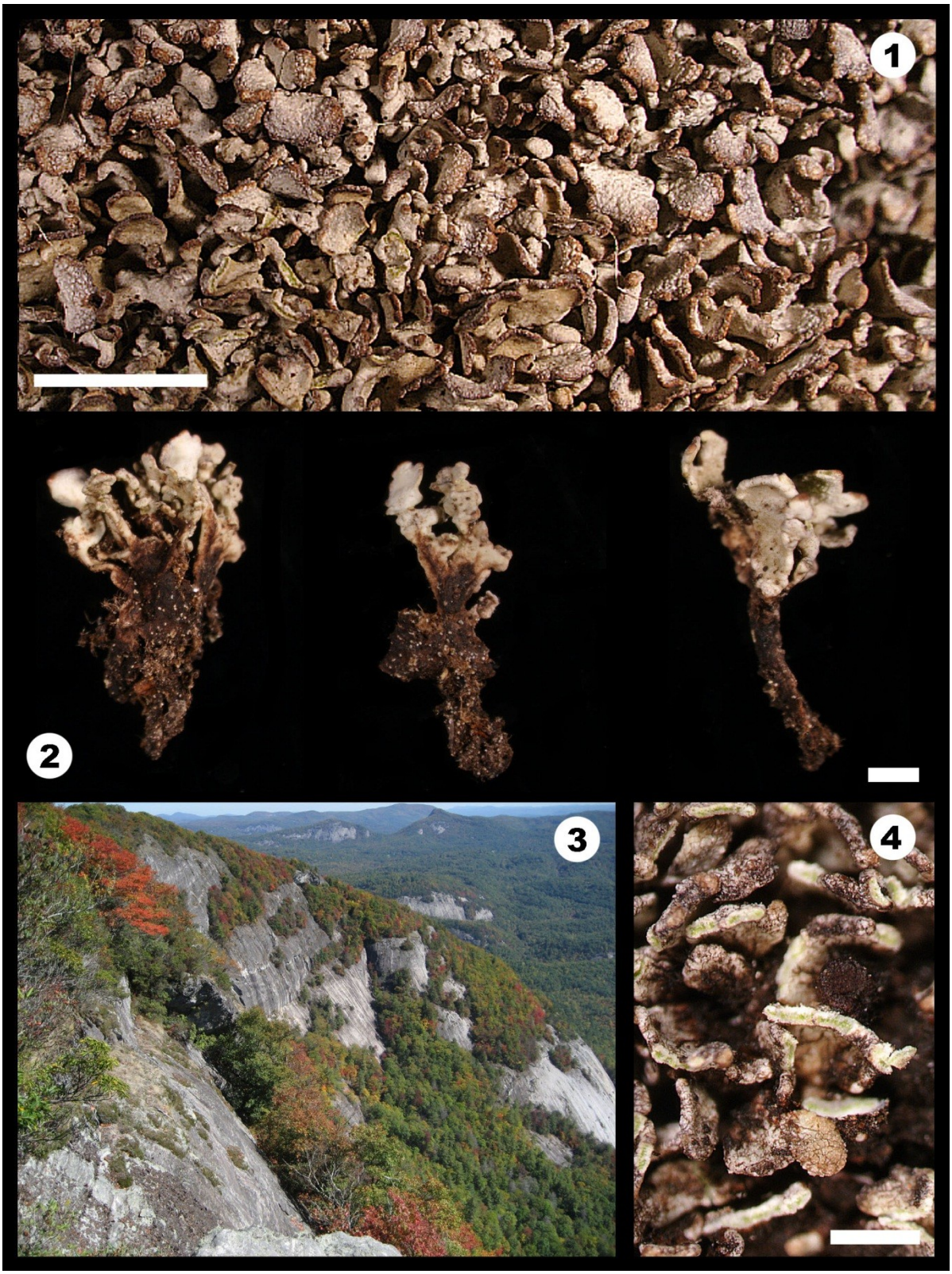

Figure 23. *Cladonia stipitata.* 1, thallus (*Harris 30810*, scale = 5 mm). 2, detail of the primary squamules (*Harris 38010*, scale = 1mm). 3, typical habitat (Jackson Co., North Carolina, USA, photo by E.A. Tripp). 4, detail of apothecium (*Lendemer 7615*, scale = 2 mm).

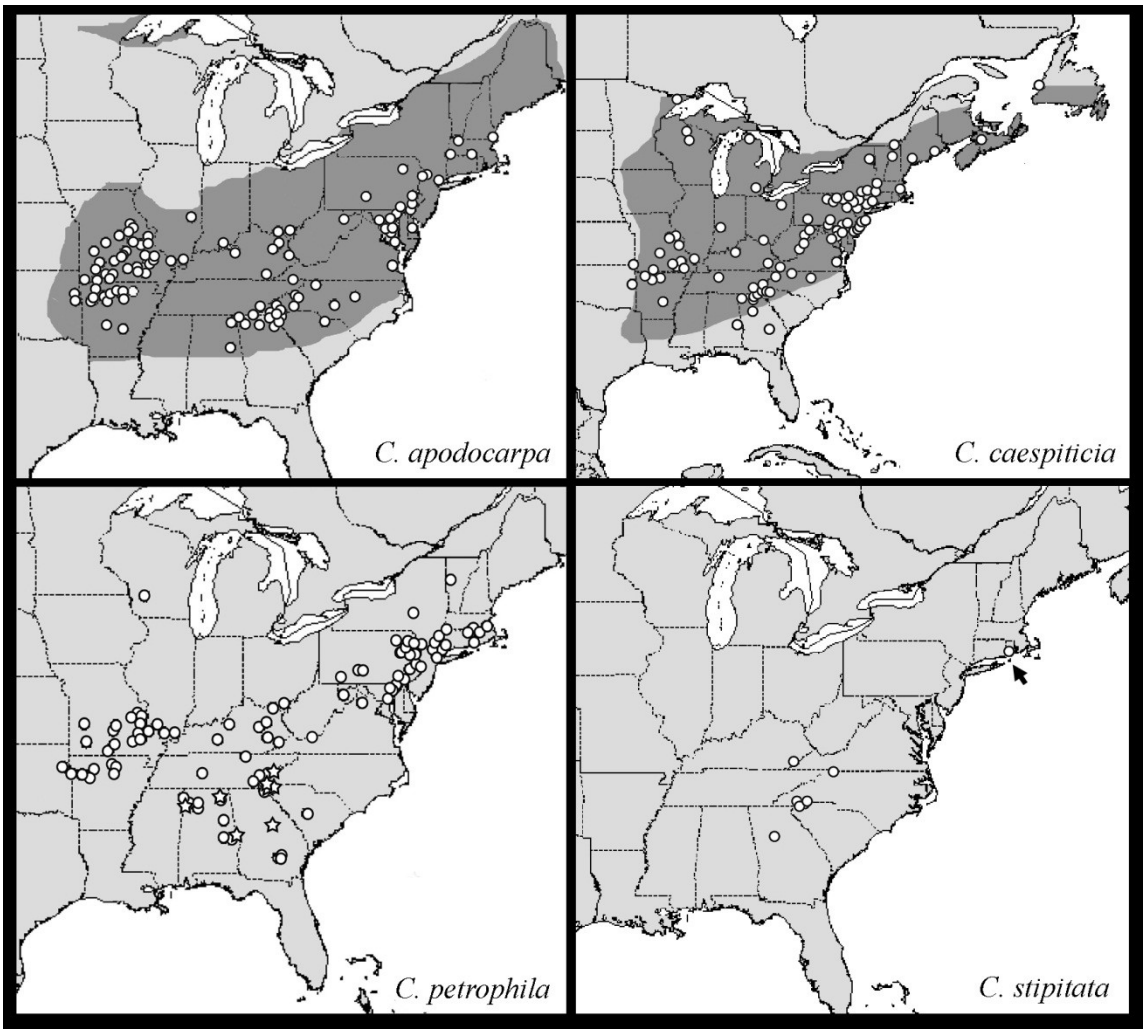

Figure 24. Geographic distributions of *C. apodocarpa* (shaded region is distribution mapped by Brodo et al. (2001)), *C. caespiticia* (shaded region is distribution mapped by Brodo et al. (2001) excluding disjunction in Nebraska), *C. petrophila* (stars = chemotype fumarprotocetraric acid lacking), and *C. stipitata* (arrow points to Rhode Island population).

CHAPTER IV – IMPLEMENTATION OF A UNIFIED TAXONOMY INTEGRATING MOLECULAR AND NON-MOLECULAR DATA IN *LEPRARIA INCANA* S.L.[7]

James C. Lendemer

Abstract. – The divaricatic acid-producing populations of *Lepraria* in North America north of Mexico are revised using traditional morphological characters, chemistry, ecology, biogeography, and ITS1, 5.8S, and ITS2 sequence data. Three taxa are accepted: *L. cryophila, L. hodkinsoniana* sp. nov., and *L. pacifica* sp. nov. Both *Lepraria crassissima* and *L. incana* are excluded from the study area. Non-cryptic, semi-cryptic, and fully-cryptic species concepts in *Lepraria* are discussed with a special emphasis on the practical integration of molecular characters into taxonomic frameworks based on non-molecular characters.

Introduction

Asexual reproduction via lichenized diaspores (i.e., specialized vegetative dispersal units produced by the thallus that contain both fungal and algal components) occurs throughout the diverse lineages that comprise lichenized fungi, and is one of the most interesting aspects of lichen biology. Species that employ this method of reproduction are defined by characters that are considered secondary to those of sexual structures (Tønsberg 1992; Kantvilas & Lumbsch 2010). Although these species comprise a significant component of the lichen biodiversity in any given area they are among the most taxonomically difficult and understudied groups of lichens (Lendemer 2011a).

[7]This chapter was published as: Lendemer, J.C. 2011. A taxonomic revision of the North American species of *Lepraria* s.l. that produce divaricatic acid, with notes on the type species of the genus *L. incana*. Mycologia 103(6): 1216-1229.

In the broad context of aesexually reproducing crustose lichens the genus *Lepraria* Ach. is a unique group, comprised of more than 70 species, that have lost the ability to reproduce sexually yet continued to speciate (Fehrer et al. 2008; Lendemer 2010; Myllys et al. 2005). This genus has been the subject of increasing study in recent years, particularly with respect to the description of new taxa and the documentation of range extensions (see summary in Lendemer (2011)). This flurry of activity has however, occurred in a highly unorganized fashion, in the absence of both a standardized taxonomic approach and a standardized morphological terminology. It has created an uneven systematic landscape where taxonomic concepts, most of which have not been corroborated with independent molecular data, vary widely and descriptions of the same taxon produced by different authors cannot be reconciled without examining authentic material.

Recognizing that these problems have created an impediment to taxonomic progress in this evolutionary interesting group, I selected a revision of *Lepraria* s.l. in North America as the topic of my dissertation. This work represents the first time in more than a century that the genus has been subjected to a complete taxonomic revision on a continental scale. After four years of study it became clear that a single large monograph would not provide the appropriate context to discuss and treat all the problems encountered in detail. As such I have chosen divide this work into a series of contributions where each problem can be treated in detail.

The first part of this series (Lendemer 2011) presented a fully illustrated standardized morphological terminology for the description of *Lepraria* thalli. The present contribution is a revision of the North American populations of *Lepraria* that produce divaricatic acid, specifically as they relate to the chemically similar taxon, *L. incana* (L.) Ach., the type species of the genus. There are three discrete sets of such populations known to occur in the region. These

are i) corticolous populations in eastern North America with thin aggregate thalli that produce divaricatic acid and zeorin, ii) saxicolous populations in eastern North America with thick well-developed thalli that produce divaricatic acid as well as nordivaricatic acid, and iii) corticolous populations in western North America that have morphologically variable thalli and produce divaricatic acid along with zeorin and accessory nordivaricatic acid as a minor constituent.

All three of the above sets of populations have been identified as *L. incana*, or as *L. crassissima* (Hue) Lettau, another species that produces divaricatic acid and was segregated from *L. incana* (Lendemer 2010). This contribution presents the results of a revision of these populations using traditional morphological characters, chemistry, ecology, biogeography, and phylogenetic analyses of ITS1, 5.8S, and ITS2 sequence data.

Materials and methods

Fieldwork and herbarium materials. – This study is based primarily on material of *Lepraria* s.l. in the herbarium of The New York Botanical Garden (NY), supplemented by material received on loan from the following herbaria: CANL, COLO, CONN, DUKE, FH, GZU, H, KANU, MIN, MSC, SBBG, and UCR. Included in the holdings of this group at NY are the >1000 specimens collected by author during his extensive field studies of *Lepraria* throughout North America. To date these total 1.5 years in the field and span the period August, 2007 to December, 2010. Through these field studies the author has been able to gather firsthand observations of ecology and morphology for all the species treated herein. A selection of the comparative specimens of other divaricatic acid-producing *Lepraria* species that were examined is presented in the Appendix.

Chemical and morphological analyses. – The chemistry and morphology of all specimens was studied using the methodology outlined in detail by Lendemer et al. (2008) with the SEM methods as corrected by Lendemer and Elix (2010). Descriptive morphological terms used throughout follow Lendemer (2011). Measurements of granule size, photobiont cell size, and hyphal thickness are given as the average (xbar) +/- 1 standard deviation (sd) and bounded by the extreme observed values, or [smallest observed]-(xbar-1sd)-xbar-(xbar+1sd)-[largest observed] followed by the number of observations (*n*). The character of thallus thickness was found to be highly variable and impossible to measure in species with thin aggregate thalli. It is expressed simply as a range of values rather than as an estimated statistic of central tendency (i.e., xbar).

Molecular data collection. – DNA extractions were performed using the DNeasy Plant Mini Kit (Qiagen) with the instructions modified to include a prolonged (~12 hour) incubation period in lysis buffer. Isolated DNA was resuspended in 100 µl of sterile water and stored at -20°C. PCR amplification was performed using the primers ITS4 and ITS5 (White et al. 1990). Amplification reactions of 25 µl contained each of the following: 2.5 µl 10× PCR Buffer (Qiagen), 2.5 µl dNTP solution (mixed to a concentration of 2 mM of each dNTP), 2.5 µl BSA solution (mixed to a concentration of 2.5 mg μl^{-1} of bovine serum albumin; Hillis et al. 1996), 0.2 µl Taq DNA Polymerase (= 1 U; Qiagen), 1 µl of each primer (in solution at a concentration of 10 µM), 9.3 µl of sterile water, 1 µl of extracted DNA, and 5 µl of Q-solution (Qiagen). PCR protocol followed Nelsen et al. (2007) and consisted of an initial denaturation of 95°C for 5 min, followed by 10 cycles of 95°C for 1 min, 62°C for 1 min, and 72°C for 1 min, then 35 cycles of 95°C for 1 min, 53°C for 1 min and 72°C for 1 min with a final extension for 7 minutes at 72°C. PCR products were studied prior to sequencing by UV examination of a 1% agarose gel on

which 1 µl of amplified PCR product had been subjected to electrophoresis and stained with ethidium bromide. Unpurified amplified PCR products were sent to the University of Washington Biochemistry DNA Sequencing Facility (BSDF) for sequencing. Sequences were assembled and edited by hand using the software package Sequencher™ 4.9 (Gene Codes Corporation, Ann Arbor, MI, USA). In order to identify contaminants, nearest sequence matches were found by searching the nucleotide collection in GenBank using BLASTn (Altschul et al. 1997).

Taxon Sampling. – The goal of this study was to elucidate how the North American *Lepraria* populations that produce divaricatic acid relate to the current circumscriptions of the two divaricatic acid-producing species reported from North America: *L. incana* s.l. and/or *L. crassissima*. In order to address this question, phylogenetic analyses were performed on a dataset consisting of sequences of the nuclear ribosomal ITS region (ITS1, 5.8S, and ITS2) of *L. incana* s.l. and its relatives. A total of twenty new sequences (*n*=20) were generated for this study (table 4). These sequences represent a geographically diverse sampling of the North American populations currently identified as *L. incana* (*n*=15) and *L. cryophila* (*n*=4). An additional sequence (*n*=1) of *L. incana* representing a European population (Germany) was generated to supplement data available in GenBank. Repeated attempts to obtain fresh material of *L. crassissima* from Europe to generate similar supplementary sequences were unsuccessful. In addition to sequences generated for this study, sequences of *L. incana* (*n*=3) and *L. crassissima* (*n*=1) were downloaded from GenBank. Additional GenBank sequences were included in the analyses as follows: i) sequences of *L. caesioalba* (de Lesd.) J.R. Laundon (*n*=5) and *L. elobata* Tønsberg (*n*=6) were included to provide representation of the clade sister to that containing *L.*

incana as recovered by Ekman and Tønsberg (2002); ii) sequences of *L. umbricola* Tønsberg (*n*=5) were included because Ekman and Tønsberg (2002) recovered the species as sister to *L. incana*; and iii) sequences of *L. jackii* Tønsberg (*n*=13) were included based on nearest sequence matches found by searching the nucleotide collection in GenBank using BLASTn (Altschul et al. 1997).

Sequence alignment and phylogenetic analyses. - Sequences were initially aligned using ClustalX (Thompson et al. 1997). The alignment was then manually adjusted using Mesquite ver. 2.0 (Maddison & Maddison 2007). Those sequences that contained clear inconsistencies with all other aligned sequences (e.g., AF517890 of *Lepraria incana*; and EU008625 of *L. nigrocincta* Diederich et al.) were assumed to have resulted from the sequencing of heterogeneous amplicon pools, and were thus excluded. Ambiguous bases and terminal gaps were then converted to missing "?" and the ambiguously aligned regions were excluded from the analysis by including them in an exclusion set. The NEXUS file was then saved with the taxa and characters compressed into a data block.

Following the methodology outlined in detail by Hodkinson and Lendemer (2011) the NEXUS file was prepared for a weighted maximum parsimony analysis with recorded ambiguously-aligned regions. INAASE 3.0 (Lutzoni et al. 2000) was used to recode the ambiguously aligned regions and to generate specific step-matrices using a transition: transversion:gap ratio of 1:2:3. STMatrix 3.0 (written by S. Zoller) was then used to generate step matrices for each of the three ITS regions included in this study. The input file for STMatrix 3.0 was produced using the procedure outlined by Gaya et al. (2003). Specifically this was done as follows: First the NEXUS file that included both ambiguously-aligned regions and constant

sites in the exclusion set was executed in PAUP* 4.0b10 (Swofford 2001). Gaps were treated as a fifth character state using the options available in the Maximum Parsimony settings tab. From the Data menu, "options/Show character status/full details/hide excluded characters" was used to generate a table showing all the nucleotide states found at each site that were *both* not constant and unambiguously aligned. The table was then opened in the display buffer and copied by hand to a blank plain text file that was then saved. The text file was then opened using a standard text editor and the table divided into three subsets (corresponding to ITS1, 5.8S, and ITS2) which were then pasted into new blank text files which were saved and named as per the corresponding ITS region. Each of these files was then opened and all extraneous text deleted, leaving only the nucleotide states for each site on each line. Each of these files was then used to generate a specific step matrix for each region of ITS via STMatrix 3.0. The full NEXUS file including recoded ambiguously-aligned regions and step matrices has been deposited in TreeBase as study #11180.

Parsimony-based phylogenetic analyses were run using weighted maximum parsimony (MP) in PAUP* 4.0b10. An initial search was made with 1000 random-addition-sequence (RAS) replicates and tree bisection reconnection (TBR) branch swapping. The MULTREES option was in effect and zero-length branches were collapsed. All equally most parsimonious trees were saved with branch lengths, and a strict consensus tree was computed for reference. In this analysis the best tree island was hit in >95% of the replicates, and 22 equally most parsimonious trees were recovered. These were identical except for the arrangement of the sequences in the clade representing *L. jackii*, which was not the focus of this study. Branch support for MP trees was estimated through bootstrap analyses (Felsenstein 1985) by performing 1000 bootstrap replicates with 5 RAS per bootstrap replicate, with all other settings as above.

For maximum likelihood (ML) and Bayesian phylogenetic analyses, MrModeltest (Nylander 2004) was run to select a proper model of nucleotide substitution. Using the Akaike Information Criterion (AIC; Akaike 1973), SYM+G was selected as the best model. Searches and bootstrap analyses were run as above using the ML PAUP block produced by MrModeltest with likelihood set as the optimality criterion. The initial ML search yielded nine trees and, as was the case for the MP search, all trees were identical except for the arrangement of the sequences within the *Lepraria jackii* clade. Bayesian analyses were performed using a MrBayes block produced through the online automated form found at the Santos Lab website (http://131.204.120.103/srsantos/mrbayes_form/index.html) and implemented with MrBayes 3.1.2 (Huelsenbeck & Ronquist *2001*) using default priors. The Markov chain Monte Carlo parameters consisted of 10000000 generations, with four chains, and a tree sampled every 100 generations. The first 10000 trees were discarded as burnin and the results were summarized as a 50% majority-rule consensus tree.

The input files used for the analyses outlined above have been deposited in the Dryad data repository (doi:10.5061/dryad.44954v8f).

Results

The goal of this study was to examine the circumscriptions of two species described from Europe, *Lepraria incana* and *L. crassissima*, specifically with respect to the three sets of ecologically and/or biogeographically discrete North American populations of *Lepraria* that also produce divaricatic acid. In order to address this question twenty new nrITS sequences were generated and integrated into a dataset with relevant available sequences from GenBank (see Taxon Sampling above). The ITS region was selected for use in this study because of its general reliability in distinguishing fungal taxa, specifically lichenized fungi, at the species level (Seifert

2009). Although there are cases where this region has proven to be not informative enough at the species level these are the exception in fungi (Begerow et al. 2010). In the present study, clades strongly supported by shared ITS molecular characters were found to correlate with patterns of non-molecular characters as well as ecology and biogeography, thus confirming the relevance and utility of this molecular marker in examining species boundaries within *Lepraria*. The results of these analyses are summarized in figure 25.

Based on the molecular phylogenetic results it is clear that neither *Lepraria crassissima* s. str. nor *L. incana* s. str. is likely to occur in North America. It is also clear that the name *L. crassissima* should not be applied to either of the two sets of chemically and/or morphologically similar populations from North America (see nos. 1 and 3 below). The usage of the European name *L. incana* for chemically and morphologically comparable North American populations requires a careful re-evaluation of current taxonomic practices, and is discussed in detail below (see no. 2).

The new sequences from North American divaricatic acid-producing *Lepraria* populations were recovered in three strongly supported clades, none of which previously had representatives in GenBank. Each of these monophyletic entities was found to be correlated with unique combinations of chemical, ecological, or morphological characters and/or biogeography (table 5). Each entity and its relationship to other known divaricatic acid-producing *Lepraria* species is discussed in detail below. A taxonomic treatment of the North American divaricatic acid-producing *Lepraria* species follows this section. The broader implications of this study on the overall taxonomy of *Lepraria* s.l. will be discussed in an upcoming publication.

Discussion

1) *Lepraria cryophila* vs. *L. crassissima*

A group of four sequences derived from saxicolous, divaricatic acid-producing populations from eastern North America was recovered as a strongly supported clade sister to that containing *L. incana s.l.* and *L. umbricola*. These populations had at one time been referred to *L. crassissima* (Hue) Lettau because they produce nordivaricatic acid in high concentration in addition to divaricatic acid, have thick thalli, and are saxicolous (Lendemer 2010). However they were recently described as a distinct taxon, *L. cryophila*, due to differences in chemistry and ecology (Lendemer 2010). Subsequent study of material of *L. crassissima* from Europe led to the discovery that the chemical and ecological differences used to separate these taxa are not clear cut as previously thought (see further discussion in the taxonomic treatment below). Nonetheless *L. cryophila* formed a monophyletic entity quite distinct from *L. crassissima* in this study. Thus *L. cryophila* represents a species that can be separated from *L. crassissima* s. str. by an allopatric distribution that is strongly correlated with chemical and ecological tendencies. In the future, additional sequences of *L. crassissima* s. str. from Europe should be generated to further test this hypothesis.

2) *Lepraria incana*, a case study in semi-cryptic species

As has been discussed above, the sequences representing *Lepraria incana* s. str. were recovered in a clade that was strongly supported as sister to a clade comprised of chemically and morphologically indistinguishable populations from eastern North America. In light of these results a pairwise distance matrix for the sequences comprising these two clades was generated (using standard Jukes-Cantor distances (Jukes & Cantor, 1969) with gaps treated as a fifth state) to examine sequence divergence. The degree of divergence observed between the two sets of sequences (average = 4.0 $\pm$ 0.1%) is significantly higher than that observed between members of

each clade (average = 0.1 ± 0.1% for North America; 0.7 ± 0.8% for Europe). It is also higher than has been observed as intraspecific variation in the lichenized ascomycetes and is comparable to that observed as interspecific variation (e.g., Nilsson et al. 2008). Considering that the divergence between these two sets of populations is correlated with different geographic distributions of a continental scale (i.e., Europe vs. North America) this would seem to represent a case of semi-cryptic speciation (Vondrák et al. 2009; Hodkinson & Lendemer 2011). While it may be easy to recognize that the eastern North American populations referred to *L. incana* actually represent a semi-cryptic species, the taxonomic implications of this conclusion are not clear cut.

Indeed, this case is very different from that of truly cryptic lichen species, where molecular characters are not correlated to any observable phenomenon (see discussions in Grube & Kroken 2009; Hodkinson & Lendemer 2011; Lendemer & Hodkinson 2009). In such cases recent workers have generally advocated a conservative taxonomic approach, refraining from the formal recognition of taxa that can only be recognized using molecular techniques (Vondrák et al. 2009, Hodkinson & Lendemer 2011). This approach is logical when one takes into account the chaos that the recognition of such taxa would cause in the context of a system that employs the type concept to determine the application of names. The vast majority of lichen type specimens are too old or too small to be analyzed with present-day molecular techniques. Although the process of epitypification could be used to overcome this hurdle, the formal recognition of such taxa is still clearly impractical at present as it is neither cost- nor time-effective to determine voucher specimens with molecular techniques on a large scale.

This case is likewise dissimilar to those where phylogenetic signal is correlated to morphological characters that have simply been overlooked by earlier workers (Argüello et al.

2007; Divakar et al. 2005; Lendemer & Hodkinson 2010; Vondrák et al. 2009). Although such instances have sometimes been referred to as involving the recognition of "cryptic species" this is conceptually incorrect. After all, the use of molecular data to uncover previously overlooked or underemphasized correlations of characters is no different from the traditional pattern of taxonomic discovery through revisionary work (Crespo & Pérez-Ortega 2009; Lendemer & Hodkinson 2010).

The problem of how to treat the two clades corresponding to *Lepraria incana* s.l. is most similar to other cases where molecular characters have been found to correlate only to discrete differences in biogeography. In these examples however, morphologically and chemically identical populations have not been recovered in a sister relationship. Rather they have been recovered in entirely different clades separated by multiple well-supported nodes (Vondrák et al. 2009; Hodkinson & Lendemer 2011). Some have given formal taxonomic recognition to these entities, recognizing that this treatment renders it impossible to assign a given specimen to a species in the absence of locality data. While this is clearly not ideal from the standpoint of practicality when naming a given specimen, it is the most parsimonious solution for reconciling inferred evolutionary history with observable phenotypic reality.

On the basis of the data available here, the most logical conclusion is clearly to formally recognize the eastern North American populations as a distinct species. Continuing to recognize a broadly defined *Lepraria incana* as including both eastern North American and European populations obfuscates two biogeographically and phylogenetically distinct entities. Indeed, formal recognition of these populations results only in more accurately and precisely defined taxonomic entities, which is ultimately the fundamental goal of taxonomy.

Further, it should be noted that this taxonomy does not actually render it impossible to apply a name to specimens that lack a geographic context. The identification of such specimens as "*Lepraria incana* s.l." or "*Lepraria incana* agg." places the specimens in their proper context based on the data available. This is no different from the use of "cf" "aff" or "?" when identifying a sterile specimen of two species that differ only in ascospore size or some other observable character which is lacking in the material. Clearly the hypothetical inadequacy of data associated with a voucher specimen should not prevent taxonomic progress. It should also be noted that it is extremely unlikely that a specimen might be encountered where the continent of its origin is unknown. Also, in the absence of a significant historical context, it is certainly difficult to justify the expense of including a specimen with such poor data in a repository for perpetuity.

An additional taxonomic implication of this treatment is that it casts doubt on the conspecificity of populations identified as *Lepraria incana* outside of Europe. Based on the results of this study it is likely that careful revision of these populations will yield additional semi-cryptic taxa or taxa with distinct but subtle chemical, ecological, or morphological characters. Until these populations receive further study however, they should continue to be referred to as *L. incana* in the absence of evidence to the contrary (Hodkinson & Lendemer 2011).

3) *Lepraria incana* in western North America

The name *Lepraria incana* has been widely applied to divaricatic acid-producing populations of *Lepraria* from western North America (e.g., Noble 1982, Knudsen & Elix 2007, Spribille 1999, Tønsberg 2004). Although many earlier reports are likely misidentifications of

other chemically distinct taxa, the more recent reports almost certainly correctly refer to a taxon that produces divaricatic acid. While collecting in western North America I was able to observe many populations that had been identified as *L. incana* in California and British Columbia. These populations displayed considerable morphological variability, with extremes resembling the thin aggregate thalli of *L. incana* and thick continuous thalli of *L. crassissima*. The majority of thalli were morphologically intermediate between these two extremes and characterized by the development of a moderately thick hypothallus. Due to this morphological variation these populations were sampled intensively at a local level (Yosemite National Park, California) as well as a broader geographic scale (British Columbia, California, and Oregon). All the sequences generated from these populations were recovered in a strongly supported clade separate from the clades comprised of sequences from both *L. incana* s. str. and *L. crassissima* s. str, as well as the other two sets of North American divaricatic acid-producing populations from eastern North America. They are formally recognized as a new species, *L. pacifica*, below.

Taxonomic Section

Summary key to the divaricatic acid-producing species of *Lepraria*

A key to the species of *Lepraria* known to produce divaricatic acid is presented below. It is intended to summarize the current state of knowledge of these taxa and facilitate the identification of specimens. As the present study clearly indicates, users should be very careful when applying names to specimens that do not fit the typical substrate or biogeographic affinities

of a given species. This key also serves to highlight where additional data, particularly molecular studies, would be useful to shed further light on the taxonomy of these species.

1. Zeorin present…2

    2. Nordivaricatic acid absent; corticolous...3

        3. Eastern North America…***L. hodkinsoniana*** **Lendemer**

        3. Europe…***L. incana*** **(L.) Ach.**

    2. Nordivaricatic acid present; corticolous or saxicolous…4

        4. Western North America; rhizohyphae absent; thallus green with the margins not ascending; typically corticolous…***L. pacifica*** **Lendemer**

        4. Europe; rhizohyphae present; thallus blue-white with the margins ascending; typically saxicolous…***L. crassissima*** **(Hue) Lettau**

1. Zeorin absent…5

    5. Hypothallus dark; corticolous; Old World (Asia, Africa)…***L. yunnaniana*** **(Hue) Zahlbr.** (incl. *L. nigrocincta* Diederich et al. from Africa)

    5. Hypothallus pale; saxicolous; New and Old World…6

        6. New World (eastern North America)…***L. cryophila*** **Lendemer**

        6. Old World (Europe)…***L. crassissima*** **(Hue) Lettau**

Accounts of the Species

1) Lepraria cryophila Lendemer, Brittonia, 62(3): 275. 2010. TYPE: **U.S.A. NORTH CAROLINA.** HAYWOOD CO.: Great Smoky Mountains National Park, vicinity of Baxter

Creek, trail to summit of Mt. Sterling (2 miles to summit), 1370–1760 m, spruce-fir-birch forest with sparse hemlock, 26.iv.2008, on vertical rock face, *J.C. Lendemer et al. 11961* (NY!, holotype).

Figure 26 (left).

Description. – **Thallus** crustose, leprose, placodioid, continuous and distinctly bi-layered, initially forming small discrete thalli which expand marginally, overlap and become confluent to form a thick crust (0.2–0.7 mm thick), off white to light blue-white in color; **hyphae** hyaline, [1.4]–(2.2)–2.8–(3.4)–[5] µm (*n*=120) in width, septate, secund, obscured by a thick layer of crystals that dissolve in KOH; **prothallus** disappearing with age; **hypothallus** a well developed layer of loosely intertwined hyphae surrounding and subtending the granules, extending outward from the edge of the thallus; **rhizohyphae** present, abundant, pale to dirty brownish; **granules** globose, [18]–(24)–30–(37)–[52] µm (*n*=120) in diameter, ecorticate, well organized and discrete, often forming compound units of multiple granules; **photobiont** green, coccoid, cells globose, [5]–(6.3)–8.0–(9.6)–[12.5] µm (*n*=120) in diameter.

A description was originally published by Lendemer (2010: 275) however it is reproduced here with improved statistical measurements and revised descriptive terminology.

Chemistry. – TLC: Divaricatic acid (major) and nordivaricatic acid (major).

Spot tests: K-, C+ pink-red, KC+ pink-red, P-, UV+ blue-white.

Ecology and distribution. – *Lepraria cryophila* is a species that typically occurs on non-calcareous rocks in high humidity microhabitats throughout eastern North America and the

Ozark Ecoregion. Occasional populations have been found on the bases of trees directly adjacent to typical thalli occurring on rock or in habitats with unusually high humidity.

Discussion. – At the time I described *Lepraria cryophila* (Lendemer 2010) I had seen only a few specimens of *L. crassissima* s. str. from Europe, including an isotype, all of which produced zeorin as a major secondary compound and occurred on calcareous rocks. As such I questioned the published reports that the concentration of zeorin was variable in *L. crassissima* and that the taxon had a wider ecological amplitude (i.e., occurring on non-calcareous rocks). Subsequently I was able to review a large number of specimens of *L. crassissima* from the type region (France, Belgium) as well as from the Canary Islands of Spain. While the specimens from the Canary Islands consistently produced zeorin as a major substance, those from France and Belgium produced zeorin in highly variable concentrations occasionally so low as to be virtually undetectable with TLC. While this variability blurs the chemical distinction separating *L. crassissima* from *L. cryophila*, the results of the phylogenetic analyses presented here clearly support them as distinct taxa.

2) Lepraria hodkinsoniana Lendemer sp. nov.

Mycobank #561127.

Figure 26 (center).

TYPE: **U.S.A. NEW JERSEY.** ATLANTIC CO.: Wharton State Forest, E of Nesco, NW of Batsto, N of Pleasant Mills Cemetery, 3–10 m, mixed *Pinus rigida-Quercus* forest with sparse *Betula*, 24.x.2004, on *Quercus*, *J.C. Lendemer 3358 = Lich. East. N. Amer. Exs. IV: 185*

(NY!, holotype; ASU, B, BG, CANB, CHR, DOV, FH, GZU, H, M, MIN, S, TNS, TU, UPS, isotypes).

Lepraria incana (GenBank JF739383) distributis America orientalis non Europa et synapomorphis molecularibus in acido desoxyribonucleinico nrITS ab aliis adenio ad positionem 548, cystosino ad positionem 47, guanino ad positiones 209 et 491, thymino 163, 206, 237, 261, et 515 (GenBank JF739366) differt.

Description. – **Thallus** crustose, leprose, aggregated, discontinuous and not stratified, initially composed of isolated granules that divide to form aggregations, eventually merging to form a thin crust (<0.1 mm thick), greenish-blue in color; **hyphae** hyaline, [1.7]–(2.3)–3–(3.8)–[7] µm (*n*=180) in width, septate, secund, obscured by a thick layer of crystals that dissolve in KOH; **prothallus** persistent; **hypothallus** indistinct, poorly developed or absent; **rhizohyphae** absent; **granules** globose, [15]–(25)–33–41–[60] µm (*n*=180) in diameter, ecorticate initially and eventually forming a poorly developed pseudocortex, well organized and discrete; **photobiont** green, coccoid, cells globose, [5]–(6.8)–8.8–(10.9)–[16] µm (*n*=180) in diameter.

Etymology. – The epithet honors Brendan P. Hodkinson, friend and colleague, and fellow lichenologist of the Mid-Atlantic region of eastern North America.

Chemistry. – This species is characterized by the presence of divaricatic acid and zeorin. Atranorin is also frequently detected, however this character is not consistent and does not correlate with substrate or geographic distribution. Occasionally nordivaricatic acid has been

detected as a trace accessory with divaricatic acid. Such occurrences are rare and may represent cases where an unusually large subsample was extracted for chemical analysis.

TLC: Divaricatic acid, zeorin, ± atranorin, ± traces of nordivaricatic acid.

Spot tests: K-, C-, KC- or very weakly KC+ pink, P-, UV+ blue-white.

Ecology and distribution. – *Lepraria hodkinsoniana* is restricted to eastern North America where it occurs primarily on the bark of hardwoods, especially *Acer* and *Quercus*. Occasionally the species has been found on conifers, and very rarely on non-calcareous rocks in habitats with high humidity. The species is most common in the Mid-Atlantic Coastal Plain, where it is frequently associated with *L. caesiella* R.C. Harris in mesic upland hardwood forests. It is however widely distributed with populations throughout the Appalachian Mountains, the Ozark Ecoregion, and the Great Lakes Region. In many respects the distribution of this species mirrors that of *Punctelia caseana* Lendemer & Hodkinson (see Lendemer & Hodkinson 2010).

Discussion. – *Lepraria hodkinsoniana* is morphologically and chemically identical to *L. incana*, another corticolous species with divaricatic acid and zeorin that is ubiquitous in central and northern Europe. In the phylogenetic analyses conducted during this study the sequences derived from eastern North American populations of *L. incana* were recovered in a strongly supported clade sister to a clade comprised of those derived from European populations of *L. incana*. Since these monophyletic groups are strongly correlated with biogeography the most parsimonious taxonomic solution is the recognition of two geographically defined cryptic species.

The new species is one of two *Lepraria* species known from eastern North America that produce divaricatic acid. The other species, *L. cryophila*, differs in having a well-developed hypothallus with pale rhizohyphae and ascending thallus margins, the production of a high concentration of nordivaricatic acid in addition to divaricatic acid, a lack of zeorin, and occurrence on non-calcareous rocks in high humidity microhabitats.

Selected additional specimens examined (atranorin absent unless indicated). – **CANADA. NOVA SCOTIA:** Kejimkujik National Park, 3.v.2009, substrate unknown, *T. McMullin 3480* (NY). **U.S.A. GEORGIA.** MCINTOSH CO.: Sapelo Island, on 16.xii.2009, *Quercus*, *J.C. Lendemer 29045* (NY). **ILLINOIS.** ALEXANDER CO.: Shawnee National Forest, Grapevine Trail Picnic and Camping Area, 6.xi.2004, on *Pinus*, *R.C. Harris 50531* (NY; at+). JACKSON CO.: Shawnee National Forest, along W side of Cedar Lake, 6.xi.2004, on *Sassafras, R.C. Harris 50446* (NY), on *Salix*, *R.C. Harris 50452* (NY; at+). UNION CO.: Shawnee National Forest, N side of Hamburg Hill, 6.xi.2004, on *Liriodendron*, *R.C. Harris 50464* (NY). **MAINE.** WASHINGTON CO.: Town of Steuben, Dyer Neck, 26.vi.2006, on *Betula*, *R.C. Harris 52984* (NY; at+). **MARYLAND.** CECIL CO.: Elk Neck State Forest, Black Hill Tract, 30.xi.2009, on *Quercus*, *J.C. Lendemer 19963* (NY, at+), SE shore of Octoraro Creek, 0–1 mi S of Porters Bridge, 26.v.2009, on *Acer*, *J.C. Lendemer 17803* (NY). KENT CO.: Millington State Wildlife Management Area, 18.iv.2004, on *Acer*, *J.C. Lendemer 6184 & W. Knapp* (NY, PH). **MICHIGAN.** CHEBOYGAN CO.: Reese's Bog, 13.vi.2002, on wood, *W.R. Buck 41995* (NY; at+). GOGEBIC CO.: Ottawa National Forest, Bobcat Lake, 3.viii.2003, on *Tsuga*, *C.M. Wetmore 89388* (MIN, NY). **MINNESOTA.** HUBBARD CO.: Itasca State Park, 8.viii.1975, on *Pinus*, *W.R. Buck 377* (NY). **NEW YORK.** BRONX CO.: The New York Botanical Garden,

1.i.2001, on *Quercus*, *A. Amtoft 320* (NY; at+). CLINTON CO.: Town of Mooers, 18.x.1996 on *Acer*, *R.C. Harris 39058* (NY; at+). **PENNSYLVANIA.** BERKS CO.: French Creek State Park, 19.xi.2006, on *Acer*, *J.C. Lendemer 8014* (NY). CUMBERLAND CO.: Michaux State Forest, Big Pond Rd., 2.vi.2009, on *Quercus, J.C. Lendemer 18304* (NY, at+). MONROE CO.: Tannersville Bog TNC Preserve, 26.vii.2009, on *Acer*, *J.C. Lendemer 18478* (NY). PIKE CO.: Delaware Water Gap National Recreation Area, 23.iv.2004, on stump, *R.C. Harris 49481* (NY; at+). YORK CO.: SGL #83, Sawmill Run, 27.v.2009, on *Acer*, *J.C. Lendemer 17903* (NY); Bridge No. 6, Mill Creek, 27.v.2009, on *Juglans*, *J.C. Lendemer 17937* (NY). **NEW JERSEY.** ATLANTIC CO.: Wharton State Forest, N of Pleasant Mills Cemetery, 24.x.2004, on *Quercus*, *J.C. Lendemer 3358* (NY, PH; at+). CAMDEN CO.: Winslow Wildlife Management Area, 4.ii.2009, on *Quercus*, *J.C. Lendemer 15361* (NY, at+). CUMBERLAND CO.: Peaslee Wildlife Management Area, 2.ii.2009, on *Quercus*, *J.C. Lendemer 15170* (NY). GLOUCESTER CO.: Glassboro Wildlife Management Area, 4.ii.2009, on *Acer*, *J.C. Lendemer 15301* (NY, at+). OCEAN CO.: Manchester Wildlife Management Area, 5.ii.2009, on *Quercus*, *J.C. Lendemer 15373* (NY, at+). **RHODE ISLAND.** WASHINGTON CO.: Marion Eppley Sanctuary, 16.ix.2006, on rotten tree, *R.C. Harris 53144* (NY). **SOUTH CAROLINA.** AIKEN CO.: The Hitchcock Woods, 14.iii.2010, on *Nyssa*, *J.C. Lendemer 22149* (NY). EDGEFIELD CO.: Sumter National Forest, along FS 634, 17.iii.1997, on *Betula*, *R.C. Harris 40249* (NY). **VIRGINIA.** SUFFOLK CITY: Dismal Swamp National Wildlife Refuge, vicinity of canal bridge over Jericho Ditch, 12.xii.2009, on *Ilex*, *J.C. Lendemer 20482 & B. Hodkinson* (NY).

3) Lepraria pacifica Lendemer sp. nov.

Mycobank #561128.

Figure 26 (right).

TYPE: **U.S.A. CALIFORNIA.** MARIPOSA CO.: Yosemite National Park, S side of Merced River, downstream of Phono Bridge, W end of Yosemite Valley, 1172 m, mesic north-facing talus slope, with mixed conifer forest, 22.ix.2009, on *Calocedrus*, *J.C. Lendemer 19765* (NY!, holotype).

Sicut *Lepraria hodkinsoniana* sed acidum nordivaricaticum continente et distributis solum America occidentalis.

Description. – **Thallus** crustose, leprose, aggregated, discontinuous and not stratified to continuous and weakly stratified, initially composed of isolated granules that divide to form aggregations, eventually merging to form a crust of variable thickness (0.1–0.4–(0.7) mm), greenish-blue in color; **hyphae** hyaline, [1.7]–(2.2)–2.8–(3.3)–[4] µm (*n*=120) in width, septate, secund, obscured by a thick layer of crystals that dissolve in KOH; **prothallus** persistent and readily visible; **hypothallus** typically indistinct, poorly developed or absent; **rhizohyphae** absent; **granules** globose, [16]–(24)–32–(40)–[60] µm (*n*=120) in diameter, ecorticate initially and eventually forming a poorly developed pseudocortex, well organized and discrete; **photobiont** green, coccoid, cells globose, [5]–(6.1)–7.5–(8.9)–[13] µm (*n*=120) in diameter.

Etymology. – The epithet “pacifica” refers to the geographic distribution of the species, which is restricted to mountain ranges more-or-less near the Pacific Coast in western North America.

Chemistry. – *Lepraria pacifica* is characterized by the presence of divaricatic and zeorin with nordivaricatic acid as an accessory in low, but highly variable, concentrations. The C+ and KC+ spot test reactions vary in intensity depending on the concentration of the latter substance. Comparative TLC of *L. crassissima*, *L. cryophila*, and *L. pacifica* revealed nordivaricatic acid to be present in consistently higher concentrations in the former two species than the latter one. Anthraquinone pigments were also found to be present in populations of *L. pacifica* from an isolated redwood forest in the Santa Lucia Mountains of central California. This population was not studied with molecular methods and has since been extirpated by a wildfire (Lendemer & Knudsen 2010).

TLC: Divaricatic acid, zeorin, traces of nordivaricatic acid, ± an unidentified anthraquinone pigment.

Spot tests: K- (or K+ purple when pigment is present), C± pink, KC+ pink, P-, UV+ blue-white.

Ecology and distribution. – The vast majority of collections of *Lepraria pacifica* were found on conifer taxa endemic to western North America. It is the dominant *Lepraria* species in the redwood forests of the Coastal Range and the Sierra Nevada Mountains in central and northern California, becoming less frequent farther north in coastal British Columbia where it is replaced by the ecologically similar *L. torii* T. Sprib. & Pérez-Ortega. Tønsberg (2004) reported *L. incana* from southern California where it likely occurs as disjunct populations in montane habitats. I have reviewed only one specimen from that region and it represents *L. pacifica*.

Discussion. – *Lepraria pacifica* has previously been confused with *L. crassissima* and *L. incana* because of its chemistry. Based on the results of the phylogenetic analyses presented here it is the only species of *Lepraria* occurring in western North America that produces divaricatic acid. Although the species typically develops a characteristic thick thallus this character varies with the degree of development of the hypothallus.

The new species is chemically similar to *Lepraria crassissima* in constantly producing nordivaricatic acid as an accessory to divaricatic acid and zeorin, however it is biogeographically, ecologically, and morphologically distinct from that taxon. *Lepraria crassissima* is a primarily saxicolous, European species with a thallus comparable to that of *L. cryophila* in having both a well-developed hypothallus and weft of rhizohyphae as well as ascending thallus margins. In contrast *L. pacifica* is a primarily corticolous species of western North America that produces a thallus of variable thickness and usually lacks rhizohyphae and ascending thallus margins. Occasional specimens (e.g., *Buck 53979*) from humid habitats can develop atypical thalli that are morphologically similar to *L. crassissima*. The results of the phylogenetic analyses conducted during this study did not reject the inclusion of such specimens in *L. pacifica*. Morphologically *L. pacifica* is most similar to robust forms of *L. incana*, a European species. *Lepraria incana* typically has a thinner thallus however, and rarely produces nordivaricatic acid in quantities detectable with TLC.

Selected additional specimens examined (specimens with anthraquinones as indicated). – **CANADA. BRITISH COLUMBIA.** QUEEN CHARLOTTE ISLANDS: Kunghit Island, Rose Harbour, 4.vii.1971, on *Thuja*, *I.M. Brodo 17682 & P.Y. Wong* (NY). SQUAMISH-LILLOOET DIST.: Stawamus Chief Provincial Park, 11.v.2009, on rotting wood, *W.R. Buck 54755* (NY),

Murrin Provincial Park, on *Thuja*, 11.v.2009, *W.R. Buck 54783* (NY). VANCOUVER CITY: University of British Columbia Endowment Lands, 9.viii.1980, on *Alnus*, *T. Ahti 38566* (H, NY), on *Tsuga*, *T. Ahti 38572* (H, NY), Pacific Spirit Regional Park, Spanish Bank Creek, 9.v.2009, on *Acer*, *W.R. Buck 54629* (NY). **U.S.A. CALIFORNIA.** MARIPOSA CO.: Yosemite National Park, 1 km E of Hodgdon Meadow on Old Big Oak Flat Rd., 19.ix.2009, on *Abies, J.C. Lendemer 19541* (NY), on *Calocedrus, J.C. Lendemer 19545* (NY); Yosemite National Park, bottom of Tuolumne Grove, 19.ix.2009, on burnt log, *J.C. Lendemer 19579-A* (NY). MONTEREY CO.: Santa Lucia Mtns., Lime Kiln State Park, 11.ix.2008, on burnt redwood, *K. Knudsen 10274.2* (NY, UCR), 4.ix.2008, on redwood, *K. Knudsen 10200* (NY, UCR; athraq+), Central Coast, Point Lobos State Reserve, 14.v.2009, on *Cupressus*, *K. Knudsen 11125* (NY, UCR). SANTA CRUZ CO.: Henry Cowell Redwoods State Park, 16.vii.2008, on *Sequoia*, *W.R. Buck 53979* (NY), The Forest of Nisene Marks State Park, 13.vii.2008, on *Sequoia*, *W.R. Buck 53923* (NY). **OREGON.** COOS CO.: South Slough National Estuarine Research Reserve, ix.2008, on *Chamaecyparis*, *B. McCune 29769* (NY). LINN CO.: W side of Clear Lake, i.2009, on wood, *B. McCune 29887* (NY). **WASHINGTON.** THURSTON CO.: between Bald Hill and Bald Hill Lake, 20 km SW of town of Yelm, iii.2010, *B. McCune et al. 30504* (NY).

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The Nature Conservancy. The curators of the herbaria cited in the methodology section are thanked for providing access to specimens cited herein. Sean Beeching, Malcolm Hodges, and Klaus Kalb are also thanked for providing loans of material from their personal herbaria.

Appendix – Comparative Material Examined

1) *Lepraria crassissima (Hue) Lettau*, Feddes Repert. Spec. Nov. Regni Veg. 61: 125. 1958.

Crocynia crassissima Hue, Bull. Soc. Bot. France, sér. 4, 24: 393. 1924. TYPE: **FRANCE:** Docelles (Vosges), au Fluxelier et a l'Encerf, ad basim rupium locis umbrosis, *sine date*, *J. Harmand s.n. = Lichenes Galici Praeceipi Exs. 400* (FH! [divaricatic, nordivaricatic, zeorin (major)], isotype).

Selected specimens examined (zeorin concentration indicated in brackets). – **BELGIUM. PROV. NAMUR:** Vallée de l'Hermeton, 25.vii.1999, on calcareous schist, *E. Sérusiaux* (LG [minor]), Falmignoul, aux ruines de Château Thierry, 13.vi.1998, on calcareous rock, *D. Ertz & E. Sérusiaux s.n.* (LG [minor]). **PROV. LUXEMBOURG:** Sante-Cécile, Vallée de la Semois, 5.vii.1997, on siliceous rock, *E. Sérusiaux s.n.* (LG [minor]). **FRANCE. DEPT. PYRÉNÉES-ATLANTIQUES:** Vallée d'Ossau, 8.vii.2000, on granite, *E. Sérusiaux s.n.* (LG [minor]). **NORWAY. SØR-TRØNDELAG:** Trondheim, 22.viii.1983, on rock wall, *T. Tønsberg 8122* (NY [major]). **SPAIN. CANARY ISLANDS:** Madeira, between Ponta do Sol and Porto Moniz, 8.vi.1992, on siliceous rock, *P. Diederich 4928* (LG [major]), La Palma, Los Tilos, 19–22.vii.1997, substrate unknown, *E. Sérusiaux s.n.* (LG [major]).

2) *Lepraria incana* (L.) Ach., Method. Lich., p. 4. 1803.

Byssus incana L., Sp. Pl. 2: 1169. 1753. TYPE: British Isles, drawing in Dillenius, *Hist. Musc.* tab. I fig. 3. 1742 "1741", holotype. TYPOTYPE: on bark, *Hist. Musc.* tab. I fig. 3 (OXF[n.v.] [divaricatic and zeorin *fide* Laundon (1992)]).

Selected specimens examined (atranorin absent unless indicated). – **AUSTRIA. STEIERMARK:** Schladminger Tauern, 5.vii.1996, on *Picea*, *H. Mayrhofer 13.028* (NY, at+). **DENMARK.** Allingåbro, 16.v.1969, on *Pinus*, *S. Svane 47* (NY). **ESTONIA. IDA-VIRU CO.:** Kurtna Landscape Reserve, 12.vi.2008, on stump, *A. Suija 107* (NY, at+). **GERMANY. OBERPFALZ:** Forest NO von Gimpertshausen, 20.xi.1993, on *Fagus*, *A. Leopold s.n.* (NY), wenige km S von Parsberg zwischen Nürnberg und Regensburg, 27.x.2007, on *Picea*, *K. Kalb s.n.* (NY[ex Kalb-37129]). **RHEINLAND-PFALZ:** Kreis Cochem, 30.i.2010, on *Quercus*, *W.R. Buck 55943* (NY), Kreis Rhein-Hunsrück, 31.i.2010, on *Alnus, W.R. Buck 55965* (NY). **LATVIA.** Kemeri National Park, 28.ix.1996, on mosses, *J. Motiejūnaitė 2952* (NY, at+). **LITHUANIA. VILNIUS CO.:** Pabradė military forest lot, 28.vi.1995, on *Betula*, *J. Motiejūnaitė 2272* (NY, at+), environs of Tamošava village, vii.1995, on *Picea*, *J. Motiejūnaitė 2118* (NY, at+). **ENGLAND.** Cambridge, Wicken Sedge Fen, 28.i.1991, on *Salix*, *J.R. Laundon 3232* (NY).

3) *Lepraria yunnaniana* (Hue) Zahlbr. *in* Handel-Mazzeti, Symbolae Sinicae 3: 244. 1930.

Crocynia yunnaniana Hue, Bull. Soc. Bot. Fr. 71: 396. 1924. TYPE: **CHINA. YUNNAN.** "sur la terre, près les rochers de You-foui, au-dessous de Mo-So-yn", 17.vi.1887, *abbé Delavay 2995* (PC[n.v.], holotype; BM[n.v.] [divaricatic and nordivaricatic (tr.) *fide* Laundon (2008)] isotype).

Lepraria nigrocincta Diederich, Sérusiaux & Aptroot *in* Aptroot, Diederich, Sérusiaux & Sipman, Biblthca Lichenol., 64: 78. 1997. TYPE: **BURUNDI.** galerie forestière de la Siguvyaye un peu en aval du pont de la route Rumonge-Bururi, 1750 m, 20.ix.1974, dans le bas d'un gros tronc de *Carapa*, *J. Lambinon 74/1266* (LG! [divaricatic, nordivaricatic], holotype)

Specimens examined. – **CHINA. YUNNAN PROV.:** Lijiang Pref., Laojunshan Mt., 16.vii.2010, corticolous, *B. Goffinet 10077* (CONN, NY). **RWANDA. GIKONGORO PROV.:** Nyungwe Forest, Marais Rwasenkoko, 31.iii.2005, corticolous, *E. Sérusiaux s.n.* (LG, NY).

Taxon	GenBank	isolate	Collector	Herbarium	Locality
L. caesioalba	EU008612	N/A	M.P. Nelsen 3969	WIS	U.S.A.
	EU008611	N/A	M.P. Nelsen 3967	WIS	U.S.A.
	EU008610	N/A	M.P. Nelsen 3966	WIS	U.S.A.
	EU008609	N/A	M.P. Nelsen 3965	WIS	U.S.A.
	AF517901	N/A	T. Tønsberg 27312	BG	Norway
L. crassissima	AF517902	N/A	T. Tønsberg 28881	BG	Norway
L. cryophila	JF739379	NY975	J.C. Lendemer 24459	NY-1216110	U.S.A. Pennsylvania, Center Co.
	JF739382	NY962	J.C. Lendemer 24714	NY-1216216	U.S.A. Pennsylvania, Warren Co.
	JF739380	NY961	J.C. Lendemer 24710	NY-1216220	U.S.A. Pennsylvania, Warren Co.
	JF739381	NY959	J.C. Lendemer 23965	NY-1205728	U.S.A. Pennsylvania, Elk Co.
L. caesioalba	EF619547	N/A	S. Slavikova-Bayerova 3529	PRA	Ukraine
	EF619546	N/A	S. Slavikova-Bayerova 3528	PRA	Ukraine
	EF619545	N/A	S. Slavikova-Bayerova 3449	PRA	Ukraine
	AF517910	N/A	T. Tønsberg 28152	BG	Czech Republic
	EF619544	N/A	A. Orange 15453-2	PRA	United Kingdom
	AF517909	N/A	T. Tønsberg 28201	BG	Germany
L. hodkinsoniana	JF739365	NY245	J.C. Lendemer 15373	NY-1078987	U.S.A. New Jersey, Ocean Co.
	JF739367	NY224	J.C. Lendemer 15170	NY-1078648	U.S.A. New Jersey, Cumberland Co.
	JF739368	NY238	J.C. Lendemer 15361	NY-1078998	U.S.A. New Jersey, Camden Co.
	JF739364	NY671	J.C. Lendemer 20482	NY-1152860	U.S.A. Virginia, Suffolk Co.
	JF739366	NY552	J.C. Lendemer 18478	NY-1107055	U.S.A. Pennsylvania, Monroe Co.
L. incana	JF739383	NY808	W.R. Buck 55965	NY-1167675	Germany, Rheinland-Pfalz
	AF517891	N/A	S. Ekman 3581	BG	Sweden
	AF517899	N/A	T. Tønsberg 28281	BG	Norway
L. jackii	EF619576	N/A	A. Orange 15175	PRA	United Kingdom, Scotland
	EF619573	N/A	A. Orange 15172	PRA	United Kingdom, Scotland
	EF619575	N/A	A. Orange 14998	PRA	United Kingdom, Scotland
	EF619572	N/A	A. Orange 15180	PRA	United Kingdom, Scotland
	EF619571	N/A	A. Orange 14997	PRA	United Kingdom, Scotland
	EF619569	N/A	Zelinkova & Halda s.n.	hb. Zelinkova	Czech Republic
	AF517912	N/A	T. Tønsberg 28150	BG	Czech Republic
	AF517911	N/A	T. Tønsberg 28127	BG	Germany
	EF619568	N/A	Peska s.n.	hb. Peska	Czech Republic
	EF619574	N/A	A. Orange 14994	PRA	United Kingdom, Scotland
	EF619566	N/A	S. Slavikova-Bayerova 3730	PRA	Ukraine
	EF619567	N/A	Gruberova s.n.	PRA	Czech Republic
	EF619570	N/A	S. Slavikova-Bayerova 3730	PRA	Ukraine
L. pacifica	JF739369	NY738	A.M Fryday 9285	MSC	U.S.A. California, Mariposa Co.

	JF739370	NY600	J.C. Lendemer 19524	NY-1133207	U.S.A. California, Mariposa Co.
	JF739373	NY590	J.C. Lendemer 19568	NY-1133223	U.S.A. California, Mariposa Co.
	JF739374	NY601	J.C. Lendemer 19562	NY-1133230	U.S.A. California, Mariposa Co.
	JF739376	NY599	J.C. Lendemer 19545	NY-1133185	U.S.A. California, Mariposa Co.
	JF739377	NY588	J.C. Lendemer 19566	NY-1133225	U.S.A. California, Mariposa Co.
	JF739378	NY744	A.M. Fryday 9285	MSC	U.S.A. California, Mariposa Co.
	JF739371	NY533	W.R. Buck 54751	NY-1136393	Canada. British Columbia, Squamish-Lillooet Distr.
	JF739372	NY651	W.R. Buck 53979	NY-1028759	U.S.A. California, Santa Cruz Co.
	JF739375	NY320	B. McCune 29887	NY-1079488	U.S.A. Oregon, Linn Co.
L. umbricola	AF517897	N/A	T. Tønsberg 24838	BG	Norway
	AF517898	N/A	T. Tønsberg 26087	BG	Norway
	EF619583	N/A	A. Orange 15001	PRA	United Kingdom
	EF619582	N/A	A. Orange 15174	PRA	United Kingdom
	EF619584	N/A	A. Orange 15250	PRA	United Kingdom

Table 4. Information for voucher specimens used to generate sequences included in this study.

		L. crassissima	L. cryophila	L. hodkinsoniana	L. incana	L. pacifica	L. yunnaniana
Chemistry							
	divaricatic	major	major	major	major	major	major
	nordivaricatic	major	major	absent or trace	absent or trace	major or minor	major
	zeorin	major to minor	absent	major	major	major	absent
Substrate		calcareous rocks	non-calcareous rocks	hardwoods rarely conifers	hardwoods and conifers	conifers rarely hardwoods	corticolous
Biogeography		Europe	North America (Appalachian +Great Lakes + Ozarks)	North America (Appalachians + Piedmont + Mid-Atlantic Coastal Plain)	Europe	North America (western Coastal Ranges and Sierra Nevadas)	Africa and Asia; South America (?)
Hypothallus	development	Well developed	Well developed	Absent/poorly developed	Absent/poorly developed	Absent/poorly developed	Well developed
	color	pale	pale	pale	pale	pale	dark
Rhizohyphae		Present	Present	Absent	Absent	Absent/poorly developed	Absent

Table 5. Tabular comparison the divaricatic acid producing species of *Lepraria*.

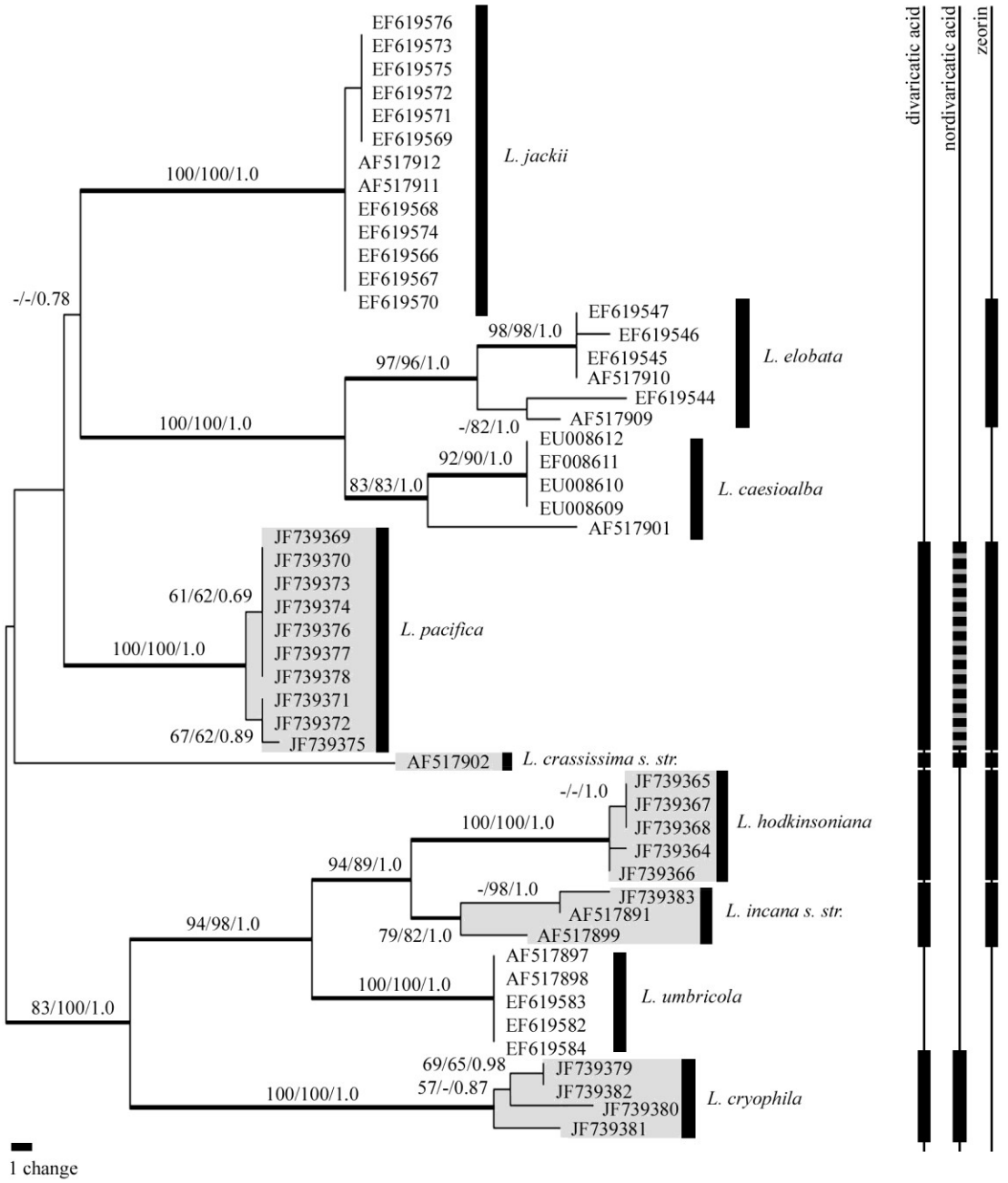

Figure 25. Phylogeny of divaricatic acid producing *Lepraria* species (shaded clades) and selected other species, inferred from ITS sequence data and displayed as one of 22 equally most parsimonious trees with midpoint rooting. Support was evaluated using maximum parsimony (MP) and maximum likelihood (ML) bootstrap proportions (BP) and Bayesian posterior probabilities (B-PP). Support values >50% are displayed at each node in the following format: MP-BP/ML-BP/B-PP. Branches with MP-BP and ML-BP support >70% as well as BP-PP support >95% are thickened. Secondary chemistry is included on the far right, where thickened lines represent the presence of a substance and gray hatching indicates consistent presence as a minor accessory.

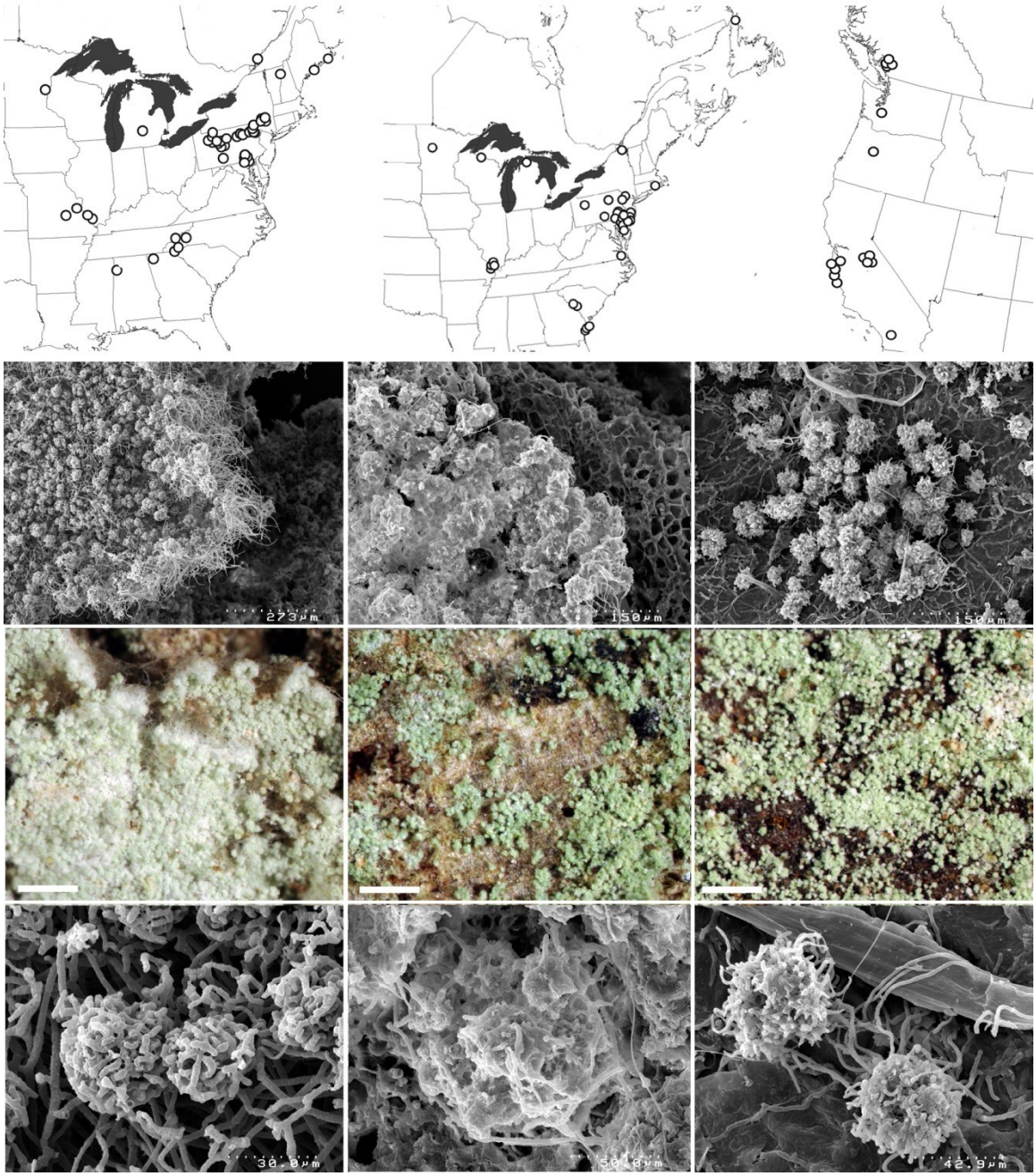

Figure 26, Overview of *Lepraria cryophila* (left), *L. hodkinsoniana* (center), and *L. pacifica* (right). From top to bottom, geographic distribution based on specimens examined, SEM of gross morphology of thallus, LM of gross morphology of thallus, SEM of granules. Scale bars as indicated, except for LMs where scale = 1.0 mm.

CHAPTER V – IMPLEMENTATION OF A UNIFIED TAXONOMY INTEGRATING MOLECULAR AND NON-MOLECULAR DATA IN *LEPRARIA NORMANDINOIDES* S.L.

James C. Lendemer

Abstract. - *Lepraria normandinoides* is currently circumscribed to include three chemotypes that differ in the presence/absence of two structurally similar depsides: fumarprotocetraric acid and protocetraric acid. Populations of these chemotypes are sympatric and morphologically identical. A molecular phylogeny was inferred from ITS1, 5.8S, and ITS2 sequence data generated from a geographically broad sampling of populations of *L. normandinoides*. Two strongly-supported clades were recovered that correlate to the depside producing chemotypes. Consequently *L. normandinoides* is restricted to the protocetraric acid producing chemotype and the fumarprotocetraric acid chemotype is formally described as *L. oxybapha*. Acid deficient populations are tentatively retained in *L. normandinoides*.

Introduction

The genus *Lepraria* Ach. comprises more than 70 described species representing a morphologically diverse and evolutionarily heterogeneous assemblage of asexually reproducing lichens with leprose thalli (Saag et al. 2009). As has been summarized in several publications, a recent flood of research has thrown the species- and genus-level taxonomy of the group into considerable disarray (Lendemer 2011, 2011b). Unfortunately the problems currently plaguing *Lepraria* not only serve to obfuscate its true alpha and beta diversity but also render it impossible to use the group to address relevant biological questions about the evolution of asexual reproduction in the Lecanoromycetes.

In an effort to remedy this situation I undertook a revision of the genus in North America north of Mexico as the subject of my doctoral dissertation. Twenty-seven *Lepraria* species had been reported from the region as of 2009 (Esslinger 2010); however, recent studies have revealed considerably more diversity than previously thought (Lendemer 2010, 2011b). Indeed, using a taxonomic approach that integrates molecular and non-molecular characters it has been demonstrated that the type species of the genus, *L. incana* (L.) Ach., is polyphyletic and that the name actually applies to three species in North America (none of which corresponds to *L. incana* s. str.). Here the same approach is used to examine the circumscription of *L. normandinoides* Lendemer & R.C. Harris, a species with three chemotypes that was separated from *L. nivalis* J.R. Laundon using a combination of chemical, ecological, and morphological characters (Lendemer & Harris 2007).

Materials and methods

Non-molecular materials and methods. – As in previous contributions on *Lepraria* s.l. by the author, this study is based primarily on material in the herbarium of The New York Botanical Garden (NY), supplemented by material received on loan from the following herbaria: CANL, COLO, CONN, DUKE, FH, GZU, H, KANU, MIN, MSC, SBBG, UCR, and UTEP. This includes >1000 specimens collected by the author during his extensive field studies of *Lepraria* throughout North America. To date these total 1.5 years in the field and span the period August, 2007 to June, 2011. Through these field studies the author has been able to gather firsthand observations of ecology and morphology for all the species known to occur in North America.

Chemical and morphological analyses were carried out using the methodology outlined in detail by Lendemer et al. (2008) with the SEM methods as corrected by Lendemer and Elix (2010). Descriptive morphological terminology follows Lendemer (2011) Measurements of

granule size, photobiont cell size, and hyphal thickness are given as the average (xbar) ± 1 standard deviation (sd) and bounded by the extreme observed values, or [smallest observed]-(xbar-1sd)-xbar-(xbar+1sd)-[largest observed]. The number of observations (*n*) is also provided.

Molecular data collection. – DNA extractions were performed using the DNeasy Plant Mini Kit (Qiagen) with the instructions modified to include a prolonged (~12 hour) incubation period in lysis buffer. Isolated DNA was resuspended in 100 µl of sterile water and stored at -20°C. PCR amplification was performed using the primers ITS4 and ITS5 (White et al. 1990). Amplification reactions of 25 µl contained each of the following: 2.5 µl 10× PCR Buffer (Qiagen), 2.5 µl dNTP solution (mixed to a concentration of 2 mM of each dNTP), 2.5 µl BSA solution (mixed to a concentration of 2.5 mg μl^{-1} of bovine serum albumin; Hillis et al. 1996), 0.2 µl Taq DNA Polymerase (= 1 U; Qiagen), 1 µl of each primer (in solution at a concentration of 10 µM), 9.3 µl of sterile water, 1 µl of extracted DNA, and 5 µl of Q-solution (Qiagen). PCR protocol followed Nelsen et al. (2007) and consisted of an initial denaturation of 95°C for 5 min, followed by 10 cycles of 95°C for 1 min, 62°C for 1 min, and 72°C for 1 min, then 35 cycles of 95°C for 1 min, 53°C for 1 min and 72°C for 1 min with a final extension for 7 minutes at 72°C. PCR products were studied prior to sequencing by UV examination of a 1% agarose gel on which 1 µl of amplified PCR product had been subjected to electrophoresis and stained with ethidium bromide. Unpurified amplified PCR products were sent to the University of Washington Biochemistry DNA Sequencing Facility (BSDF) for sequencing. Sequences were assembled and edited by hand using the software package Sequencher™ 4.9 (Gene Codes Corporation, Ann Arbor, MI, USA). In order to identify contaminants, nearest sequence matches

were found by searching the nucleotide collection in GenBank using BLASTn (Altschul et al. 1997).

Taxon Sampling. – The goals of this study were to 1) determine whether the chemotypes of *Lepraria normandinoides* represent monophyletic entities, and 2) examine the circumscription of *L. normandinoides* with respect to other chemically similar species with placodioid thalli. To address these issues phylogenetic analyses were performed on a dataset comprised of sequences of the nuclear ITS region (ITS1, 5.8s, and ITS2) of the three chemotypes of *L. normandinoides*, and selected additional sequences from GenBank (see below). A total of thirteen (*n*=13) new sequences were generated for this study including a geographically diverse sampling of the fumarprotocetraric acid chemotype (*n*=4) and protocetraric acid chemotype (*n*=8). Multiple attempts to sequence specimens of the acid deficient chemotype led to only a single usable sequence (*n*=1). In addition to the above sequences the following were downloaded from GenBank: 1) sequences of *L. normandinoides* (EU008628, EU008629) that were unidentified to species but nonetheless included in prior published studies; 2) sequences of *L. isidiata* (*n*=6) and *L. nivalis* (*n*=2), both of which have placodioid thalli and are chemically comparable to *L. normandinoides*; and 3) sequences of *L. sylvicola* (*n*=3), a chemically and morphologically distinct species that was recovered among the nearest sequence matches using BLASTn.

Sequence alignment and phylogenetic analyses. – Sequences were initially aligned using ClustalX (Thompson et al. 1997). The alignment was then manually adjusted using Mesquite ver. 2.0 (Maddison & Maddison 2007). Sequences containing clear inconsistencies with all other aligned sequences were assumed to have resulted from the sequencing of heterogeneous

amplicon pools, and thus excluded (see Hodkinson & Lutzoni 2009). Ambiguous bases and terminal gaps were converted to missing "?" and the ambiguously aligned regions were excluded from the analysis by including them in an exclusion set. The NEXUS file was then saved with the taxa and characters compressed into a data block.

Following Hodkinson and Lendemer (2011) the NEXUS file was prepared for a weighted maximum parsimony analysis with recorded ambiguously-aligned regions. INAASE 3.0 (Lutzoni et al. 2000) was used to recode the ambiguously aligned regions and to generate specific step-matrices using a transition: transversion:gap ratio of 1:1:1. STMatrix 3.0 (written by S. Zoller; *Miadlikowska* et al. 2002) was then used to generate step matrices for each of the three ITS regions. The input file for STMatrix 3.0 was produced using the procedure outlined by Gaya et al. (2003) and described in greater detail by Lendemer (2011b).

Parsimony-based phylogenetic analyses were run using weighted maximum parsimony (MP) in PAUP* 4.0b10. An initial search was made with 1000 random-addition-sequence (RAS) replicates and tree bisection reconnection (TBR) branch swapping. The MULTREES option was in effect and zero-length branches were collapsed. All equally most parsimonious trees were saved with branch lengths, and a strict consensus tree was computed for reference. In this analysis the best tree island was hit in 99% of the replicates, and 36 equally most parsimonious trees were recovered. These were identical except for the arrangement of the sequences within each of the clades representing the chemotypes of *L. normandinoides*. Branch support for MP trees was estimated through bootstrap analyses (Felsenstein 1985) by performing 1000 bootstrap replicates with 5 RAS per bootstrap replicate, with all other settings as above. For maximum likelihood (ML) and Bayesian phylogenetic analyses, MrModeltest (Nylander 2004) was run to select a proper model of nucleotide substitution. Using the Akaike Information *Criterion* (*AIC;*

Akaike 1973), K80+G was selected. Searches and bootstrap analyses were run as above using the ML PAUP block produced by MrModeltest with likelihood set as the optimality criterion. The initial ML search yielded eight trees and, as was the case for the MP search, all trees were topologically equivalent except for the arrangement of the sequences within each of the clades representing the chemotypes of *L. normandinoides*. Bayesian analyses were performed using a MrBayes block produced through the online automated form found at the Santos Lab website (http://131.204.120.103/srsantos/mrbayes_form/index.html) and implemented with MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). The model parameters produced by MrModeltest were pasted directly into the MrBayes block. The Markov chain Monte Carlo parameters consisted of 10,000,000 generations, with four chains, and a tree sampled every 100 generations. The first 10,000 trees were discarded as burnin and the results were summarized as a 50% majority-rule consensus tree.

The input files used for the analyses outlined above have been deposited in the Dryad data repository (http://dx.doi.org/10.5061/dryad.g0164d60).

Results

The results of the maximum parsimony, maximum likelihood, and Bayesian inference analyses outlined above are summarized in figure 27. The sequences of *Lepraria isidiata*, *L. nivalis*, and *L. sylvicola* were each recovered in strongly supported clades. These results support the current taxonomic circumscriptions of these species. Concurrently they reiterate the utility of nrITS sequence data in testing species boundaries within *Lepraria* s.l., notably recovering as monophyletic, taxa that were circumscribed based on a suite of correlated non-molecular characters (Lendemer 2011b; Slavíková-Bayerová & Fehrer 2007).

The sequences of *Lepraria normandinoides*, the taxon that was the focus of this study, were recovered in two strongly supported clades that correspond to the fumarprotocetraric and protocetraric chemotypes presently included within the species. Interestingly, the lone sequence representing the acid deficient chemotype of *L. normandinoides* was nested within the clade otherwise composed of sequences of the protocetraric acid chemotype. This strong correlation between chemical and molecular characters clearly indicates that *L. normandinoides* as presently defined actually comprises two distinct monophyletic entities. The taxonomic consequences of this result are discussed further below. Although this study found that *L. normandinoides* is actually comprised of two discrete monophyletic entities, it further supports the distinction between those entities and *L. nivalis* that was proposed by Lendemer and Harris (2007).

Discussion

General remarks

Lepraria is an example of a genus where lichenologists resorted almost exclusively to chemotaxonomy when delimiting species due to a perceived paucity of other characters (Lendemer 2011), despite contemporary evidence that this practice did not necessarily result in the circumscription of monophyletic taxonomic entities (Ekman & Tønsberg 2002, Tretiach et al. 2009). The inadequacies of this approach were highlighted by Lendemer and Harris (2007) who utilized an integrated suite of biological, chemical, ecological, and micromorphological characters to distinguish the taxon that is the focus of the present study from the chemically similar *L. nivalis* in which it had previously been included. While the present study clearly supports the use of a suite of independent characters above one single character *a priori,* it also serves to highlight the limitations of this methodology. Indeed, the suite of characters cited above enabled Lendemer and Harris (2007) to distinguish *L. normandinoides* from *L. nivalis*. However

the same suite of characters led those authors to include three chemotypes that differed in the presence/absence of two biosequentially related substances, fumarprotocetraric acid and protocetraric acid, within a single taxon. This decision emphasized a shared suite of characters above a single difference. The addition of molecular characters provided an independent dataset whose correlation with the chemical characters supports the further division of *L. normandinoides* into two taxa.

Interestingly this appears to be first recorded case where molecular phylogenetic analyses confirm the distinctness of two morphologically identical sympatric chemotypes that produce substances which are biosequentially nearly identical (differing in a single bond). It should be noted that in the context of the discussions of *Lepraria* species concepts by Lendemer (2011b) the present case neither represents cryptic nor semi-cryptic species. Rather, the molecular phylogenetic analyses undertaken here were found to correlate with differences in chemistry that were initially underemphasized. This study led to a reevaluation of the weight that should be placed upon these chemical characters when considering *L. normandinoides* s.l. This methodology is no different from that of a typical taxonomic revision where successive evaluation of new materials and data generated from new tools leads to the redefinition of earlier concepts (Lendemer & O'Brien 2011).

What should be done with the acid deficient populations?

The formal splitting of *Lepraria normandinoides* into two species defined by chemical and molecular characters logically leads to the question of how to classify the acid deficient populations of this taxon that are known to exist. Such populations are presently known only from the northern fringes of the range of *L. normandinoides*, however they are entirely sympatric

with both fumarprotocetraric acid and protocetraric acid producing populations that are morphologically and ecologically indistinguishable. While the lone sequence of an acid deficient population included in this study was recovered as nested within a clade comprised of sequences from protocetraric acid producing populations it is probable that the loss of depside production could also occur in fumarprotocetraric acid producing populations. Thus it is unlikely that all the populations of *L. normandinoides* s.l. that lack depsides represent cases where *L. normandinoides* s. str. has lost the ability to produce protocetraric acid. The inclusion of these populations in *L. normandinoides* s. str. here represents a pragmatic approach to reconciling the results of molecular phylogenetic analyses with observable reality. While some may consider the existence of acid deficient populations as evidence that the current broad circumscription of *L. normandinoides* should be retained, such a solution would obfuscate correlated differences in molecular and chemical characters. Indeed, acid deficient populations represent <3% of the total number of specimens of *L. normandinoides* s.l. examined, and occur in a limited geographic area relative to the total range of the taxon. Thus the vast majority (>95%) of populations of *L. normandinoides* can be identified with certainty as *L. normandinoides* or *L. oxybapha* using chemical characters alone.

The above raises the question of how lichenologists should view acid deficient populations of a given species. Should the absence of a specific substance be viewed as a discrete characteristic difference? Or should it be viewed as the absence of a character in the same way as albino forms of species? Clearly neither approach should be adopted *a priori* and rather each case should be evaluated individually using an independent dataset as has been done here.

Taxonomic Section

1) *Lepraria normandinoides* Lendemer & R.C. Harris, Opuscula Philolichenum 4: 45. 2007. TYPE: **U.S.A. NORTH CAROLINA.** TRANSYLVANIA CO.: Nantahala National Forest, Shower Falls, along Cold Mountain Road, south slope of Panthertail Mountain, Lake Toxaway Quad., 3500 ft., small roadside waterfall over gneiss, 29.v.2006, on gneiss in overhang, *J.C. Lendemer et al. 7001 = Lich. East. N. Amer. Exs. V: 221* (NY!, holotype; ASU!, B!, BG!, CANB!, FH!, GZU!, H!, M!, MIN!, S!, TNS!, TU!, isotypes).

Mycobank #510776.

Description. – **Thallus** crustose, leprose, placodioid with crisped margins, continuous and distinctly bi-layered, initially forming small discrete thalli which expand marginally, overlap, and become confluent to form a thick crust (0.2–0.5 mm thick), bone white to light blue-white; **hyphae** hyaline or pigmented, [1.8]–(2.3)–*2.9*–(3.7)–[4.8] µm (*n*=58), septate, secund, obscured by a layer of POL+ crystals that do not dissolve in KOH; prothallus disappearing with age; **hypothallus** a variably developed layer of loosely intertwined hyphae surrounding and subtending the granules, extending outward from the edge of thallus; **rhizohyphae** present, typically abundant, pigmented brown; **granules** globose, [19]–(24)–*38*–(53)–[84] µm (*n*=52), ecorticate, well organized and discrete, not forming compound units, sometimes separating from the hypothallus and then producing abundant projecting hyphae; **photobiont** green, coccoid, cells globose, [6]–(7.9)–*9.8*–(11.6)–[13.2] µm (*n*=72).

Chemistry. – Atranorin, roccellic acid, +/- protocetraric acid. Spot tests: K+ yellow, C-, KC-, P+/- orange/red, UV-.

Ecology and distribution. – *Lepraria normandinoides* is a North American endemic that is widely distributed throughout temperate eastern North America with rare disjunct populations extending westward into the Greater Sonoran Desert Region and Black Hills of South Dakota (figure 28). The species occurs in high humidity low-light habitats on sheltered non-calcareous rock faces and overhangs as well as the bark of hardwoods and conifers. Acid deficient populations of *L. normandinoides* are rare and so far known only from the northern/boreal portions of its geographic range.

Discussion. – *Lepraria normandinoides* is a common and conspicuous species that is widespread in eastern North America. Due to its appearance, as well as the thickness of the thallus which facilities easy collection with minimal substrate, the species is somewhat less impacted by collector bias when compared to other sympatric species with less conspicuous thalli. Historically the species was confused with *L. lobificans* auct. (i.e., *L. finkii* (Hue) R.C. Harris) until Laundon (1989) restricted the use of that name to material producing atranorin and stictic acids. Laundon (1989) then described material with atranorin and protocetraric or fumarprotocetraric acids that been referred to *L. lobificans* as a new taxon, *L. nivalis*. *Lepraria normandinoides* was included within Laundon's broad concept of *L. nivalis* until Lendemer and Harris (2007) recognized it was chemically, ecologically, and morphologically distinct.

In the field the only species likely to be confused with *Lepraria normandinoides* are those which also have placodioid thalli (Lendemer 2011), specifically *L. eburnea* J.R. Laundon,

L. finkii, and *L. vouauxii* (Hue) R.C. Harris. However, none of these species has thalli that develop a marginal lip and all produce different secondary compounds (atranorin, zeorin, stictic acid aggregate in *L. finkii* and dibenzofurans of the pannaric acid aggregate in *L. vouauxii*). Although one chemotype of *L. nivalis* is comparable to *L. normandinoides* in producing protocetraric acid, it differs ecologically in occurring on calcareous substrates and morphologically in lacking brown rhizohyphae. *Lepraria lanata* Tønsberg, a species endemic to the high elevations of the southern Appalachians, is chemically similar to *L. normaninoides* but differs markedly in having massive aggregations of granules (>100 μm in diameter) which resemble small cotton balls.

Selected specimens examined ("" denotes depside deficient collections).* – **U.S.A. ALABAMA.** CLAY CO.: Talladega National Forest, Chinnabee Silent Trail, 24.ix.1992, on rock, *R.C. Harris 28390* (NY). DeKALB CO.: DeSoto State Park, 4.x.1998, on rock, *W.R. Buck 34741* (NY). JACKSON CO.: Buck's Pocket State Park, 3.x.1998, on rock, *R.C. Harris 42378* (NY). **ARKANSAS.** FRANKLIN CO.: Ozark National Forest, Ozark Highlands National Recreational Trail, 8.vi.2000, on rock, *W.R. Buck 37275* (NY). NEWTON CO.: Ozark National Forest, E of FSR 1463, 28.iii.2006, on sandstone, *J.C. Lendemer et al. 6557* (NY, PH). **CONNECTICUT.** WINDHAM CO.: Town of Ashford, Yale Forest, along Boston Hollow Rd. ~0.6 mi NE of jct w/ Eastford Rd., 24.vii.2002, on rock, *R.C. Harris 46097* (NY). **GEORGIA.** COFFEE CO.: Broxton Rocks Ecological Preserve, 9.ii.2003, on *Magnolia*, *R.C. Harris 47050* (NY). COLUMBIA CO.: Heggie's Rock TNC Preserve, 8.xi.2008, on rock, *G.B. Perlmutter et al. 1639* (NY). RABUN CO.: Lake Burton Wildlife Management Area, 17.ix.2006, on rock, *J.C. Lendemer et al. 7657* (NY). ROCKDALE CO.: Panola Mountain State Park, 17.iv.2007, on

granite, *J.C. Lendemer et al. 8984* (NY). **ILLINOIS.** POPE CO.: Shawnee National Forest, Burden Falls, 26.v.1997, on rock, *L.R. Phillippe 28777* (NY). RANDOLPH CO.: Piney Creek Ravine Nature Preserve, 7.xi.2004, on sandstone, *R.C. Harris 50566* (NY). **KENTUCKY.** BATH CO.: Daniel Boone National Forest, Tater Knob, 10.x.1995, on *Nyssa*, *R.C. Harris 36882* (NY). LETCHER CO.: Bad Branch Nature Preserve, 14.ix.1991, on rock, *R.C. Harris 27023* (NY). **MAINE.** HANCOCK CO.: T10SD, slope of Tunk Mountain, 8.vii.2008, on rock, *R.C. Harris 54743* (NY); T10SD, Maine Public Reserve Lands, 0–0.25 mi N of ME82 directly across from Tunk Lake Access, 10.vi.2010, on rock, *J.C. Lendemer 23028* (NY*); Little Deer Isle, 8.xi.2005, on rock, *T. Harris 194* (NY*). OXFORD CO.: Berry Ledge, Paris, 29.ix.1931, on rock, *J.C. Parlin 9897* (NY). WASHINGTON CO.: T10SD, Donnell Pond Public Reserve Lands, Black Mountain, 10.vi.2010, on *Betula*, *J.C. Lendemer 29957* (NY). **MARYLAND.** CECIL CO.: New Valley, along Basin Run, 1.xii.2009, on rock, *J.C. Lendemer 20017* (NY). BALTIMORE CO.: Gunpowder Falls, 18.ix.1090, on rock, *C.C. Plitt 328* (NY). WASHINGTON CO.: Sideling Creek, 4.vi.1990, on rock, *C.F. Reed 136003-A* (NY). **MASSACHUSETTS.** BERKSHIRE CO.: Bartholomew's Cobble, 28.iii.1981, on rock, *R.C. Harris 13540* (NY). **MINNESOTA.** ST. LOUIS CO.: Voyageurs National Park, E side of Browns Bay, 13.vii.1979, on rock, *C.M. Wetmore 39989* (NY*). **MISSISSIPPI.** TISHOMINGO CO.: Tishomingo State Park, 27.ix.1992, on rock, *R.C. Harris 28584* (NY). **MISSOURI.** IRON CO.: E slope of Royal Gorge, 27.iv.1988, on rock, *R.C. Harris 21781* (NY). MADISON CO.: Amidon Memorial Conservation Area, 21.x.2001, on rock, *R.C. Harris 45058* (NY). NEWTON CO.: Wildcat Glade Natural Area, 6.xi.2002, on rock, *W.R. Buck 42997* (NY). **NEW JERSEY.** BERGEN CO.: Palisades, *sine date*, on rock, *C.F. Austin s.n.* (NY). MORRIS CO.: Ledgewood, 10.viii.1914, on rock, *C. Barbour 3* (NY). **NEW MEXICO.** SIERRA CO.:

Black Range, along North Percha Creek, 8.vii.1995, on rock, *R.D. Worthington* (NY, UTEP). **NEW YORK.** ESSEX CO.: Town of Keene, Chapel Pond, 19.ix.2004, on rock, *R.C. Harris 50039* (NY). JEFFERSON CO.: Wellesley Island State Park, 20.ix.1983, on mosses over rock, *R.C. Harris 16746* (NY). ROCKLAND CO.: Bear Mountain State Park, Iona Island Bird Sanctuary, 18.vii.2010, on rock, *J.C. Lendemer 23681 & A. Moroz* (NY). ULSTER CO.: Ice Caves Mountain National Landmark, 14.v.1994, on rock, *R.C. Harris 32840* (NY). WESTCHESTER CO.: Croton Dam Rd., 0.5 mi S of NY129, 27.vii.1975, on *Carya*, *C.R. Prince 75-65* (NY). **NORTH CAROLINA.** CLAY CO.: Nantahala National Forest, Southern Nantahala Wilderness, 17.ix.2006, on *Acer*, *J.C. Lendemer et al. 7736* (NY). JACKSON CO.: Bad Creek Trail to Ellicott's Rock, 28.ix.1989, on *Quercus*, *R.C. Harris 24866* (NY). MACON CO.: Nantahala National Forest, McDowell Mt., 12.viii.1994, on rock, *R.C. Harris 33149* (NY). SWAIN CO.: Great Smoky Mountains National Park, Straight Fork Rd., 6.viii.2009, on rock, *J.C. Lendemer 19159 & E.A. Tripp* (NY). TRANSYLVANIA CO.: Gorges State Park, 9.viii.2005, on *Quercus rubra*, *J.C. Lendemer 4586 & E.A. Tripp* (NY). **OHIO.** GALLIA CO.: Wayne National Forest, above Symmes Creek, 19.v.2006, on sandstone, *J.C. Lendemer et al. 7437* (NY). SCIOTO CO.: Shawnee State Forest, along Pond Lick Run, 21.v.2005, on sandstone, *J.C. Lendemer 7161* (NY). **OKLAHOMA.** CHEROKEE CO.: J.T. Nickel Family Nature and Wildlife Preserve, 30.x.2000, on rock, *W.R. Buck 38448* (NY). LOVE CO.: Lake Murray State Park, 24.iv.2004, on sandstone, *S. Strawn & S. Strawn 92* (NY). **PENNSYLVANIA.** CARBON CO.: Hickory Run State Park, 19.v.2009, on rock, *J.C. Lendemer 17573 & R.C. Harris* (NY). HUNTINGDON CO.: Trough Creek State Park, 22.iv.2008, on rock, *W.R. Buck 53550* (NY). MONROE CO.: Tannersville Bog TNC Preserve, 24.xi.2009, on rock, *J.C. Lendemer 19552 & V. Doyle* (NY). TIOGA CO.: Tioga State Forest, E shore of Gleason Hollow Run, 14.v.2009, on

rock, *J.C. Lendemer 16925* (NY). WYOMING CO.: Bowmans Creek Ledges, 20.vii.2008, on rock, *J.C. Lendemer 13510* (NY). YORK CO.: Apollo County Park, 9.viii.2009, on rock, *J.C. Lendemer 19455* (NY). **RHODE ISLAND.** PROVIDENCE CO.: Town of Lincoln, Lime Rock Nature Preserve, 17.ix.2006, on rock, *R.C. Harris 53210* (NY). **SOUTH CAROLINA.** GREENVILLE CO.: Caesars Head State Park, 14.iii.1997, on base of tree, *R.C. Harris 40035* (NY). **SOUTH DAKOTA.** MINNEHAHA CO.: Palisades State Park, 10.v.2004, on quartzite, *M.K. Advaita 3491* (NY). **TENNESSEE.** BLOUNT CO.: Great Smoky Mountains National Park, Rich Mountain Gap, 30.vi.2010, on *Quercus*, *J.C. Lendemer et al. 23584* (NY). COCKE CO.: Great Smoky Mountains National Park, Gabes Mountain Trail, 5.viii.2009, on *Quercus*, *J.C. Lendemer 19078 & E.A. Tripp* (NY). MONROE CO.: Cherokee National Forest, Citico Creek Wilderness, 6.viii.1994, on *Acer*, *R.C. Harris 32915* (NY). SCOTT CO.: Big South Fork National River and Recreation Area, 7.viii.1994, on rock, *R.C. Harris 32967* (NY). **VIRGINIA.** GRAYSON CO.: Jefferson National Forest, Comers Creek, 5.iv.2008, on hardwood, *G.B. Perlmutter 1291* (NY). PATRICK CO.: Blue Ridge Parkway, Rock Castle Gorge Loop Trail, 6.x.1995, on rock, *R.C. Harris 36656* (NY). **WEST VIRGINIA.** POCAHONTAS CO.: Monongahela National Forest, upper slopes of Spruce Mountain, 20.x.2007, on *Acer*, *J.C. Lendemer 9936 & A. Moroz* (NY). WYOMING CO.: near New Richmond, 30.v.1967, on rock, *C.F. Reed 74818* (NY).

2) *Lepraria oxybapha* Lendemer sp. nov.

Mycobank #564049.

Figure 30.

Ut in *Lepraria normandinoides* sed acido protocetrarico nullo et acido fumarprotocetrarico continente differt.

TYPE: **U.S.A. PENNSYLVANIA.** SULLIVAN CO.: Ricketts Glen State Park, S slopes above Big Run, 1 mi W of jct w/ PA 487, 2067 ft., *Tsuga/Betula/Acer* forest with cold air talus slope and large sheer cliffs, 19.ix.2010, on rock in overhang, *J.C. Lendemer 25637 & J. Kunsman* (NY!, holotype).

Description. – **Thallus** crustose, leprose, placodioid with crisped margins, continuous and distinctly bi-layered, initially forming small discrete thalli which expand marginally, overlap, and become confluent to form a thick crust (0.2–0.5 mm thick), bone white to light blue-white in color; **hyphae** hyaline or pigmented, [2.1]–(2.6)–*3.3*–(4.0)–[4.8] µm (*n*=39), septate, secund, obscured by a layer of POL+ crystals that do not dissolve in KOH; prothallus disappearing with age; **hypothallus** a variably developed layer of loosely intertwined hyphae surrounding and subtending the granules, extending outward from the edge of thallus; **rhizohyphae** present, typically abundant, pigmented brown; **granules** globose, [24]–(35)–*48*–(61)–[80] µm (*n*=45), ecorticate, well organized and discrete, not forming compound units, sometimes separating from the hypothallus and then producing abundant projecting hyphae; **photobiont** green, coccoid, cells globose, [5]–(7.7)–*9.6*–(11.5)–[13.2] µm (*n*=39).

Etymology. – The epithet “oxybapha” refers to the saucer shaped “lobes” produced at the margins of the thallus.

Chemistry. – Atranorin, roccellic acid, fumarprotocetraric acid, and traces of protocetraric acid. Spot tests: K+ yellow, C-, KC-, P+ orange/red, UV-.

Ecology and distribution. – *Lepraria oxybapha* is a North American endemic whose range primarily correlates to an Appalachian-Ozarks distribution (Brodo et al. 2001). Although it is widespread throughout those regions it is most frequent in the northern/central Appalachians, becoming rarer further south and in the Ozarks (figure 29). Rare disjunct populations occur westward in the Great Plains and the Greater Sonoran Desert Region. The species is restricted to high humidity low-light habitats where it occurs on the bark of conifers and hardwoods as well as sheltered non-calcareous rock faces and overhangs.

Discussion. – *Lepraria oxybapha* is a distinctive North American species that can be recognized by its thalli which develop a marginal lip, brown rhizohyphae on the lower surface, occurrence on trees and non-calcareous substrates, and the presence of fumarprotocetraric acid in addition to atranorin and roccellic/angardianic acid. *Lepraria normandinoides* is morphologically identical but differs chemically in the presence or absence of protocetraric acid instead of fumarprotocetraric acid as a major substance. *Lepraria torii*, a species whose distribution is primarily bi-coastal in boreal North America, with occasional disjunct occurrences throughout the boreal forests, also produces roccellic/angardianic acid and fumarprotocetraric acids and thus could be confused with *L. oxybapha*. That species is rarely sympatric with *L. oxybapha*, typically does not produce atranorin, and has a distinctive green aggregate thallus that lacks a marginal lip.

Selected specimens examined. – **U.S.A. ARKANSAS.** STONE CO.: Ozark National Forest, City Rock Bluff Special Interest Area, 18.iv.2005, on sandstone, *R.C. Harris 51247* (NY). **GEORGIA.** COFFEE CO.: Broxton Rocks Ecological Preserve, 16–17.xii.1993, on rock, *R.C. Harris 32693* (NY). TOWNS CO.: Southern Nantahala Wilderness, Chattahoochee National Forest, Hightower Gap to Rich Knob, 11.xi.2007, on rock, *J.C. Lendemer et al. 10935* (NY). **KENTUCKY.** LETCHER CO.: Bad Branch Nature Preserve, 14.ix.1991, on rock, *R.C. Harris 27016* (NY). **LOUISIANA.** NATCHITOCHES PARISH: Kisatchie National Forest, Longleaf Trail Vista, 27.iii.1982, on sandstone, *J. Pruski 2472* (NY). **MAINE.** HANCOCK CO.: T28MD, SE slopes of Lead Mountain, 11.vi.2010, on *Fagus*, *J.C. Lendemer 23199* (NY). **MASSACHUSETTS.** BERKSHIRE CO.: Town of Pittsfield, Pittsfield State Forest, 4.vi.2010, on *Betula*, *J.C. Lendemer 22459* (NY). **MISSOURI.** SHANNON CO.: Ozark National Scenic Riverways, Rocky Falls, 22.ix.1990, on rock, *R.C. Harris 25576* (NY). **NEW YORK.** GREENE CO.: Town of Hunter, Stony Clove Notch, 13.ix.2008, on *Betula*, *J.C. Lendemer 14083-A* (NY). ULSTER CO.: Town of Shandaken, Slide Mountain Wilderness Area, 13.ix.2008, on *Betula*, *J.C. Lendemer 14011* (NY). **NORTH CAROLINA.** CLAY CO.: Nantahala National Forest, Buck Creek, 10.xi.2007, on *Betula*, *J.C. Lendemer et al. 10453* (NY). HAYWOOD CO.: Great Smoky Mountain National Park, around Caldwell Fork, 4.viii.2009, on dead tree, *E.A. Tripp 631 & J.C. Lendemer* (NY). MADISON CO.: 3 mi S of Hot Springs, 27.viii.1968, on rock, *W.L. Culberson 14276* (NY). **OKLAHOMA.** GREER CO.: Quartz Mountain Arts & Conference Center and Nature Park, 10.iv.2007, on moss over rock, *C.A. Morse 15061 & D. Ladd* (NY). OTTAWA CO.: N of E0060 Rd. on W side of Spring River, 31.x.2000, on rock, *R.C. Harris 44436* (NY). **PENNSYLVANIA.** BRADFORD CO.: State Game Lands No. 36, 18.v.2009, on *Betula*, *J.C. Lendemer 17349 & R.C. Harris* (NY). MONROE CO.: Tobyhanna State Park,

27.vii.2009, on *Acer*, *J.C. Lendemer 18627* (NY). TIOGA CO.: Tioga State Forest, slopes above jct US15/I-99, 12.v.2009, on rock, *J.C. Lendemer 16495 & D. Atha* (NY). WAYNE CO.: Lacawac Sanctuary, 25.vii.2009, on *Betula*, *J.C. Lendemer 18451* (NY). WYOMING CO.: State Game Lands No. 57, Henry Lott Rd., 21.vii.2008, on rock, *J.C. Lendemer 13626* (NY). YORK CO.: W-shore of Susquehanna River, 0.5 mi S of jct River Rd. & PA372, 28.v.2009, on *Betula*, *J.C. Lendemer 17978* (NY). **TENNESSEE.** BLOUNT CO.: Great Smoky Mountains National Park, Gabes Mountain Trail, 5.viii.2009, on rock, *J.C. Lendemer 19059 & E.A. Tripp* (NY). SEVIER CO.: Great Smoky Mountains National Park, Chimney Tops Trail, 28.vi.2010, on *Liriodendron*, *R.C. Harris 56182* (NY). **TEXAS.** LLANO CO.: sine loc., 30.i.1931, on granite, *E. Whitehouse 48* (NY). **VERMONT.** ESSEX CO.: Town of Victory, Victory State Forest, Umpire Mountain, 16.v.2008, on rock, *R.C. Harris 54350* (NY). ORLEANS CO.: Town of Westfield, Green Mountains, Hazen's Notch, 13.v.2005, on rock, *R.C. Harris 51347* (NY). **VIRGINIA.** GILES CO.: Mountain Lake Biological Station, 7.x.1995, on *Betula*, *R.C. Harris 36718* (NY). SMYTH CO.: Jefferson National Forest, Whitetop Mountain, 5.iv.2008, on *Sorbus*, *R.C. Harris 54112* (NY).

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Taxon	GenBank	NY-isolate	Collector	Herbarium	Substrate	Locality
L. normandinoides		NY1096	*J.C. Lendemer 27166*	NY-1217984	saxicolous	U.S.A. Vermont, Orleans Co.
	EU008629	n/a	*M.P. Nelsen 3959*	WIS	unknown	U.S.A. North Carolina
		NY555	*J.C. Lendemer 19340*	NY-1118395	saxicolous	U.S.A Pennsylvania, Lancaster Co.
		NY391	*J.C. Lendemer 7909*	NY-873614	saxicolous	U.S.A. North Carolina, Clay Co.
		NY397	*J.C. Lendemer 13510*	NY-1069214	saxicolous	U.S.A. Pennsylvania, Wyoming Co.
		NY924	*J.C. Lendemer 23681*	NY-1148496	saxicolous	U.S.A. New York, Rockland Co.
		NY646	*J.C. Lendemer 19827*	NY-1133911	saxicolous	U.S.A. Pennsylvania, Union Co.
		NY1033	*J.C. Lendemer 25561*	NY-1217133	saxicolous	U.S.A. Pennsylvania, Centre Co.
		NY1034	*J.C. Lendemer 24310*	NY-1206243	*Quercus*	U.S.A. Pennsylvania, Cameron Co.
		NY500	*J.C. Lendemer 16925*	NY-1106534	saxicolous	U.S.A. Pennsylvania, Tioga Co.
L. oxybapha		NY1032	*J.C. Lendemer 25637*	NY-1217271	saxicolous	U.S.A. Pennsylvania, Sullivan Co.
		NY1031	*J.C. Lendemer 24602*	NY-1216329	*Fraxinus*	U.S.A. Pennsylvania, Forest Co.
		NY888	*J.C. Lendemer 23199*	NY-1148377	*Fagus*	U.S.A. Maine, Hancock Co.
		NY1029	*J.C. Lendemer 24713*	NY-1216217	saxicolous	U.S.A. Pennsylvania, Warren Co.
	EU008628	n/a	*West & Westad s.n.*	WIS	unknown	U.S.A. Wisconsin
		NY543	*J.C. Lendemer 18627*	NY-1117630	*Acer*	U.S.A. Pennsylvania, Monroe Co.

Table 6. Information for voucher specimens of *Lepraria normandinoides* s.l. used to generate sequences included in this study.

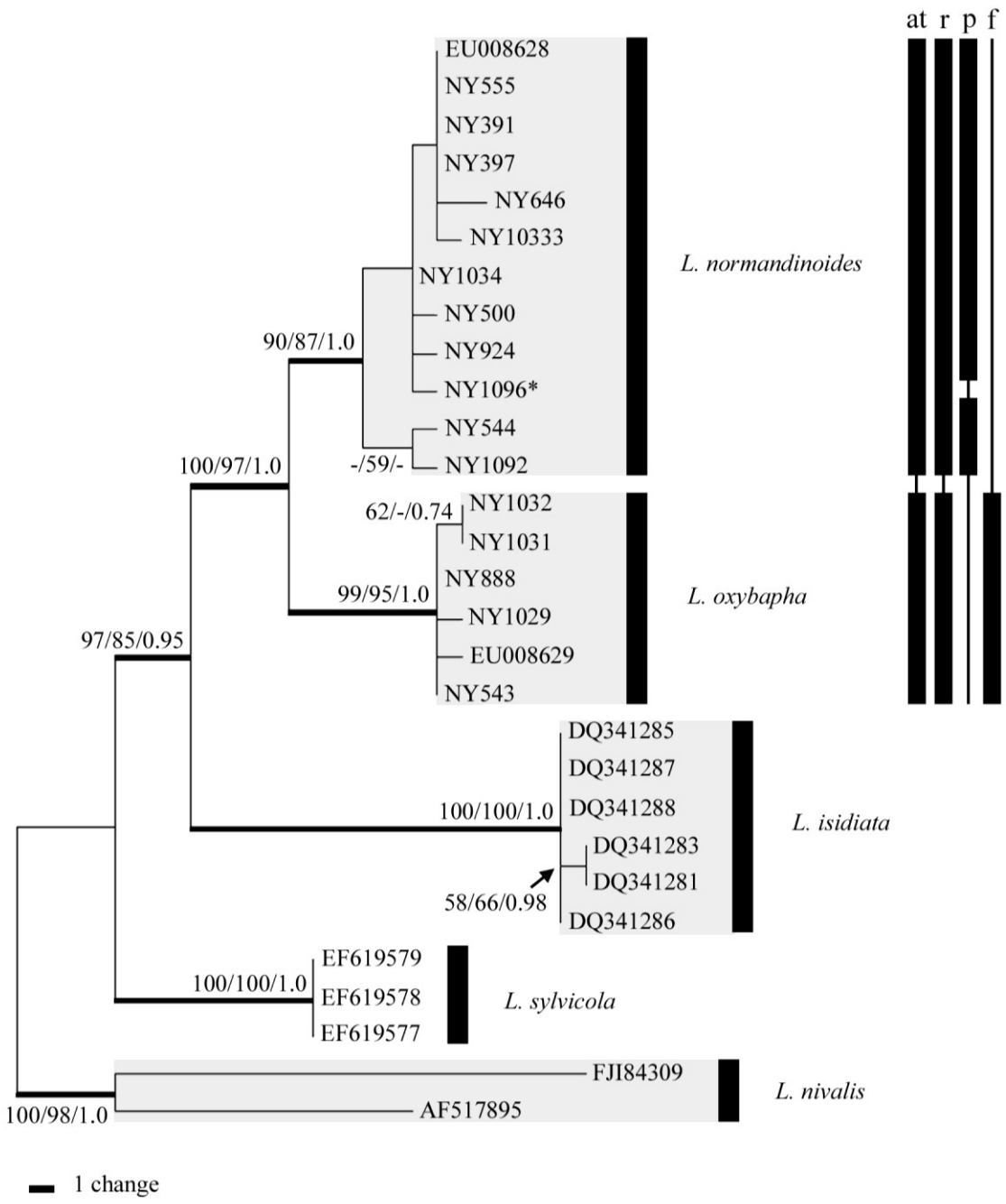

Figure 27. Phylogeny of fumar/protocetraric acid producing *Lepraria* species with placodioid thalli (shaded clades) with *L. sylvicola* included to expand sampling, inferred from ITS sequence data and displayed as one of 36 equally most parsimonious trees with midpoint rooting. Support was evaluated using maximum parsimony (MP) and maximum likelihood (ML) bootstrap proportions (BP) and Bayesian posterior probabilities (B-PP). Support values >50% are displayed at each node in the following format: MP-BP/ML-BP/B-PP. Branches with MP-BP and ML-BP support >70% as well as BP-PP support >95% are thickened. Chemical data for *L. normandinoides* s.l. are provided on the far right, with thickened lines indicating the presence of a substance a given voucher specimen (at = atranorin, r = roccellic acid, p = protocetraric acid, f = fumarprotocetraric acid).

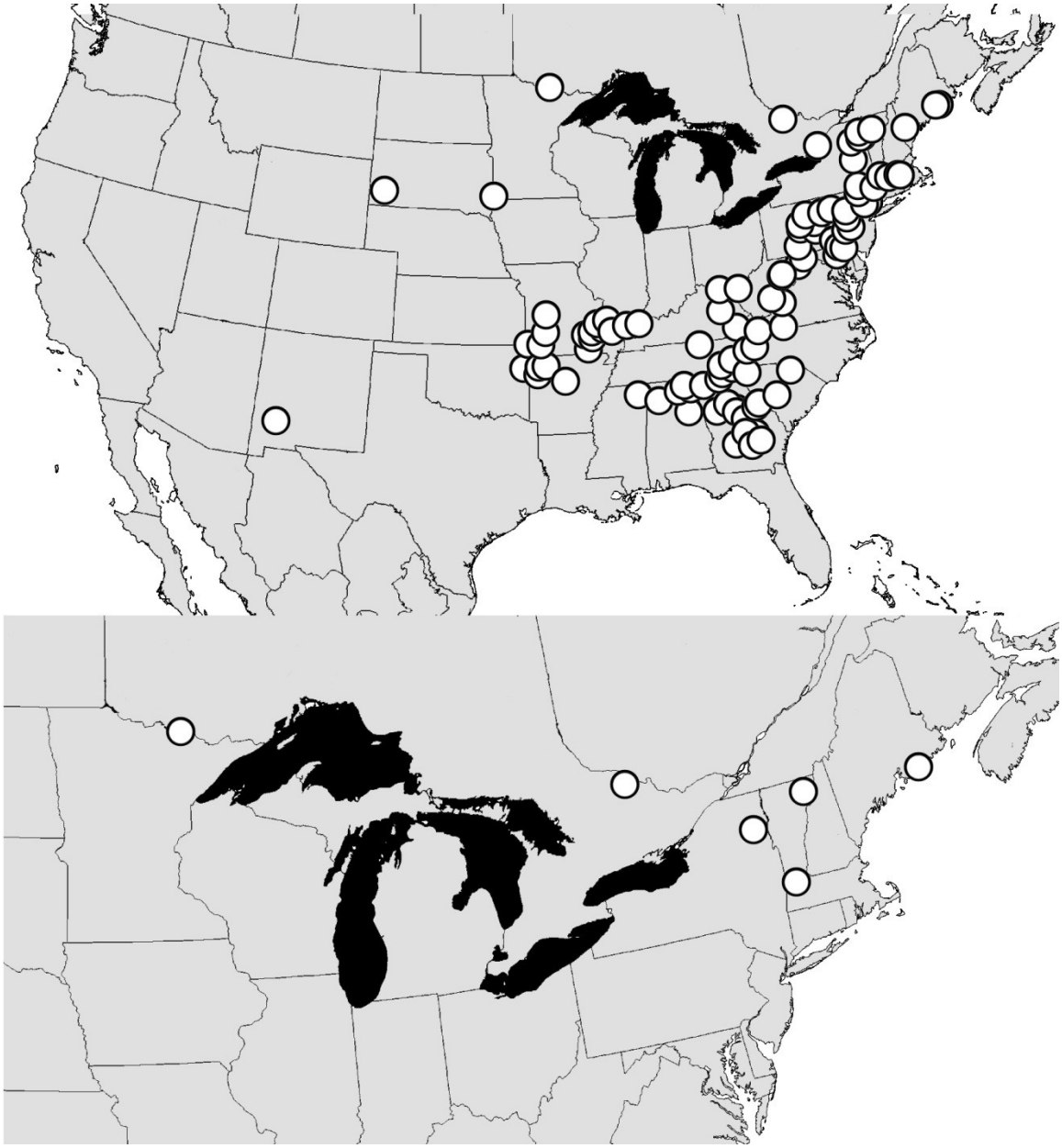

Figure 28. Geographic distribution of *Lepraria normandinoides* s. str. based on specimens examined for this study (protocetraric producing chemotype above, protocetraric deficient chemotype below).

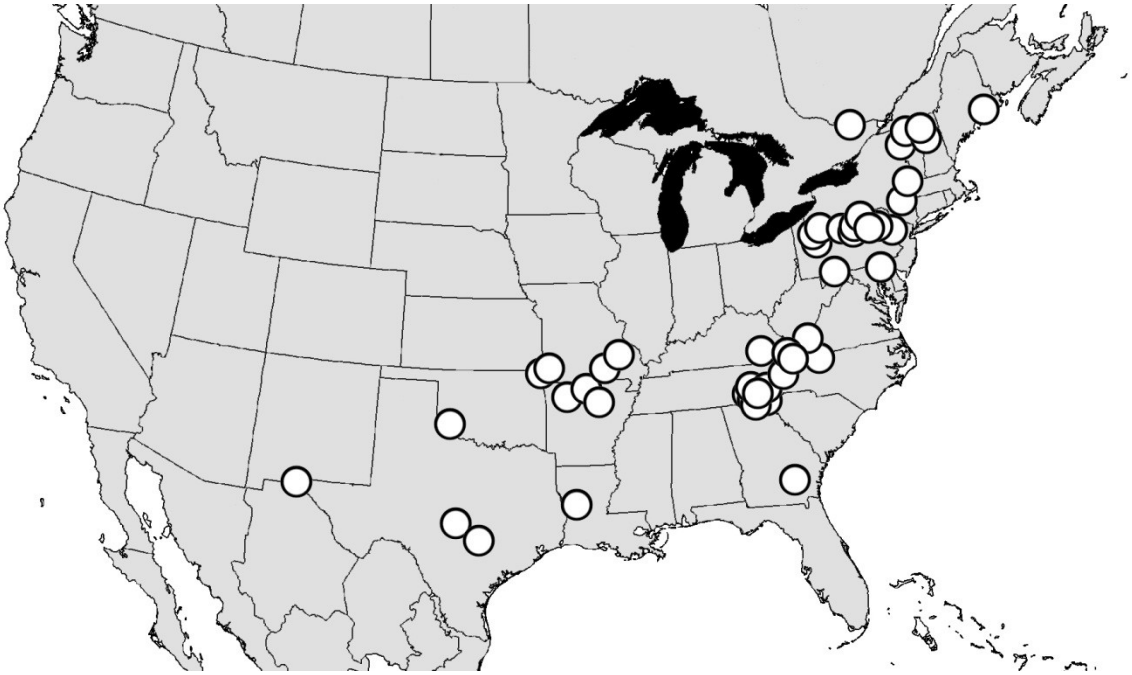

Figure 29. Geographic distribution of *Lepraria oxybapha* based on specimens examined for this study.

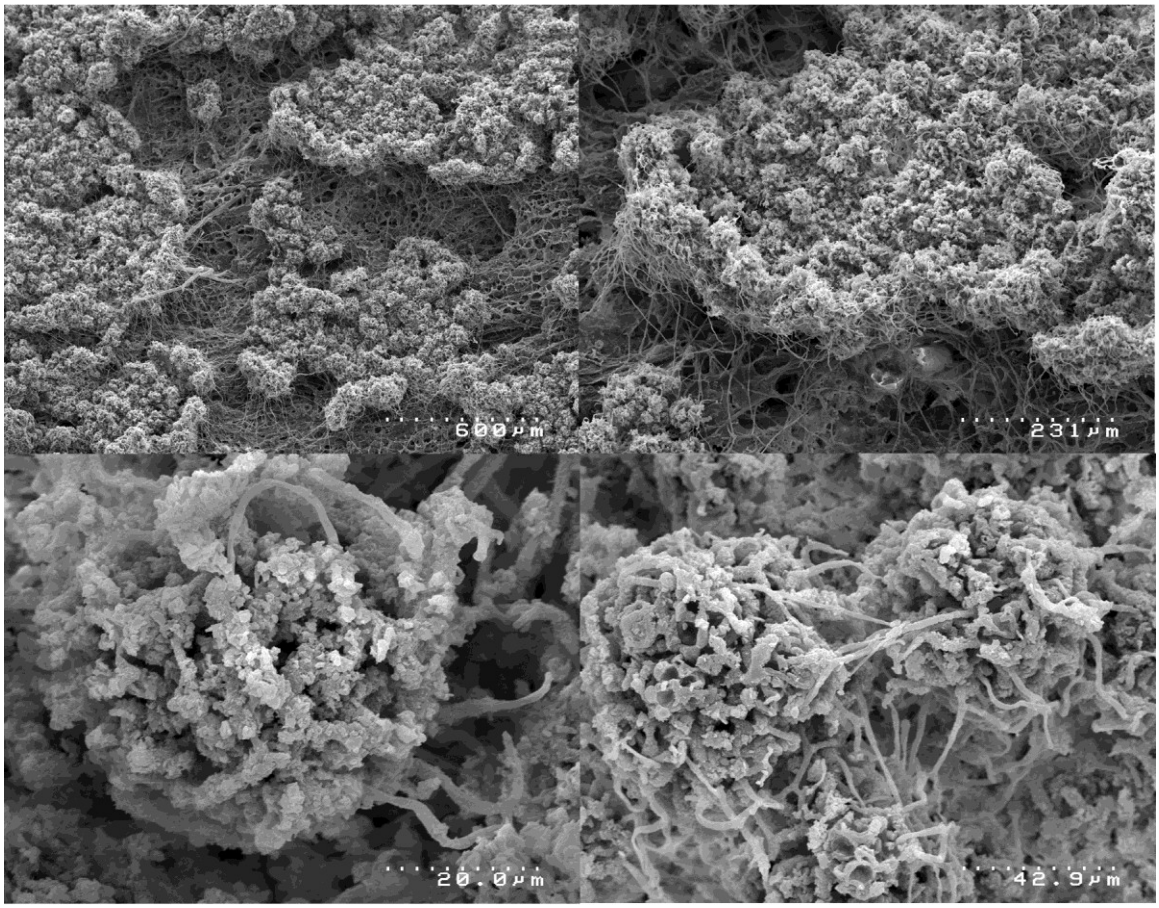

Figure 30. Scanning electron micrographs illustrating the morphology of *Lepraria oxybapha* (all from *Lendemer 9763*, NY). A, macromorphology of thalline margins. B, detail of thalline margin illustrating raised "lip". C, detail of a granule. D, two granules in the context of the hypothallus. Scales as indicated.

CHAPTER VI – IMPLEMENTATION OF A UNIFIED TAXONOMY INTEGRATING MOLECULAR AND NON-MOLECULAR DATA IN *LEPRARIA CAESIELLA* S.L.

James C. Lendemer

Abstract. – *Lepraria caesiella* as currently circumscribed includes two sets of populations with different thallus morphologies. The populations are chemically identical but differ ecologically and are rarely sympatric. A phylogeny was inferred from ITS1, 5.8S, and ITS2 sequence data generated from a geographically broad sampling of the populations with each thallus type. Two strongly-supported clades were recovered that correlate to the two different morphotypes present within the species. Thus, *L. caesiella* is restricted to populations with aggregate thalli. Populations with placodioid thalli are formally described as a new species, *L. harrisiana*. A detailed taxonomic treatment of both taxa is presented.

Introduction

The lichen genus *Lepraria* Ach. is a polyphyletic grouping of more than seventy species of sterile asexually reproducing crustose lichens with leprose thalli (Lendemer 2011; Saag et al. 2009). Members of the genus are common and conspicuous components of the crustose lichen biota throughout temperate regions of the world where they often dominate high humidity, low light habitats (Laundon 1992). Taken broadly, the genus presents a unique opportunity to study the repeated evolution of the leprose growth form and asexual reproduction in the Lecanoromycetes. Further, the core of *Lepraria*, which comprises a monophyletic group within the family Stereocaulaceae (Ekman & Tønsberg 2002), presents an equally unique opportunity to study the long term effects of sterility.

Unfortunately, the chaotic state of taxonomy and descriptive morphology in the genus has precluded the above studies from taking place (Lendemer 2011). To resolve this problem I undertook a revision of *Lepraria* in North America north of Mexico as the topic of my dissertation. The goal of this project was to first introduce a standardized morphological descriptive scheme that could be employed to describe all the species in the genus (Lendemer 2011). This was followed by the use a multi-faceted approach to integrate molecular and non-molecular characters to resolve the delineation of the North American members of the genus. The species with divaricatic acid, including the generic type *L. incana* (L.) Ach., were treated by Lendemer (2011b). The species with placodioid thalli that produce atranorin, fumarprotocetraric and protocetraric acid were treated by Lendemer (in rev.). The present study uses this methodology to examine *L. caesiella* R.C. Harris, which is one of the most common and widespread species in eastern North America. Specifically, it aims to resolve whether the two chemically identical morphotypes presently included within the species correspond to monophyletic entities that should be formally recognized.

Materials and methods

Non-molecular materials and methods. – As in previous contributions on *Lepraria* s.l. by the author, this study is based primarily on material in the herbarium of The New York Botanical Garden (NY), supplemented by material received on loan from the following herbaria: CANL, COLO, CONN, DUKE, FH, GZU, H, KANU, MIN, MSC, SBBG, UCR, and UTEP. This includes >1000 specimens collected by the author during his extensive field studies of *Lepraria* throughout North America. To date these total 1.5 years in the field and span the period August, 2007 to August, 2011. Through these field studies the author has been able to gather firsthand observations of ecology and morphology for all the species known to occur in North America.

Chemical and morphological analyses were carried out using the methodology outlined in detail by Lendemer et al. (2008) with the SEM methods as corrected by Lendemer and Elix (2010). Descriptive morphological terminology follows Lendemer (2011). Measurements of granule size, photobiont cell size, and hyphal thickness are given as the average (xbar) ± 1 standard deviation (sd) and bounded by the extreme observed values, or [smallest observed]–(xbar-1sd)–xbar–(xbar+1sd)–[largest observed]. The number of observations (*n*) is also provided.

Molecular data collection. – Only subsamples used for chemical analyses with Thin Layer Chromatography were used in downstream molecular analyses. This was done to avoid any obvious contamination from admixed thalli of chemically different species. DNA extractions were performed using the DNeasy Plant Mini Kit (Qiagen) with the instructions modified to include a prolonged (~12 hour) incubation period in lysis buffer. Isolated DNA was resuspended in 100 µl of sterile water and stored at -20°C. PCR amplification was performed using the primers ITS4 and ITS5 (White et al. 1990). Amplification reactions of 25 µl contained each of the following: 2.5 µl 10× PCR Buffer (Qiagen), 2.5 µl dNTP solution (mixed to a concentration of 2 mM of each dNTP), 2.5 µl BSA solution (mixed to a concentration of 2.5 mg μl^{-1} of bovine serum albumin; Hillis et al. 1996 [not in lit cit]), 0.2 µl Taq DNA Polymerase (= 1 U; Qiagen), 1 µl of each primer (in solution at a concentration of 10 µM), 9.3 µl of sterile water, 1 µl of extracted DNA, and 5 µl of Q-solution (Qiagen). PCR protocol followed Nelsen et al. (2007) and consisted of an initial denaturation of 95°C for 5 min, followed by 10 cycles of 95°C for 1 min, 62°C for 1 min, and 72°C for 1 min, then 35 cycles of 95°C for 1 min, 53°C for 1 min and 72°C for 1 min with a final extension for 7 minutes at 72°C. PCR products were studied prior to

sequencing by UV examination of a 1% agarose gel on which 1 µl of amplified PCR product had been subjected to electrophoresis and stained with ethidium bromide. Unpurified amplified PCR products were sent to the University of Washington Biochemistry DNA Sequencing Facility (BSDF) for sequencing. Sequences were assembled and edited by hand using the software package Sequencher^{TM} 4.9 (Gene Codes Corporation, Ann Arbor, MI, USA). In order to identify contaminants, nearest sequence matches were found by searching the nucleotide collection in GenBank using BLASTn (Altschul et al. 1997).

Taxon Sampling. – The goals of this study were to 1) determine whether the chemically identical morphotypes of *Lepraria caesiella* represent distinct monophyletic entities, and 2) examine the circumscription of *L. caesiella* with respect to other sympatric corticolous species that are morphologically comparable in having aggregate thalli, or chemically similar in the production of zeorin. To address these points, phylogenetic analyses were performed on a dataset comprised of sequences of the nuclear ITS region (ITS1, 5.8s, and ITS2) of the morphotypes of *L. caesiella* and selected additional sequences from GenBank (see below) supplemented by additional new sequences of *L. elobata* Tønsberg and *L. friabilis* Lendemer. A total of twenty seven (*n*=27) sequences were generated for this study, including fifteen (*n*=15) representing a geographically diverse sampling of the morphotypes of *L. caesiella*. Eight (*n*=8) of these represented the morphotype with an aggregate thallus while seven (*n*=7) represented the morphotype with a placodioid thallus. In order to compare *L. caesiella* with *L. friabilis*, a morphologically identical but chemically distinct species that is partially sympatric with *L. caesiella* s.l., seven (*n*=7) new sequences were generated from populations of that species, including the holotype. As *L. elobata*, another morphologically identical but chemically distinct

species that is partially sympatric with *L. caesiella*, was to be included in this study, an additional five (*n*=5) sequences were generated from North American populations of that taxon to supplement those available from GenBank which were all derived from European populations. In addition to the above sequences the following were downloaded from GenBank: 1) sequences of *L. caesiella* from previous studies (*n*=2), 2) reference sequences of *L. elobata* (*n*=6), and 3) reference sequences of *L. atlantica* Orange (*n*=4) another chemically distinct but morphologically comparable species that was recovered among the nearest sequence matches using BLASTn.

Sequence alignment and phylogenetic analyses. – Sequences were first aligned using ClustalX (Thompson et al. 1997). The alignment was then manually adjusted using Mesquite ver. 2.0 (Maddison & Maddison 2007). Sequences containing clear inconsistencies with all other aligned sequences were assumed to have resulted from the sequencing of heterogeneous amplicon pools, and thus excluded. Ambiguous bases and terminal gaps were converted to missing "?" and the ambiguously aligned regions were excluded from the analysis by including them in an exclusion set. The NEXUS file was then saved with the taxa and characters compressed into a data block.

Following Hodkinson and Lendemer (2011b) the NEXUS file was prepared for a weighted maximum parsimony (MP) analysis with recorded ambiguously-aligned regions. INAASE 3.0 (Lutzoni et al. 2000) was used to recode the ambiguously aligned regions and to generate specific step-matrices using a transition: transversion: gap ratio of 1:1:1. STMatrix 3.0 (written by S. Zoller an F. Lutzoni; Miadlikowska et al. 2002) was then used to generate step matrices for each of the three ITS regions. The input file for STMatrix 3.0 was produced using

the procedure outlined by Gaya et al. (2003) and described in greater detail by Lendemer (2011b). The alignment used for MP analyses described below comprised 634 characters, of which 10 represented recoded ambiguously aligned regions and 97 were parsimony informative.

Parsimony-based phylogenetic analyses were run using weighted MP in PAUP* 4.0b10. An initial search was made with 1000 random-addition-sequence (RAS) replicates and tree bisection reconnection (TBR) branch swapping. The MULTREES option was in effect and zero-length branches were collapsed. All equally most parsimonious trees were saved with branch lengths, and a strict consensus tree was computed for reference. In this analysis the best tree island was hit in 98.6% of the replicates, and 6 equally most parsimonious trees were recovered, all of which were identical. Branch support for MP trees was estimated through bootstrap analyses (Felsenstein 1985) by performing 1000 bootstrap replicates with 5 RAS per bootstrap replicate, with all other settings as above.

For maximum likelihood (ML) an initial set of analyses were performed on the dataset with ambiguously-aligned regions recoded in PICS-Ord following Lücking et al. (2011). For this set of analyses the original ambiguously-aligned regions were manually deleted using Mesquite and the alignment was then saved in an extended phylip format. Recoded ambiguously aligned regions generated in PICS-Ord were then concatenated with the end of the alignment using the alt-copy/paste function of MSWord. A topology search and bootstrap analysis of 1000 replicates each, using the GTRGAMMA model, were performed using the windows executable version of RAxML 7.2.6. Using the same program, the bootstrap bipartitions were mapped to the best tree recovered in the topology search and these were visualized in FigTree 1.2.3 (Rambaut 2009). The clades comprising sequences of *L. atlantica* and *L. elobata* which were strongly supported with MP received markedly lower support with ML-RAxML. (It should be noted that this issue

was also encountered by Fehrer et al. (2008).) Thus an additional set of analyses were performed using PAUP on a version of the dataset without PICS-Ord characters included. For these analyses, as well as for Bayesian phylogenetic analyses, MrModeltest (Nylander 2004) was used to select a proper model for nucleotide substitution. Using the Akaike Information Criterion (AIC; Akaike 1973), SYM+G was selected. Searches and bootstrap analyses were run as above using the ML PAUP block produced by MrModeltest with likelihood set as the optimality criterion. Bayesian analyses were performed using a MrBayes block produced through the online automated form found at the Santos Lab website (http://131.204.120.103/srsantos/mrbayes_form/index.html) and implemented with MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). The model was implemented by selecting six substitution rates with gamma-shaped rate variation and equal state frequencies. Otherwise default priors were used. The Markov chain Monte Carlo parameters consisted of 10,000,000 generations, with four chains, and a tree sampled every 100 generations. The first 10,000 trees were discarded as burnin and the results were summarized as a 50% majority-rule consensus tree.

The original alignment file as well as MP, ML, and BI input files can be downloaded from the Dyrad Repository (http://dx.doi.org/10.5061/dryad.70m919n6).

Results

The results of the molecular phylogenetic analyses outlined above are summarized in figure 31. In each of the analyses conducted for the present study the sequences of *Lepraria caesiella* s.l. were recovered in two strongly supported clades that correspond to the two morphotypes presently included within the species because of their chemical similarity. Thus, *L. caesiella* as presently defined includes two phylogenetically distinct entities that can be separated on the basis of their thallus morphology. As such the name *L. caesiella* is restricted to

populations with aggregate thalli, the thallus type represented in the holotype of that name. Whereas the populations with placodioid thalli are formally described as a new species, *L. harrisiana*. These two taxa are treated in detail in the taxonomic section below. It is noteworthy that these analyses further confirm the distinctions between *L. caesiella* s. str. and *L. friabilis*, which are morphologically similar but chemically distinct, as outlined by Lendemer et al. (2008). The sequences of *L. atlantica* and *L. elobata* were consistently recovered as monophyletic groups that were strongly supported with maximum parsimony. Interestingly, the level of support of these clades differed significantly between the maximum likelihood and Bayesian inference analyses that were performed. Previous studies have recovered these taxa as monophyletic with strong support (Slavíková-Bayerová & Fehrer 2007; Fehrer et al. 2008) and they are otherwise correlated to suites of non-molecular characters.

The above results mirror those of prior studies that have repeatedly affirmed the value of nuITS sequence data in circumscribing species both within *Lepraria* s.l. (Lendemer 2011b, Lendemer in rev., Slavíková-Bayerová & Fehrer 2007) and more broadly across other groups of lichenized fungi (Hodkinson & Lendemer 2011; Lendemer & Hodkinson 2009, 2010). There are cases where nuITS sequence data alone have been found to be insufficient to unequivocally resolve hypothetical boundaries of species of lichenized fungi that were circumscribed based on non-molecular characters (Lendemer & Hodkinson 2009). Such cases are, however, disproportionately few when placed in the broader context of studies, such as the present one, where the results of phylogenetic analyses of an independent dataset comprised of nuITS sequence data can be correlated to suites of non-molecular characters to circumscribe monophyletic taxonomic units.

Some may consider the variable support that different methods of phylogenetic inference recovered for *Lepraria atlantica* and *L. elobata* to be evidence that nuITS sequence data alone is insufficient to resolve these taxa as distinct. Previous authors have also encountered the same problem with *L. atlantica* (Slavíková-Bayerová & Fehrer 2007) and concluded that it was due to the fact that most of the informative characters were included in ambiguously aligned regions that were excluded from the dataset. The present study provides some support for this argument because both taxa were recovered with strong support with maximum parsimony analyses where the ambiguously aligned regions were included as recoded characters. Interestingly *L. elobata* was recovered with weak support in a maximum likelihood analysis in PAUP that excluded ambiguously aligned regions, while it was not supported in another analysis using RAxML that included those regions as recoded characters. The opposite was true for *L. atlantica*, which was poorly supported when ambiguously aligned regions were excluded, but then strongly supported when they were recoded and included. That these different methods of inference obtained radically different levels of support, reinforces the importance of examining the same dataset with more than one method. It also reinforces the use of non-molecular characters to reconcile the results of phylogenetic inference with observable phenotypic reality.

Discussion

The genus *Lepraria* presents an excellent opportunity to examine the robustness of chemotaxonomy as a tool for circumscribing monophyletic entities at the species level (Lendemer 2011, Lendemer in rev.). Indeed, the widespread assumption that the genus was devoid of morphological characters (Laundon 1992; Fehrer et al. 2008) led workers to emphasize chemical characters over those of biogeography, ecology, and morphology (Lendemer 2011). It has long been recognized that this practice does not consistently result in the recognition of

monophyletic entities (Lendemer 2011b). Regardless, a chemotaxonomic approach was favored because it relied on discrete presence/absence characters rather than vegetative morphological characters whose interpretation was often perceived to be subjective.

Lepraria s.l. is one of the few groups of lichenized fungi where there have been multiple attempts to use molecular phylogenetic analyses to confirm the monophyly of species delineated by non-molecular characters (Lendemer 2011b, in rev.; Fehrer et al. 2008; Slavíková-Bayerová & Fehrer 2007; Tretiach et al. 2009). Interestingly, these previous studies of have produced conflicting results regarding the utility of chemotaxonomy. Some studies have supported the use of chemical characters to delineate species (Lendemer in rev.; Fehrer et al. 2008; Slavíková-Bayerová & Fehrer 2007). Others however, have recovered well supported monophyletic entities that were chemically variable (Tretiach et al. 2009). Based on these studies it is clear that within *Lepraria* s.l. chemical characters alone cannot *a priori* be used to circumscribe monophyletic taxonomic entities. Rather, a case by case approach is necessary in which the robustness of chemical characters is confirmed with independent correlated characters. It should be noted that comparable studies of other taxa representing the diverse lineages that constitute lichenized fungi have produced similarly conflicting results (see discussion in Hodkinson and Lendemer (2011)). Collectively these results serve to highlight the inherent dangers associated with the unfettered use of chemotaxonomy in the absence of correlated characters (molecular, or non-molecular).

The present study focuses on *Lepraria caesiella*, a common species that is widely distributed throughout eastern North America. At the time it was first described, *L. caesiella* was circumscribed to exclude a group of populations that were chemically identical to the type but differed in having thick well-delimited thalli. This morphology coupled with the production of a fatty acid in addition to atranorin led the describing author to compare these populations to *L.*

rigidula (de Lesd.) Tønsberg (Harris *in* Lendemer 2005). Subsequently these populations were subsumed within *L. caesiella* on account of their shared chemical characters.

When the author began his revision of *Lepraria* s.l. the first step he took was to review all the known species in the genus to synthesize a standardized morphological terminology and descriptive scheme (Lendemer 2011). When the author reviewed *L. caesiella* s.l. it became clear that the differences between the two morphotypes of *L. caesiella* corresponded to the two fundamentally different types of thallus organization that occur in the genus. Thus, testing the monophyly of these morphotypes with molecular data represented an opportunity to examine the validity of this newly introduced method of morphological characterization. That these morphotypes were here recovered as well supported and distinct monophyletic entities in this study, lends support to the new system.

The results pertaining to *Lepraria elobata* also merit comment here. It is noteworthy that one sequence from a North American population (NY647) formed a well-supported clade with two sequences generated from European populations. When Lendemer (2011) studied the North American and European populations of *L. incana* (L.) Ach. he found that comparable differences in geographic distribution corresponded to monophyletic entities. The nuITS sequences of these entities were as divergent from each other as any other two species of *Lepraria* included in that study. They were thus recognized as distinct semi-cryptic species and the North American populations were formally described as a new species. The results for *L. elobata* obtained in the present study confirm that species of *Lepraria* can have widely separated inter-continental distributions. Thus, morphologically and chemically identical sets of allopatric populations that are separated by significant geographic barriers do not necessarily represent semi-cryptic species.

Taxonomic Section

1) ***Lepraria caesiella*** **R.C. Harris** ***in*** **Lendemer**, Opusc. Philolichenum 2: 51. 2005. TYPE: **U.S.A. PENNSYLVANIA.** MONROE CO.: Delaware Water Gap National Recreation Area, Community Drive Wetlands, ca. 1 mi S of Shoemakers, ca. 2 mi S of Bushkill, 24.iv.2004, on large dead birch (*Betula*), *Lendemer et al. 2304 = Lich. East. N. Amer. Exs. IV: 172* (NY!, holotype; ASU!, B!, BG!, DOV!, FH!, GZU!, H!, MIN!, NDA!, S!, TU!, isotypes).

Mycobank #356801.

Figure 32.

Description. – **Thallus** crustose, leprose, aggregated, discontinuous and not stratified, initially composed of isolated granules that divide to form aggregations, eventually merging to form a thin crust (<0.1 mm thick), gray-blue in color; **hyphae** hyaline, [1.8]–(2.4)–3.1–(3.7)–[5] µm (*n*=42) in width, septate, second, obscured by a thick layer of POL+ crystals that dissolve in KOH and recrystalize as thin bent needles; **prothallus** persistent; **hypothallus** absent; **rhizohyphae** absent; **granules** globose, [24]–(35)–47–(59)–[76] µm (*n*=96) in diameter, ecorticate, well organized and discrete, remaining distinct and not forming compound units; **photobiont** green, coccoid, cells globose, [6]–(7.9)–9.8–(11.8)–[15.6] µm (*n*=96) in width

Chemistry. – Atranorin, zeorin, pallidic acid. Spot tests: K+ yellow, C-, KC-, P+ weak yellow, UV-.

Ecology and distribution. – *Lepraria caesiella* is common and widely distributed throughout temperate and boreal northeastern North America, extending southward in the Appalachian Mountains (figure 34). Disjunct saxicolous populations also occur in the Ozark

Ecoregion. The species occurs on a wide diversity of corticolous substrates, including both conifers and hardwoods. Though the majority of the corticolous collections examined are from hardwoods, particularly oaks (*Quercus*), saxicolous populations are also often encountered on non-calcareous rocks. These saxicolous populations are ecologically distinct from other sympatric *Lepraria* species with aggregate thalli because they occur on dry, protected rock faces that are sheltered from rain but still exposed to some light. Most other species occur either on rock faces fully exposed to the elements, or in shaded overhangs that are completely protected and always shaded.

Discussion. – *Lepraria caesiella* is chemically comparable to *L. harrisiana*, described below, which differs morphologically in having a placodioid thallus. Although previously considered conspecific, the two entities are strongly supported as distinct in molecular phylogenetic analyses of nuITS sequence data. They also have somewhat non-overlapping geographic distributions and substrate preferences.

There are only six other *Lepraria* species with aggregate thalli known from North America that are likely to be confused with *L. caesiella*. Of these, only *L. elobata* consistently produces zeorin. However, it is chemically distinct from *L. caesiella* in producing stictic acid in addition to atranorin and zeorin. It also does not produce pallidic acid. Although unreported in the protologue, *L. salazinica* Tønsberg, a rare saxicolous species of the Appalachian Mountains, also can produce zeorin in low concentrations (Lendemer unpublished data). It differs from *L. caesiella* in consistently producing salazinic acid in addition to atranorin. *Lepraria jackii* Tønsberg, a northern boreal species in North America, also produces atranorin and fatty acids, however, it does not produce zeorin and the fatty acids are jackinic and norjackinic acids not

pallidic acid. A further species, *L. friabilis*, occurs on conifers in the coastal plain of southeastern North America. Although morphologically similar to *L. caesiella* s. str., and originally considered to be sympatric with it (Lendemer et al. 2008) the present study has revealed all the presumed sympatric populations actually represent *L. harrisiana*, which is described below. *Lepraria friabilis* also differs from *L. caesiella* in producing fumarprotocetraric acid rather than zeorin and pallidic acid.

Lepraria humida Slav.-Bay. & Orange is a saxicolous species of shaded overhangs that was described from Europe and will be reported from North America in a separate publication (Lendemer in rev.). Like *L. jackii*, *L. humida* does not produce pallidic acid or zeorin.

Members of the *Lepraria neglecta* group could also be confused with *L. caesiella*, however, none of the chemotypes in that group is known to produce zeorin and pallidic acid. Outside of North America, populations with zeorin and accessory porphyrilic acid were described from Greenland as *L. alpina* var. *zeorinica* L. Saag (Saag et al. 2007). Similarly, populations with zeorin and accessory stictic acid from Greenland were described as *L. caesioalba* var. *groenlandica* L. Saag (Saag et al. 2007). While the fatty acids roccellic acid or rangiformic acid were also reported from some populations of these taxa, pallidic acid was not reported from either of them.

The lichen most likely to be confused with *Lepraria caesiella* is actually *Lecanora nothocaesiella* Lendemer & R.C. Harris, a sorediate member of the *Lecanora subfusca* group. Indeed, in my revision of *Lepraria* species from numerous herbaria I have encountered many specimens of *L. nothocaesiella* misidentified as *L. caesiella*. *Lecanora nothocaesiella* differs from *L. caesiella* in lacking pallidic acid and in its thallus morphology. The thallus of *L. nothocaesiella* is not truly leprose, but rather appears such because it produces diffuse eroding

soralia that are immersed in the substrate. It also typically produces a conspicuous, shiny, film-like prothallus with darkened areas near the growing edge of the thallus. Species of *Lepraria* never produce this type of prothallus because the hyphae of the thallus rest on, rather than penetrate and grow through, the substrate (Lendemer 2011).

Selected specimens examined (of 377 total). – **CANADA. NEW BRUNSWICK.** CHARLOTTE CO.: Pennfield Parish, W of Jake Lee Rd., 1.v.2011, on *Betula papyrifera*, *J.C. Lendemer 28023-A* (NY). **ONTARIO.** BRUCE CO.: Bruce Peninsula National Park, 19.ix.2008, on dead *Picea*, *R.C. Harris 54884* (NY). LANARK CO.: 0–0.2 mi N of Peneshula Rd., 0.6 mi W of jct. w/ Cedar Cove Rd., 21.v.2011, on *Pinus strobus*, *J.C. Lendemer 28196 & R.E. Lee* (NY). **QUEBEC.** GATINEAU CO.: Gatineau Park, near Ramsey Lake, 30.vii.1978, on *Tsuga*, *T. Ahti 34648 & I.M. Brodo* (H, NY). PONTIAC MNP.: Gatineau Park, Church Hill, 22.v.2011, on *Tsuga*, *J.C. Lendemer 28332 & C. Freebury* (NY). **U.S.A. ARKANSAS.** FRANKLIN CO.: Ozark National Forest, Boston Mountains Ranger District, Shores Lake, 17.x.2005, on sandstone, *J.C. Lendemer 5605* (NY). **CONNECTICUT.** LITCHFIELD CO.: Town of Norfolk, Holleran Swamp Preserve, 20.ix.2003, on *Acer*, *R.C. Harris 48065* (NY). WINDHAM CO.: Town of Westford, Yale Myers Forest, Boston Hollow, 19.ix.2009, on *Tsuga*, *R.C. Harris 55660* (NY). **DELAWARE.** SUSSEX CO.: Ellendale State Forest, 25.x.1989, on *Ilex*, *R.C. Harris 24913* (NY). **KENTUCKY.** PERRY CO.: Daniel Boone National Forest, Old Field Branch of Leatherwood Creek, 6.x.2001, on *Tsuga*, *R.C. Harris 44971* (NY). **MAINE.** YORK CO.: Town of Saco, Ferry Beach State Park, 15.ix.2002, on *Betula papyrifera*, *R.C. Harris 46335* (NY). **MARYLAND.** CECIL CO.: Elk Neck State Forest, Black Hill Tract, 30.xi.2009, on *Quercus*, *J.C. Lendemer 19967* (NY). **MASSACHUSETTS.** BERKSHIRE CO.: Town of Hancock,

Pittsfield State Forest, 4.vi.2010, on *Acer*, *J.C. Lendemer 22492* (NY). PLYMOUTH CO.: Town of Carver, Myles Standish State Forest, 31.vii.2010, on *Picea*, *W.R. Buck 56448* (NY). **MICHIGAN.** JACKSON CO.: E of Updike Rd., ~.4 mi N of I-94, 25.vi.1979, on *Quercus*, *R.C. Harris 13098* (NY). ONTONAGON CO.: Ottawa National Forest, Lathrop Creek, 10.viii.2003, on *Tsuga*, *C.M. Wetmore 89965* (MIN, NY). **NEW JERSEY.** ATLANTIC CO.: Wharton State Forest, N of Pleasant Mills Cemetery, 24.x.2004, on *Sassafras, J.C. Lendemer 3355* (NY). BURLINGTON CO.: Wharton State Forest, Batsto Natural Area, 9.x.2004, on *Quercus*, *J.C. Lendemer 3181* (NY). CAMDEN CO.: Wharton State Forest, 0–0.5 mi W of CR 536 (Chew Rd.), 6.ii.2009, on *Quercus*, *J.C. Lendemer 15487* (NY). CUMBERLAND CO.: Peaslee Wildlife Management Area, 2.ii.2009, on *Quercus*, *J.C. Lendemer 15095* (NY). GLOUCESTER CO.: Winslow Wildlife Management Area, 6.ii.2009, on *Pinus, J.C. Lendemer 15441* (NY). HUNTERDON CO.: Mitchell property, 4.xi.1992, on *Crataegus*, *R.C. Harris 28993* (NY). MONMOUTH CO.: Allaire State Park, 5.ii.2009, on *Quercus*, *J.C. Lendemer 15417* (NY). OCEAN CO.: Manahawkin Wildlife Management Area, 2.xii.2009, on *Quercus*, *J.C. Lendemer 20067* (NY). SALEM CO.: Parvin State Park, 26.xii.2008, on *Quercus*, *J.C. Lendemer et al. 15012* (NY). WARREN CO.: Jenny Jump State Forest, 11.ix.1992, on *Tsuga*, *R.C. Harris 27924* (NY). **NEW YORK.** ESSEX CO.: SW side of Chapel Pond, 19.ix.2004, on *Acer*, *J.C. Lendemer 3093* (NY). JEFFERSON CO.: Wellesley Island State Park, 20.ix.1983, on *Tsuga*, *R.C. Harris 16751* (NY). SUFFOLK CO.: 1.5 km W of Promised Land, 22.iv.1984, on *Pinus*, *R.C. Harris 17967* (NY). ST. LAWRENCE CO.: J.F. Dubuar Forest Trail, 6.ix.1981, on dead *Picea*, *R.C. Harris 13971* (NY). ULSTER CO.: along Esopus Creek, 13.ix.1980, on *Pinus strobus*, *R.C. Harris 13460* (NY). WESTCHESTER CO.: Mianus Gorge, 14.viii.1976, on bark, *C.R. Prince 76-48* (NY). **NORTH CAROLINA.** GRAHAM CO.: Nantahala National Forest, Hooper Bald,

1.x.1997, on *Betula alleghaniensis*, *R.C. Harris 41064* (NY). SWAIN CO.: Great Smoky Mountains National Park, terminus of Lakeshore Drive to jct. of Lakeshore Trail and White Oak Branch Trail, 29.vi.2010, on *Pinus*, *J.C. Lendemer et al. 23421* (NY). TRANSYLVANIA CO.: Gorges State Park, 12.viii.2005, on *Betula lenta*, *J.C. Lendemer 4958 & E. Tripp* (NY). **OHIO.** GALLIA CO.: Wayne National Forest, Symmes Creek Natural Area, 19.v.2006, on *Quercus*, *R.C. Harris 52648* (NY). SCIOTO CO.: Shawnee National Forest, above Turkey Creek Lake, 18.v.2006, on *Acer*, *J.C. Lendemer 7525* (NY). **PENNSYLVANIA.** CLARION CO.: Cook Forest State Park, 6.ix.2010, on *Tsuga*, *J.C. Lendemer 24542* (NY). ELK CO.: Moshannon State Forest, Barr Hollow, 31.viii.2010, on rock, *J.C. Lendemer 23974* (NY). FRANKLIN CO.: Michaux State Forest, N-slopes of Rocky Mountain, 1.vi.2009, on *Quercus*, *J.C. Lendemer 18206* (NY). MONROE CO.: Tannersville Bog TNC Preserve, 24.xi.2009, on *Tsuga*, *J.C. Lendemer 19940-A & V. Doyle* (NY). NORTHUMBERLAND CO.: Wildlife Preserve, ~2.5 mi W of Alaska, 20.v.2009, on *Quercus*, *R.C. Harris 55331* (NY). PHILADELPHIA CO.: Fairmount Park, Forbidden Drive, 3.iii.2007, on *Acer*, *J.C. Lendemer 8603 & A. Moroz* (NY). SULLIVAN CO.: Ricketts Glen State Park, 19.ix.2010, on *Tsuga*, *J.C. Lendemer 25661 & J. Kunsman* (NY). TIOGA CO.: Tioga State Forest, Asaph Wild Area, 11.v.2009, on *Tsuga*, *J.C. Lendemer 16435 & D. Atha* (NY). UNION CO.: Bald Eagle State Park, Joyce Kilmer Natural Area, 14.ix.2010, on *Tsuga*, *J.C. Lendemer 25411* (NY). YORK CO.: Apollo County Park, 9.xiii.2009, on *Betula*, *J.C. Lendemer 19429* (NY). **RHODE ISLAND.** WASHINGTON CO.: Marion Eppley Wildlife Sanctuary, 16.ix.2006, on *Nyssa*, *R.C. Harris 53142* (NY). **TENNESSEE.** BLOUNT CO.: Great Smoky Mountains National Park, White Oak Sinks, 13.x.2010, on *Tsuga*, *J.C. Lendemer et al. 26884* (NY). COCKE CO.: Great Smoky Mountains National Park, Gabes Mountain Trail, 5.viii.2009, on *Tsuga*, *J.C. Lendemer 19034 & E. Tripp*

(NY). **VERMONT.** ADDISON CO.: Green Mountain National Forest, Bristol Cliffs Wilderness Area, 20.x.2010, on *Betula lenta*, *J.C. Lendemer 27367 & M. Sundue* (NY). CALEDONIA CO.: Town of Wheelock, Wheelock Farm, 22.x.2010, on *Abies, J.C. Lendemer 27547 & M. Sundue* (NY). CHITTENDEN CO.: Town of Huntington, Lower Gorge Preserve, 17.x.2010, on *Tsuga*, *J.C. Lendemer 27089 & M. Sundue* (NY). ESSEX CO.: Town of Averill, Quimby Country resort property, 19.v.2008, on *Betula papyrifera*, *R.C. Harris 54586* (NY). ORLEANS CO.: Town of Craftsbury, Craftsbury Outdoor Center, 16.v.2005, on *Abies*, *R.C. Harris 51478* (NY). **VIRGINIA.** GILES CO.: Pond Drain, 7.x.1995, on *Betula alleghaniensis*, *R.C. Harris 36700* (NY). PATRICK CO.: Blue Ridge Parkway, Rock Castle Gorge Loop Trail, 6.x.1995, on *Quercus*, *R.C. Harris 36648* (NY). **WEST VIRGINIA.** PRESTON CO.: 2 mi N of Terra Alta, 287.vi.1963, on trees, *C.F. Reed 62858* (NY). POCAHONTAS CO.: Watoga State Park, 30.ix.2000, on *Tsuga*, *R.C. Harris 43932* (NY). TUCKER CO.: Monongahela National Forest, Olsen's Bog, 22.iv.2001, on *Betula lenta*, *R.C. Harris 44871* (NY). **WISCONSIN.** BAYFIELD CO.: Chequamegon National Forest, Rainbow Lake, 29.v.2004, on *Tsuga*, *C.M. Wetmore 90460* (MIN, NY). ONEIDA CO.: Town of Minocqua, Patterson Hemlocks State Natural Area, 29.iv.2002, on *Tsuga*, *R.C. Harris 45977* (NY).

2) *Lepraria harrisiana* Lendemer sp. nov.

Mycobank #563739.

Figure 33.

TYPE: **U.S.A. NORTH CAROLINA.** CAMDEN CO.: Dismal Swamp State Park, S side of Kim Saunders Ditch, at 5 mi marker, Atlantic white cedar (*Chamaecyparis thyoides*)

stand with blow-downs and mixed hardwoods (*Magnolia, Acer, Nyssa*), 10.xii.2009, on *Chamaecyparis*, *J.C. Lendemer 20191 & S. Williams* (NY!, holotype).

Description. – **Thallus** crustose, leprose, placodioid, continuous and typically distinctly bi-layered, initially forming small discrete thalli which expand marginally, overlap and become confluent to form a thick crust (0.2–0.4 mm thick), light greenish-blue in color, often with a slightly yellowish hue as the thallus ages in the herbarium; **hyphae** hyaline, [1.2]–(2.2)–2.9–(3.5)–[4.8] µm (*n*=68) in width, septate, secund, obscured by a thick layer of POL+ crystals that dissolve in KOH; **prothallus** disappearing with age; **hypothallus** a thin, often poorly developed layer of loosely intertwined hyphae surrounding and subtending the granules, extending outward from the edge of the thallus; **rhizohyphae** present, pale to dirty brownish; **granules** globose, [19]–(27)–38–(48) –[72] µm (*n*=124) in diameter, ecorticate, well organized and discrete, often forming compound units of multiple granules; **photobiont** green, coccoid, cells globose, [6] –(7.8)–9.6–(11.4)–[14.4] µm (*n*=97) in diameter.

Chemistry. – Atranorin, zeorin, pallidic acid. Spot tests: K+ yellow, C-, KC-, P+ weak yellow, UV-.

Etymology. – The epithet honors Richard C. Harris (1939–present), a lichenologist who has made tremendous contributions to our knowledge of the crustose lichen biota of eastern North America where the new species occurs (Buck 2009). Harris described *Lepraria caesiella*,

the species from which *L. harrisiana* has been segregated. He was also among the first modern lichenologists to study *Lepraria* s.l., revising extensive herbarium materials and proposing new combinations (e.g., Harris 1985) before the revisions of Laundon (1992) and Tønsberg (1992).

Ecology and distribution. – *Lepraria harrisiana* is widespread in eastern North America in high humidity habitats, particularly those associated with bodies of water (figure 34). It is widely distributed in conifer swamps and riparian forests throughout both the Gulf and Atlantic Coastal Plains of southeastern North America, occurring from Massachusetts to Georgia and westward to at least Alabama. Disjunct populations also occur at high elevations of the southern Appalachian Mountains, riparian conifer forests along the edges of Ozarks and in the Ouachita Mountains, oceanic forests of the Canadian Maritimes, and conifer swamps of the Great Lakes Region. As geographically disparate as this distribution may appear, it is not illogical considering the recent biogeographic patterns outlined by Lendemer et al. (in press.) for other lichens that occur in the southern Appalachian Mountains. Interestingly, saxicolous populations of *L. harrisiana* have not been encountered and the majority of the collections are from coniferous hosts (*Picea, Pinus, Thuja,* especially *Chamaecyparis*). Whereas the chemically similar *L. caesiella* rarely occurs on conifers, and rather is most often found on the bark of hardwoods and on non-calcareous rocks.

Discussion. – *Lepraria harrisiana* was first recognized as distinct by Harris (in Lendemer 2005) who referred to it as a possible form of *L. rigidula* in the discussion of the protologue of *L. caesiella*. Subsequently, I lumped that form with *L. caesiella*, attributing the differences in thallus morphology to ecological factors. While developing a standardized set of descriptive

morphological terms for *Lepraria* thalli (Lendemer 2011), it became clear that the inclusion of two morphotypes in *L. caesiella* was untenable. Indeed, it would have meant that *L. caesiella* would have been circumscribed as having both an aggregate and a placodioid thallus type. These were the two primary types of *Lepraria* thalli outlined by Lendemer (2011). Molecular phylogenetic analyses of nuITS sequence data confirmed that the two morphotypes represent well-supported monophyletic entities that should be treated as distinct species.

Although *Lepraria harrisiana* is typically easily recognized by its thallus type and chemistry, some specimens with poorly developed thalli can be difficult to separate from *L. caesiella*. However, the white hypothallus of *L. harrisiana* is always evident, even if it is thin and poorly developed. A careful search of the thallus margin will also typically reveal portions with a well developed weft of hyphae protruding outward (figure 33). It should be noted that this highlights the need to collect large, ample specimens in order to determine *Lepraria* (and sterile *Lecanora*) species with confidence.

Surprisingly, there are relatively few other *Lepraria* species that are likely to be confused with *L. harrisiana*. Only *L. caesiella* and *L. pallida* Sipman are chemically similar in producing atranorin, zeorin, and pallidic acid. The former taxon differs in having an aggregate thallus. While the latter taxon, described from South America, Madagascar, and the Seychelles by Sipman (2004), has a *normandinoides*-type placodioid thallus (see figure 5 herein and Lendemer (2011)).

The new species could be confused with *Lepraria aurescens* Orange & Wolseley and *L. squamatica* Elix, two morphologically similar species that also occur in southeastern North America. Both are easily distinguished chemically (*L. aurescens* produces thamnolic acid and *L. squamatica* produces squamatic acid). Further they appear to be allopatric with *L. harrisiana*,

occurring further south in northern and central Florida (Lendemer 2010). *Lepraria rigidula*, the species which Harris originally compared to *L. harrisiana*, differs in producing nephrosteranic acid, lacking zeorin and pallidic acid, and in having an aggregate thallus.

Selected specimens examined. – **CANADA. NEW BRUNSWICK.** CHARLOTTE CO.: Grand Falls Flowage on St. Croix River, 20.iv.2011, on *Tsuga*, *J.C. Lendemer 27854* (NY), on *Betula*, *J.C. Lendemer 27786* (NY). **ONTARIO.** BRUCE CO.: Bruce Peninsula National Park, 20.ix.2008, on *Pinus*, *J.C. Lendemer 14283* (NY). FRONTENAC CO.: Arcol Road, 24.ii.2011, on *Pinus strobus*, *C. Lewis 508* (NY). OTTAWA-CARLTON CO.: City of Ottawa, Stony Swamp, 19.v.2011, on *Thuja*, *J.C. Lendemer 28152 & I.M. Brodo* (NY). **U.S.A. ALABAMA.** CLAY CO.: Cheaha State Park, 24.ix.1992, on *Pinus*, *R.C. Harris 28298* (NY). COVINGTON CO.: Conecuh National Forest, Solon Dixon Forestry Education Center, 14.iv.2007, on *Pinus*, *J.C. Lendemer et al. 9430* (NY). MARSHALL CO.: Lake Guntersville State Park, 13.viii.2005, on *Pinus*, *J.C. Lendemer 4872* (NY). **ARKANSAS.** FAULKNER CO.: Cove Creek Natural Area, 7.x.2010, on *Pinus*, *J.C. Lendemer et al. 26201* (NY). GARLAND CO.: Ouachita National Forest, W side of North Fork Ouachita River, 6.x.2010, on *Pinus*, *J.C. Lendemer et al. 26116* (NY). **CONNECTICUT.** WINDHAM CO.: Town of Windham, Windham Bog, 20.ix.2009, on *Chamaecyparis*, *R.C. Harris 55730* (NY). **FLORIDA.** ESCAMBIA CO.: along CR 99a AT Brushy Creek, 8.xii.1993, on *Taxodium*, *R.C. Harris 31907* (NY). SANTA ROSA CO.: Blackwater River State Forest, 9.xii.1993, on *Taxodium*, *R.C. Harris 32004* (NY). **GEORGIA.** DAWSON CO.: Amicalola Falls State Park, 21.xii.2009, on *Pinus echinata*, *M.F. Hodges s.n.* (NY). ROCKDALE CO.: Panola Mountain State Park, 17.iv.2007, on *Pinus*, *J.C. Lendemer et al. 9002* (NY). TATNALL CO.: Big Hammock Natural Area, 20.xii.2009, on *Taxodium*, *J.C.*

Lendemer 21368 & S.Q. Beeching (NY). **ILLINOIS.** ALEXANDER CO.: Shawnee National Forest, Grapevine Trail Picnic and Camping Area, 6.xi.2004, on *Pinus*, *R.C. Harris 50521* (NY). **KENTUCKY.** BULLITT CO.: Bernheim Arboreum and Research Forest, 28.iii.2002, on *Pinus virginiana*, *D. Ladd 23610 & M. Ladd* (NY). **MAINE.** HANDCOCK CO.: T.10 SD, N of ME 182, 27.vii.2006, on *Tsuga*, *R.C. Harris 53071* (NY). WASHINGTON CO.: Town of Cutler, Cutler Coastal Public Lands, 8.vi.2010, on conifer, *J.C. Lendemer 22755* (NY). **MARYLAND.** CAROLINE CO.: Idylwild State Wildlife Management Area, 18.iv.2006, on *Acer*, *J.C. Lendemer 6226 & W. Knapp* (NY). WICOMICO CO.: Sharptown Dunes, 19.iv.2006, on *Quercus*, *J.C. Lendemer et al 6291* (NY). **MISSISSIPPI.** SCOTT CO.: Bienville National Forest, Bienville Pines Scenic Area, 29.ix.1992, on *Pinus*, *R.C. Harris 28713* (NY). **MISSOURI.** CARTER CO.: Mark Twain National Forest, vicinity of Big Barren Creek Natural Area, 9.x.2010, on *Pinus*, *J.C. Lendemer 26576 & D. Ladd* (NY). **NEW JERSEY.** ALTANTIC CO.: Wharton State Forest, Batsto Natural Area, 15.iii.2000, on *Chamaecyparis*, *R.C. Harris 43787* (NY). BURLINGTON CO.: Wharton State Forest, W shore of Batsto River, 13.xi.2004, on *Pinus rigida*, *J.C. Lendemer 3522 & R.F. Lendemer* (NY). CAMDEN CO.: Wharton State Forest, 0–0.5 mi W of CR 536 (Chew Rd.), 6.ii.2009, on *Pinus*, *J.C. Lendemer 15477* (NY). CAPE MAY CO.: Peaslee Wildlife Management Area, 2.ii.2009, on *Chamaecyparis*, *J.C. Lendemer 15208* (NY). CUMBERLAND CO.: Peaslee Wildlife Management Area, 2.ii.2009, on log, *J.C. Lendemer 15116* (NY). GLOUCESTER CO.: Glassboro Wildlife Management Area, 4.ii.2009, on *Quercus*, *J.C. Lendemer 15302* (NY). OCEAN CO.: Manchester Wildlife Management Area, 5.ii.2009, on *Gaylussacia*, *J.C. Lendemer 15366* (NY). SALEM CO.: Parvin State Park, 26.xii.2008, on *Pinus*, *J.C. Lendemer et al. 15018* (NY). **NEW YORK.** SUFFOLK CO.: Cranberry Bog Nature Preserve, 20.ix.1986, on *Chamaecyparis*, *R.C. Harris 19438-B*

(NY). ST. LAWRENCE CO.: Twin Lakes, 6.ix.1981, on *Tsuga*, *R.C. Harris 13900* (NY). **NORTH CAROLINA.** ORANGE CO.: Johnston Mill Nature Preserve, 7.iii.2009, on *Pinus taeda*, *G.B. Perlmutter et al. 1690* (NY). MACON CO.: Sunset Rocks, Highlands, 9.vi.1981, on *Pinus*, *R.C. Harris 13658* (NY). SURRY CO.: Pilot Mountain State Park, 26.viii.2006, on *Pinus rigida, G.B. Perlmutter 587 & J. Pearson* (NY). TRANSYLVANIA CO.: Gorges State Park, 9.viii.2005, on *Pinus*, *J.C. Lendemer 4617 & E.A. Tripp* (NY). WILKES CO.: Stone Mountain State Park, 23.ix.1993, on *Pinus*, *R.C. Harris 30793* (NY). **PENNSYLVANIA.** WAYNE CO.: Dripping Cliffs TNC Preserve, 2.vii.2008, on *Tsuga*, *J.C. Lendemer 12373* (NY). **SOUTH CAROLINA.** AIKEN CO.: The Hitchcock Woods, 14.iii.2010, on *Pinus*, *J.C. Lendemer 22069* (NY). McCORMICK CO.: Sumter National Forest, Stevens Creek Heritage Preserve, 11.iii.2010, on *Pinus*, *R.C. Harris 55848* (NY). **TENNESSEE.** BLOUNT CO.: GSMNP, Rich Mountain Gap at Ace Gap Trail, 30.vi.2010, on *Pinus*, *J.C. Lendemer et al. 23601* (NY). COCKE CO.: GSMNP, Gabes Mountain Trail, 5.viii.2009, on *Tsuga*, *E.A. Trip 656 & J.C. Lendemer* (NY). SEVIER CO.: GSMNP, Chimney Tops Trail, 28.vi.2010, on *Betula*, *J.C. Lendemer et al. 23372* (NY). **VERMONT.** ESSEX CO.: Town of Averill, Quimby Country resort property, 19.v.2008, on *Abies*, *R.C. Harris 54600* (NY). **VIRGINIA.** CHEASPEAKE CITY: Dismal Swamp National Wildlife Refuge, E of Laurel Ditch, 13.xii.2009, on *Pinus*, *J.C. Lendemer et al. 20572* (NY). SUFFOLK CO.: Dismal Swamp National Wildlife Refuge, vicinity of canal bridge over Jerico Ditch, 12.xii.2009, on *Fagus*, *J.C. Lendemer 20437 & B.P. Hodkinson* (NY). **WISCONSIN.** ONEIDA CO.: Town of Woodruff, Northern Highland State Forest, 28.iv.2002, on *Tsuga*, *R.C. Harris 45954* (NY).

Acknowledgments

Thanks to Irwin Brodo and an anonymous reviewer for reviewing the manuscript. Richard Harris and Bill Buck are also thanked for reviewing early drafts of the manuscript prior to submission. Fieldwork carried out by the author, during which specimens used in this study were collected, was funded in part by The City University of New York (CUNY), The National Park Service (Great Smoky Mountains National Park), The Southern Appalachian Botanical Society (SABS), The Torrey Botanical Society (TBS), The Western Pennsylvania Conservancy (WPC). Laboratory work and loan handling was facilitated by The New York Botanical Garden (NYBG) and funded in part by a grant from The California Lichen Society (CALS). In addition to the above funding sources, supplementary fieldwork and laboratory studies were made possible by a Dissertation Improvement Grant from the National Science Foundation (NSF-DDIG Award #11104333). Sean Beeching, Irwin and Fenja Brodo, Colin Freebury, Malcolm Hodges, Rob E. Lee, Michael Sundue, and Signa and Floyd Williams, are particularly thanked for facilitating permitting and other aspects of my fieldwork. The Humboldt Research Institute in Steuben, Maine hosted the author during his study there in summer of 2010. Finally, the curators and collections staff of the institutions cited in the materials and methods section are thanked for loaning specimens and answering various information queries.

	GenBank	Isolate	Collection	Locality	Substrate	Herbarium
*bolded vouchers = seen by the author						
L. atlantica	AF517887	N/A	*Orange 11829*	Ireland	?	BG
	EF619550	N/A	*Orange 15462*	United Kingdom	?	PRA
	EF619549	N/A	*Orange 15456*	United Kingdom	?	PRA
	EF619548	N/A	*Orange 15459*	United Kingdom	?	PRA
L. caesiella	JQ070280	NY464	***Lendemer 17484***	U.S.A. Pennsylvania	*Acer*	NY-1105936
	JQ070281	NY231	***Lendemer 15095***	U.S.A. New Jersey	*Pinus*	NY-1079171
	JQ070284	NY241	***Lendemer 15441***	U.S.A. New Jersey	*Pinus*	NY-1078768
	JQ070286	NY232	***Lendemer 15097***	U.S.A. New Jersey	*Pinus*	NY-1079170
	JQ070283	NY977	***Lendemer 23794***	U.S.A. Pennsylvania	rock	
	JQ070285	NY1024	***Lendemer 24652***	U.S.A. Pennsylvania	*Acer*	NY-1216277
	JQ070287	NY635	***Lendemer 19967***	U.S.A. Maryland	*Quercus*	NY-1133862
	JQ070282	NY1107	***Lendemer 25566***	U.S.A. Pennsylvania	*Betula*	NY-1217868
	EU008608	N/A	***Nelsen 3968***	U.S.A. Pennsylvania	*Acer*	WIS
	EU008607	N/A	***Nelsen 3962***	U.S.A. Massachusetts	*Tsuga*	WIS
L. sp. nov.	JQ070288	NY218	***Lendemer 15125***	U.S.A. New Jersey	*Pinus*	NY-1078696
	JQ070289	NY219	***Lendemer 15165***	U.S.A. New Jersey	*Pinus*	NY-1078653
	JQ070290	NY246	***Lendemer 15294***	U.S.A. New Jersey	*Pinus*	NY-1078815
	JQ070291	NY663	***Lendemer 20503***	U.S.A. Virginia	*Pinus*	NY-1152941
	JQ070292	NY227	***Lendemer 15367***	U.S.A. New Jersey	*Pinus*	NY-1078992
	JQ070293	NY661	***Lendemer 20191***	U.S.A. New Jersey	*Chamaecyparis*	NY-1152438
	JQ070294	NY667	***Lendemer 20437***	U.S.A. Virginia	*Fagus*	NY-1152904
L. elobata	EF619546	N/A	*Slavikoba-Bayerova 3528*	Ukraine	?	PRA
	EF619547	N/A	*Slavikova-Bayerova 3529*	Ukraine	?	PRA
	EF619545	N/A	*Slavikova-Bayerova 3449*	Ukraine	?	PRA
	AF517910	N/A	*Tonsberg 28152*	Czech Republic	?	BG
	EF619544	N/A	*Orange 15453-2*	United Kingdom	?	PRA
	JQ070298	NY647	***Harris 55713***	U.S.A. Connecticut	rock	NY-1104177
	AF517909	N/A	*Tonsberg 28201*	Germany	?	BG
	JQ070295	NY610	***Buck 55092***	U.S.A. Utah	*Selaginella*	NY-1199479
	JQ070296	NY580	***Lendemer 19675***	U.S.A. California	rock	NY-1133087
	JQ070297	NY677	***Lendemer 9804***	U.S.A. New York	rock	NY-953120
	JQ070299	NY1058	***Lendemer 27625***	U.S.A. Vermont	*Abies*	NY-1218778
L. friabilis	JQ070273	NY711	***Lendemer 21369***	U.S.A. Georgia	*Taxodium*	
	JQ070274	NY13	***Lendemer 9063***	U.S.A. Alabama	*Taxodium*	NY-974484
	JQ070275	NY653	***Lendemer 21753***	U.S.A. Georgia	*Pinus*	NY-1104342
	JQ070276	NY659	***Lendemer 20269***	U.S.A. North Carolina	*Taxodium*	NY-1152728

JQ070277	NY662	***Lendemer 20283***	U.S.A. North Carolina	*Fagus*	NY-1152714
JQ070278	NY669	***Lendemer 21344***	U.S.A. Georgia	*Taxodium*	NY-1150273
JQ070279	NY670	***Lendemer 20360***	U.S.A. North Carolina	*Oxydendrum*	NY-1152599

Table 7. Voucher information and GenBank accession numbers for the molecular component of this study.

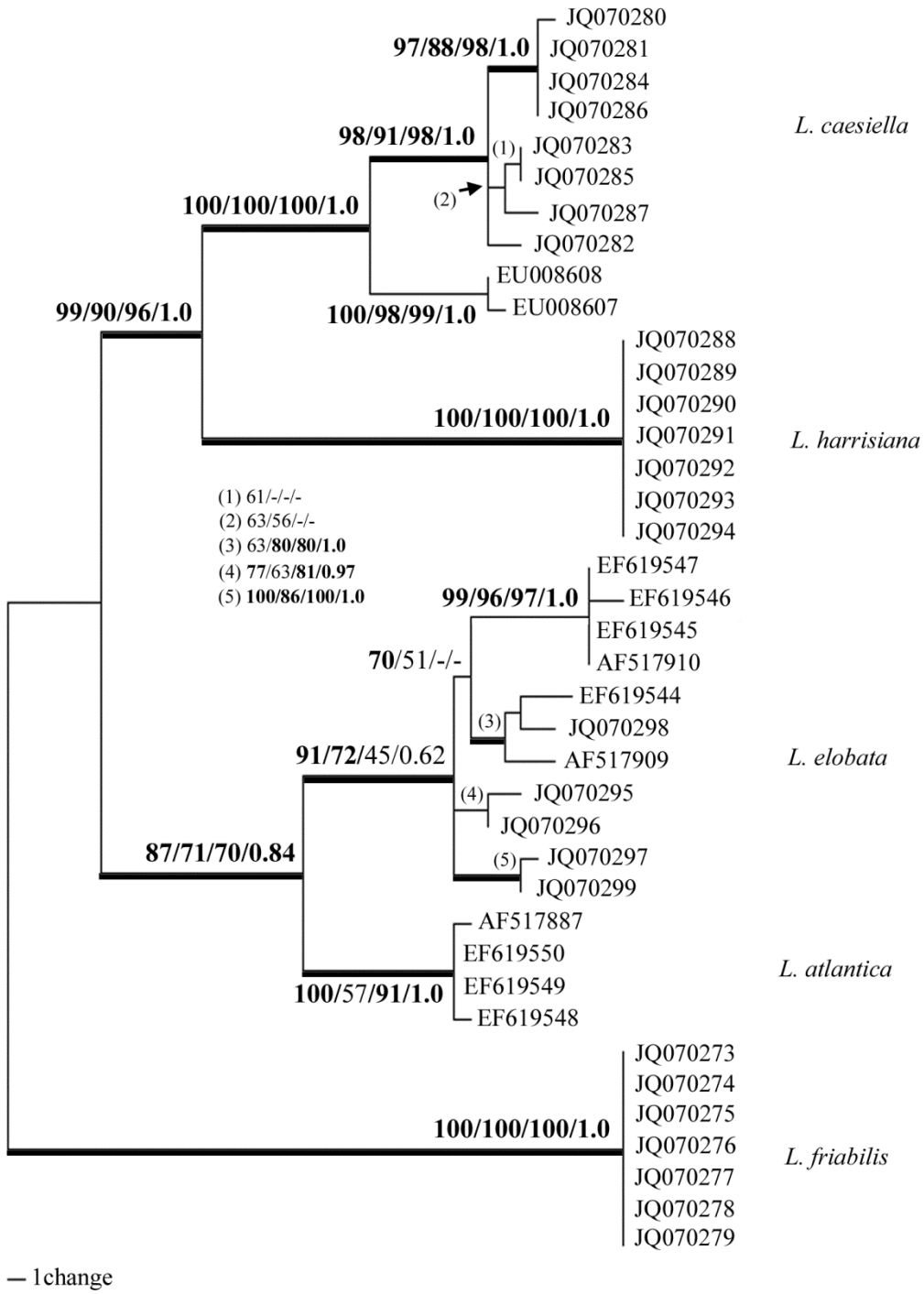

Figure 31. Phylogeny of *Lepraria caesiella* s.l. and selected species of *Lepraria* with aggregate thalli, inferred from ITS sequence data and displayed as one of six equally most parsimonious trees with midpoint rooting. Support was evaluated using maximum parsimony (MP) and maximum likelihood (ML) bootstrap proportions (BP) and Bayesian posterior probabilities (B-PP). Support values are displayed at each node in the following format: MP-BP/ML-BP[PAUP]/ML-BP[RAxML]/B-PP. Thickened branches are those that were recovered with MP-BP support ≥70% and ML-BP support ≥70% in either of the two types of ML analyses performed. Support values of >70% for MP-BP and ML-BP, and 95% for B-PP are bolded.

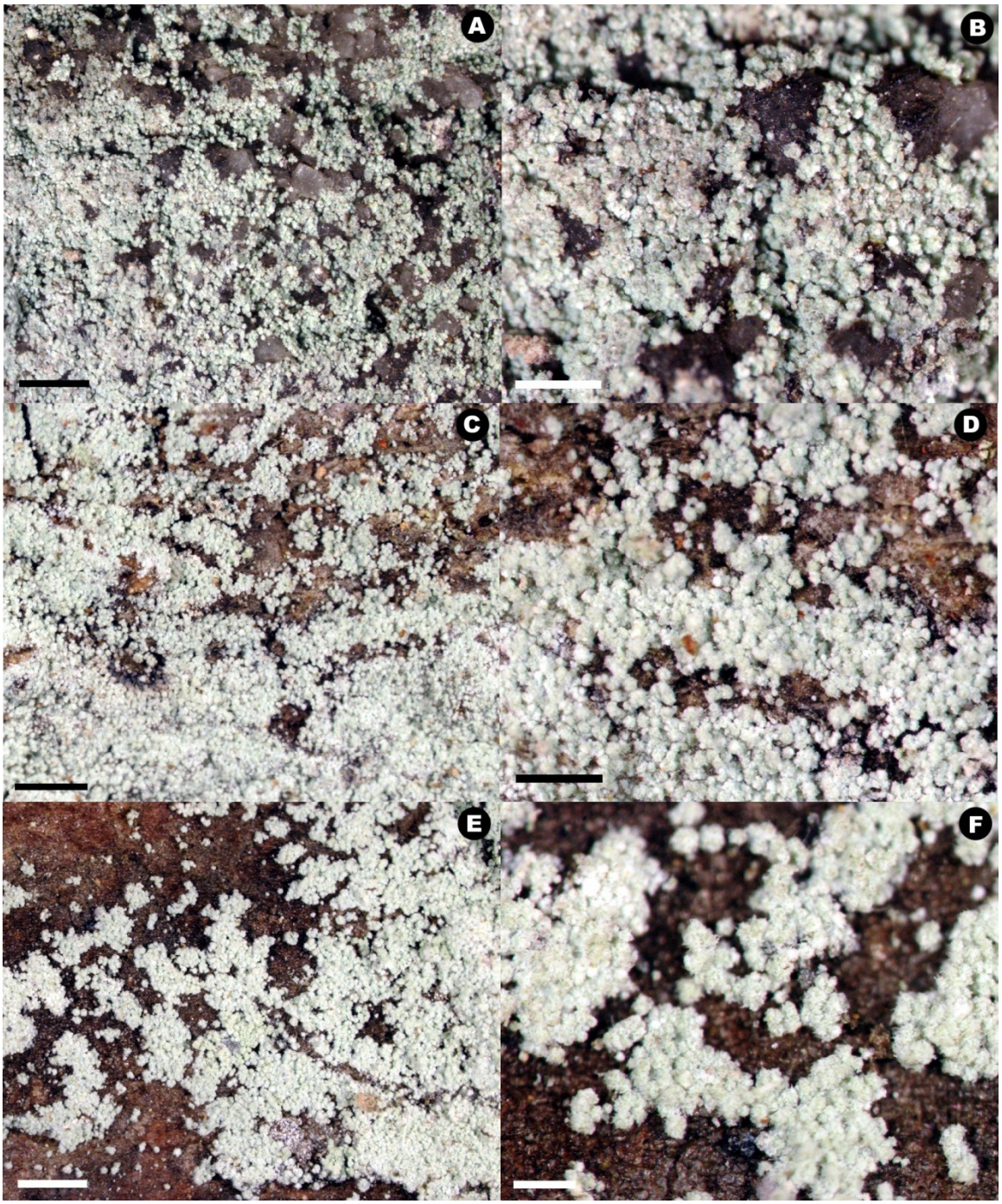

Figure 32. *Lepraria caesiella*, gross morphology of the thallus (A, C, E) and detail of granule aggregations (B, D, F). A–B, *Lendemer 29374*. C–D, *Lendemer 19967*. E–F, *Lendemer 15441*. Scales = 1.0 mm: A, C, E; 0.5 mm: B, D; 0.25 mm: F.

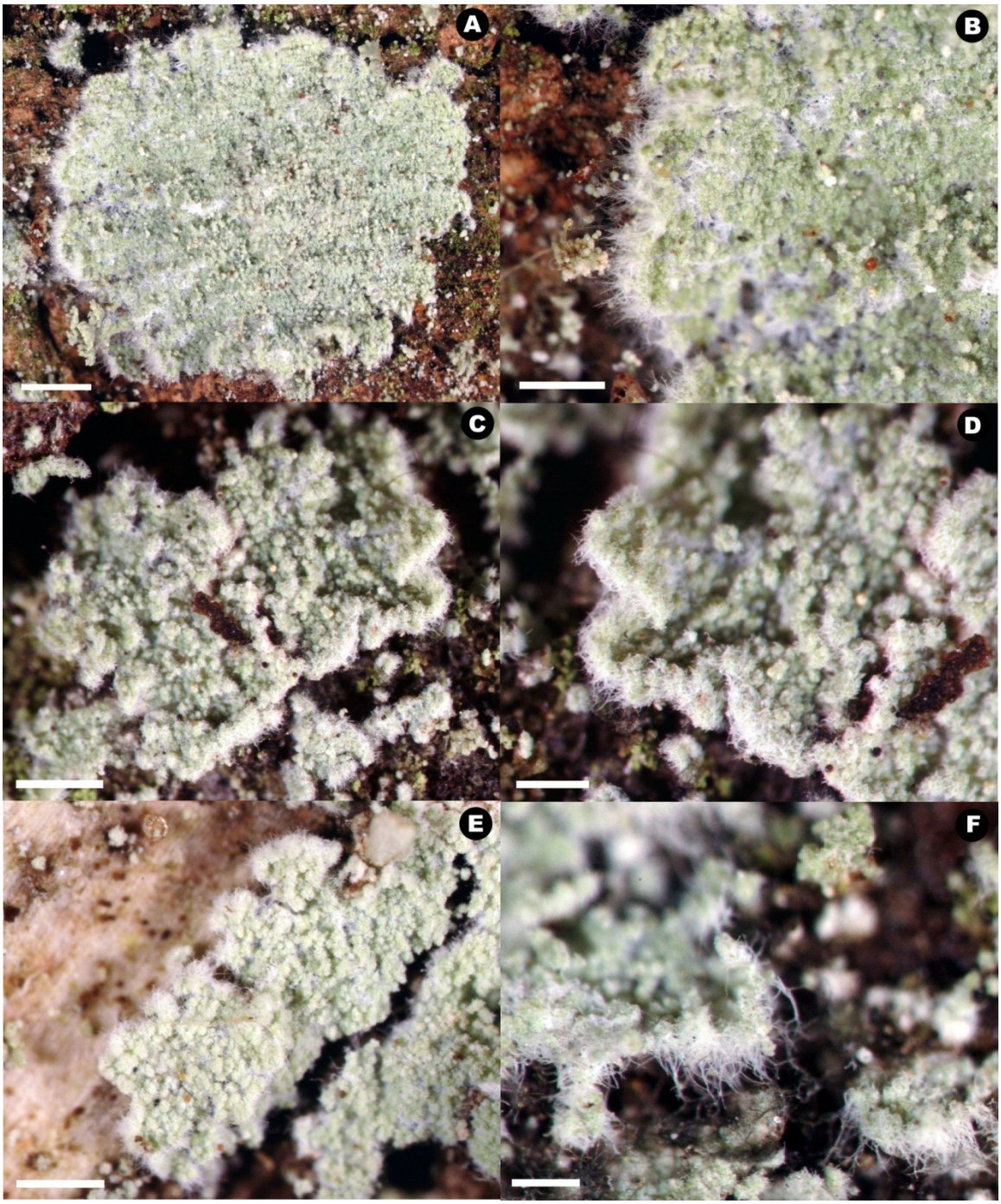

Figure 33. *Lepraria harrisiana*, gross morphology of the thallus (A, C, E) and details of the upper surface and margins (B, D, F). A–B, *Lendemer 20191*. C, D and F, *Lendemer 15165*. E, *Lendemer 15125*. Scales = 1.0 mm: A; 0.5 mm: B, C, E; 0.25 mm: D; 0.2 mm: F.

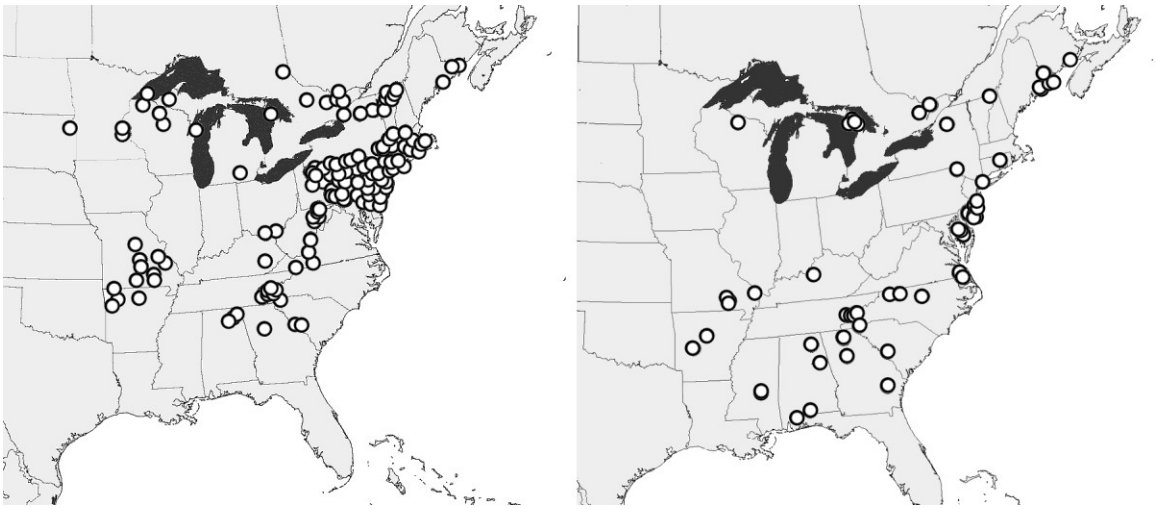

Figure 34. Geographic distributions of *Lepraria caesiella* (left) and *L. harrisiana* (right) based on specimens examined for this study.

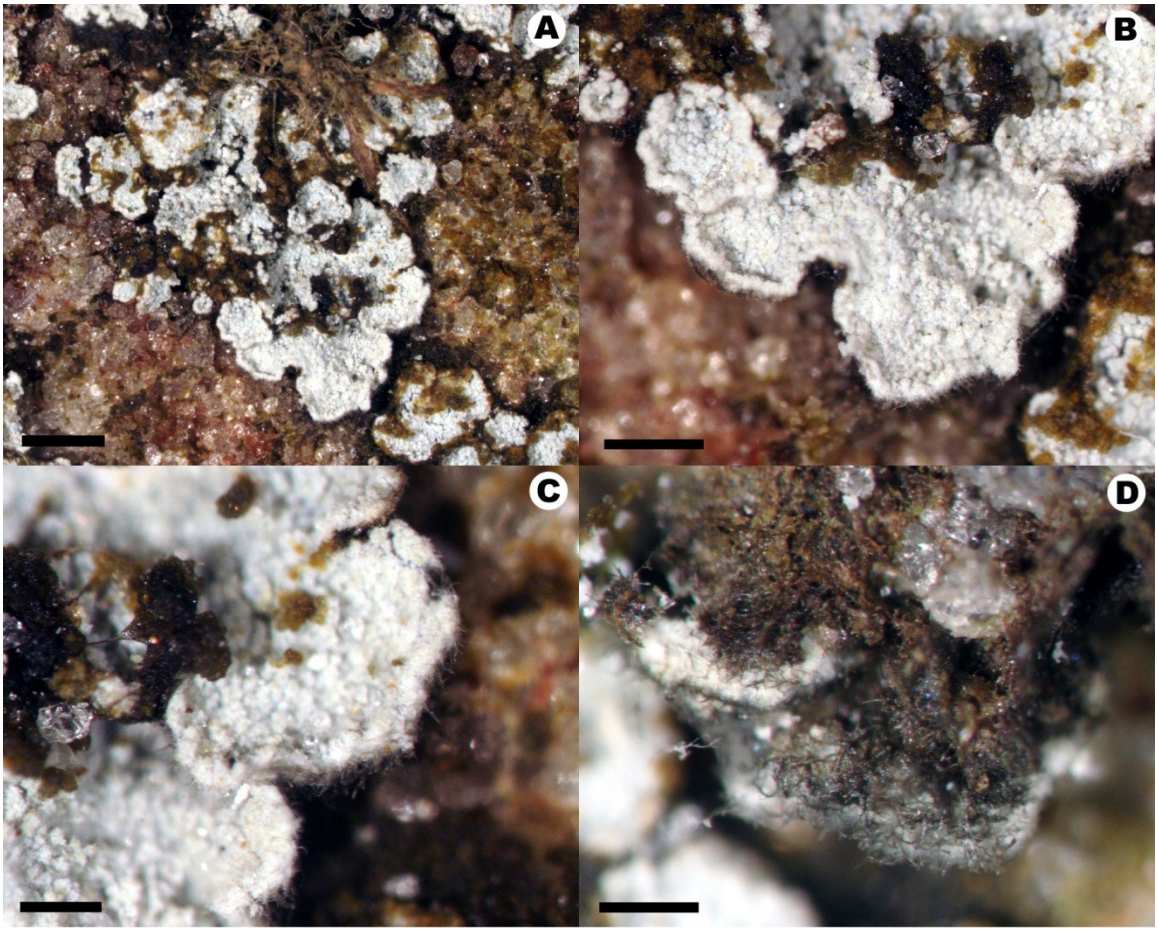

Figure 35, *Lepraria pallida* (all from holotype, *Sipman 40945*, B). A, macromorphology of the thallus. B–C, detail of thallus surface and margins. D, detail of lower surface illustrating dark rhizohyphae. Scales = 1.0 mm: A; 0.5 mm: B; 0.25 mm: C–D.

LICHEN TAXONOMY FOR THE 21ST CENTURY: A REVISION OF THE GENUS *LEPRARIA* S.L. IN NORTH AMERICA NORTH OF MEXICO

by

JAMES C. LENDEMER

VOLUME 2

A dissertation submitted to the Graduate Faculty in Biology (Plant Sciences) in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

2012

CHAPTER VII – IMPLEMENTATION OF A UNIFIED TAXONOMY INTEGRATING MOLECULAR AND NON-MOLECULAR DATA IN *LEPRARIA NEGLECTA* S.L.

James C. Lendemer

Abstract. - A revision of the *Lepraria neglecta* group in North America is presented that provides new insight into the validity of the widespread use of chemotaxonomy to circumscribe taxa of lichenized fungi. The *L. neglecta* group is a distinctive entity within the sterile, asexually reproducing lichen genus *Lepraria* whose constituent populations are united by their occurrence in exposed habitats and the development of a pseudocortex on the granules of the thallus. Previous studies have concluded that the group represents a monophyletic entity; however the question of how to classify the chemical variability exhibited within the group has remained unresolved. A phylogeny was inferred from ITS1, 5.8S, and ITS2 sequence data generated from a geographically and chemically broad sampling of populations within the *L. neglecta* group. The inferred phylogeny did not recover the chemotypes within the *L. neglecta* group as monophyletic entities. Thus, all the names applied to members of the group are placed in synonymy with *L. neglecta* (these are *L. alpina* (basionym *Crocynia alpina*), *L. alpina* var. *zeorinica*, *L. angardiana, L. borealis, L. caesioalba* (basionym *Crocynia caesioalba*), *L. caesioalba* var. *groenlandica*, *L. gelida*, *L. svalbardensis*, and *L. zonata*). The results of these molecular phylogenetic analyses also 1) did not support the distinction of *L. salazinica* from *L. elobata*, thus that name is placed in synonymy here, and 2) elucidated the occurrence of *L. humida* in North America.

Introduction

The lichen genus *Lepraria* Ach. represents a unique group of more than 70 sterile, asexually reproducing species that have been classified together because of their shared vegetative morphology rather than shared evolutionary history (Nelsen et al. 2008; Lendemer 2011). Although the genus is polyphyletic and comprised of several phylogenetically disparate elements, the majority of taxa belong to a core group, centered around the generic type *L. incana* (L.) Ach., that has affinities to the genus *Stereocaulon* Hoffm. and the family Stereocaulaceae (Ekman & Tønsberg 2002).

Sterile, asexually reproducing crustose lichens are widely considered to be among the most taxonomically difficult and neglected groups of lichenized fungi (Fryday & Coppins 1997; Harris & Lendemer 2010; Kantvilas & Lumbsch 2011; Lendemer 2011a). Taxa that reproduce in this manner have evolved in nearly all the diverse lineages that constitute lichenized fungi. The genus *Lepraria* is perhaps the best known example because its constituent taxa are ubiquitous in some temperate regions of the world (Laundon 1992). The absence of sexual reproductive structures in *Lepraria* has led some to consider the genus devoid of morphological characters (Laundon 1992). Thus taxonomy in the group has relied largely upon chemotaxonomy and gross differences in macromorphology. Unfortunately studies of the genus during the last decade have been plagued by a lack of standardized species concepts and descriptive morphological terms (see summary in Lendemer 2011). This created a taxonomic impasse complicated by immense confusion and conflict in the literature, which could only be overcome by a large scale revision of the entire group across a broad geographic region where species representing all the known lineages were likely to occur.

To address the above, the author undertook a revision of the genus in North America north of Mexico as the subject of his dissertation. After extensive field studies coupled with

detailed morphological studies and revision of herbarium specimens a standardized morphological scheme was developed and implemented (Lendemer 2011). Subsequently a series of studies involving molecular phylogenetic analyses were undertaken to examine, confirm, and refine the delineations of the species present in the study area. Each study has been published separately because the characters salient to the circumscription of each taxon or group of taxa have varied considerably and presented a logical venue to discuss broader issues of species delineation in lichenized fungi.

The present study examines *Lepraria neglecta* s.l., one of the most common taxa in North America. In a broad sense the name *L. neglecta* applies to a complex of populations that occur in exposed microhabitats, have aggregate thalli (figure 36) in which the granules develop a pseudocortex (figure 37) (Lendemer 2011), and exhibit remarkable chemical variability. Despite an absence of independent data, recent authors have proceeded to formally describe the chemical variation (i.e., chemotypes) in this group at various taxonomic ranks and with variable taxonomic concepts. Mirroring the situation in *Lepraria* as a whole, the group has become taxonomically unworkable due to a fundamental lack of conceptual consistency. This study aims to resolve the taxonomy of the group using an integrated approach that attempts to correlate the results of molecular phylogenetic analyses with independent chemical, morphological, ecological, and biogeographical characters.

Materials and methods

Non-molecular materials and methods. – As in previous contributions on *Lepraria* s.l. by the author, this study is based primarily on material in the herbarium of The New York Botanical Garden (NY), supplemented by material received on loan from the following herbaria: B, BM, BG, CANL, COLO, CONN, DUKE, FH, GZU, H, KANU, MIN, MSC, SBBG, UCR, and

UTEP. This includes >1000 specimens collected by the author during his extensive field studies of *Lepraria* throughout North America. To date these total 1.5 years in the field and span the period August, 2007 to August, 2011. Through these field studies the author has been able to gather firsthand observations of ecology and morphology for all the species known to occur in North America. Chemical and morphological analyses were carried out using the methodology outlined in detail by Lendemer et al. (2008) with the SEM methods as corrected by Lendemer & Elix (2010). Descriptive morphological terminology follows Lendemer (2011).

Molecular data collection. – Subsamples used for DNA extraction were specifically those that were used in upstream chemical analyses (TLC). Only those samples that exhibited no contamination in TLC analyses were used for downstream molecular analyses. Mixtures of *Lepraria* species with aggregate thalli can be impossible to detect microscopically or macroscopically, and the impact of this phenomenon on molecular phylogenetic analyses has previously been discussed by Fehrer et al. (2008). It should be noted that traditionally when TLC revealed the existence of mixed chemotypes in a given sample it has been assumed to be the result of contamination from a mixed population. However, this is based on the preconceived notion that only one primary substance could be present in a given individual. During this study numerous collections with both fumarprotocetraric and alectorialic acids were encountered in eastern North America. There was, however, no evidence that a mixed population of *L. caesioalba* and *L. neglecta* was involved (i.e., only a single discrete thallus rosette was sampled). Although these samples were excluded from downstream molecular studies, it would be valuable for a future study to examine this situation further.

DNA extractions were performed using the DNeasy Plant Mini Kit (Qiagen) with the instructions modified to include a prolonged (~12 hour) incubation period in lysis buffer. Isolated DNA was resuspended in 100 µl of sterile water and stored at -20°C. PCR amplification was performed using the primers ITS4 and ITS5 (White et al. 1990). Amplification reactions of 25 µl contained each of the following: 2.5 µl 10× PCR Buffer (Qiagen), 2.5 µl dNTP solution (mixed to a concentration of 2 mM of each dNTP), 2.5 µl BSA solution (mixed to a concentration of 2.5 mg μl^{-1} of bovine serum albumin; Hillis et al. 1996), 0.2 µl Taq DNA Polymerase (= 1 U; Qiagen), 1 µl of each primer (in solution at a concentration of 10 µM), 9.3 µl of sterile water, 1 µl of extracted DNA, and 5 µl of Q-solution (Qiagen). PCR protocol followed Nelsen et al. (2007) and consisted of an initial denaturation of 95°C for 5 min, followed by 10 cycles of 95°C for 1 min, 62°C for 1 min, and 72°C for 1 min, then 35 cycles of 95°C for 1 min, 53°C for 1 min and 72°C for 1 min with a final extension for 7 minutes at 72°C. PCR products were studied prior to sequencing by UV examination of a 1% agarose gel on which 1 µl of amplified PCR product had been subjected to electrophoresis and stained with ethidium bromide. Unpurified amplified PCR products were sent to the University of Washington Biochemistry DNA Sequencing Facility (BSDF) for sequencing. Sequences were assembled and edited by hand using the software package Sequencher™ 4.9 (Gene Codes Corporation, Ann Arbor, MI, USA). In order to identify contaminants, nearest sequence matches were found by searching the nucleotide collection in GenBank using BLASTn (Altschul et al. 1997).

Taxon Sampling. – The goals of this study were to 1) determine whether the morphologically identical chemotypes of *Lepraria neglecta* s.l. represent discrete monophyletic entities, 2) determine whether the two morphotypes exhibited within *L. neglecta* s.l. represent

discrete monophyletic entities, and 3) generally examine the circumscription of *L. neglecta* and its relationship to other taxa belonging to the broad *L. neglecta* group as defined by Fehrer et al. (2008). To study these questions, phylogenetic analyses were performed on a dataset comprised of sequences of the nuclear ITS region (ITS1, 5.8s, and ITS2) derived from a diverse sampling of populations of *L. neglecta* s.l., additional selected sequences downloaded from GenBank, and several sequences that were generated to expand taxon sampling and geographic representation of the outgroups. In total, forty-six (n = 46) new sequences were generated for this study. Thirty-five (n = 35) of these represented a geographically diverse sampling of as many chemotypes of *L. neglecta* s.l. as could be obtained. Eleven (n = 11) sequences were generated to enhance outgroup representation (see below).

Taxon sampling and outgroup selection were informed by Fehrer et al. (2008). In order to address the questions related to the circumscription of *Lepraria neglecta* s.l., all available GenBank sequences of *L. alpina*, *L. borealis*, *L. caesioalba*, and *L. neglecta* were included in the dataset. Several partially identified sequences whose placement in *L. neglecta* s.l. was indicated by blast searches and Fehrer et al. (2008) were also included. Four sequences (n = 4) of *L. granulata* were included to improve sampling within the "*L. neglecta*-core group" of Fehrer et al. (2008). Based on that publication twelve sequences of *L. elobata* (six newly generated and six from GenBank) were also included because that species was found to be sister to the "*L. neglecta*-core group". Three sequences (n = 3) were generated from a single specimen of the rare North American species *L. salazinica*. These were included because of the morphological and chemical similarly of the species to the others included in the dataset. Sequences of *L. humida* and *L. atlantica* were also downloaded from GenBank. These taxa were selected as the outgroup because they formed the closest sister group to a broadly defined *L. neglecta*-group (i.e., *L.*

nelgecta-core + *L. elobata*). Three new sequences (*n* = 3) of *L. humida* from heretofore unrecognized North American populations of the species were included to increase geographic representation in the outgroup.

Sequence alignment and phylogenetic analyses. – Sequences were first aligned using ClustalX (Thompson et al. 1997). The alignment was then manually adjusted using Mesquite ver. 2.0 (Maddison & Maddison 2007). Sequences containing clear inconsistencies with all other aligned sequences were assumed to have resulted from the sequencing of heterogeneous amplicon pools, and thus excluded. Ambiguous bases and terminal gaps were converted to missing "?" and the ambiguously aligned regions were excluded from the analysis by including them in an exclusion set. The NEXUS file was then saved with the taxa and characters compressed into a data block.

Following Hodkinson & Lendemer (2011) the NEXUS file was prepared for a weighted maximum parsimony analysis with recorded ambiguously-aligned regions. INAASE 3.0 (Lutzoni et al. 2000) was used to recode the ambiguously aligned regions and to generate specific step-matrices using a transition:transversion:gap ratio of 1:1:1. STMatrix 3.0 (written by S. Zoller; Miadlikowska et al. 2002) was then used to generate step matrices for each of the three ITS regions. The input file for STMatrix 3.0 was produced using the procedure outlined by Gaya et al. (2003) and described in greater detail by Lendemer (2011b).

Parsimony-based phylogenetic analyses were run using weighted maximum parsimony (MP) in PAUP* 4.0b10. An initial search was made with 1000 random-addition-sequence (RAS) replicates and tree bisection reconnection (TBR) branch swapping. The MULTREES option was in effect and zero-length branches were collapsed. All equally most parsimonious trees were

saved with branch lengths, and a strict consensus tree was computed for reference. In this analysis two equally best tree islands were hit in two of the replicates (one time for each island). Two equally most parsimonious trees were recovered, which differed only in the arrangement of the sequences within several clades and the presence/absence of a node resolving the clade comprised of AF517901 and AF517894 as belonging to the larger clade representing *L. neglecta* s.l. Since the two best tree islands were each hit in only a single replicate, branch support for MP trees was estimated through bootstrap analyses (Felsenstein 1985) by performing 1000 bootstrap replicates with 100 RAS per replicate and all other settings as above.

For maximum likelihood (ML) analyses were performed on the dataset with ambiguously-aligned regions recoded in PICS-Ord following Lücking et al. (2011). Ambiguously-aligned regions were manually deleted using Mesquite and the alignment was then saved in an extended phylip format. Recoded ambiguously aligned regions generated in PICS-Ord were then concatenated with the end of the alignment using the alt-copy/paste function of MSWord. A topology search and bootstrap analysis of 1000 replicates each, using the GTRGAMMA model, were performed using the windows executable version of RAxML 7.2.6. Using the same program, the bootstrap bipartitions were mapped to the best tree recovered in the topology search and these were visualized in FigTree 1.2.3 (Rambaut 2009). The tree recovered by the ML search was topologically equivalent to the most parsimonious tree, which recovered the node resolving AF517901 and AF517894 as members of a monophyletic *L. neglecta* s.l. Thus the best tree from the ML search is displayed in Fig. 3.

For Bayesian phylogenetic analyses, MrModeltest (Nylander 2004) was run to select a proper model of nucleotide substitution. Using the Akaike Information Criterion (*AIC;* Akaike 1973), GTR+I+G was selected. These analyses were performed using a MrBayes block produced

through the online automated form found at the Santos Lab website (http://131.204.120.103/srsantos/mrbayes_form/index.html) and implemented with MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). The GTR+I+G model was implemented by selecting six substitution rates and gamma-shaped rate variation with a proportion of invariable sites. Otherwise the default priors were used. The Markov chain Monte Carlo parameters consisted of 10,000,000 generations, with four chains, and a tree sampled every 100 generations. The first 10,000 trees were discarded as burnin and the results were summarized as a 50% majority-rule consensus tree.

Results

The results of the molecular phylogenetic analyses described above are summarized in figure 38. In each of these analyses the sequences representing *Lepraria atlantica*, *L. granulata*, and *L. humida* were recovered as strongly supported monophyletic groups. The sequences representing *L. elobata* were recovered as a strongly supported monophyletic group with maximum parsimony and maximum likelihood, however, this clade was not supported with Bayesian inference. The three sequences of *L. salazinica* were resolved as a strongly supported monophyletic group embedded within *L. elobata*. The implications of this result are discussed in further detail in the following section.

The so called "core neglecta group" as defined by Fehrer et al. (2008), which comprises *Lepraria neglecta* s.l. and *L. granulata*, was also supported with all three methods of phylogenetic inference. Interestingly, all the sequences representing the diverse array of chemotypes presently included in *L. neglecta* s.l. were recovered as a monophyletic group that was well supported with maximum parsimony and Bayesian inference, but marginally so with maximum likelihood (MP:78, ML:69, BI: 0.98). Within *L. neglecta* s.l. multiple clades were

recovered, some with high levels of support in all three analyses; however, this phylogenetic signal correlated with neither chemical nor morphological characters. Rather, the phylogenetic structure within *L. neglecta* s.l. largely appears to be defined by geography (see table 8), however, this correlation is far from absolute.

The above results agree with those obtained by Fehrer et al. (2008) from analyses of a dataset with comparable taxon sampling, but a more limited geographic scope. That study focused on characterizing the species outside of *Lepraria neglecta* s.l. and the present study confirms those characterizations. Remarkably, the lack of correlation between the results of the molecular phylogenetic analyses performed here and the chemical and morphological variation found within *L. neglecta* s.l. does not conflict with any prior studies. In fact the assumption that chemical characters would correlate to phylogenetic structure within the group was based on analyses of datasets with inadequate taxon sampling (e.g., Ekman & Tønsberg 2002). No prior studies of *L. neglecta* s.l. have included a broad sampling of different chemotypes across a large geographic area. Instead, by employing a limited sampling strategy of one or two sequences for a given chemotype, this lack of correlation was undetected.

Discussion

General Remarks

Lepraria is an example of a group that has long been considered to be devoid of morphological characters despite both historical and contemporary evidence to the contrary (Lendemer 2011). Recent studies have not only elucidated morphological characters in the group (Lendemer 2011), but also confirmed their value with independent molecular phylogenetic analyses (Lendemer 2011b, in rev., in rev.A). Perhaps the most important aspect of this study is that it reaffirms the ability of morphological characters to circumscribe monophyletic species of

lichenized fungi. In this case, the unique morphology of *L. neglecta* s.l. presents an easily observed synapomorphy for a clade comprised of numerous chemically variable populations. The fact that careful study can reveal phylogenetically salient morphological characters in a group with a reputation like *Lepraria* effectively refutes the notion that morphology is unable to properly characterize the alpha-level diversity of lichenized fungi as has been suggested by Lumbsch & Leavitt (2011).

The present study also represents yet another example where molecular phylogenetic analyses of nrITS sequence data have effectively and efficiently resolved species boundaries both within *Lepraria* s.l. (Fehrer et al. 2008; Tretiach et al. 2009; Lendemer 2011b, in rev., in rev.A) and lichenized fungi as a whole (Arup 2006, 2006a; Amtoft et al. 2008; Nordin et al. 2008; Arup & Åkelius 2009; Lendemer & Hodkinson 2009; Vondrák et al. 2009; Gaya et al. 2011; Lendemer & Hodkinson 2010; Lendemer & O'Brien 2011; Šoun et al. 2011; Vondrák et al. 2011). This study also highlights the danger of *a priori* using one type of data (be it chemical, morphological, ecological, biogeographical, or molecular) to circumscribe species. Indeed it specifically refutes the ability of chemotaxonomy to circumscribe monophyletic entities. Instead it strongly reiterates the inherent value of using suites of correlated characters to delineate monophyletic species (Lendemer & Hodkinson 2009; Hodkinson & Lendemer 2011a).

The results of this study clearly illustrate that within *Lepraria* some species are chemically uniform (e.g., *L. atlantica*, *L. granulata*, and *L. humida*) while others exhibit varying degrees of chemical variation (i.e., *L. elobata* and *L. neglecta*). Unfortunately chemotaxonomy is a practice that has been widely applied, with increasing levels of detail, within *Lepraria* s.l. and across the diverse lineages of lichenized fungi. This is largely because of an increasing perception that morphological characters were not effective in circumscribing monophyletic

species (Lumbsch & Leavitt 2011). Historically, spot tests were used to detect the presence/absence of secondary compounds, and then more sensitive crystal tests were employed. Eventually thin layer chromatography (TLC) became commonplace, followed by high performance thin layer chromatography (HPTLC), mass spectrometry, and high performance liquid chromatography (HPLC). As such, greater levels of chemical detail have been integrated into taxonomic schemes of lichenized fungi as methods of chemical analysis have increased in sensitivity, precision, and accuracy.

This shift has effectively created a system in which the vast majority of the consumers of taxonomic products (e.g., monographs and revisions) lack the training and/or resources to adequately document our vanishing biodiversity. In essence, this has had the inversely proportional effect of placing a greater and greater burden on a smaller and smaller group of workers. Further, in the absence of analyses of independent datasets, chemotaxonomy has led in many cases to a grossly uneven misrepresentation of biological diversity wherein minor chemical differences are accorded species status. This was certainly the case for *Lepraria neglecta* s.l. Somewhat paradoxically, chemical uniformity is also frequently used to explain observed morphological and biogeographical variability. Clearly, the present situation is untenable and calls for radical change.

A not so new circumscription for Lepraria neglecta s.l.

The names *Lepraria caesioalba* and *L. neglecta* have long been used for a group of chemically disparate populations of *Lepraria* that grow in exposed habitats. These populations are morphologically characterized by the development of a pseudocortex that gives the granules a hard, compact appearance (Lendemer 2011). In his seminal revision of *Lepraria*, Laundon

(1992) applied the name *L. neglecta* to populations that produce alectorialic acid and *L. caesioalba* to populations that produce atranorin, fumarprotocetraric acid, and roccellic acid. Since Laundon considered the production of dibenzofurans indicative of genus level divergence in *Lepraria s.l.* he also applied the name *Leproloma cacuminum* (A. Massal.) Laundon to populations that were morphologically concordant with those above, but chemically divergent in the production of porphyrilic acid in addition to atranorin and roccellic acid (Laundon 1989).

Over time, further study led to the discovery of additional morphologically identical chemotypes. Initially only additional depside-producing chemotypes (e.g., those with stictic acid or psoromic acid) were reported (Ekman & Tønsberg 2002). However, subsequent authors then noted the existence of variants of the depside-producing chemotypes that produced different fatty acids (Tønsberg 2004). More recently, authors have discovered the existence of further variants of the above chemotypes that produce the dibenzofuran porphyrilic acid and/or the terpenoid zeorin (Saag et al. 2007; Saag et al. 2009).

Thus a multitude of chemotypes have been documented that combine the features of what had previously been considered discrete entities. Despite a lack of confirmation from an independent dataset, authors have largely relied on chemotaxonomy, formally recognizing each chemotype as its own taxon, as a method to resolve the taxonomy of this group. Unfortunately this proliferation of nomenclature has created a taxonomic impasse and resulted in conflicting and confusing accounts in the literature (Kukwa & Zhurbenko 2010). For instance, it is not clear whether the presence of porphyrilic acid precludes the placement of a population in *L. caesioalba* even though it also produces stictic acid, atranorin, and a fatty acid. Similarly, populations that produce atranorin and rangiformic acid are classified as *L. borealis* while those that produce roccellic acid instead of rangiformic acid are retained as a chemotype of *L.*

caesioalba (Tønsberg 2004). How one determines what substance determines the taxonomic affinity of a sample is clearly subjective.

As has been discussed above, the chemotypes of *Lepraria neglecta* s.l. were not recovered as monophyletic entities in the molecular phylogenetic analyses carried out for this study. Instead, the phylogenetic structure recovered in these analyses roughly correlated to large scale geographic regions. This supports the hypothesis that within *L. neglecta* s.l. chemical variation occurs at the population level and that the same chemotype has evolved independently in different sets of allopatric populations. Indeed, extensive sampling of *L. neglecta* s.l. from throughout North America failed to reveal any correlation between distributional patterns and chemical variation (figure 39).

In addition to the chemical variation within *Lepraria neglecta* s.l. there is significant variation in thallus morphology, specifically in the size of the granules. This led Lendemer (2011) to define two morphological subtypes within the group: one for populations with granules 20–50μm in diameter, and one for populations with granules ≥100μm in diameter. The molecular phylogenetic analyses carried out for this study also did not support the distinction between these thallus types. This supports the hypothesis that the large granules found in *alpina*-type thalli actually represent an extreme of a morphological continuum.

The populations that comprise *Lepraria neglecta* s.l. are unique within the broader context of *Lepraria*. While all other species of *Lepraria* occur in sheltered or protected microhabitats, *L. neglecta* s.l. typically occurs in habitats that are exposed to the elements. Perhaps the extreme morphological and chemical variation found in the group, as well as the considerable genetic divergence between sets of populations, are responses or adaptations to living in exposed habitats. In the context of the aforementioned variation, it is of utmost

importance to remember that the populations of this group are united by a morphological synapomorphy: the development of a pseudocortex on the granules. Clearly, the results of the analyses carried out for this study indicate that the diverse populations of *L. neglecta* s.l. are best recognized as single variable species. Not only does this solution result in the delineation of a morphologically, ecologically, and genetically well characterized taxon, but it also resolves the impasse created by a proliferation of names in a confusing taxonomic scheme. Thus, in the taxonomic section below all the names currently used for the chemotypes of this group are placed in synonymy with the oldest name, *L. neglecta*.

Are there cryptic species within Lepraria neglecta ?

The topic of cryptic species in lichenized fungi has received considerable attention in print in recent years (for discussions see: Printzen & Ekman 2004; Crespo & Pérez-Oretga 2009; Vondrák et al. 2009; Crespo & Lumbsch 2010; Lendemer 2011b; Lendemer & O'Brien 2011; Lumbsch & Leavitt 2011). The use of molecular phylogenetic analyses to evaluate and circumscribe species of lichens is now widespread enough that our community has begun to form a consensus as to what does, and does not, constitute a cryptic species. The preponderance of the taxonomic literature indicates that when molecular phylogenetic analyses lead to the re-evaluation of previously overlooked or underemphasized observable characters the resultant taxa are not to be considered cryptic species Lendemer (2011b). This is logical since detailed morphological and chemotaxonomic studies have been carried out on only a small fraction of lichenized fungi.

In light of the above, authors have generally reserved the terms "semi-cryptic" and "cryptic" for unusual cases where genetic differences do not correlate to observable phenotypic

reality. The term "semi-cryptic" has been applied to cases where phylogenetically distinct entities can be recognized by differences in ecology or biogeography (see summary in Lendemer (2011b)). The term "cryptic" has been rarely used because there have been few cases uncovered where highly divergent phylogenetic entities are supported by molecular characters alone. Interestingly this may be the case within *L. neglecta* because multiple, albeit poorly supported, divergent clades were recovered within the taxon. Whether cryptic species are involved is however, impossible to determine based on the present dataset because sequences from only one gene were used. Future studies with an increased sampling may refute or support this hypothesis.

If cryptic species are indeed present within *Lepraria neglecta* one must strongly question whether they should be formally described. Those who favor this approach argue that it is the only way to accurately document, and ultimately conserve, lichen biodiversity (Lumbsch & Leavitt 2011). The author, however, contends that this practice would be detrimental to lichen taxonomy, ecology, and conservation/management. The formal recognition of truly cryptic species would lead to a situation where populations cannot be identified with certainly in the absence of molecular data.

Is Lepraria salazinica conspecific with L. elobata ?

Lepraria elobata is an infrequent taxon that is widespread in boreal/northern North America and is characterized by its aggregate thallus and the production of atranorin, zeorin, and stictic acid. Tønsberg (2007) described *L. salazinica* from several populations in the southern Appalachian Mountains that were morphologically identical to *L. elobata* but differed in the production of atranorin, roccellic acid, and salazinic acid. While conducting fieldwork for this study the author encountered a single population of *L. salazinica* in the central Appalachian

Mountains of Maryland. Revision of herbarium specimens led to the discovery of two more collections from the mid-Atlantic Region. Interestingly, extensive fieldwork in the southern Appalachian Mountains failed to uncover any additional populations.

Chemical analysis of the voucher from Maryland revealed the presence of zeorin in low concentrations in all three thallus fragments that were collected. Sequences were generated from each fragment and were recovered as a strongly supported monophyletic group embedded within a clade comprised of sequences of *L. elobata* from North America and Europe. These results clearly do not reject the inclusion of the Maryland population in *L. elobata*. Examination of the holotype of *L. salazinica* confirmed the presence of low concentrations of zeorin, although this substance was not reported in the protologue. Given these results, the formal separation of *L. salazinica* from *L. elobata* is untenable. Thus, *L. salazinica* is placed in synonymy with *L. elobata* in the taxonomic section below and the concept of *L. elobata* is expanded to encompass the production of salazinic acid or stictic acid.

Lepraria humida, another Lepraria with an inter-continental distribution

Previous molecular phylogenetic analyses of sequences purported to represent the same *Lepraria* taxon from different continents have produced conflicting results. Lendemer (2011b) recovered sequences of morphologically and chemically identical populations of *L. incana* from North America and Europe as well-supported monophyletic entities whose nrITS sequences were as divergent as any other two species of *Lepraria* examined to date (see also Fehrer et al. (2008)). Thus the North American populations were formally described as a new taxon, *L. hodkinsoniana* Lendemer.

Conversely Lendemer (in rev.A) did not recover sequences of *Lepraria elobata* from North American and European populations as highly divergent monophyletic groups. As such the previously hypothetical inter-continental distribution of *L. elobata* was supported with analyses of an independent molecular dataset. Interestingly the same results were obtained in this study for sequences derived from North American populations of *L. humida*. Since *L. humida* has not previously been reported from North America these populations represent a new record for the continent. They also provide additional evidence that large scale geographic distributions of *Lepraria* species must be evaluated on a case by case basis. They cannot *a priori* be assumed to have, or not to have, inter-continent distributions.

Taxonomic Section

The synonymies required in light of the results of this study are formally proposed below. Detailed taxonomic treatments of the taxa treated here, and those with which they have previously been confused, will be published in an upcoming monograph of *Lepraria* s.l. in North America north of Mexico (Lendemer in prep.). Secondary metabolite data from TLC analyses conducted by the author on type specimens is included throughout the synonymies below.

1) *Lepraria elobata* Tønsberg, Sommerfeltia, 14: 197. 1992. TYPE: **NORWAY:** Vest-Agder, Kristiansand, Hamresand, 5 m, 28.ix.1991, on *Pinus sylvestris*, *T. Tønsberg 17404* (BG!, holotype).

Syn. nov. *Lepraria salazinica* Tønsberg, Opuscula Philolichenum, 4: 52. 2007. TYPE: **U.S.A. NORTH CAROLINA.** SWAIN CO.: Great Smoky Mountains National Park, Balsam

Mountain, along Balsam Mountain Rd., 4 miles (along the road) from the gate (near the end of Heintooga Rd.), 1420–1450 m, 20.vi.2002, saxicolous under overhang, *T. Tønsberg 30941* (BG!, holotype).

2) *Lepraria neglecta* (Nyl.) Erichsen, Flechtenfl. Nordwestdeustchl., 394. 1957.

Lecidea neglecta Nyl., Not. Sällsk. Fauna et Fl. Fenn. Förh., Nyl. Ser., 1: 233. 1859. TYPE: **FINLAND:** Uusimaa, near Helsinki, 1858, on small moss cushions covering granitic rocks, *W. Nylander s.n.* (H[n.v.], lectotype [selected by Laundon 1992]).

Crocynia neglecta (Nyl.) Hue, Bull. Soc. Bot. France, 81: 376. 1924.

Crocynia alpina B. de Lesd., Bull. Soc. Bot. France, 61: 85. 1914 . TYPE: **U.S.A. COLORADO.** LARIMER CO.: Rocky Mountain National Park, Toll Pass, 12050 ft, alpine tundra, in snow accumulation sites with good drainage, 21.viii.1979, *Willard s.n.= Lich. Exs. COLO 609* (UPS[n.v.], neotype [selected by Baruffo et al. 2006]; DUKE!, NY!, isoneotypes).

Lepraria alpina (B. de Lesd.) Tretiach & Baruffo *in* Baruffo et al., Nova Hedwigia, 83: 390. 2006.

Lepraria alpina var. *zeorinica* L. Saag, Mycotaxon, 102: 68. 2007. TYPE: **GREENLAND:** Qingertivaq, 550 m, 30.vii.1970, on mosses and soil (humus), *E. Hansen ESH-70.0032* (C[n.v.], holotype; TU[n.v.], isotype).

Lepraria zeorinica (L.) Kukwa, Mycotaxon, 108: 361. 2009.

Lepraria angardiana Øvstedal, Nova Hedwigia, 37: 687. 1983. TYPE: **ANTARCTICA:** Dronning Maud Land, Sverdrupfjella, Sørhausane, 31.xii.1970, on soil, *Angard s.n.* (BG[n.v.], holotype).

Leproloma angardianum (Øvstedal) J.R. Laundon, Lichenologist, 21: 19. 1989.

Lepraria borealis Loht. & Tønsberg *in* Lohtander, Ann. Bot. Fenn., 31: 224. 1994. TYPE: **NORWAY. NORD-TRØNDELAG:** Namsskogan, Børgefjell Nat. Park, Namskroken, N bank of river Namsen E of Mattisflya, 340 m, 23.viii.1975, muscicolous on rock, *T. Tønsberg 1519* (BG!, holotype; H!, isotype).

Lepraria cacuminum sensu Lohtander, Ann. Bot. Fenn., 32: 52. 1995.

Leproloma cacuminum sensu Laundon, Lichenologist, 24: 345. 1992.

Crocynia caesioalba B. de Lesd., Bull. Soc. Bot. France, 61: 84. 1914. TYPE: **FRANCE:** Seine-et-Marne, Forêt de Fontainebleau, Jul 1913, very common on mosses and lichens on sandstone rocks, *Bouly de Lesdain* s.n. (E[n.v.], holotype; BM, GL, PC [all n.v.], isotypes).

Leproloma caesioalba (B. de Lesd.) M. Choisy, Bull mens. Soc. Linn. Lyon, 19: 12. 1950.

Lepraria caesioalba (B. de Lesd.) J.R. Laundon, Lichenologist, 24: 324. 1992.

Lepraria caesioalba var. *groenlandica* L. Saag, Mycotaxon, 102: 73. 2007. TYPE: **GREENLAND:** Qaanaaq, Thule, 28.vi.1986, on soil (humus) and mosses, *E. Hansen ESH-86.0321* (C[n.v.], holotype;TU[n.v.], isotype).

Lepraria gelida Tønsberg & Zhurb., Graphis Scripta, 18: 64. 2006. TYPE: **NORWAY. SVALBARD:** Spitzbergen, SSE of Wijdefjorden, E of Austfjorden, SE of Finnekroken, the W-facing foothills of Finlandsveggen, 20–30 m, 5.vii.2002, on slightly sloping ground in *Dryas octopetala* heath, *T. Tønsberg 31049* (BG!, holotype).

Lepraria svalbardensis Tønsberg *in* Øvstedal et al., Sommerfeltia, 33: 213. 2009. TYPE: **NORWAY. SVALBARD:** Spitsbergen, the W-facing foothill of Mt. Platåberget, uphill from the road between Longyearbyen and Bjørndalen, 70–75 m, 26.vi.2003, muscicolous in dry niche in scree, *T. Tønsberg 31851* (BG[n.v.], holotype; UDGA[n.v.], isotype).

Lepraria zonata Brodo, Bull. N.Y. St. Mus. Sci. Surv., 410: 263. 1968. TYPE: **U.S.A. NEW YORK.** SUFFOLK CO.: Wading River, 11.viii.1962, on vertical surface of partially shaded boulder, *I.M. Brodo 3869* (MSC!, holotype).

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Taxon	GenBank	Isolate	NY-Barcode	Collection	Locality	
*bolded collections indicated vouchers seen by the author						
L. atlantica	EF619548	N/A	PRA	Orange 15459	United Kingdom	
	AF517887	N/A	BG	Orange 11829	Ireland	
	EF619550	N/A	PRA	Orange 15462	United Kingdom	
	EF619549	N/A	PRA	Orange 15456	United Kingdom	
L. elobata	AF517910	N/A	BG	Tonsberg 28152	Czech Republic	
	EF619547	N/A	PRA	Slavikova-Bayerova 3529	Ukraine	
	EF619545	N/A	PRA	Slavikova-Bayerova 3449	Ukraine	
	EG619546	N/A	PRA	Slavikova-Bayerova 3528	Ukraine	
	AF517909	N/A	BG	Tonsberg 28201	Germany	
		NY647	NY-1104177	**Harris 55713**	U.S.A. Connecticut	rock
	EF619544	N/A	PRA	Orange 15453-2	United Kingdom	
		NY1058	NY-1218778	**Lendemer 27625**	U.S.A. Vermont	Abies
		NY29	NY-953120	**Lendemer 9804**	U.S.A. New York	rock
		NY677	NY-953120	**Lendemer 9804**	U.S.A. New York	rock
		NY421	NY-1103536	**Lendemer 17830**	U.S.A. Maryland	rock
		NY422	NY-1103536	**Lendemer 17830**	U.S.A. Maryland	rock
		NY423	NY-1103536	**Lendemer 17830**	U.S.A. Maryland	rock
		NY580	NY-1133087	**Lendemer 19675**	U.S.A. California	rock
		NY610	NY-1199479	**Buck 55092**	U.S.A. Utah	*Selaginella*
L. granulata	DQ914540	N/A	PRA	Slavikova-Bayerova 3325	Bulgaria	
	DQ914541	N/A	PRA	Slavikova-Bayerova 3230	Bulgaria	
	DQ914542	N/A	PRA	Slavikova-Bayerova 3324	Bulgaria	
	DQ914539	N/A	PRA	Slavikova-Bayerova 3243	Bulgaria	
L. humida		NY519	NY-1103934	**Harris 55530**	U.S.A. Maine	rock
		NY1064	NY-1219442	**Lendemer 27018**	U.S.A. North Carolina	rock
		NY1063	NY-1217940	**Lendemer 27072**	U.S.A Vermont	rock
	EF619554	N/A	PRA	Orange 15454-a	United Kingdom	
	EF619556	N/A	PRA	Orange 15764	United Kingdom	
	EF619555	N/A	PRA	Orange 15453-1	United Kingdom	
	EF619553	N/A	PRA	Orange 15457	United Kingdom	
	EF619551	N/A	PRA	Orange 15759	United Kingdom	
	EF619552	N/A	PRA	Orange 15243	United Kingdom	
	DQ401101	N/A	PRA	Orange 15754	United Kingdom	
L. neglecta	AF517901	N/A	BG	Tonsberg 27312	Norway	
	AF517894	N/A	BG	Tonsberg 28455	Norway	
		NY934	NY-1148148	**Root 2373**	U.S.A. Oregon	soil
		NY933	NY-1148175	**Root 2379**	U.S.A. Oregon	soil
		NY321	NY-1079489	**McCune 28216**	U.S.A. Oreon	mosses
		NY305	NY-976499	**McCune 29620**	U.S.A. Oregon	rock

	NY322	NY-1079493	**McCune 28484**	U.S.A. Oregon	mosses
	NY127	NY-1068849	**Lendemer 14960**	U.S.A. California	rock
	NY117	NY-1068824	**Lendemer 14986**	U.S.A. California	rock
	NY604	NY-1107514	**Lendemer 19735**	U.S.A. California	mosses
	NY61	NY-973302	**Lendemer 11397**	U.S.A. California	soil
	NY129	NY-1068766	**Lendemer 14788**	U.S.A. California	soil
	NY142	NY-1068722	**Lendemer 14830**	U.S.A. California	conifer
	NY605	NY-1107498	**Lendemer 19751**	U.S.A. California	conifer
	NY586	NY-1133336	**Lendemer 19632**	U.S.A. California	mosses
	NY932	NY-1148150	**Root 2381**	U.S.A. Oregon	soil
	NY135	NY-1068597	**Lendemer 14677**	U.S.A. California	rocks
	NY581	NY-1133173	**Lendemer 19584**	U.S.A. California	rocks
AF517908	N/A	BG	Tonsberg 28249	Norway	
	NY602	NY-1133078	**Lendemer 19684**	U.S.A. California	mosses
AF517919	N/A	BG	Tonsberg 28977	U.S.A.	
	NY582	NY-1133144	**Lendemer 19616**	U.S.A. California	rock
	NY125	NY-1068609	**Lendemer 14665**	U.S.A. California	conifer
AF517888	N/A	BG	Tonsberg 28476	Norway	
	NY525	NY-1136449	**Buck 55113**	U.S.A. Utah	soil
AF517905	N/A	BG	Tonsberg 28680	Canada	
	NY607	NY-1133071	**Lendemer 19691**	U.S.A. California	soil
	NY587	NY-1107333	**Kalb 36707**	Australia, Osterreich	soil
	NY1117	NY-1218321	**Lendemer 26073**	U.S.A. Arkansas	rock
EU008612	N/A	WIS	**Nelsen 3969**	U.S.A. Pennsylvania	hardwood
	NY465	NY-1105782	**Lendemer 17220**	U.S.A. Pennsylvania	hardwood
	NY463	NY-1105968	**Lendemer 17453**	U.S.A. Pennsylvania	hardwood
	NY612	NY-1104219	**Harris 55753**	U.S.A. New York	hardwood
	NY986	NY-1217284	**Lendemer 25722**	U.S.A. Pennsylvania	hardwood
EU008611	N/A	WIS	**Nelsen 3967**	U.S.A. Pennsylvania	hardwood
	NY1115	NY-1218204	**Beeching 10499**	U.S.A. Georgia	rock
EU008609	N/A	WIS	**Nelsen 3965**	U.S.A. Pennsylvania	rock
	NY980	NY-1205604	**Lendemer 23751**	U.S.A. Pennsylvania	hardwood
	NY603	NY-1107514	**Lendemer 19735**	U.S.A. California	mosses
AF517893	N/A	BG	Tonsberg 28388	Norway	
	NY783	NY-1151627	**Lendemer 16108**	Chile, Prov. Bio Bio	mosses
	NY793	NY-1151626	**Lendemer 16109**	Chile, Prov. Bio Bio	rocks
	NY777	NY-1151606	**Lendemer 16040**	Chile, Prov. Bio Bio	mosses
	NY595	NY-1107461	**McCune 30079**	U.S.A. Oregon	mosses
	NY334	NY-1079668	**Rosentreter 16535**	U.S.A. Idaho	soil

Table 8. GenBank numbers and associated voucher information DNA sequences used in this study.

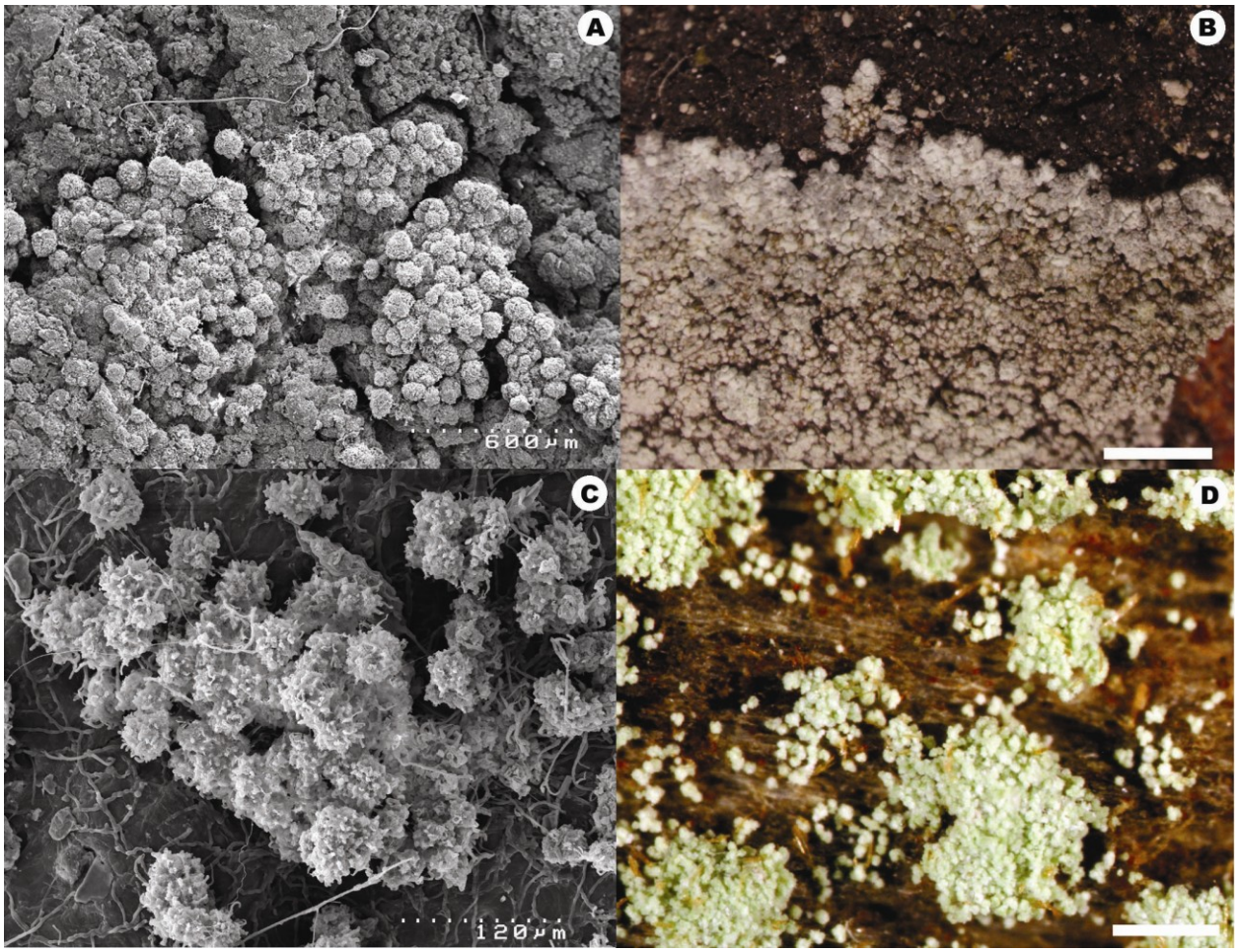

Figure 36. Scanning electron (left) and light (right) micrographs illustrating external morphology of *L. neglecta* s.l. (A & B) compared to that of another species with a typical aggregate thallus (*L. pacifica*, C & D). A, *Lendemer 17098* (NY), scale as indicated. B, *Lendemer 17710A* (NY), scale = 1.0 mm. C, *Lendemer 19325* (NY), scale as indicated. D, *Lendemer 19566* (NY), scale = 0.5 mm.

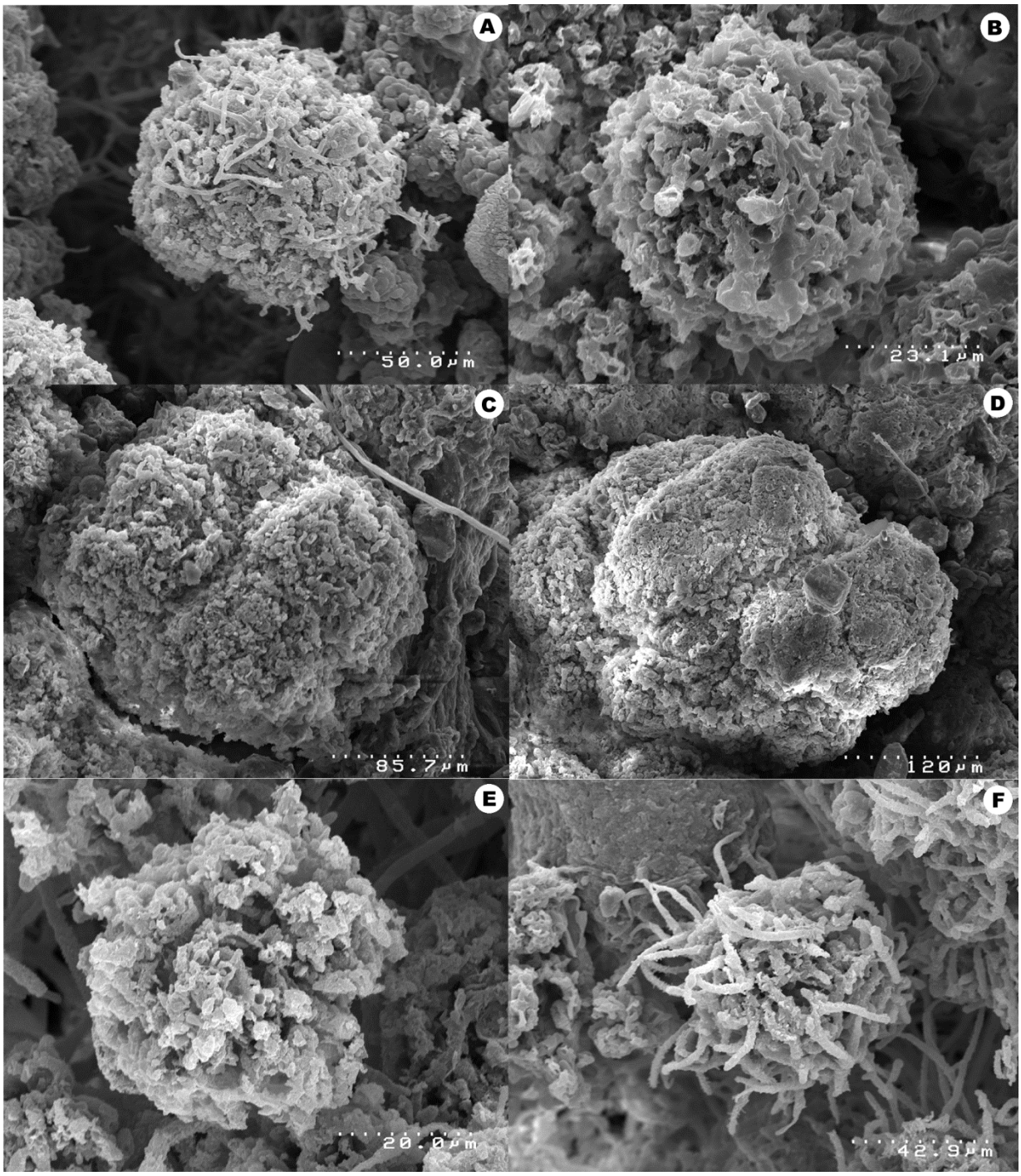

Figure 37. Scanning electron micrographs illustrating development of a pseudocortex on the granules of *L. neglecta* s.l. (A–D) and comparative images of granules from the unrelated species *L. squamatica* (E) and *L. cryophila* (F). A–B, young ecorticate granule in a *caesioalba*-type thallus (right) juxtaposed with mature granule with a well developed pseudocortex (left; both *Lendemer 17098*, NY). C–D, young ecorticate granule in an *alpina*-type thallus (right) juxtaposed with mature granule with a well developed pseudocortex (left; both *Buck 55129B-831*, NY). E, typical granule from the thallus of *L. squamatica* (*Elix 37755*, NY). F, typical granule from the thallus of *L. cryophila* (*Lendemer11961*, NY). All scales are as indicated.

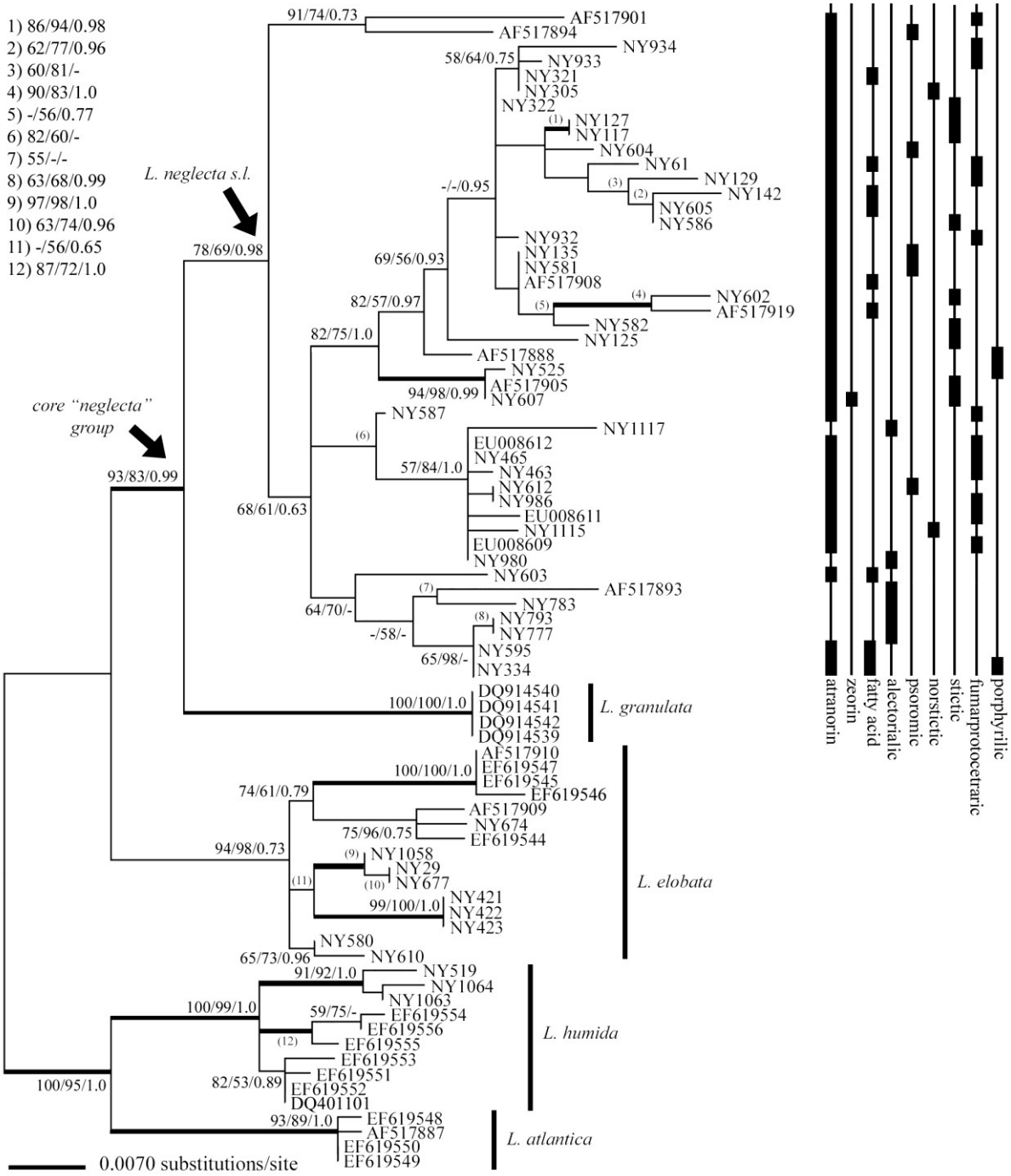

Figure 38. Phylogeny of *Lepraria neglecta* s.l. and its relatives, inferred from ITS sequence data and displayed as the most likely tree obtained from the ML topology search. Support was evaluated using maximum parsimony (MP) and maximum likelihood (ML) bootstrap proportions (BP) and Bayesian posterior probabilities (B-PP). Support values are displayed at each node in the following format: MP-BP/ML-BP/B-PP. Thickened branches are those that were recovered with MP-BP and ML-BPP support ≥70% and B-PP support ≥0.95. The chemotype of the voucher for each sequence of *L. neglecta* s.l. is scored as thickened lines to the right of the tree. (Note that atranorin can sometimes be detected as a minor accessory in the alectorialic acid chemotype and thus is scored as present.)

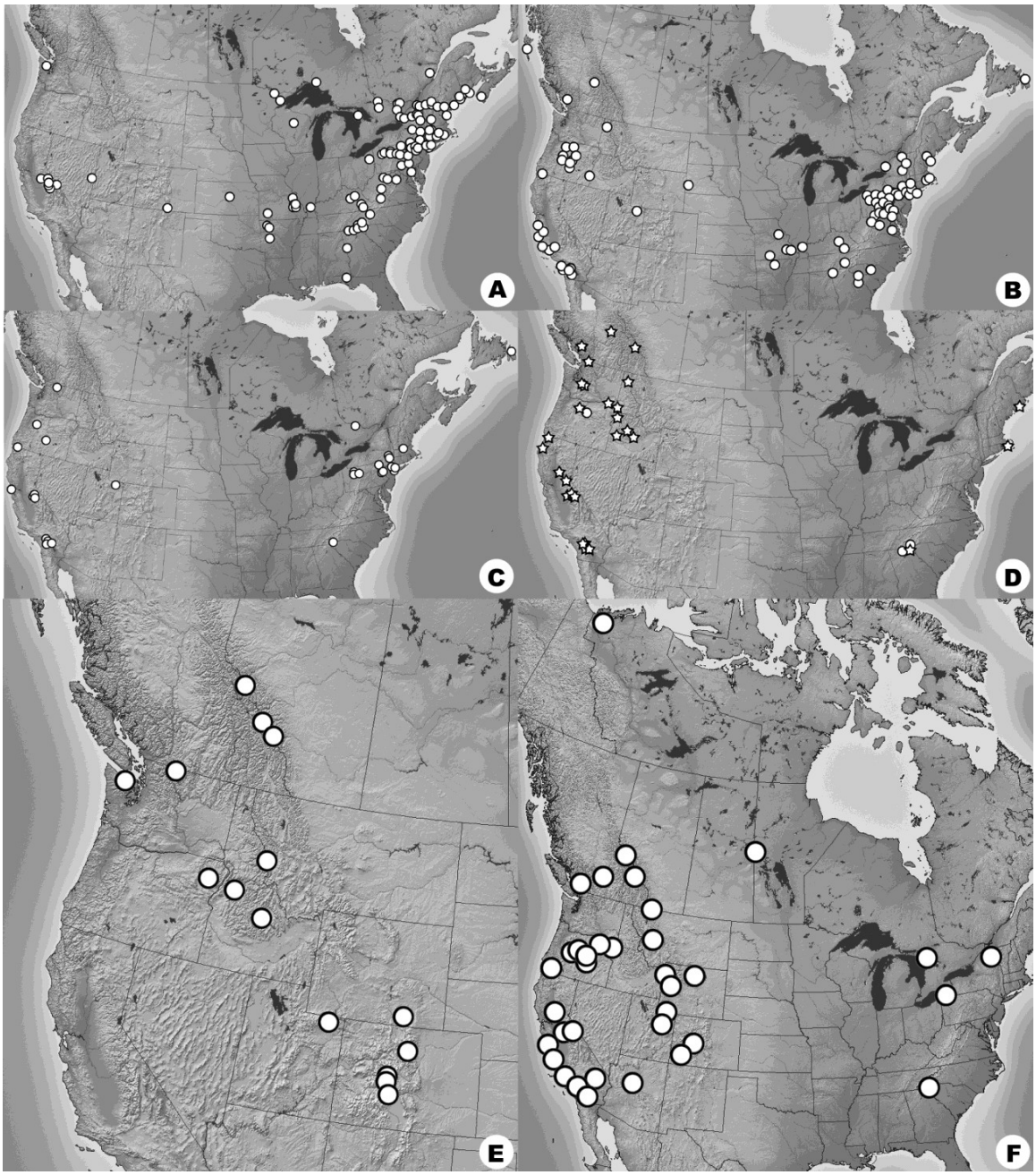

Figure 39. Geographic distributions of the chemotypes of *Lepraria neglecta* s.l. in North America based on specimens examined for this study. A, alectorialic acid chemotype (*L. neglecta* s.str.). B, fumarprotocetraric acid chemotype (*L. caesioalba*). C, psoromic acid chemotype (unnamed). D, stictic acid (stars, *L. svalbardensis*) and norstictic acid (circles, unnamed) chemotypes. E, porphyrilic acid chemotype (*L. alpina*). F, fatty acid chemotypes (populations with roccellic/angardianic acid were unnamed, those with rangiformic/jackinic acid were referred to *L. borealis*).

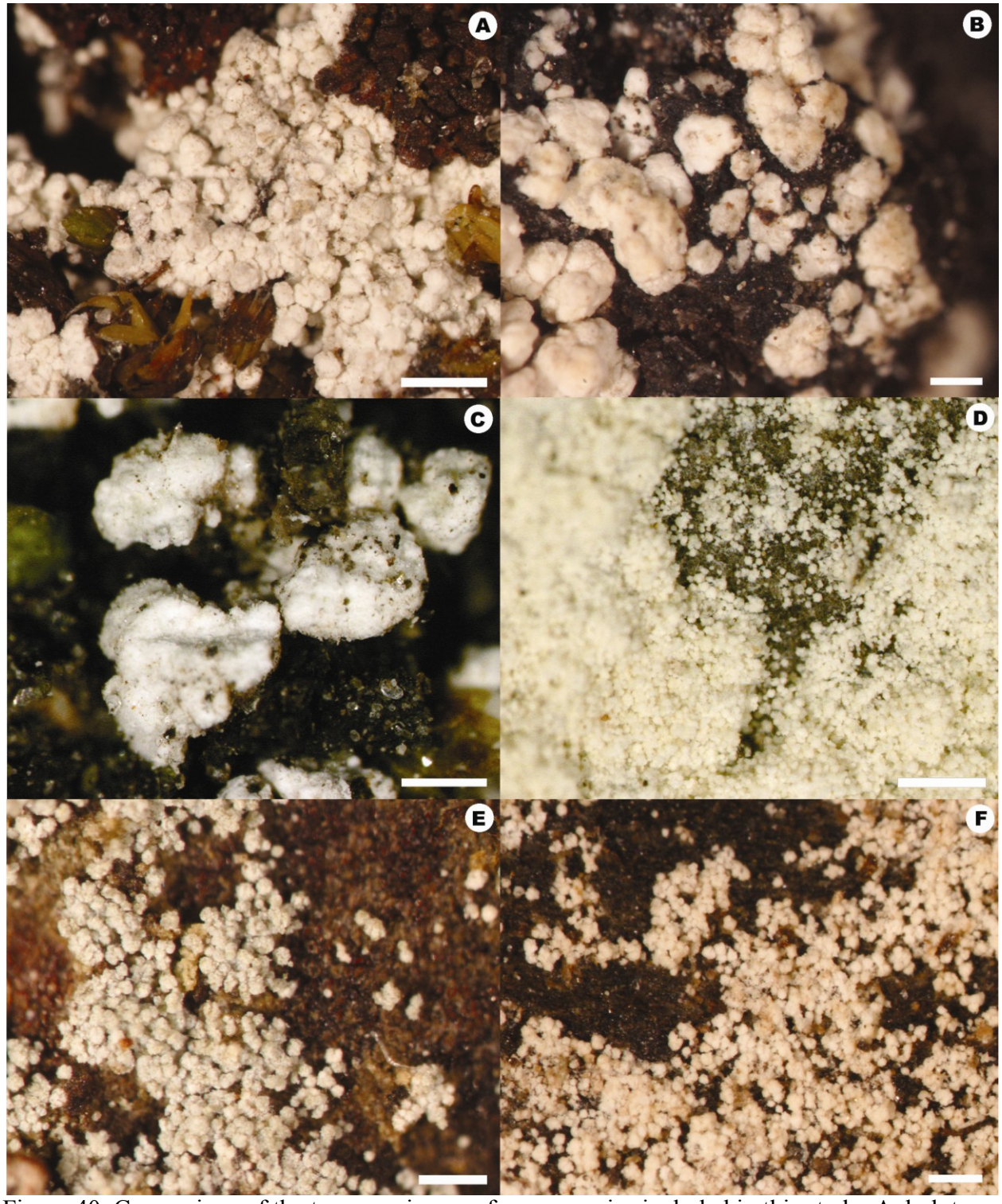

Figure 40. Comparison of the type specimens of some species included in this study. A, holotype of *L. borealis* (*Tønsberg 1519*, BG; scale = 0.5 mm). B, isolectotype of *L. alpina* (*Willard s.n.*, NY; scale = 0.25 mm). C, holotype of *L. gelida* (*Tønsberg 31049*; scale = 0.5 mm). D, holotype of *L. jackii* (*Tønsberg 6176*, BG; scale = 0.5 mm). E, holotype of *L. elobata* (*Tønsberg 17404*, BG; scale = 0.25 mm). F, holotype of *L. salazinica* (*Tønsberg 30941*, BG; scale = 0.25 mm).

CHAPTER VIII – NOT ALL STERILE CRUSTOSE LICHENS RESEMBLE THEIR CONGENERS MORPHOLOGICALLY: *LECANOGRAPHA INSOLITA*, A CASE STUDY[8]

James C. Lendemer and Kerry Knudsen

Abstract. – *Lecanographa insolita* is described as new to science based on collections from the Channel Islands of southern California and an isolated coastal redwood forest in central California, U.S.A. Placement of this normally sterile species in the genus *Lecanographa* is supported by secondary chemistry and the anatomy of sterile ascoma found on a single thallus. The species has a non-filament forming coccoid photobiont rather than *Trentepohlia*, an apparently unique feature within the genus *Lecanographa* as well as the broader context of the Roccellaceae. Comparison is drawn to other members of the Roccellaceae that have byssoid thalli.

Introduction

Members of the lichen family Roccellaceae Chevall. form a major component of the lichen biota along the Pacific Coast of North America from central California south through Baja California, Mexico. The family *sensu* Lumbsch and Huhndorf (2007) is represented by 16 genera and more than 50 species that have evolved to occupy nearly every available habitat (e.g., corticolous, saxicolous, and lichenicolous) and whose morphologies span the full continuum of lichen growth forms (e.g., crustose to fruticose) and an array of reproductive strategies (e.g., sexual and asexual) (Egea & Torrente 1992, 1993, 1994; Ertz & Diederich 2008; Ertz & Egea 2007; Sparrius 2004; Sundin & Tehler 1996; Tehler 1983, 1985, 2002; Tehler et al. 1995, 1997,

[8]This chapter was published as: Lendemer, J.C. and K. Knudsen. 2010. *Lecanographa insolita*, an unusual new species of Roccellaceae from western North America. The Bryologist 113: 350-355.

2009). Many of these species are endemic to the Pacific Coast of North America and even within this region are rare or have restricted geographic distributions (*ibid*.). Considering the high diversity of this family in the region and the restricted distribution of many of the taxa it is not surprising that during recent fieldwork in poorly collected areas several populations of an undescribed saxicolous species of *Lecanographa* were found. The species is described here as *L. insolita*.

Materials and methods

All specimens were studied dry using a Bausch & Lomb StereoZoom 7 dissecting microscope and subjected to chemical analysis using standard spot tests (reagents are abbreviated following Brodo et al. (2001)) and Thin Layer Chromatography (TLC). TLC was carried out using solvent systems A and C following the standardized methods of Culberson & Kristinsson (1970). A fragment of the new species was also sent to Jack Elix for more detailed chemical analysis with High Performance Liquid Chromatography (HPLC). A sample of the holotype of *Lepraria lecanorica* was used for comparison and as a standard for lecanoric acid. Collections were also examined with a Scanning Electron Microscope (SEM) following the methods outlined by Lendemer et al. (2008). Images were assembled in Adobe Photoshop 4.0 and that program was used to improve contrast in some of the illustrations provided here.

Taxonomic Section

***Lecanographa insolita* Lendemer & K. Knudsen sp. nov.**

Mycobank #516769

Figures 41 & 43.

. Ab *Lecanactis latebrarum* erythrinicum et *Trebouxia* continens, acido leprarico et *Trentepohlia* nullo differt.

TYPE: **U.S.A. CALIFORNIA.** SANTA BARBARA CO.: Transverse Range, Channel Islands National Park, Santa Rosa Island, Lobos Canyon at road crossing, Santa Rosa Island North Quad., 96 m, oaks, coastal sage shrubs, 13.vi.2009, on exposed sandstone in canyon wall, *K. Knudsen 11401* (NY!, holotype; UCR!, isotype).

Description. – Thallus saxicolous, crustose, leprose, indeterminate, without lobes, initiating from individual granules that through division form diffuse patches or heaps that in turn become confluent, eventually overlapping and forming a continuous colony of varying thickness (0.5–2.0 mm thick) with a thin layer of lichenized granules subtended by a loosely connected matrix of necrotic granules laced with irregularly branching interconnecting hyphae and heavily interspersed Is this a correct word?) with large crystals of calcium oxalate (POL+, not dissolving in K or HNO_3); greenish in color due to the algae in the uppermost layer of granules; hypothallus a thin network of hyphae underneath the granules and extending outward from the edge of thallus; granules 12–25 µm in diameter, irregular and ecorticate, often consistent of a single layer of gelatinized hyaline hyphae surrounding the algal core; readily dividing; hyphae hyaline, septate, 2.5–4.0 µm wide, irregularly branching. Ascoma rare (seen only in *Knudsen 7586*, UCR), known only in an immature state (lacking a well-developed hymenium with asci and ascospores), round, sessile, not constricted at the base, emarginate or with a narrow margin when young that is quickly excluded with age; disc with a thin bluish-white pruina; exciple brown, composed of indistinct hyphae, lacking crystals, K–; hypothecium

brown, K–; hymenium hyaline, poorly developed with profusely branched paraphysoids; asci and ascospores unknown. Pyncidia unknown. Photobiont a coccoid green alga (*Trebouxia*), cells globose, 8.5–12.2 μm in diameter.

Chemistry. – Erythrin (major), isoerythrin (minor), lecanoric acid (minor), methyl lecanorate (minor), additional unidentified accessory substances are also often detected by TLC. Spot tests: K–, C+ strong red, KC+ strong red, P–, UV–.

Etymology. – The epithet "insolita," meaning "strange" or "unusual" denotes the different photobiont of this species compared to the rest of the genus and family.

Distribution. – Presently *Lecanographa insolita* is known only from a handful of populations at two geographically restricted localities along the Pacific Coast of western North America (figure 42A).

Ecology. – *Lecanographa insolita* occurs on sandstone in Lobos Canyon on Santa Rosa Island in a mixed saxicolous community including *Lepraria xerophila* Tønsberg, *L. santamonicae* K. Knudsen & Elix, *Niebla ceruchoides* Rundel & Bowler, *Caloplaca stantonii* W.A. Weber ex Arup, and *Leprocaulon quisquiliare* (Leers) M. Choisy. Thalli are particularly well developed to their maximum thickness on a sunny, dry west-facing slope in the canyon bottom. Nearby on *Quercus agrifolia* is the only population of *Roccella gracilis* Bory on the island. The rare saxicolous *Arthonia madreana* Egea & Torrente, endemic to Santa Rosa Island, also occurs in Lobos Canyon. *Lecanographa insolita* at this location is exposed to regular fog

occurrences. The thalli in the populations on Santa Rosa Island also infrequently exhibited tunnels or burrows created by an unknown species of pseudoscorpion.

The populations at Limekiln State Park in Monterey County occurred in deep confluent canyon bottoms within a few kilometers of the Pacific Ocean in a coast redwood forest (*Sequoia sempervirens* (Lamb. *ex* D. Don) Endl.) in deep shade with high relative humidity from perennial streams. The specimens from this location are thinner than those from Santa Rosa Island and always occurred alone on volcanic rock. These populations probably did not survive the Chalk Fire of 2008.

Both locations where *Lecanographa insolita* occurs have high annual relative humidity. In the development of southern California's Mediterranean climate since the end of the Pleistocene, with long arid summers, both locations represent relictual microhabitats and both are close to the Pacific Ocean. Further populations will probably be found at sites with similar habitats elsewhere in the region.

Discussion. – Initially we referred several poorly developed specimens of this taxon to *Lepraria lecanorica* Tønsberg on account of the thick C+ red, sterile thallus, and coccoid photobiont (Kocourková et al. 2008). When additional populations from the Channel Islands with better developed thalli were studied we became uncomfortable with this identification and examined the type material of *L. lecanorica* and confirmed it represented a separate corticolous taxon.

We then reexamined the chemistry and discovered that our material differed from *Lepraria lecanorica* in the production of erythrin as a major substance rather than as a trace accessory to lecanoric acid. While erythrin is restricted to members of the Roccellaceae

(Follmann & Huneck 1969; note *Chiodecton cretacea* Zahlbr. is now placed in the Roccellaceae as *Dirina cretacea* (Zahlbr.) Tehler (Tehler 1983)) we still favored placement of the taxon in *Lepraria* because that genus is well known for its high degree of chemical diversity (Knudsen & Elix 2007, Knudsen et al. 2007, Saag et al. 2009).

Finally a single thallus with abundant apothecia resembling those of *Lecanographa* Egea & Torrente was discovered in the collections from the island at UCR. This discovery clearly excluded placement of the species in *Lepraria* and suggested further comparison with members of the Roccellaceae was needed. Review of the relevant literature revealed that some species of Roccellaceae have a thick *Lepraria*-like or byssoid thalli (Egea & Torrente 1994, Jørgensen & Tønsberg 1988, Thor 1990, Sparrius 2004) and that the only aberrant feature of our material that precluded placement in the family was the presence of a coccoid *Trebouxia* photobiont rather than *Trentepohlia*. While we recognize that the inclusion of this new species in *Lecanographa* significantly emends the circumscription of the genus and family to include lichens with a non-*Trentepohlia* photobiont, this is not out of line with current generic and familial concepts in lichens that place emphasis on similarities in the mycobiont rather than photobiont. In fact, this type of broad circumscription with respect to photobiont is mirrored in several other lichen families including the closely related Arthoniaceae Reichenb. *ex* Reichenb. (e.g., Grube 1998).

Within the Roccellaceae, *Lecanographa insolita* is most similar in thallus morphology to *Lecanactis latebrarum* (Ach.) Arn., a sterile species with lepraric acid that occurs on shaded siliceous rocks in humid habitats in Europe (Jørgensen & Tønsberg 1988; Wirth 1995 [color illustration]). The new species differs from *L. latebrarum* in chemistry (erythrin vs. lepraric acid), geographic distribution (central coastal California vs. Europe), and photobiont (*Trebouxia* vs. *Trentepohlia*). Interestingly both *Lecanactis latebrarum* and *Lecanographa insolita* have a

mycobiont with thick rugose hyphae, a character emphasized and illustrated by Jørgensen and Tønsberg (1988) that stands in contrast to the comparatively narrow and smooth hyphae found in *Lepraria* species (Lendemer et al. 2008; Lendemer 2011).

Lecanographa insolita can also be distinguished from the genera of Roccellaceae with byssoid thalli (e.g., *Ancistrosporella* G. Thor and *Sagenidium* Stirt.) treated by Egea and Torrente (1994) by its chemistry (erythrin vs. gyrophoric acid and lepraric acid with gyrophoric acid, respectively (*fide* Egea & Torrente (1994)) and the nature of the photobiont. Unfortunately the apothecia found in *L. insolita* were immature or abortive and thus the characters of the asci and ascospores, both primary features used in distinguishing genera in the crustose members of the Roccellaceae (Egea & Torrente 1994, Sparrius 2004), remain unknown. We have chosen to describe the species in *Lecanographa* on the basis of chemistry (the presence of erythrin is shared with other members of the genus) and the superficial similarity of the apothecia to other sympatric species of that genus, notably *L. dimelaenoides* (Egea & Torrente) Egea & Torrente and *L. hypothallina* (Zahlbr.) Egea & Torrente. We admit, however, further study with molecular methods may reveal *L. insolita* occupies an isolated position in the Roccellaceae and should be segregated in its own genus.

Additional specimens examined. – **U.S.A. CALIFORNIA.** MONTEREY CO.: Santa Lucia Mountains, Lime Kiln State Park, Hare Creek Trail, Lopez Point Quad., 30 m, 36°00’38”N, 121°31’W, redwood forest, 16.viii.2008, on shaded rock outcrop, *K. Knudsen 10165* (NY!, UCR!), 4.ix.2008, *K. Knudsen 10197* (NY!, UCR!); Santa Lucia Mountains, Lime Kiln State Park, main trail past split to Lime Kiln and Waterfall trails, 40 m, 36°00’N, 121°31’W, redwood forest, 21.vii.2008, on shaded rock, *K. Knudsen 9941 & J. Kocourková*

(NY!, UCR!). SANTA BARBARA CO.: data as for the type, 16.x.2006, *K. Knudsen 7549 & S. Baguskus* (UCR!), *K. Knudsen 7587 & S. Baguskus* (NY!, UCR!).

Comparative material of Lepraria lecanorica examined. – **U.S.A. ARIZONA.** COCONINO CO.: SSW of Flagstaff, Cononino National Forest, uphill from Hwy 89, 35˚00.30'N, 111˚44.04'W, dry W-facing slope with *Pseudotsuga menziesii* and *Pinus*, 18.iv.2001, on shaded base of *Quercus*, *T. Tønsberg 28991* (BG, holotype).

Acknowledgments

The work of Kerry Knudsen was supported by a co-operative agreement between Channel Islands National Park and the University of California at Riverside. The work of the James Lendemer was supported by The New York Botanical Garden (NYBG) and The City University of New York (CUNY). The first author thanks Lehman College of CUNY for use of the SEM and Mike Baxter for help in preparation of the samples that were examined using the SEM. We thank Jana Kocourková for the photograph of the type locality of *Lecanographa insolita*. We also thank Harrie Sipman and Richard Harris for helpful discussion as well as Tor Tønsberg and the staff of BG for loaning the type of *Lepraria lecanorica*. Special thanks to Jack Elix for identification of the primary metabolite in the new species and helpful suggestions regarding taxa with a similar chemistry. Additional special thanks go to John Hall for confirmation of the identity of the photobiont in the new species.

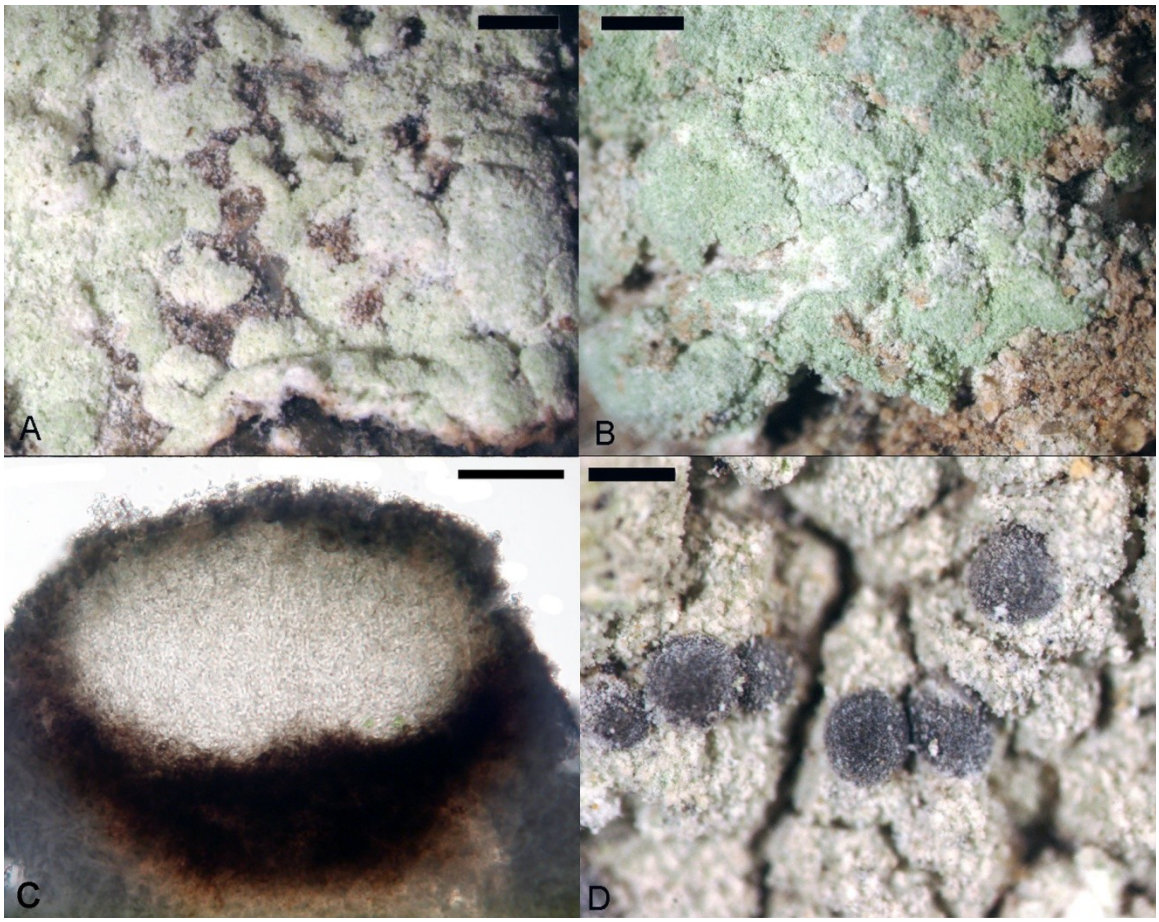

Figure 41. Macroscopic morphology of *Lecanographa insolita*. (beginning at top left). A. Poorly developed sterile thallus from shaded site (*Knudsen 10165*, NY; scale = 1.0 mm). B. Surface of well developed thallus (*Knudsen 7586*, UCR; scale = 1.0 mm). C. section of apothecium (*Knudsen 7586*, NY; scale = 50 µm). D. Apothecia (*Knudsen 7586*, UCR; scale = 0.5 mm).

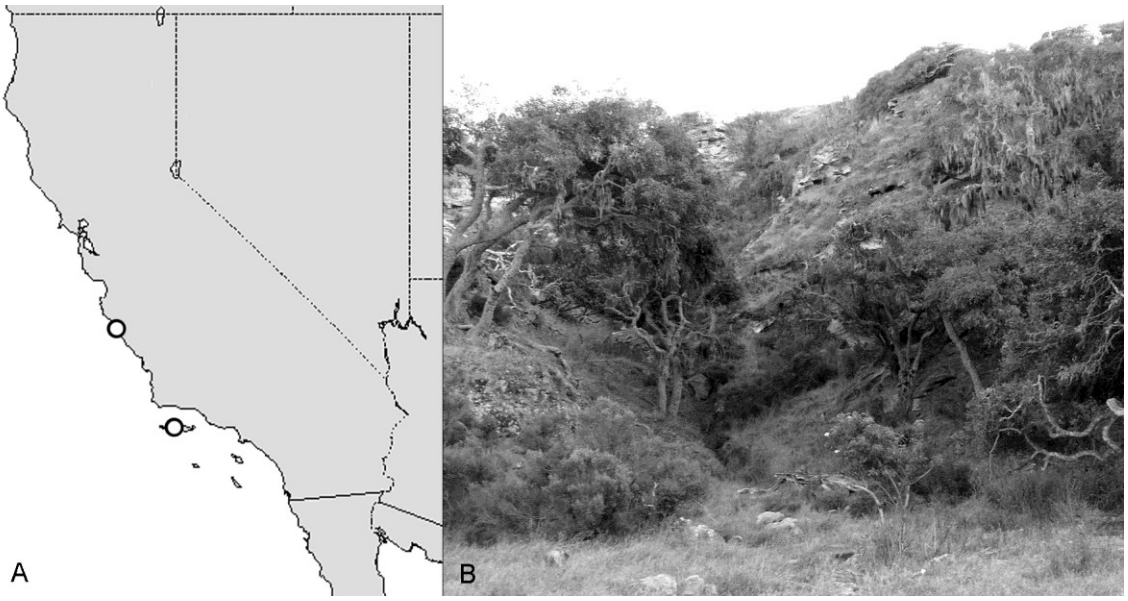

Figure 42. A. Geographic distribution. B. Type locality of *Lecanographa insolita*.

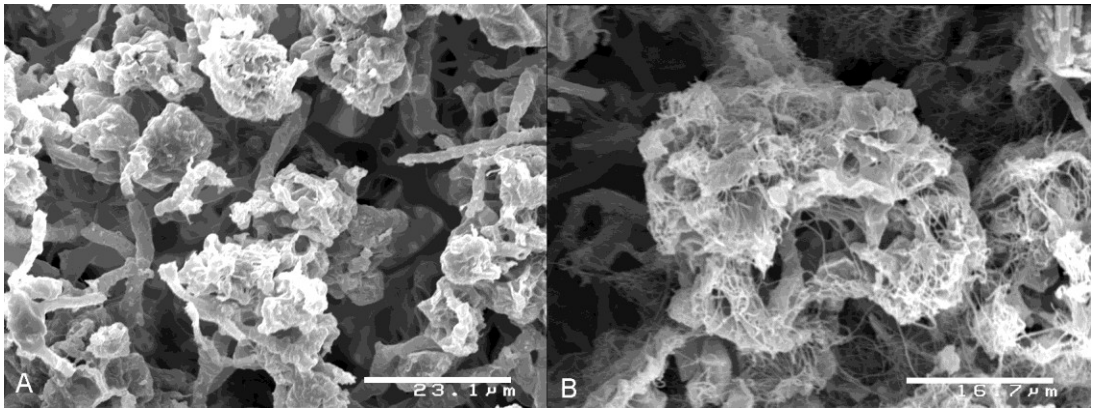

Figure 43. Scanning electron micrographs of *Lecanographa insolita* (both from holotype). A. Granules and thick roughened hyphae of thallus. B. Detail of granule with network of associated bacteria.

CHAPTER IX – IMPLEMENTATION OF A SYSTEMATIC METHODOLOGY THAT INTEGRATES MOLECULAR AND NON-MOLECULAR CHARACTERS TO PLACE AND CIRCUMSCRIBE SPECIES OF STERILE ASEXUALLY REPRODUCING LICHENS

James C. Lendemer and Brendan P. Hodkinson

Abstract. – Crustose asexually reproducing lichens represent a large component of lichen biodiversity that is often overlooked and underestimated; as a result, there remains remarkable potential for new discovery in this taxonomically-neglected polyphyletic group. For this study, we analyze *ITS* and *mtSSU* rDNA sequences in conjunction with chemical and anatomical data in order to understand the systematic placement of an enigmatic sterile lichen species that has been referenced in literature for over half a decade, but has remained undescribed due to our inability to integrate it into a higher-level taxonomic framework using morphology alone. Here we demonstrate the utility of a systematic methodology that integrates molecular and non-molecular characters to place and circumscribe species of asexually reproducing lichens that are typically sterile. Based on our analyses, the new species, ***Caloplaca reptans***, shows phylogenetic and morphological affinities to a broad group of *Caloplaca* species with gray thalli including the type species of the genus, *C. cerina*. This study highlights the fact that the family Teloschistaceae is morphologically more diverse than previously understood, and contains elements that cannot easily be placed in known 'species groups.'

Introduction

Lichens represent a subset of the fungal kingdom that has evolved a unique lifestyle that involves the capture and propagation of algae and/or cyanobacteria. Although many lichens reproduce through the dispersal of sexual diaspores (i.e., ascospores), asexual reproduction is not uncommon and occurs in nearly every one of the diverse lineages that comprise lichens (Bowler

and Rundel 1975). This type of reproduction can occur through either the dispersal of purely fungal diaspores (e.g., conidia) or lichenized diaspores (e.g., isidia or soredia). The latter comprise a morphologically diverse array of structures that have evolved to facilitate the co-dispersal of both the fungal and algal components of the lichen.

The majority of crustose lichens that reproduce exclusively via lichenized diaspores are undescribed or otherwise poorly understood (Kantvilas and Lumbsch 2010; Harris and Lendemer 2010). Therefore, they represent a large component of lichen biodiversity that is currently overlooked and underestimated. Historically they have been rarely collected as most lichenologists perceive them as impossible to identify because of their frequent sterility (Fryday and Coppins 1997). The last decade has seen a marked increase in attempts to collect and define these taxa; however, they are still considered to be among the most taxonomically neglected and difficult groups of lichens (Fryday and Coppins 1997; Kantvilas and Lumbsch 2010; Harris and Lendemer 2010; Tønsberg 1992).

Satisfactory classification of these taxa using strictly non-molecular tools is almost impossible as they normally lack the sexual structures (i.e., apothecia) whose morphological characters inform generic and familial placement. In the past, unequivocal placement of such taxa in a genus or family has only been possible when one encountered a fertile individual. Such events are rare, however, and one can spend decades accumulating dozens, even hundreds of collections of a morphologically well characterized taxon before a fertile individual is found (Harris 1990; Kantvilas and Lumbsch 2010). Often, fertile individuals are never found, and systematic placement is a "best guess" based on the instinct of the taxonomist and correlated characters such as chemistry, ecology, and photobiont (Timdal 2001).

Taxa that reproduce exclusively (or almost exclusively) through vegetative diaspores occur in nearly all the diverse lineages that constitute lichenized fungi, necessitating extensive experience with a broad array of distantly related lichens. Even with such broad knowledge, there is no guarantee that taxonomic placement based on correlated characters is correct (Nelsen et al. 2008). Further, it renders impossible the placement of taxa that are phenotypically isolated from their congeners (Lendemer and Knudsen 2010). Clearly, traditional taxonomic methods alone do not provide a simple and convenient framework within which to evaluate and describe most of the biological diversity of the group. Instead, one can treat only a fraction of the taxa, effectively relegating the vast majority to taxonomic obscurity, excluded from the taxonomic dialogue, estimates of diversity, and most importantly, from consideration in conservation/management plans (Kantvilas and Lumbsch 2010).

One particularly distinctive sterile lichen occurs commonly on sheltered non-calcareous rocks throughout the Appalachian Mountains, and has been known for more than half a decade; however, its taxonomic placement has remained uncertain. Harris and Lendemer (2005) initially inferred placement of this taxon in the order Teloschistales, a diverse clade with two main speciose genera of crustose lichens, *Caloplaca* Th. Fr. (Teloschistaceae) and *Rinodina* (Ach.) Gray (Physciaceae). Both genera contain members that are extremely diverse in terms of morphology and chemistry. As members of the genus *Caloplaca* typically produce anthraquinones in the thallus or apothecial discs, unlike the species in question, a placement in *Rinodina* seemed logical (Harris and Lendemer 2005; Lendemer 2010b). However, only immature apothecia that lacked asci and ascospores had been found in the taxon, making definitive placement impossible since characters of these structures are the primary distinguishing features between the two genera (and families) in which the taxon could

potentially be placed. Thus, since these structures had not been observed, the generic and familial affinities of the taxon remained ambiguous.

In light of this situation, we took the opportunity to develop an approach that uses molecular data in conjunction with morphological and chemical analyses to place sterile lichen-forming fungal species in a higher-level phylogenetic and taxonomic framework. For this study we performed analyses on two gene regions (ITS and mtSSU rDNA) to infer the familial placement of this enigmatic sterile lichen-forming fungal species. Subsequently, more in-depth analyses were performed on the ITS region and the morphology of the species to evaluate its placement within the family and its relationship to described genera and species groups.

Materials and methods

Morphological and chemical analyses. – The morphology of each specimen was studied in detail with standard light microscope techniques to classify the types of diaspores present, the photobiont, and the morphology/anatomy of the thallus. Measurements of macro-morphological structures were taken from dry specimens. Measurements of micro-morphological structures and anatomical studies were carried out on sections of the thallus mounted in water. External ultrastructure was also studied using a scanning electron micrograph following the methodology outlined by Lendemer and Elix (2010). Secondary chemistry was examined using spot tests with standard reagents (Brodo et al. 2001) and thin layer chromatography was conducted using solvent C following the methodology of Culberson and Kristinsson (1970).

Molecular data generation. – Three specimens were selected for study with DNA sequence-based methods. ITS sequence data were generated as outlined by Hodkinson and Lendemer (2011) and mtSSU data were generated using the same procedures, but with the

mrSSU1/3R primer pair published by Zoller et al. (1999). Nucleotide sequences were assembled and edited using the software package Sequencher 4.9 (Gene Codes Corporation, Ann Arbor, MI, USA) and MegaBLAST searches were performed on the NCBI non-redundant nucleotide collection to screen out contaminant and/or endolichenic fungal sequences (Zhang et al. 2000). Since all sequences were found to be identical for all three specimens, only the sequence data for extract NY177 were used in downstream analyses.

Molecular phylogenetic analyses

Two-gene analyses. – Based on MegaBLAST searches, the sequences generated for the new species only shared a significant degree of similarity with members of the family Teloschistaceae. We subsequently conducted phylogenetic analyses based on the combined mtSSU and ITS data, both to confirm placement in the family and to attempt to understand its finer-scale relationships to other members of the family. Full tables of all members of Teloschistaceae represented by mtSSU and ITS were downloaded from GenBank in October, 2010. These tables were checked for overlapping species, and species represented by both genes were selected as reference taxa to be included in the dataset. For cases in which multiple representatives of a species were present, no more than two representative samples were chosen for the combined 2-gene analysis. Preliminary examination of the sequence data revealed high sequence similarity between the taxon of interest and members of Gaya et al.'s "lineage 2," so members of "lineage 1" were designated as outgroup taxa and only selected members of the outgroup were retained in downstream analyses (Gaya et al. 2003, 2008).

A 2-gene ITS+mtSSU alignment was assembled by hand using Mesquite 2.72 (Maddison and Maddison, 2009). Each alignment was adjusted manually taking into consideration rRNA secondary structure (Kjer 1995) and ambiguously-aligned regions were excluded. A preliminary RAxML (Stamatakis 2006) bootstrap analysis with 1000 replicates was run on the data matrix of unambiguously-aligned nucleotides for each of the two genes and the two trees were checked for conflict between nodes supported by bootstrap proportions (BP) >70%.

Weighted maximum parsimony (MP) analyses were conducted as outlined by Hodkinson and Lendemer (2011). One set of analyses was run without INAASE characters, while another was run with these characters integrated, using a 1:1:1 transition:transversion:gap ratio for calculating pairwise alignments and cost matrices.

For maximum likelihood (ML), we began with a NEXUS file of the dataset from the MP analyses, deleted ambiguously-aligned regions, and manually converted the file to extended phylip format. Using a Windows executable of RAxML 7.2.6, we performed a topology search and subsequent bootstrap analyses (1000 replicates each, GTRGAMMA model). The bootstrap bipartitions were then mapped to the best tree from the topology search using RAxML and the resulting tree was visualized with FigTree 1.3.1 (Rambaut 2009). Following this first round of analyses we conducted three additional bootstrap analyses (1000 replicates each) using RAxML with the following variations of the above dataset (see below for explanation of secondary structure and recoding methodologies): [1] ML dataset + secondary structure considered, [2] ML dataset + ambiguously aligned regions recoded with PICS-Ord (Lücking et al. 2011), and [3] ML dataset + secondary structure considered + ambiguously aligned regions recoded in PICS-Ord.

Secondary structure was inferred separately for each molecule (mtSSU, ITS1, 5.8S, and ITS2) using the CentroidAlifold software (Hamada et al., 2011) in the CentroidFold 0.0.8

package (http://www.ncrna.org/software/centroidfold/). ClustalW 1.83 (Thompson 1994) was used to generate a file in Clustal format (.aln) for each region. The following command was used to generate secondary structure inferences: centroid_alifold -g -1 input.aln > output. The final Vienna-formatted ('dot-bracket') model listed in each output file was used for downstream analyses. Secondary structure models were manually aligned to the full multiple-sequence alignment in TextPad and ambiguously-aligned regions were manually excised simultaneously from the nucleotide alignment and the secondary structure model. Stray brackets in the secondary structure model that no longer matched brackets that had been deleted were converted to periods immediately upon deletion of the complementary bracket. The VARNA java webstart (Darty et al. 2009) was used for visualization of the molecule and for easy identification of paired nucleotides. RAxML bootstrap analyses were run as above using S6A to model the evolution of paired sites.

PICS-Ord was run for all ambiguously-aligned sites using the recommended settings outlined in the initial version of the program manual with characters equally weighted (Lücking et al., 2011). The recoded ambiguously aligned regions were concatenated with the ML dataset and specified as multistate (vs. DNA) characters in a partitions file that was run in RAxML along with the dataset using the model parameters and number of replicates outlined above.

For Bayesian inference (BI) the dataset was analyzed using Windows versions of both MrBayes *3.1.2 (*Huelsenbeck and Ronquist 2001*)* and Phycas 1.2.0 (Lewis et al. 2010). The MrBayes block of the original NEXUS file was prepared as follows. First the dataset was partitioned into the following regions: mtSSU, ITS1, 5.8S, and ITS2; partitions were defined following the steps outlined in MrBayes online Wiki manual (http://mrbayes.sourceforge.net/wiki/index.php/Analyzing_a_Partitioned_Data_Set_3.2). The

nucleotide substitution model for each partition was then chosen using Akaike's Information Criterion (AIC; Akaike 1974) following analyses with MrModeltest (Nylander 2004). The models chosen were: GTR+I for mtSSU, HKY+G for ITS1 and ITS2, and K80+I for 5.8S. Models and priors were applied to the relevant partition as outlined in the MrBayes manual (URL cited above). Ambiguously aligned regions were then excluded from the analyses by typing "exclude" followed by the numerical ranges of the regions to be excluded in a line following the partition definitions. The remainder of the MrBayes block was produced through the online automated form found at the Santos Lab website (http://131.204.120.103/srsantos/mrbayes_form/index.html). The Markov chain Monte Carlo parameters consisted of 10,000,000 generations, with four chains, and a tree sampled every 100 generations. The first 10,000 trees were discarded as burnin and the results were summarized as a 50% majority-rule consensus tree. Two additional BI analyses were run using Phycas with the same partitions and models that were used in MrBayes. Scripts for these analyses were produced as outlined by Hodkinson (2011). The two analyses differed only in that one allowed trees with polytomies to be sampled ("mcmc.allow_polytomies = True") and the other did not ("mcmc.allow_polytomies = False").

ITS-only analyses. – In order to assess the relationship of the new species to more closely-related members of Teloschistaceae for which only the ITS locus had been sequenced, phylogenetic analyses were conducted both with and without constraints (based on the two-gene analyses) on an ITS-only dataset. MegaBLAST was used to find the top 20 hits, and the accession numbers of these hits were then put into a list separated by commas. This list was used to search the NCBI nucleotide database and the group of twenty sequences was exported as a single fasta file. The Perl script 'fasta_from_NCBI_editor.pl' (available as a supplement from the

Dryad data repository) was written and run to create a fasta file with names that could be easily used for downstream applications. Additionally, a Perl script called 'create_data_table_from_NCBI_fasta.pl' (also available as a supplement from the Dryad data repository) was used to automatically create a data table with all species identifications, GenBank Accession numbers and GenInfo Identifiers for the exported sequences. The combined ITS+mtSSU alignment was opened in Mesquite and the edited fasta file with the sequences from GenBank was imported. ITS sequences were aligned pairwise to the reference alignment in Mesquite and minor adjustments were made manually. Subsequently, the mtSSU portion of the alignment was deleted.

Ambiguously-aligned regions were redefined and the Phycas script written for the previous analysis allowing polytomies was modified for the ITS region only. MP topology and bootstrap searches were run as described above with INAASE recoding. We also performed ML topology and bootstrap searches as described above (without secondary structure or PICS-Ord) but using RAxML-HPC-MPI-SSE3 7.2.8a compiled from source on CentOS. ML analyses were run both with a backbone constraint and without one. For the backbone constraint, we used the 50% majority-rule tree for the 2-gene Phycas inference in which polytomies were allowed; this constraint tree contained all the nodes (and only the nodes) that were supported with ML-BP $\geq$ 70% in at least one of the 2-gene RAxML analyses presented. The bootstrap bipartitions were then mapped to the best tree from the topology search using RAxML-HPC 7.0.4 compiled on CentOS and the resulting tree was visualized with FigTree 1.3.1 (Rambaut 2009).

Data archiving. – All physical voucher specimens examined for this study have been deposited in the herbarium of The New York Botanical Garden (NY) or were distributed as

duplicates. An electronic data record for each NY specimen was added to the C.V. Starr Virtual Herbarium (http://sciweb.nybg.org/science2/VirtualHerbarium.asp) and cross-referenced with the physical voucher via a unique identifier number (barcode). Georeferenced locality and habitat data were incorporated into the electronic record, as was complete identification history. Using NY's modular database EMu, all digital media (e.g. micrographs and sequence data) were also appended to the relevant records. Aliquots of total extracted genomic DNA were also incorporated into the NY genomic DNA collection. Electronic records for each aliquot were created in EMu and cross-referenced via a different set of barcodes. These e-records were then also linked to the e-records for the physical vouchers. Nucleotide sequences were deposited in GenBank (see Appendix 1). Alignments and analysis files (e.g., secondary structure models, Perl scripts, Phycas scripts, tree files, etc.) are available through the Dryad Data Repository (http://datadryad.org/).

Results and Discussion

Taxonomic status of C. reptans

The phylogenetic analyses presented here place the new species within the family Teloschistaceae. Although the species was not originally hypothesized to be a member of this family, morphological characters are congruent with this placement. The species morphologically would be classified in *Caloplaca*, in either the subgenus *Pyrenodesmia* or the *C. cerina*-group, because it is crustose and lacks anthraquinones in all thalline tissues; however, placement in either group is equivocal at present due to confused and conflicting accounts of their circumscriptions in the literature (Vondrák et al. 2008a, 2008b; Šoun et al. 2011). Clearly, the super-specific taxa as currently defined in *Caloplaca* and Teloschistaceae neither correlate

with discrete suites of non-molecular characters nor sufficiently accommodate the diversity within the family.

A major taxonomic revision is underway that will entail large-scale re-delimitation of the genera within this family (Gaya et al. 2010). The results of molecular work associated with these studies will help to determine the accuracy of the current concepts of genera and species groups on a larger scale. As far as generic concepts, the genus *Caloplaca*, as currently circumscribed, is the only one in the family that could satisfactorily accommodate the combination of chemical and morphological characters seen in the new species. Although it is likely that certain members of *Caloplaca* used in this study will find a home in other genera based on molecular phylogenetic analyses (e.g., of the species used in this study, *C. flavorubescens*, *C. marina*, and *C. microthallina*), likelihood-based analyses reconstruct a clade (albeit weakly supported) containing a large set of species currently classified in *Caloplaca* that includes the type species (*C. cerina*) and the species in question (*C. reptans*), making it unlikely that the species will be shifted elsewhere.

Despite the fact that this species fits within the current concept of *Caloplaca*, it is worth noting that, even in hindsight, a placement within Physciaceae (specifically, the genus *Rinodina*) would not be unreasonable based on traditional morphological and chemical characters. Indeed, this study reinforces the necessity of using additional data for the placement of species that lack the sexual characters typically used for placing species within a higher-level taxonomic framework.

An approach for addressing the taxonomically neglected sterile crustose lichens

It is remarkable that the power of molecular data has not been harnessed broadly for the purpose of classifying sterile crustose lichens. Despite the widespread use of molecular data in lichen systematics, there remain few published cases of these data being used in this manner (Lendemer and Lumbsch 2008; Kantvilas and Lumbsch 2010). Although researchers are beginning to use molecular data to address the so-called "hidden diversity" of cryptic taxa (e.g., Aletermann 2004; Argüello et al. 2007; Vondrák et al. 2009), this is conceptually distinct from the approach taken here because sterile crustose lichens represent a visible component of the biota that has simply been neglected.

Indeed, our poor knowledge of this group is somewhat paradoxical. The species are typically easily recognizable using correlated non-molecular characters (e.g., morphology and chemistry). Nonetheless, the vast majority remain incompletely understood because they cannot be placed with certainty into a higher level systematic framework. We believe that the approach used here to place *Caloplaca reptans* in a family and genus can be applied to all sterile crustose lichens and presents a new way to make taxonomic progress in this group. There is an urgent need for such progress because sterile asexually reproducing lichens can represent up to half of the total lichen biodiversity in a given biota. To date, this large component of biodiversity has been largely neglected and thus excluded from dialogues of taxonomy and conservation.

Phylogenetic implications

Although no true conflict between highly-supported nodes could be detected in the present set of analyses, maximum parsimony methods analogous to the ones used previously to reconstruct relationships in the family Teloschistaceae (Gaya et al. 2003, 2008, 2011) give a well-supported reconstruction of the placement of *Seirophora* (i.e., sister to *C. cerina*) that is not

supported by likelihood-based analyses (figures. 44–46). Parsimony-based analyses are known to produce spurious results when there are heterogeneous rates of nucleotide substitution (Kolaczkowski and Thornton 2004; Spencer et al. 2005); the most frequently recognized symptom of this is termed 'long-branch attraction' (LBA; Felsentein 1978; Swofford et al. 2001; Anderson and Swofford 2004). While it is not obvious that LBA is a problem in this particular situation, the problem of heterogeneity may well still apply. Therefore, despite high MP-BP support, the results showing the species of *Seirophora* as more closely related to *C. cerina* than they are to *C. reptans* should be treated with skepticism. It is worth noting that the use of INAASE reinforced the MP results (i.e., MP-BP was higher for the node uniting *Seirophora* and *C. cerina*). Future studies are needed to determine whether certain recoding schemes (or recoding schemes in general) may sometimes serve to reinforce biases in parsimony-based analyses due to heterogeneous rates of nucleotide substitution.

The analyses presented here also demonstrate the weakness of polytomy-free Bayesian analyses. In the two Phycas analyses conducted on the two-gene data set, the support for nodes was consistently lower when polytomies were allowed, indicating that analyses without polytomies result in support values that are consistently inflated. This would apply to all analyses with the popular program MrBayes (Huelsenbeck and Ronquist 2001). The major problem with polytomy-free Bayesian analyses that leads to inflated support is often illustrated by the so-called 'star-tree paradox,' which is that an analysis of a data set in which there are no data to resolve relationships between taxa (i.e., one in which the resultant phylogeny should always be a 'star-tree' comprised of a single large polytomy) will invariably produce a set of trees that have resolutions that are arbitrary (Lewis et al. 2005).

Since both parsimony-based methods and polytomy-free Bayesian methods have specific weaknesses that artificially inflate support values in specific types of situations, we call into question the results of studies that evaluate nodal support using only these methods (Kolaczkowski and Thornton, 2009). Relationships previously reconstructed within Teloschistaceae using only these two basic approaches (e.g., by Gaya et al. (2003, 2008), Muggia et al. (2008), and Vondrák et al. (2008a, 2008b, 2009)) are therefore suspect, especially given that both rapid evolutionary radiations and heterogeneous rates of nucleotide substitution are likely to obscure the phylogeny of this family.

Taxonomic Section

***Caloplaca reptans* Lendemer & Hodkinson sp. nov.**

Mycobank #564050.

Figure 47.

TYPE: **U.S.A. PENNSYLVANIA.** HUNTINGDON CO.: Rothrock State Forest, 2.vii.2008, *J.C. Lendemer et al. 11745* (NY! [DNA isolate NY178], holotype; isotypes to be distributed as *Lichens East. N. Amer. Exs. X: 446* to B!, BG!, CANL!, FH!, GZU!, H!, KANU!, LD!, M!, MIN!, NDA!, S!, TU!).

Description. – *Thallus* crustose, sorediate, areolate, gray to gray-green (in deep shade) or gray-brown (exposed to the sun). *Areoles* dispersed often becoming continuous and then overgrowing each other to form a thick crust, highly variable in diameter, 80–150 µm thick, upper surface glossy, epruinose, margins minutely lobate. *Soralia* discrete, laminal, becoming excavate as evacuated by soredia. *Soredia* ecorticate, globose, *28*–35–*42* µm in diameter (*n*=41),

comprised of an algal core surrounded by single layer of fungal hyphae, pigments typically absent. *Cortex* 10–20 µm thick, paraplectenchymatous, gelatinized and comprised of one to several layers of thin-walled hyphae with cells 5–10 µm wide, uppermost layer of hyphae variably pigmented reddish-brown (K+ dark brownish-purple). *Medulla* variable in thickness, composed of photobiont cells amid loosely packed paraplectenchymatous thin-walled hyphae. *Apothecia* unknown in a mature state, deformed/immature apothecia indicate the presence of a gray thalline margin and reddish-brown apothecial discs that lack anthraquinones. *Pycnidia* infrequent to common, superficial, globose to ± ellipsoid, ~150–250 µm wide; wall paraplectenchymatous, 15–30 µm thick, brownish-red pigmented. *Conidia* hyaline, bacilliform, 3.2–*3.8*–4.5 × 1.0–*1.2*–1.4 µm (*n*=20). *Photobiont* coccoid, cells globose, *8.6*–10.–*12.0* µm in diameter (*n*=22).

Chemistry. – No substances detected, all spot tests negative.

Ecology and distribution. – Endemic to the Appalachian Mountains in eastern North America where it is widespread and common (figure 48) in moist, humid habitats, occurring on non-calcareous sheltered face rock faces and in rock overhangs.

Etymology. – "reptans" refers to the margins of the thallus that appear to creep across the substrate.

Notes. – *Caloplaca reptans* is morphologically most similar to its congeners that have gray thalli and lack anthraquinones, namely members of the *C. cerina*-group and the subgenus

Pyrenodesmia. Indeed, molecular phylogenetic analyses (figure 46) corroborate this hypothesis and place the new taxon within a largely unsupported clade containing members of these groups. Although species that reproduce through lichenized diaspores are known to occur in the aforementioned groups, none share the set of character states expressed in *C. reptans* (Vondrák et al. 2008a; Šoun et al. 2011)

In most keys to *Caloplaca* that include sterile species with gray thalli (e.g., Wetmore 1966; Wirth 1966; Fletcher and Laundon 2009), the new species would key to *C. teicholyta* (Ach.) J. Steiner on account of its sorediate thallus that appears to be marginally lobate. In addition to significant differences in molecular characters (note that the ITS sequence for the species is not amongst the top 20 BLAST hits used in the ITS-only phylogenetic analyses), *C. teicholyta* differs from *C. reptans* in having a much larger, truly placodioid thallus, coarse soredia that are formed in diffuse soralia rather than fine soredia formed in discrete soralia, and occurring on calcareous rather than non-calcareous rocks. *Caloplaca teicholyta* is also not known to occur in North America (Esslinger 2011).

The areolate thallus of *Caloplaca reptans* somewhat resembles some forms of *C. chlorina* (Flot.) Sandst., which differs in the production of anthraquinones in the apothecial disc and blastidia that bud from the thallus surface rather than soredia formed in discrete laminal soralia (Fletcher and Laundon 2009). Confusion is also possible with *C. lecanoroides* Lendemer, a corticolous species known only from the Sierra Nevada of western North America. It differs from *C. reptans* in its ecology as well as in having distinctly cupuliform soralia with bright green soredia (Lendemer et al. 2010). *Caloplaca obscurella* (J. Lahm *ex* Körb.) Th. Fr. and *C. ulcerosa* Coppins & P. James are other sorediate species with gray thalli that lack anthraquinones (Fletcher and Laundon 2009). In those species, which are both corticolous, the thallus is

composed of minute elobate areoles. *Caloplaca soralifera* Vondrák & Hrouzek is another sorediate species with a gray thallus; however, it typically occurs on calcareous rocks and the thallus is composed of elobate white pruinose areoles that dissolve into dark, almost black, soredia (Vondrák and Hrouzek 2006). *Caloplaca yuchiorum* Lendemer & C.A. Morse, recently described from non-calcareous rocks in southern North America (Lendemer and Morse 2010), differs from *C. reptans* in having a continuous thallus with marginal soralia and in the production of atranorin. In summary, *C. reptans* is not likely to be confused with any of the known sorediate *Caloplaca* species with gray thalli that lack anthraquinones. Similarly there are no other North American sorediate crustose lichens placed in other genera, including *Rinodina*, the previously hypothesized genus for the species in question (for a taxonomic account of all North American species of *Rinodina*, see Sheard (2010)), that are likely to be confused with this taxon (for summary keys of North American sterile crustose lichens, see Lendemer (2010b)).

Selected specimens examined (all from non-calcareous rocks in shade or overhangs). – **U.S.A. ALABAMA**. JACKSON CO.: Pisgah, 2.x.1999, *W.R. Buck 36328* (NY). **GEORGIA**. RABUN CO.: Tallulah Gorge State Park, 11.xi.2010, *S.Q. Beeching 10961* (NY). TOWNS CO.: Hightower Gap, 11.xi.2007, *J.C. Lendemer 10981* (NY). WHITE CO.: Hogpen Gap, 4.xii.2010, *S.Q. Beeching 11013* (NY). **MASSACHUSETTS**. BERKSHIRE CO.: Mt. Greylock Reservation, 8.v.1995, *W.R. Buck 27785* (NY). **NEW YORK**. ULSTER CO.: Town of Shandaken, 13.ix.2008, *J.C. Lendemer 14027* (NY), *J.C. Lendemer 14036* (NY, DNA isolate NY176). **NORTH CAROLINA**. CLAY CO.: Nantahala National Forest, 17.ix.2006, *J.C. Lendemer 7740* (NY). SWAIN CO.: Great Smoky Mountains National Park, 6.viii.2009, *J.C. Lendemer 19252* (NY). MITCHELL CO.: Carver's Gap, 23.vi.2011, *B.P. Hodkinson 11942*

(NY; DUKE). **PENNSYLVANIA**. BRADFORD CO.: State Game Lands No. 12, 18.v.2009, *J.C. Lendemer 17397* (NY). CARBON CO.: Hickory Run State Park, 19.v.2009, *J.C. Lendemer 17505* (NY). CENTRE CO.: Bald Eagle State Forest, 14.ix.2010, *J.C. Lendemer 25569* (NY). ELK CO.: Moshannon State Forest, 31.viii.2010, *J.C. Lendemer 23949* (NY). JEFFERSON CO.: Clear Creek State Forest, 8.ix.2010, *J.C. Lendemer 24894* (NY). LACKAWANNA CO.: Merli-Sarnoski County Park, 17.vii.2008, *J.C. Lendemer 13206* (NY). LANCASTER CO.: Scalpy Hollow Rd. & River Rd., 29.vii.2009, *J.C. Lendemer 18746* (NY). MONROE CO.: Delaware Water Gap National Recreation Area, 17.ix.2005, *J.C. Lendemer 4977* (NY). PIKE CO.: Delaware Water Gap National Recreation Area, 23.iv.2004, *J.C. Lendemer 2702* (NY). SULLIVAN CO.: Ricketts Glen State Park, 19.ix.2010, *J.C. Lendemer 25662* (NY). TIOGA CO.: Tioga State Forest, 12.v.2009, *J.C. Lendemer 16509* (NY). WAYNE CO.: State Game Lands No. 57, 21.vii.2010, *J.C. Lendemer 13666* (NY). WESTMORELAND CO.: Powdermill Run Nature Preserve, 20.x.2009, *J.C. Lendemer 19849* (NY). WYOMING CO.: Bowmans Creek Ledges, 20.vii.2008, *J.C. Lendemer 13551* (NY, DNA isolate NY177). YORK CO.: State Game Lands No. 83, 9.viii.2009, *J.C. Lendemer 19386* (NY). **TENNESSEE**. SEVIER CO.: Great Smoky Mountains National Park, 23.vi.2010, *E. Tripp 977* (NY). **WEST VIRGINIA**. FAYETTE CO.: New River Gorge, vii.2010, *D. Clark s.n.* (NY). POCAHONTAS CO.: Monongahela National Forest, 20.x.2007, *J.C. Lendemer 9910* (NY). TUCKER CO.: Blackwater Falls State Park, 23.ix.2001, *W.R. Buck 39092* (NY).

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	ITS		mtSSU	
Species	Accession No.	GenInfo Identifier	Accession No.	GenInfo Identifier
Caloplaca aractina	GU723409	296278782	-	-
	EU365845	164520639	-	-
	GU723408	296278781	-	-
	GU723413	296278786	-	-
	GU723414	296278787	-	-
	GU723415	296278788	-	-
Caloplaca cerina [FNM-183]	EU681283	197342682	EU680862	196174784
[FNM-185]	EU681284	197342683	EU680863	196174785
	HM538540	301030512	-	-
	EU365861	164520655	-	-
Caloplaca chlorina	HM538512	301030484	-	-
	HM538514	301030486	-	-
	HM538515	301030487	-	-
	HM538517	301030489	-	-
	EU365858	164520652	-	-
Caloplaca flavorubescens	AY143394	33304573	AY143403	33304587
Caloplaca haematites	GU723410	296278783	-	-
	GU723407	296278780	-	-
Caloplaca marina	EU563461	187371884	EU088309	158711867
Caloplaca microthallina	EU563464	187371887	EU088310	158711868
Caloplaca monacensis	HM538494	301030466	-	-
	HM538504	301030476	-	-
Caloplaca percrocata	HQ234598	311115503	-	-
Caloplaca reptans	**NY178**	-	**NY178**	-
Caloplaca sp.	HM538482	301030454	-	-
Caloplaca transcaspica	EU192156	158702450	-	-
Fulgensia fulgens [FNM-171]	EU681285	197342684	EU680864	196174786
[FNM-186]	EU681286	197342685	EU680865	196174787
Seirophora contortuplicata	EU639614	183585234	EU680953	196174875
Seirophora orientalis	EU681287	197342686	EU680952	196174874
Xanthomendoza borealis	EU681355	197342754	EU680943	196174865
Xanthoria elegans	AF279769	9964110	EU680922	196174844
Xanthoria sorediata	AY453647	41814643	EU680920	196174842

Table 9. GenBank accession numbers (GB) and GenInfo identifiers (GI) for the sequences used in the molecular phylogenetic analyses of this study.

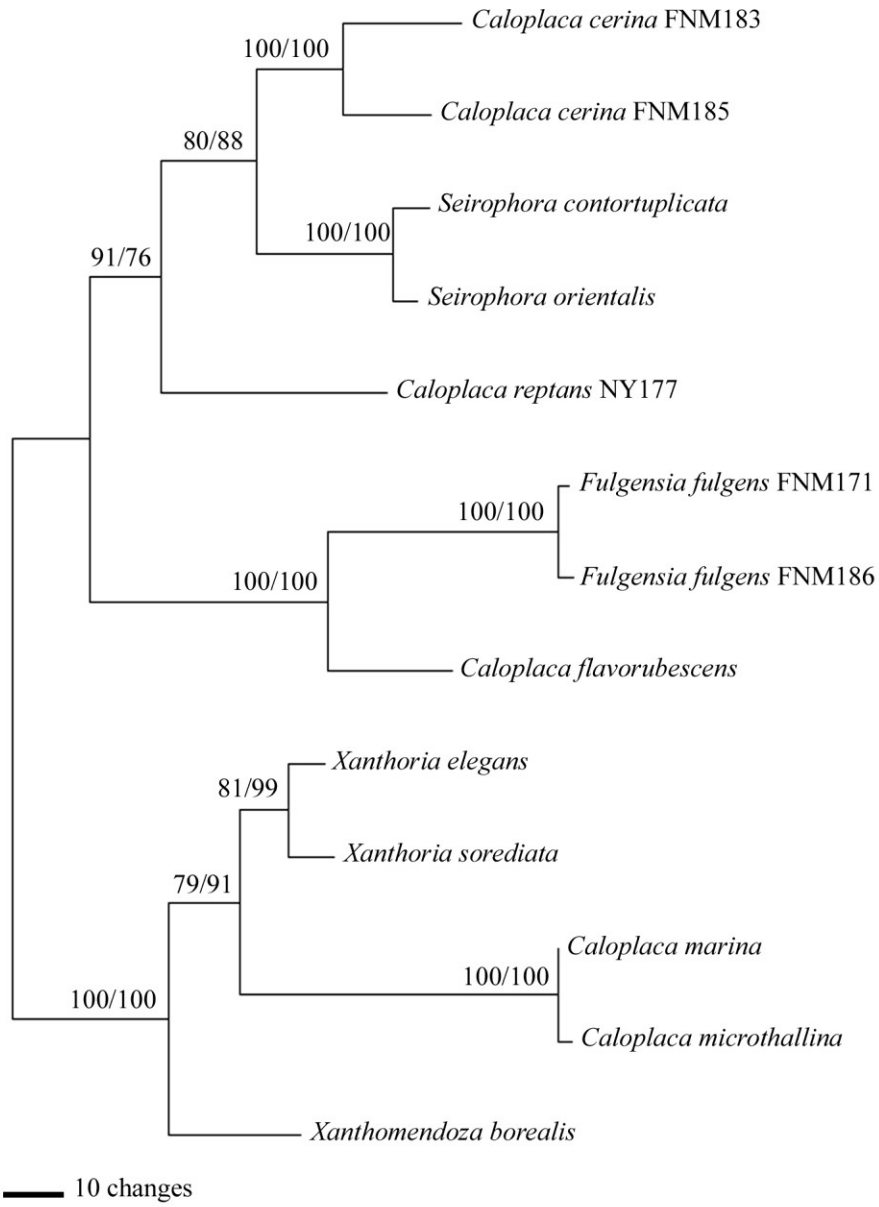

Figure 44. Weighted maximum parsimony (MP) phylogeny of Teloschistaceae including *Caloplaca reptans* based on a combined mtSSU/ITS sequence data set, inferred without ambiguously aligned (AA) regions recoded. MP-bootstrap proportions (BP) from 1000 resamplings of the dataset, both with and without AA-regions recoded using INAASE, are shown at each node as follows: MP-BP-noINAASE / MP-BP+INAASE).

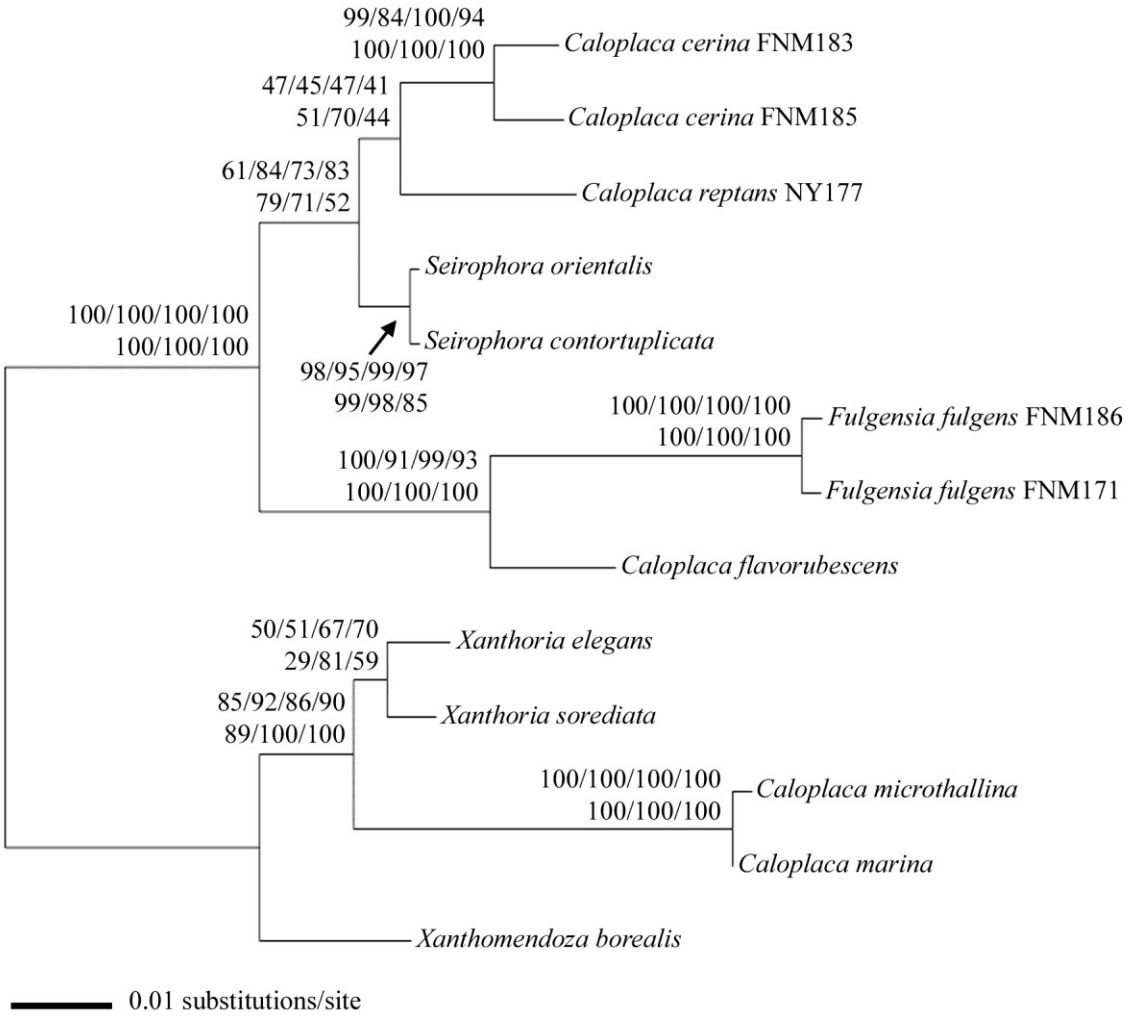

Figure 45. Maximum likelihood (ML) phylogeny of Teloschistaceae including *Caloplaca reptans* based on a combined mtSSU/ITS sequence data set, inferred without consideration of secondary structure or recoded ambiguously aligned (AA) regions. ML-bootstrap proportions (BP) from 1000 resamplings and Bayesian Posterior Probabilities (B-PP) are shown at each node. The upper values refer to ML-BP as follows: RAxML alone/RAxML+SecStr/RAxML+PICS-Ord/RAxML+SecStr+PICS-Ord. The lower values refer to B-PP as follows: MrBayes/Phycas-noPolytomies/Phycas+Polytomies.

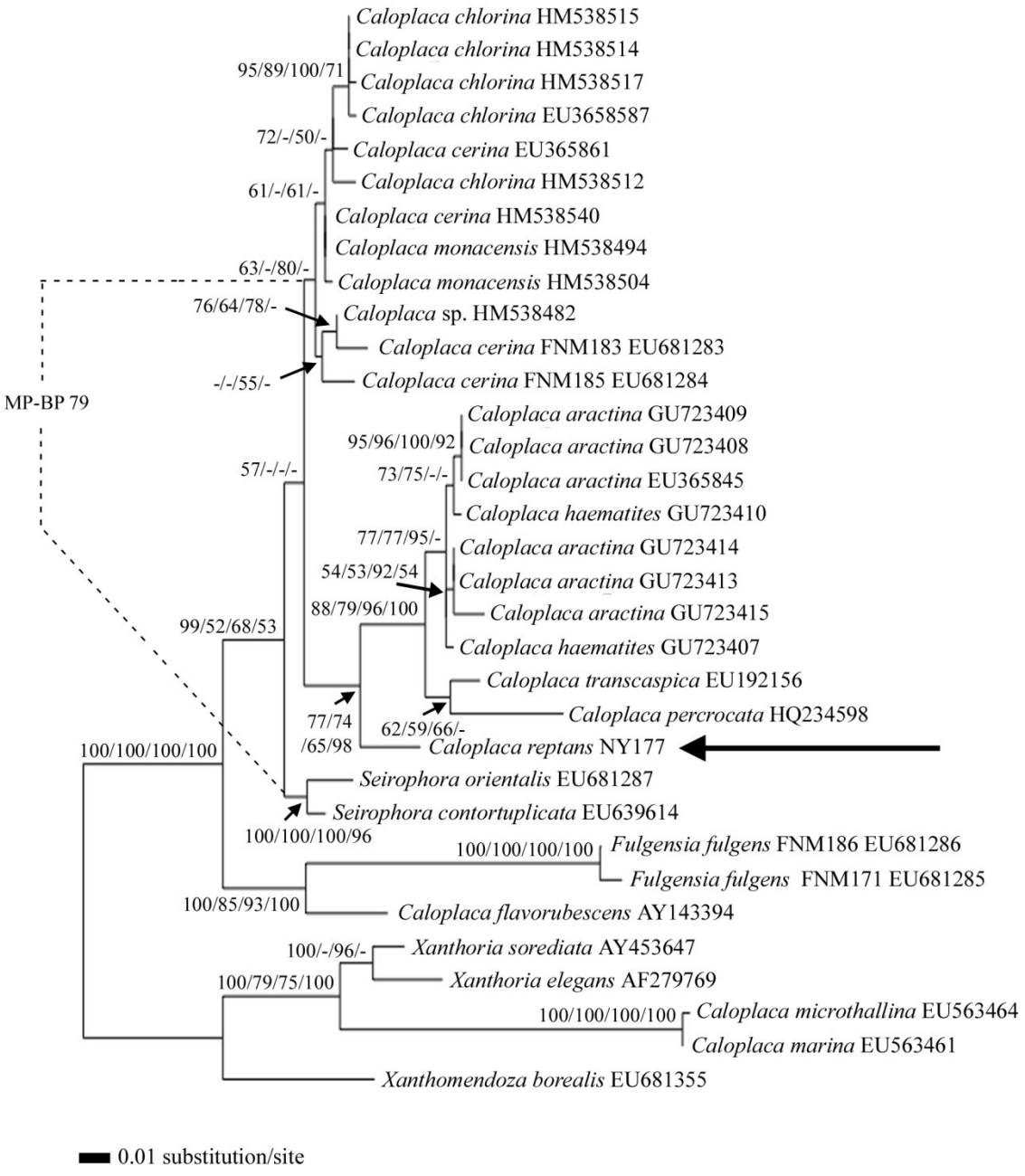

Figure 46. Constrained maximum likelihood (ML) phylogeny of Teloschistaceae including *Caloplaca reptans* and its 20 closest BLAST hits based on an ITS-only data set. ML and maximum parsimony (MP) bootstrap proportions (BP) from 1000 resamplings and Bayesian Posterior Probabilities (B-PP) are shown at each node as: ML-BP-constrained/ML-BP-unconstrained/MP-BP+INAASE/B-PP+Polytomies.

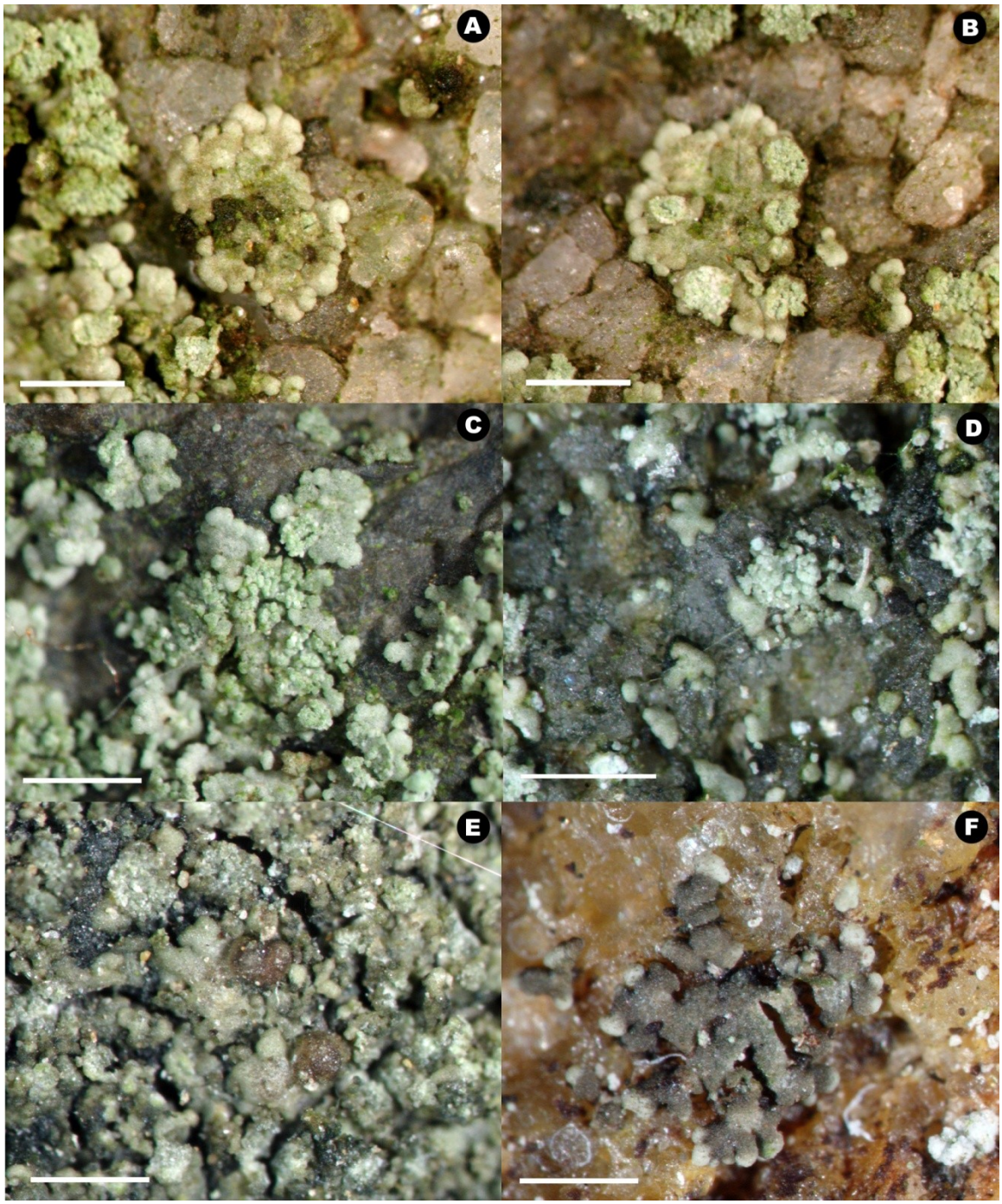

Figure 47. Morphology of *Caloplaca reptans* (all scales = 0.5 mm). A, young rosette forming thallus lacking soralia (*Lendemer 19849*). B, rosette forming thallus with discrete laminal soralia (*Lendemer 19849*). C, coalescent thalli with laminal soralia, some of which are superficially marginal (*Lendemer 25662*). D, dispersed areolate thalli, note laminal soralium on thallus in center (*Lendemer 25643*). E, deformed/aborted apothecia (*Lendemer 25569*). F, thallus with dissected margins that resemble lobes.

Figure 48. Geographic distribution of *Caloplaca reptans* as presently known.

CHAPTER X – GENERIC REALIGNMENTS FOR *LEPRARIA* AND *LEPROCAULON*

James C. Lendemer and Brendan P. Hodkinson

Abstract. – Results are presented for a series of studies that combine molecular phylogenetic analyses, utilizing both ITS and mtSSU sequence data, with morphological and chemical analyses of the diverse lineages nominally included in the sterile lichen genus *Lepraria*. 1) A core group of species representing *Lepraria* s.str. is recognized as a sister group to *Stereocaulon* in the family Stereocaulaceae. Species of *Lepraria* producing the secondary compounds argopsin, pannarin, and usnic acid are shown to belong to other lineages of lichen-forming ascomycetes. 2) Study of *Leprocaulon*, an enigmatic genus hypothesized to be related to *Lepraria*, revealed that all the described species, except for the type species, actually represent members of the *Lepraria* s. str. clade that have evolved a fruticose growth form. The correct name for the type species of *Leprocaulon* is shown to be *L. quisquiliare*, not *L. microscopicum*, and the genus is redefined to include several crustose leprose species previously placed in *Lepraria*. *Leprocaulon quisquiliare* is also shown to comprise two distinct, morphologically convergent species. The name is lectotypified and epitypified based on material from the type region (Germany) and its application restricted to populations from the Old World. Populations of *L. quisquiliare* from the New World are described as a new species, *L. americanum*. *Leprocaulon*, in its revised sense, is recognized in a new family (Leprocaulaceae) sister to the Physciaceae and including the genus *Halecania*. 3) A new genus in the family Pilocarpaceae, *Nelsenium*, is described to accommodate *Lepraria usnica*. 4) The status of *Lepraria ecorticata* is discussed in the context of several sequences of *Lepraria* not assigned to species that belong to strongly-supported clades containing members that have been placed in the genus *Lecanora*

based on sexual characters. The following nomenclatural novelties are proposed: *Lepraria albicans* comb. nov. (for *Leprocaulon albicans*), *L. arbuscula* comb. nov. (for *Leprocaulon arbuscula*), *L. congestum* comb. nov. (for *Leprocaulon congestum*), *L. gracilescens* comb. nov. (for *Leprocaulon gracilescens*), *L. pseudoarbuscula* comb. nov. (for *Leprocaulon pseudoarbuscula*), *L. subalbicans* comb. nov. (for *Leprocaulon subalbicans*), *L. tenellum* comb. nov. (for *Leprocaulon tenellum*); Leprocaulaceae fam. nov. (type genus *Leprocaulon* Nyl.), *Leprocaulon adhaerens* comb. nov. (for *Lepraria adhaerens*), *L. americanum* sp. nov. (for New World populations of *Leprocaulon quisquiliare*), *Leprocaulon coriense* comb. nov. (for *Lepraria coriensis*), *L. knudsenii* sp. nov. (for a leprarioid saxicolous taxon from western North America with usnic acid and zeorin), *L. santamonicae* comb. nov. (for *Lepraria santamonicae*), *L. terricola* comb. nov. (for *Lepraria terricola*), *L. textum* comb. nov. (for *Lepraria texta*); *Nelsenium* gen. nov. (type species, *N. usnicum*), *N. usnicum* comb. nov. (for *Lepraria usnica*).

Introduction

Lichen-forming fungi comprise more than 10% of ascomycete biodiversity and, together with mushroom-forming fungi, represent some of the most readily visible and publicly accessible groups of organisms studied by mycologists. Remarkably, despite their "high profile" nature, many aspects of lichen biology and taxonomy remain poorly understood and critically understudied. These two factors converge noticeably in a highly speciose assemblage of lichens that are referred to as sterile asexually reproducing crustose lichens, or more simply "sterile crusts." These taxa do not represent a monophyletic group, but rather are treated together because they have all evolved to reproduce primarily through the dispersal of lichenized diaspores. Lichenized diaspores are specialized dispersal units that include multiple members of the lichen ecosystem (i.e., fungal, algal, and bacterial components) (Arnold et al. 2009,

Hodkinson & Lutzoni 2009, Hodkinson et al. 2011), not only the primary fungal symbiont. Species that employ this mode of reproduction are typically sterile (i.e., do not produce sexual reproductive structures) and have evolved in nearly all the diverse lineages that comprise lichen-forming fungi. Little is understood about the selection pressures and biological processes that have led to the rampant repeated evolution of "sterile crusts" in lichens. Similarly, the species that reproduce in this manner are considered amongst the most taxonomically neglected and difficult groups of lichens. This is because they typically lack the sexual characters that are needed to conclusively elucidate their phylogenetic affinities within the broad context of lichen-forming fungi.

That the so called "sterile crusts" are so poorly understood is troubling for a multitude of reasons. Clearly, enhanced knowledge of their taxonomy and phylogenetic relationships would contribute towards understanding their development and reproductive biology. Such progress would almost certainly lead to sweeping new insights into processes of biology and speciation, not just in lichens but also in fungi as a whole. It is similarly distressing that we are in the midst of a biodiversity crisis, yet have only preliminary understanding of a group that can comprise as much as half the lichen diversity in a given biota.

In an effort to overcome the taxonomic impasse in this group, we developed a set of procedures to to phylogenetically place and phenotypically define sterile crustose lichens so that they can be formally described (Hodkinson & Lendemer in rev.). Concurrently, the first author undertook a revision of one of the most unusual members of this group, the lichen genus *Lepraria* Ach., as the topic of his dissertation. *Lepraria* represents a heterogeneous assemblage of approximately 60 sterile crustose species that have leprose thalli and are thought to be related to the genus *Stereocaulon* (Ekman & Tønsberg 2002; Lendemer 2011). The genus is noteworthy

because despite an apparent lack of sexual reproduction, the group has continued to diversify both chemically and morphologically (Fehrer et al. 2008).

The issues highlighted above also apply to, and are magnified in, the genus *Lepraria*. Although the genus is widely acknowledged to be polyphyletic, an alternative taxonomy has not been proposed in modern times. Also unresolved is the status of an enigmatic group of species with sterile fruticose thalli classified in the genus *Leprocaulon* Nyl. Here, we present the results of an extensive series of analyses aimed at finally resolving the circumscription of *Lepraria* s.l., and its relationship to *Leprocaulon*. This study has yielded a number of remarkable and surprising discoveries with significant biological implications and unexpected taxonomic consequences.

Materials and methods

Non-molecular materials and methods. – As in previous contributions on *Lepraria* s.l. by the first author, this study is based primarily on material in the herbarium of The New York Botanical Garden (NY), supplemented by material received on loan from the following herbaria: CANL, COLO, CONN, DUKE, FH, GZU, H, KANU, MIN, MSC, SBBG, UCR, and UTEP. This includes >1000 specimens collected by the first author during his extensive field studies of *Lepraria* throughout North America. To date these total 1.5 years in the field and span the period of August, 2007 to August, 2011. Through these field studies the author has been able to gather firsthand observations of ecology and morphology for all the species known to occur in North America.

Chemical and morphological analyses were carried out using the methodology outlined in detail by Lendemer et al. (2008) with the SEM methods as corrected by Lendemer and Elix (2010). For Leprarioid taxa, descriptive morphological terminology follows Lendemer (2011)

and measurements of granule size, photobiont cell size, and hyphal thickness are given as the average (xbar) ± 1 standard deviation (sd) and bounded by the extreme observed values, or [smallest observed]-(xbar-1sd)-xbar-(xbar+1sd)-[largest observed]. The number of observations (*n*) is also provided. For Leprocauloid taxa, descriptive morphological terminology follows Lamb and Ward (1974), and measurements are expressed as simple ranges.

Molecular data collection. – Subsamples used for extraction were specifically those that were used in upstream chemical analyses (TLC). Only those samples that exhibited no contamination in TLC analyses were used for downstream molecular analyses. DNA extractions were performed using the DNeasy Plant Mini Kit (Qiagen) with the instructions modified to include a prolonged (~12 hour) incubation period in lysis buffer. Isolated DNA was resuspended in 100 µl of sterile water and stored at -20°C. PCR amplification was performed using the primers ITS4 and ITS5 (White et al. 1990) and mtSSU1 and mtSSU3R (Zoller et al. 1999). Amplification reactions of 25 µl contained each of the following: 2.5 µl 10× PCR Buffer (Qiagen), 2.5 µl dNTP solution (mixed to a concentration of 2 mM of each dNTP), 2.5 µl BSA solution (mixed to a concentration of 2.5 mg μl^{-1} of bovine serum albumin; Hillis et al.), 0.2 µl Taq DNA Polymerase (= 1 U; Qiagen), 1 µl of each primer (in solution at a concentration of 10 µM), 9.3 µl of sterile water, 1 µl of extracted DNA, and 5 µl of Q-solution (Qiagen). PCR protocol for both genes followed Nelsen et al. (2008). PCR products were studied prior to sequencing by UV examination of a 1% agarose gel on which 1 µl of amplified PCR product had been subjected to electrophoresis and stained with ethidium bromide. Unpurified amplified PCR products were sent to the University of Washington Biochemistry DNA Sequencing Facility (BSDF) for sequencing. Sequences were assembled and edited by hand using the software

package Sequencher™ 4.9 (Gene Codes Corporation, Ann Arbor, MI, USA). In order to identify contaminants, nearest sequence matches were found by searching the nucleotide collection in GenBank using BLASTn (Altschul et al. 1997).

Taxon Sampling. – The goals for this study were to 1) to examine the affinities of the diverse lineages that comprise *Lepraria* s.l., 2) to circumscribe *Lepraria* s. str., and 3) to resolve the taxonomy and systematic placement of any taxa found to have affinities outside of *Lepraria* s. str. To address the above questions we sampled extensively throughout *Lepraria* s.l., generating 140 new mtSSU and 21 nrITS sequences (note some of the latter were used in other simultaneously published studies). In addition to sequences of *Lepraria* s.l., we generated sequences of other taxa with sterile leprose thalli (e.g., *Lecanora thysanophora* R.C. Harris), taxa considered to have phylogenetic affinities to the *Lepraria* s.l. (i.e., species of *Leprocaulon* Nyl.), unusual taxa that we considered potentially affiliated with *Lepraria* s.l. (e.g., *Lecanora phrygnitis* Tuck.), and crustose species of the sister group to *Lepraria* s.l. (i.e., *Stereocaulon nivale* (Follmann) Fryday).

In order to determine the affinities of the newly generated sequences, and circumscribe clades for subsequent more detailed revision, we integrated the above sequences into the Lecanoromycetidae dataset of Schmull et al. (2011). This dataset includes diverse taxa from the lineages that comprise the Lecanoromycetidae. We also downloaded all available sequences of *Lepraria* species from GenBank.

Sequence alignment and phylogenetic analyses

i. Mapping the phylogenetically dispersed representatives of Lepraria *s.l.* – In order to ascertain the placement of the diverse lineages that comprise *Lepraria* s.l., we used RAxML 7.2.6 to map the sequences newly generated for this study onto the Lecanoromycetidae phylogeny generated by Schmull et al. (2011). First, the nucleotide alignment associated with that study was downloaded from the second author's website: http://sites.google.com/site/brendanhodkinson/publications/Schmull_et_al_2011_Lecidea_213.nex (a similar, reformatted version of the alignment is stored in TreeBASE (http://treebase.org/) under study 11062 (http://purl.org/phylo/treebase/phylows/study/TB2:S11062)). All non-mtSSU sites were manually pruned out of the alignment, along with taxa not represented by sequences from the mtSSU region, using Mesquite 2.0 (Maddison & Maddison 2007). (All other alignment manipulations discussed in this section were performed with this program unless otherwise noted.) The mtSSU sequences generated for this study, in addition to the sequences of *Lepraria* and *Leprocaulon* from GenBank, were then aligned with those in the pruned dataset using the online interface of MAFFT v.6 (Katoh 2011). Ambiguously aligned regions were then excluded by defining them as part of an exset. Prior to exporting the alignment several sequences were removed from the dataset as follows: 1) four sequences of *Lepraria coriensis* (EU601743-6) and one of *L. usnica* (EU601747) generated by Nelsen et al. (2008) were excluded due to their extremely short length, 2) sequences of *Buellia punctata*, *Myochroidea porphyrospoda*, *Pseudocyphellaria crocata*, and *Xanthoparmelia conspersa* were excluded because they were included in the Schmull et al. (2011) alignment but excluded from the final tree included with the alignment file. The ambiguously aligned regions were then manually deleted and the alignment exported in extended phylip format for use with RAxML.

The Lecanoromycetidae topology published by Schmull et al. (2011) and included in the downloaded alignment file was then used as a reference tree to map the newly generated sequences for this study. For this procedure we utilized the final RAxML topology included in the alignment file published by those authors, with the taxa lacking mtSSU sequences pruned from the tree using Mesquite. The pruned tree was then saved as a separate file with a translation table, and manually converted to standard newick format for use in RAxML. It should be noted that in addition to the taxa listed by Schmull et al. (2011) as lacking mtSSU sequences in their dataset, we also pruned *Coccocarpia dominguensis* and *Lecidoma demissum* from the tree. This was because, in that work, although GenInfo identifiers were listed for mtSSU sequences of those taxa, they were not actually included in the alignment.

After completing the above, we used the alignment in phylip format, and the reference tree in newick format, as input files for RAxML and invoked the "-f y" command to map the sequences onto the tree. The results of this procedure were visualized in FigTree 1.2.3 (Rambaut 2009) and used to inform the taxon sampling of the series of analyses described in detail below.

ii. The Lepraria *s. str. clade*. – The majority of sequences generated for this study were mapped in a clade within the family Stereocaulaceae, corresponding to *Lepraria* s.str. To examine the relationships within sterile leprose members of this family we pruned the mtSSU data from "I" to only those sequences (newly generated and from GenBank) mapped in the families Stereocaulaceae and Cladoniaceae. All additional mtSSU sequences of *Stereocaulon* available in GenBank were also included (see Table 1) in the dataset. No ambiguously aligned regions were found in the alignment except at either end. These were excluded and any remaining terminal gaps were converted to missing "?" as were all polymorphisms/uncertainties.

A version of the alignment with the ends manually deleted was saved as an extended phylip file and used as the input for maximum likelihood (ML) analyses in RAxML. This consisted of a topology search and bootstrap analyses each with 1000 replicates using the model GTRGAMMA. The bootstrap proportions were then mapped to the best tree recovered from the topology search using the RAxML "-f b" command, and the resulting file was viewed in FigTree.

As part of its routine processes, RAxML produces a reduced alignment file from which all fully duplicative (i.e., identical) sequences have been pruned. The sequences from this reduced file were used as the basis to manually create a reduced nexus file from the original alignment in Mesquite. The reduced nexus file was then used as the input for another ML analysis as described above, as well as additional maximum parsimony (MP) and Bayesian inference (BI) analyses in PAUP* 4.0b10 and MrBayes 3.1.2 respectively. For MP analyses the ends and all constant sites were defined as part of an exset. An initial topology search was made with 1000 random-addition-sequence (RAS) replicates and tree bisection reconnection (TBR) branch swapping. The MULTREES option was in effect and zero-length branches were collapsed. All equally most parsimonious trees were saved with branch lengths. Bootstrap analyses were then conducted with 1000 replicates with 5 RAS per replicate and all other settings as above. The results were summarized in PAUP as a 50% majority rule consensus tree. For BI analyses MrModeltest (Nylander 2004) was run to select an appropriate model of nucleotide substitution. Using Akaike Information Criterion (AIC; Akaike 1973), GTR+I+G was selected. These analyses were performed using a MrBayes block produced through the online automated form found at the Santos Lab website (http://131.204.120.103/srsantos/mrbayes_form/index.html) and implemented with MrBayes

3.1.2 (Huelsenbeck & Ronquist 2001*)*. The ends of the alignment were defined as part of an exset. A general-time-reversible model of nucleotide substitution assuming a gamma-shaped rate variation with a proportion of invariable sites was implemented and the program's default priors were used. The Markov chain Monte Carlo parameters consisted of 10,000,000 generations, with four chains, and a tree sampled every 100 generations. The first 10,000 trees were discarded as burnin and the results were summarized as a 50% majority-rule consensus tree.

iii. The Halecania *clade*. – A group of sequences, mainly derived from western North American species of *Lepraria* s.l. whose chemistry is discordant with *Lepraria* s.str. (see results below) was mapped to the order Teloschistales in "I." Thus to resolve the placement of these sequences we pruned the dataset from "I" to include only members of the order Teloschistales (i.e., the newly generated sequences, Teloschistaceae, Physciaceae, Megalosporaceae and Letrouitiaceae). Three mtSSU sequences of *Lepraria coriensis* from GenBank were also added because Nelsen et al. (2008) had recovered them in a clade that was sister to Physciaceae (with members of Teloschistaceae being outside of the clade that included both *L. coriensis* and Physciaceae). The ambiguously aligned regions were then defined as part of an exclusion set (note this exclusion set differs from that of the dataset in "I"). This alignment was then used to prepare an extended phylip input file for use by RAxML as above in "I." The ML analyses were performed and results visualized as outlined in "II" above. The alignment was then also prepared for MP and BI analyses as outlined in "II." The analyses were also carried out in the same manner as above, using a complete rather than reduced alignment (GTR+I+G was selected by AIC and implemented for BI). It should be noted that although we included the mtSSU GenBank sequence of *Speerschneidera euploca* (Tuck.) Trevis. in the above analyses (since it had been

included in previous studies of *Lepraria* (Nelsen et al. 2008)), we excluded it from the taxonomic conclusions of this study. While MegaBLAST analyses of the sequences putatively derived from this sample reveal the similarity of the two sequenced mitochondrial loci to other sequences from members of the order Teloschistales, the nuclear LSU sequence (AY300862) is shown to have a 100% maximum identity to a sequence from a member of Ramalinaceae (AY756347), which is the family were *S. euploca* has traditionally been classified. Additional study is needed to confirm that a contaminant or chimera is not involved.

In order to examine the species-level relationships within this clade we also conducted an additional set of analyses using sequence data from the less conserved nuclear ITS region. For these analyses we compiled an alignment of ITS sequences from the same samples included in the mtSSU analyses above as well as additional accessions of each putative species. We also included sequences of *Lepraria adhaerens* based on prior results from BLASTn searches. After manual inspection and correction of the alignment produced by MAFFT we then defined all ambiguously aligned regions as part of an exclusion set. A subsequent series of MP, ML, and BI analyses were then carried out in the same manner as the mtSSU dataset (however HKY+G was selected by AIC and implemented for BI)

iv. The Lepraria usnica *clade*. – The sequence of *Lepraria usnica* from GenBank used in "I" above was mapped within the family Pilocarpaceae, a position already recovered as strongly supported by Nelsen et al. (2008). To confirm this placement, we pruned the alignment from "I" to only representatives of the Pilocarpaceae and the sequence of *L. usnica* used in "I." The procedures for creating the input files and running the ML, MP, and BI analyses were identical to those used above in "iii" (GTR+I+G was selected by AIC and implemented for BI).

v. The sequences mapped with members of Lecanora *s. str.* – In "I" above, two sets of sequences of *Lepraria* s.l. were mapped with sequences of *Lecanora* s.l. newly generated for this study. Unfortunately the phylogenetic relationships within the family Lecanoraceae remain poorly understood and resolved at present (Schmull et al. 2011). Although we speculate that future studies will lead to the division of this group, we attempted to confirm this placement of these sequences within *Lecanora* s.l. For these analyses we pruned the alignment from "I" to the sequences of Lecanoraceae and the sequences mapped in "Lecanora-1" and "Lecanora-2" in "I" above. We then prepared input files and conducted ML, MP, and BI analyses as above in "iii" (HKY+I+G was selected by AIC and implemented for BI).

Results and discussion

The results of the procedures and analyses performed for this study indicate that radical changes to the generic circumscription of *Lepraria* s.l. are needed. Concurrently, they provide remarkable new insights into two poorly understood aspects of the biology of lichen-forming fungi, namely the repeated evolution of asexual reproduction and divergent thalline growth forms. *Lepraria* has long been recognized as a polyphyletic assemblage of taxa, linked by their shared thalline macromorphology and asexual reproductive strategy (Ekman & Tønsberg 2002; Nelsen et al. 2008). However, the conceptual problems that have plagued the taxonomy of the group for the last decade, coupled with the lack of large scale molecular phylogenetic studies, have prevented previous workers from proposing an alternate taxonomy (see Lendemer 2011 for a summary). In this study, we not only confirmed the polyphyly of *Lepraria*, but used mtSSU sequence data to infer placement of the diverse lineages within the genus into four different families of lichen-forming fungi in the Lecanoromycetidae (figure 49). We then conducted

molecular phylogenetic analyses of mtSSU and ITS sequence data to resolve the genus level taxonomy of these lineages and finally arrive at a taxonomy for *Lepraria* s.l. that reflects the evolutionary history of its constituent taxa. Each of the four elements of *Lepraria* s.l. labeled in figure 49 is discussed in greater detail below.

I. The "*Lepraria* s. str. clade"

Our first step in resolving the taxonomy of *Lepraria* s.l. was to conduct molecular phylogenetic analyses of a dataset comprising the mtSSU sequences mapped within the Stereocaulaceae ("Lepraria s. str." in figure 49) together with the other representative sequences of that family from GenBank. These analyses revealed that a core group of *Lepraria* species, including the type of the genus, *L. incana* (L.) Ach., was consistently recovered as a monophyletic group sister to *Stereocaulon* (figure 50). In our analyses, this clade was poorly supported by ML and BI methods of inference; however, it was strongly supported by MP (MP: 90; ML: 57&54; BI: 0.64). Nonetheless, the monophyly of *Lepraria* has been strongly supported in previous studies of the Lecanoromycetes and is widely accepted (Miadlikowska et al. 2006; Schmull et al. 2011).

Our results indicate that while these two genera share a common ancestor, the crustose leprose growth form of *Lepraria* s.str. (figures 50A–B) is not likely derived from the fruticose growth form of *Stereocaulon* (figure 50D). In fact, early diverging members of *Stereocaulon* appear to comprise the taxa with a crustose growth form that were previously considered morphologically aberrant and placed in other genera or families. These were only recently recognized as members of *Stereocaulon* on the basis of shared sexual characters (Fryday & Coppins 1996, Fryday & Glew 2003, Timdal 2002). Large scale molecular phylogenetic studies

of *Stereocaulon* are desperately needed to resolve the deeper nodes within this clade. Such studies would likely shed more light on the evolution of different growth forms in the group.

Without a doubt, the most remarkable result of our analyses of mtSSU sequence data from the Stereocaulaceae is that atranorin-producing members of the fruticose asexually reproducing genus *Leprocaulon* were recovered as nested within the core clade of *Lepraria* s.str. (figure 50). In fact, the mtSSU sequences of these taxa were 100% identical to those of some members of the *L. neglecta* group. Interestingly, this relationship is affirmed when one considers that the granules of the *L. neglecta* group and the phyllocladia produced in these species of *Leprocaulon* both have a well-developed pseudocortex. Previous studies have revealed that the development of a pseudocortex on the granules of members of the *L. neglecta* group is a unique morphological synapomorphy within *Lepraria* (Lendemer in rev.b).

Although the above species of *Leprocaulon* have long been considered to belong to the Stereocaulaceae, and allied to *Lepraria*, their placement has nonetheless remained unresolved (Lamb & Ward 1974). Here we have shown that, in fact, these taxa are derived from *Lepraria* and represent an independent evolution of the fruticose growth form in the Stereocaulaceae. This derivation of a fruticose thallus (figure 50C) from a crustose thallus (figures 50A–B) in *Lepraria* clearly mirrors that of *Stereocaulon*. Based on the above, we here transfer the five atranorin-producing species of *Leprocaulon* to *Lepraria*. Surprisingly, the type species of *Leprocaulon*, *L. microscopicum*, actually belongs to a previously unrecognized lineage sister to the family Physciaceae in the suborder Physciineae (figure 51). This is discussed in further detail below.

As circumscribed here, *Lepraria* s. str. comprises the members of *Lepraria* s.l. that do not produce the secondary compounds argopsin, pannarin, and usnic acid. The disposition of the species that produce those substances is detailed below. The genus now also includes all the

members of *Leprocaulon*, except for the type species, *Leprocaulon microscopicum*. This species shares the chemistry of some of the species here excluded from *Lepraria* s.l. (e.g., *L. knudsenii* and *L. texta*) as it also produces usnic acid and zeorin.

In addition to revealing a monophyletic *Lepraria* s.str. (that now includes most members of *Leprocaulon*), our molecular phylogenetic analyses of mtSSU from Stereocaulaceae showed the *Lepraria* clade as a whole to have relatively short branch-lengths and a remarkably low degree of divergence between mtSSU sequences across the clade when compared with other groups of lichen-forming fungi (e.g., *Stereocaulon*). This result may be attributable to the fact that *Lepraria* s. str. is comprised entirely of asexually reproducing taxa (Schön et al. 1998). However, analyses including additional loci and a phylogenetically broader sampling of reference taxa are needed in order to investigate this possibility further. These preliminary results highlight the fact that the genus *Lepraria* holds great promise as a model system for studying the genetic/genomic consequences of long-term asexuality.

Taxonomic consequences

***Lepraria albicans* (Th. Fr.) Lendemer & Hodkinson comb. nov.**

Mycobank #54051.

Stereocaulon albicans Th. Fr., Stereoc. Pilophor. Comm. 36. 1857. TYPE: "in Peruvia, unde specimina attulerunt Haenke (Herb. Fries) et Gaudichaud (a Cel. Montagne benign communicatum)".

Leprocaulon albicans (Th. Fr.) Nyl. *in* Hue, Nouv. Arch. Mus. Hist. Nat., sér. 3, 4: 134. 1892.

Nomenclatural note. – The combination *Leprocaulon albicans* was not validly published by Hue (1890: 248) because he did not actually associate the generic and specific epithets. It was validated in the publication cited above.

***Lepraria arbuscula* (Nyl.) Lendemer & Hodkinson comb. nov.**

Mycobank #564052.

Stereocaulon arbuscula Nyl., Syn. Lich. 1: 253. 1860. TYPE: "In Himalaya, Sikkim, altitudine 9000–10000 pedum (in regione temperate), ex coll. J.D. Hook. et Thoms. no. 2160".

Leprocaulon arbuscula (Nyl.) Nyl. *in* Hue, Nouv. Arch. Mus. Hist. Nat., sér. 3, 4: 134. 1892.

Nomenclatural note. – The combination *Leprocaulon arbuscula* was not validly published by Hue (1890: 248) because he did not actually associate the generic and specific epithets. It was validated in the publication cited above.

***Lepraria congesta* (Nyl.) Lendemer & Hodkinson comb. nov.**

Mycobank #564053.

Stereocaulon congestum Nyl., Ann. Sci. Nat. Bot., sér. 4, 11: 210. 1859. TYPE: “in Peruvia, Casapi, D. Matthews, ex. hb. Hooker”.

Leprocaulon congestum (Nyl.) I. M. Lamb & A. M. Ward, J. Hattori Bot. Lab. 38: 519. 1974.

***Lepraria gracilescens* (Nyl.) Lendemer & Hodkinson comb. nov.**

Mycobank #564054.

Stereocaulon gracilescens Nyl., Ann. Sci. Nat. Bot., sér. 4, 11: 210. 1859. TYPE: “In Peruvia, prov. Carabaya, Wedd[ell].”.

Leprocaulon gracilescens (Nyl.) I. M. Lamb & A. M. Ward, J. Hattori Bot. Lab. 38: 523. 1874.

***Lepraria pseudoarbuscula* (Asahina) Lendemer & Hodkinson comb. nov.**

Mycobank #564055.

Stereocaulon pseudoarbuscula Asahina, J. Jap. Bot. 19: 282. 1943. TYPE: **JAPAN. HONSHU:** Prov. Musashi, Titibu, 1933, *Y. Asahina 1249* (CAN or TNS [hb. Asahina], lectotype).

Leprocaulon pseudoarbuscula (Asahina) I. M. Lamb & A. M. Ward, J. Hattori Bot. Lab. 38: 533. 1974.

Typificational note. – Lamb and Ward (1974) lectotypified the name *Stereocaulon pseudoarbuscula* using *Asahina 1249*, but did not state which of the two cited specimens was to be considered the lectotype. Since we have not examined either specimen we refrain from forming a "second-step" lectotypification here.

***Lepraria subalbicans* (I. M. Lamb) Lendemer & Hodkinson comb. nov.**

Mycobank #564056.

Stereocaulon subalbicans I. M. Lamb *in* Imshaug, Bryologist 60: 220. 1957. TYPE: **CHILE. COQUIMBO PROV.:** La Serena, Cerro Los Loros, 1940, ad terram nudam lectum, *R. Santesson 2530* (FH, holotype).

Leprocaulon subalbicans (I. M. Lamb) I. M. Lamb & A. M. Ward, J. Hattori Bot. Lab. 38: 534. 1974.

***Lepraria tenella* (Tuck.) Lendemer & Hodkinson comb. nov.**

Mycobank #564057.

Stereocaulon tenellum Tuck., U.S. Explor. Exped. 17: 123. 1862. TYPE: "near Lima, Peru," [Wilkes Expedition 1838–1842].

Leprocaulon tenellum (Tuck.) Nyl., Lich. Japon. 19. 1890.

II. The "*Halecania* clade"

As has been discussed above, the majority of species currently placed in *Leprocaulon* actually represent fruticose members of *Lepraria* s. str. A conclusion supported by both morphological and molecular characters. The genus *Leprocaulon* was described by William Nylander (*in* Lamy 1878). He did this to accommodate an unusual fruticose lichen that produces pseudopodetia that are similar to those of *Stereocaulon*, but which also lack the cephalodia typical of that genus and are covered with ecorticate granules rather than corticate phyllocladia (Lamb & Ward 1974). Clearly the absence of sexual reproductive structures coupled with the presence of ecorticate granules is what led Nylander, and many subsequent authors, to conclude this lichen was more closely related to *Lepraria* than *Stereocaulon*.

While this conclusion was largely accurate, our molecular phylogenetic analyses have revealed that *Leprocaulon microscopicum*, the type species of *Leprocaulon*, actually belongs to a heretofore unrecognized lineage (labeled "*Halecania*-clade" in figure 49; see figure 51 for detail). This same lineage was recovered in previous phylogenetic analyses that demonstrated it to be within the Teloschistales (and also illustrated that a placement within the Physciineae would be reasonable), by showing it to be part of a well-supported clade with the Physciaceae, while members of the Teloschistaceae were outside of that well-supported clade (Nelsen et al. 2008). However, the taxonomic significance of this was not previously recognized, likely because sample size was small and certain sequences were of suspect origin (e.g., those from *Speerschneidera euploca*). (Readers should note that throughout this section we use the name *L. microscopicum* to prevent confusion, but in the taxonomic section below and in the figures, we use the nomenclaturally correct name for this taxon, *L. quisquiliare*.) Previous studies have recovered *L. microscopicum* in a phylogenetic position outside of the Stereocaulaceae (i.e., unrelated to *Lepraria* s. str.) but have not resolved its placement (Ekman & Tønsberg 2002). Our

analyses recovered *L. microscopicum* in a strongly supported clade with several other members of *Lepraria* s.l. whose unusual secondary chemistries and morphologies have led authors to doubt their placement within that genus (Knudsen & Elix 2007, Lendemer 2010). The taxa that comprise this clade all have primarily Mediterranean distributions. They are also characterized by the production of usnic acid, argopsin, or pannarin – three substances that do not occur in *Lepraria* s.str. as circumscribed here.

Remarkably, the crustose leprose and sterile fruticose growth forms found in *Lepraria* s. str. as circumscribed here, are also found in the newly recognized clade containing *Leprocaulon microscopicum*. Our molecular phylogenetic analyses of ITS sequence data recovered seven distinct entities (six molecular clades and one isolated sequence) with their own specific combinations of morphological characters, chemical attributes and biogeographical affinities in this group (figures 52 & 53). Four have crustose leprose thalli (*L. adhaerens*, *L. knudsenii*, *L. santamonicae*, and *L. texta*), two have sterile fruticose thalli (*L. americanum* and *L. microscopicum* [= *L. quisquiliare*]), and one has an unusual modified crustose leprose thallus (*L. terricola*). The sterile fruticose thalli of *L. americanum* and *L. microscopicum* are morphologically similar to those species of *Leprocaulon* now placed in *Lepraria* (i.e., compare figure 50C with figure 53C–D & G).

Two of the aforementioned species are new to science and are formally described below. One of these, *Leprocaulon knudsenii*, is described to accommodate a crustose leprose species that commonly occurs on shaded non-calcareous rocks in California. The other, *L. americanum*, is a semi-cryptic species described to accommodate the New World populations of *L. microscopicum*, which our analyses have revealed is a distinct taxon likely restricted to the Old World (figures 51 & 52). (For detailed discussions of semi-cryptic species in lichen-forming

fungi, the reader should refer to the following publications (Lendemer 2011; Lendemer & O'Brien 2011; Lendemer in rev.).)

The above clade is referred to as the "*Halecania*-clade" (figure 49) because it was recovered in a strongly supported sister relationship to *Halecania alpivaga* (figure 51), a relationship confirmed by the discovery of lecanorine apothecia with *Halecania*-type asci and ascospores in several populations of *Lepraria adhaerens* and *L. santamonicae*. Here, we formally recognize the clade containing *Leprocaulon microscopicum* and its relatives, as well as *Halecania*, as a new family named Leprocaulaceae. It should be noted that *H. alpivaga* is the only member of *Halecania* with mtSSU sequence data available in GenBank, thus further study is needed to determine if all the members of that genus belong this lineage. It should also be noted that a similar relationship was recovered by Nelsen et al. (2008) when attempting to place *Lepraria coriensis* (here confirmed as a member of the "*Halecania*-clade" and combined into *Leprocaulon*) into a higher level taxonomic framework using mtSSU sequence data. Unfortunately, the limited taxon sampling within *Lepraria* s.l. coupled with the lack of *Leprocaulon microscopicum*, led those authors to question the validity of their findings due to a lack of shared morphological and chemical characters.

Taxonomic consequences

Leprocaulaceae Lendemer & Hodkinson fam. nov.

Mycobank #564058.

Type genus: *Leprocaulon* Nyl.

Diagnosis. – A morphologically diverse family sister to the Physciaceae that is comprised of primarily sterile, asexually reproducing lichens that produce pannarin, argopsin, and usnic acid. Fertile taxa have *Halecania*-type asci, lecanorine apothecia, and hyaline ascospores that resemble those of *Lecania*.

***Leprocaulon* Nyl. *in* Lamy,** Bull. Soc. Bot. France 25: 352. 1878.

Type species: *L. nanum* (Ach.) Nyl. (= *L. quisquiliare* (Leers) M. Choisy)

***Leprocaulon adhaerens* (K. Knudsen, Elix & Lendemer) Lendemer & Hodkinson comb. nov.**

Mycobank #564059.

Lepraria adhaerens K. Knudsen, Elix & Lendemer, Opuscula Philolichenum 4: 5. 2007. TYPE: **U.S.A. CALIFORNIA.** SAN DIEGO CO.: Torrey Pines State Park, 107 m, thin-soiled opening in maritime chaparral on sandstone bluffs, 13.iv.2005, growing over *Rinodina intermedia*, *Lepraria xerophila*, bryophytes, and soil, *K. Knudsen et al. 2700* (UCR!, holotype).

***Leprocaulon americanum* Lendemer & Hodkinson sp. nov.**

Mycobank #564060.

TYPE: **U.S.A. CALIFORNIA.** SAN DIEGO CO.: South Coast, Torrey Pines State Park, trail to Flat Rock, Del Mar Quad., alt. 15 m, Coastal strand, on sandy soil, 1.vi.2008, *K. Knudsen 9715* (NY!, holotype; UCR!, isotype).

Description. – **Thallus** dimorphic, primary thallus a persistent granular crust, and secondary thallus of fruticose pseudopodetia; **primary thallus** crustose, leprose, composed of granules dividing to form aggregations, eventually merging and forming a continuous crust of variable thickness (0.1–0.3 mm thick), yellow-green in color; **prothallus** absent or poorly developed; hypothallus absent; **rhizohyphae** absent; **granules** globose, 30–80 μm in diameter, ecorticate, discrete, often forming irregular compound units; **secondary thallus** of erect pseudopodetia with adherent ecorticate granules resembling those of the primary thallus; **pseudopodetia** solid, 0.2–0.8 cm tall, with a cord of gelatinized hyphae, typically simple though sometimes with irregular branching, initially opaque throughout and then with the lower portions turning dark blue-black with age; **hyphae** hyaline, 2–4 μm in width, septate, obscured by a thick layer of POL+ crystals that dissolve in KOH; **photobiont** green, coccoid, cells globose, 8–12 μm in diameter.

Chemistry. – Usnic acid and zeorin. Spot tests: K-, C-, KC+ golden yellow, P-, UV-.

Ecology and distribution. – The new species is rare but widely distributed in coastal regions with a Mediterranean climate along the Pacific Coast in the New World. It is often locally abundant, occurring on soil, rocks, and organic matter in sheltered rock microhabitats.

Discussion. – The name *Leprocaulon americanum* is here introduced to accommodate populations from the New World that were previously referred to *L. quisquiliare*. The molecular

phylogenetic analyses conducted for this study have shown that the name *L. quisquiliare* should be restricted to populations occurring in the Old World.

Selected additional specimens examined. – **CHILE. VIII REGIÓN DEL BÍO-BÍO.** ÑUBLE PROV.: Rada de Buchupuero, N end of Buchupuero, vicinity of Iglesia de Piedra, ~12.07 km N of Cobquecura, alt. 0–10 m, black sand beach with extensive volcanic remnants, 6.iv.2009, on soil, *J.C. Lendemer 15937-A & A. Moroz* (CONC, NY). **U.S.A. CALIFORNIA.** SANTA BARBARA CO.: Santa Rosa Island, Torrey Pine forest, along Torrey Pine Rd., alt. 26.7 m, 15.x.2006, on soil near edge of pine needle duff, *K. Knudsen 7432* (NY, UCR). VENTURA CO.: Santa Monica Mountains, N slope of Conejo Mountain, above Conejo Creek, alt. 99 m, conejo volcanics with coastal sage scrub, 12.i.2008, on soil on vertical rock walls, *J.C. Lendemer 11445 & K. Knudsen* (NY).

Leprocaulon coriense **(Hue) Lendemer & Hodkinson comb. nov.**

Mycobank #564061.

Crocynia coriensis Hue, Bull. Soc. Bot. France 71: 386. 1924. TYPE: **SOUTH KOREA:** Cheju-do ["Quelpaert"], Hongo, on moss in rocky depression, x.1906, *R. P. Faurie 645* (hb. B. de Lesdain(?), holotype destroyed(?); KYO [n.v.], isotype).

Lecanora coriensis (Hue) J. R. Laundon, Nova Hedwigia 76: 97. 2003.

Lepraria coriensis (Hue) Sipman, Herzogia 17: 28. 2004.

Leprocaulon knudsenii **Lendemer & Hodkinson sp. nov.**

Mycobank #564062.

TYPE: **U.S.A. CALIFORNIA.** ORANGE CO.: Peninsular Range, Santa Ana Mountains, lower Fremont Canyon, on slope of small side canyon, Black Star Canyon Quad., alt. 247 m, burnt chaparral, in sheltered crevice on sandstone outcrop, 3.xii.2007, *K. Knudsen 9256* (NY!, holotype; UCR!, isotype).

Description. – **Thallus** crustose, leprose, aggregated, discontinuous and not stratified, initially composed of isolated granules that divide to form aggregations, eventually merging to form a thin crust (<0.1–0.2 mm thick), yellow-green in color; **hyphae** hyaline, [1.5](–2.4–)2.5 (–3.5–)[4] µm (*n*=96) in width, septate, secund, obscured by a thick layer of POL+ crystals that dissolve in KOH; **prothallus** persistent; **hypothallus** absent; **rhizohyphae** absent; **granules** globose, [30]–(35)–45–(55)–[81] µm (*n*=96) in diameter, ecorticate, well organized and discrete, remaining distinct and not forming compound units; **photobiont** green, coccoid, cells globose, [6]–(9.6)–12.1–(14.6)–[20.4] µm (*n*=96) in width.

Etymology. – The new species honors Kerry Knudsen (b. 1950), a lichenological colleague who has worked extensively in southern California and described many of the species transferred here to *Leprocaulon*.

Chemistry. – Usnic acid and zeorin. Spot tests: K-, C-, KC+ golden yellow, P-, UV-.

Ecology and distribution. – The new species is widely distributed in the mountain ranges of central and southern California, U.S.A. It occurs on non-calcareous rocks in sheltered microhabitats including overhangs and shaded vertical faces.

Discussion. – *Leprocaulon knudsenii* is a sterile member of the Leprocaulaceae with a crustose leprose thallus that produces usnic acid and zeorin. Historically the species would have been placed in *Lepraria*, however,7 the molecular phylogenetic analyses conducted for this study indicate it that is a member of the genus *Leprocaulon* as delineated here. The only other member of *Lepraria* s.l. with an aggregate thallus that produces usnic acid and zeorin is *L. ecorticata* (J.R. Laundon) Kukwa. Based on the results presented here, we exclude *L. ecorticata* from western North America pending further study. As is discussed below (section IV) the application the name *L. ecorticata* is not well understood, and the phylogenetic affinities of the type specimen (from the United Kingdom) remain unknown.

Selected additional specimens examined. - **U.S.A.: CALIFORNIA.** MARIPOSA CO.: Yosemite National Park, S shore of the Merced River, alt. 535 m, foothill woodland, on rock, 20 Sep 2009, *J.C. Lendemer 19641* (NY). MARIN CO.: Mount Tamalpais State Park, S of parking lot on Panoramic Drive, alt. 602 m, scattered bay and oak wooded grassland surrounding serpentine barren, on shaded boulder, 29 Jan 2006, *J.C. Lendemer 5840 & K. Knudsen* (NY). RIVERSIDE CO.: San Bernardino National Forest, San Jacinto Mountains, N-facing slope above North Fork San Jacinto River, alt. 739 m, moist decomposing granite outcrops, on rock, 11 Jan 2008, *J.C. Lendemer 11482 & K. Knudsen* (NY). SANTA BARBARA CO.: Santa Cruz Island, Prisoners Canyon, alt. 14 m, *Quercus agrifolia* woodland in riparian habitat, on volcanic rock in

shade, 16 Jan 2007, on volcanic rock in shade, *K. Knudsen et al. 8582* (NY). VENTURA CO.: Santa Monica Mountains, S of Agoura Rd., Agoura Hills, alt. 226 m, moist rock outcrop along road, on rock, 12 Jan 2008, *J.C. Lendemer 11460 & K. Knudsen* (NY).

Leprocaulon quisquiliare **(Leers) M. Choisy**, Bull. Mens. Soc. Linn. Soc. Bot. Lyon 19: 166. 1950.

Lichen quisuiliaris Leers, Fl. Herborn. 264. 1775. TYPE: Micheli, Nova Plantarum Genera, tab. 53, fig. 8. 1729 (**illustration here selected as lectotype**). EPITYPE: **GERMANY. REINLAND-PFALZ:** Kreis-Cochem, Mosel Valley, just N of village of Pommern in vicinity of chapel, alt. 200 m, S-facing basic schist slope, 30.i.2010, *W.R. Buck 55960* (NY!, epitype **selected here**).

Stereocaulon quisquiliare (Leers) Hoffm., Deutschl. Fl. 2: 130. 1796.

Syn. *Lichen nanus* Ach., Lich. Suec. Prod. 206. 1798. TYPE: "Suecia", without date or collector (H-ACH 1757-A [photo!], **lectotype selected here**).

Stereocaulon nanum (Ach.) Ach., Meth. Lich. 315. 1803.

Leprocaulon nanum (Ach.) Nyl. *in* Lamy, Bull. Soc. Bot. France 25: 352. 1878.

Syn. *Lichen microscopicus* Vill., Hist. Pl. Dauphiné 3: 946. 1789. TYPE: Micheli, Nova Plantarum Genera, tab. 53, fig. 8. 1729 (**illustration here selected as lectotype**).

Stereocaulon microscopicum (Vill.) Frey, Rabenh. Krypt.-Fl., ed. 2, 9: 89. 1932.

Leprocaulon microscopicum (Vill.) Gams *ex* D. Hawksw. *in* Hawksworth & Skinner, Trans. Proc. Torquay Nat. Hist. Soc. 16: 128. 1974.

Nomenclatural note. – The name *Leprocaulon microscopicum* has been applied to this taxon since it was taken up by Lamb and Ward (1974) in their monograph of *Leprocaulon*. However, as these authors noted, the earliest name for this taxon is actually *L. quisquiliare* (Leers) M. Choisy (based on *Lichen quisquiliaris* Leers). Lamb and Ward (1974: 530) refrained from using *L. quisquiliare* because they considered the name a *nomen confusum*, largely on the basis of the fact that the protologue consisted of a mixture of elements and they had not reviewed any type material. These authors also indicated that *L. quisquiliare* would be proposed for formal rejection; however, no formal proposal appears to have been published and the name does not presently appear in any of the appendices of the *Code* (McNeill et al. 2006). As such, we here take up the name *L. quisquiliare*, select the original illustration published by Micheli (1729) as the lectotype, and select one of the vouchers used for sequencing in this study as an epitype to affix the name to a physical specimen that clearly represents the taxon as currently understood. Since then name *Lichen microscopicus* has not been previously typified, we have chosen to select the same Micheli illustration to serve as the lectotype of that name. Since *Lichen microscopicus* and *L. quisquiliare* are now based on the name type, they become obligate homotypic synonyms. While we could propose the name *L. microscopicum* for conservation, we assert that to do so would be a perversion of a system clearly intended to stabilize the nomenclature of species with economic importance or use in the applied sciences.

Leprocaulon santamonicae **(K. Knudsen & Elix) Lendemer & Hodkinson comb. nov.**

Mycobank #564063.

Lepraria santamonicae K. Knudsen & Elix, Bryologist 110: 115. 2007. TYPE: **U.S.A. CALIFORNIA.** LOS ANGELES CO.: eastern end of the Santa Monica Mountains, Giffith Park, 207 m, mesic chaparral mixed with coastal sage scrub, 27.xi.2005, on shaded W-facing vertical roadcut on shallow soil and mosses over rock, *K. Knudsen et al. 4380* (UCR!, holotype; NY!, isotype).

Leprocaulon terricola **(Lendemer) Lendemer & Hodkinson comb. nov.**

Mycobank #564064.

Lepraria terricola Lendemer, Brittonia 62: 285. 2010. TYPE: **U.S.A. CALIFORNIA.** SANTA BARBARA CO.: Santa Rosa Island, Channel Islands National Park, upper Cherry Canyon below Main Road, Santa Rosa Island North Quad., 245 m, bishop pines, manzanita, oaks, 26.x.2008, on shaded soil of ravine, *K. Knudsen 10606* (NY!, holotype; UCR!, isotype).

Leprocaulon textum **(K. Knudsen, Elix & Lendemer) Lendemer & Hodkinson comb. nov.**

Mycobank #564065.

Lepraria texta K. Knudsen, Elix & Lendemer *in* Knudsen & Elix, Lichen Flora of the Greater Sonoran Desert Region 3: 387. 2007. TYPE: **U.S.A. CALIFORNIA.** VENTURA CO.: Santa Monica Mountains, Party Rock, high above Carlisle Valley (National Park Service property), 698 m, north side of outcrop, 8.ii.2006, on Conejo volcanics, *K. Knudsen et al. 5153* (UCR!, holotype).

III – The "Pilocarpaceae-clade"

When Nelsen et al. (2008) used mtSSU sequence data to infer the higher level systematic placement of *Lepraria usnica* Sipman, they recovered the species as a member of the family Pilocarpaceae. In their analyses, this placement was strongly supported, and we again affirm this here (figure 54). The authors did not, however, formally describe a new genus to accommodate this taxon. Here we establish the genus *Nelsenium* and provide the necessary combination to place *L. usnica* in its proper phylogenetic context. Based on the available literature, *L. usnica* is a corticolous species that occurs in the Paleotropics (Sipman 2003). We have not studied this species in detail and assert that it should be included in any future revision of the species with usnic acid that were previously considered to belong to *Lepraria* s.l. (see sections "II" and "IV" for further discussion).

Taxonomic consequences

***Nelsenium* Lendemer & Hodkinson gen. nov.**

Mycobank #564066.

Type species: *N. usnicum* (Sipman) Lendemer & Hodkinson

Description. – The description of *Lepraria usnica* published by Sipman (2003) serves as the description for this unispecific genus.

***Nelsenium usnicum* (Sipman) Lendemer & Hodkinson comb. nov.**

Mycobank #564067.

Lepraria usnica Sipman, Biblioth. Lichenol. 86: 179. 2003. TYPE: **SINGAPORE:** Sembawan Park, on N-coast, alt. ca. 2 m, grassland with scattered trees and shrubs, on tree trunk (*Cassia fistula* 80 cm diam.) within reach from the ground, 25 Nov. 2000, *H. Sipman 46399 & B.C. Tan* (B [n.v.], holotype; SINU [n.v.], isotype).

IV – The "Lecanora-1" and "Lecanora-2" clades

As has been detailed above, all the usnic acid-producing species we have studied that are currently placed in *Lepraria* s.l. actually belong to other unrelated lineages in the Lecanoromycetes (figure 49). In addition to those taxa whose systematic position has been resolved within the families Leprocaulaceae and Pilocarpaceae, several sequences representing potentially undescribed species were mapped in clades containing members of the genus *Lecanora* Ach. (figure 49). Our additional molecular phylogenetic analyses including other representatives of the Lecanoraceae strongly support the conclusion that these species are all closely related to other species that have been placed in *Lecanora* based on morphological analyses of sexual characters (figure 55).

The aforementioned sequences represent populations of *Lepraria* s.l. (figure 56) from eastern North America that produce usnic acid and zeorin, as well as a population from California that produces only usnic acid. It is likely that many lichenologists would have applied names such as *L. ecorticata* (J.R. Laundon) Kukwa to these populations. We have refrained from doing so here because in recent years these names, particularly *L. ecorticata*, have been widely applied to biogeographically discordant elements that we suspect may be taxonomically heterogeneous (Kukwa 2006a). To further complicate matters, *L. ecorticata* was originally described as member of the genus *Lecanora* (Laundon 2003), and if it truly belongs in that

genus, then it needs to be revised in light of the many other *Lecanora* species with sorediate or leprose thalli that produce usnic acid. This is highlighted by the fact that mtSSU sequences of an unassigned *Lepraria* voucher were 100% identical to two newly generated reference sequences of *Lecanora thysanophora* R.C. Harris ("Lecanora-1" of Figures 1 and 7). That taxon is typically sterile, has a crustose leprose thallus, and also produces usnic and zeorin among other substances (Harris et al. 2000). Similarly, mtSSU sequences of two other unassigned *Lepraria* vouchers were recovered in a clade with *Lecanora phryganitis* Tuck. ("Lecanora-2" of figures 49 & 55). *Lecanora phryganitis* is an unusual species that is narrowly endemic to central California and has a fruticose thallus whereas the vast majority of *Lecanora* species have crustose thalli (Brodo et al. 2001).

While leaving the unassigned sequences of *Lepraria* s.l. recovered in the family Lecanoraceae as taxonomically unresolved is not ideal, we believe it represents the most pragmatic approach. This group clearly requires further study, and it would be unfortunate to make formal changes without a thorough understanding of the taxa involved. Similar actions in the past led to the current taxonomic and conceptual chaos in *Lepraria* that is only now being resolved (Lendemer 2011, 2011b, in rev., in rev.a, in rev.b).

Conclusions

The present study represents the culmination of nearly one decade of study of *Lepraria* s.l. by the first author, coupled with a half decade long collaboration between the authors to develop a set of molecular phylogenetic "best practices" to resolve the taxonomy of enigmatic sterile crustose lichens (Hodkinson & Lendemer in rev.). Here, we have initiated the disintegration of *Lepraria* s.l. into evolutionarily discrete units placed in their proper phylogenetic context within the higher level taxonomy of lichen-forming fungi. Using different

combinations of analyses of mtSSU and ITS sequence data, we have defined a restricted *Lepraria* s.str., uncovered a previously unrecognized family in the order Teloschistales, recircumscribed the enigmatic genus *Leprocaulon*, recognized a new sterile leprose genus in the family Pilocarpaceae, and highlighted a significant need for new studies of the family Lecanoraceae. These changes represent significant new insights into the occurrence of persistent sterility and evolution of asexual reproduction via lichenized diaspores in the lichen-forming ascomycetes. Concurrently, these evolutionary inferences have shed considerable light on the complicated story of the evolution of macroscopic growth forms in lichens. Particularly noteworthy is the recognition of the development of morphologically convergent sterile fruticose growth forms in two highly divergent lineages comprised otherwise of crustose leprose species. While the present study is certainly not the last word on *Lepraria*, we feel it represents a great step forward.

Acknowledgments

Bill Buck, Richard Harris, and Kerry Knudsen are thanked for reviewing drafts of the manuscript prior to submission and for their extensive discussion of various problems associated with this study. Kerry Knudsen is specifically thanked for encouraging the first author to pursue studies of the taxa now placed in *Leprocaulon*. Teuvo Ahti and Leena Myllys (H) are thanked for locating and digitizing the lectotype of *Leprocaulon nanum*. David Hawksworth is thanked for providing a copy of his 1974 publication in an obscure British serial. Fieldwork carried out by the first author during which specimens used in this study were collected was funded in part by The City University of New York (CUNY), The National Park Service (Great Smoky Mountains National Park), The National Science Foundation (NSF), The Southern Appalachian Botanical Society (SABS), The Torrey Botanical Society (TBS) and The Western Pennsylvania

Conservancy (WPC). Laboratory work and loan handling was facilitated by The New York Botanical Garden (NYBG) and funded in part by a grant from The California Lichen Society (CALS). In addition to the above funding sources, supplementary fieldwork and laboratory studies were made possible by a Doctoral Dissertation Improvement Grant from the National Science Foundation (NSF-DDIG Award DEB-1110433). During the course of this research, BPH was supported by the National Science Foundation under awards EF-1115086 (North American Lichens and Bryophytes), DEB-1011504 (A Phylogenetic Characterization of the Lichen Microbiome) and EF-0832858 (NIMBioS). Sean Beeching, Ernie and Fenja Brodo, Colin Freebury, Malcolm Hodges, Rob E. Lee, Jennifer Staniforth, Michael Sundue, and Signa and Floyd Williams, are thanked for facilitating permitting and other aspects of the first author's fieldwork. The Humboldt Research Institute in Steuben, Maine hosted the first author during his study there in summer of 2010. Finally, the curators and collections staff of the institutions cited in the materials and methods section are thanked for loaning specimens and answering various information queries.

	nrITS	mtSSU	Isolate	Colletion data	Voucher Barcode	Locality
Lepraria larrainiana		xxx	NY775	*Lendemer 15937*	NY-1219403	Chile, BioBio Prov.
Lepraria normandinoides		xxx	NY646	*Lendemer 19827*	NY-1133911	U.S.A. Pennsylvania
Lepraria normandinoides		xxx	NY544	*Lendemer 18674*	NY-1118078	U.S.A. Pennsylvania
Lepraria normandinoides		xxx	NY500	*Lendemer 16925*	NY-1106534	U.S.A. Pennsylvania
Lepraria oxybapha		xxx	NY888	*Lendemer 23199*	NY-1148377	U.S.A. Maine
Lepraria oxybapha		xxx	NY543	*Lendemer 18627*	NY-1117630	U.S.A. Pennsylvania
Lepraria oxybapha		xxx	NY199	*Morse 15061*	NY-973219	U.S.A. Oklahoma
Lepraria vouauxii		xxx	NY526	*Buck 55225*	NY-1136506	U.S.A. Utah
Lepraria xerophila		xxx	NY27	*Knudsen 7362*	NY-1133498	U.S.A. California
Lepraria xerophila		xxx	NY14	*Knudsen 8737*	NY-1133499	U.S.A. California
Lepraria vouauxii		xxx	NY668	*Lendemer 20381*	NY-1152850	U.S.A. Virginia
Lepraria vouauxii		xxx	NY1189	*Lewis 512*	NY-1222012	Canada, Ontario
Lepraria membranacea		xxx	NY1188	*Lendemer 28306-A*	NY-1223363	Canada, Quebec
Lepraria membranacea		xxx	NY1125	*Lewis 450*	NY-1218432	Canada, Ontario
Lepraira friabilis		xxx	NY1110	*Lendemer 26011*	NY-1218379	U.S.A. Arkansas
Lepraria cryophila		xxx	NY1119	*Lewis 565*	NY-1222227	Canada, Ontario
Lepraria xerophila		xxx	NY28	*Knudsen 7624*	NY-1133500	U.S.A. California
Lepraria normandinoides		xxx	NY924	*Lendemer 23681*	NY-1148496	U.S.A. New York
Lepraria oxybapha		xxx	NY1127	*Lendemer 25983*	NY-1199209	U.S.A. Arkansas
Lepraria vouauxii		xxx	NY1123	*Lendemer 26102*	NY-1218292	U.S.A. Arkansas
Lepraria vouauxii		xxx	NY936	*Harris 56396*	NY-1198796	U.S.A. Tennessee
Lepraria vouauxii		xxx	NY1162	*Lendemer 26899*	NY-1220426	U.S.A. North Carolina
Lepraria vouauxii		xxx	NY1155	*Tripp 1304*	NY-1220011	U.S.A. Alabama
Lepraria vouauxii		xxx	NY506	*Lendemer 16662*	NY-1106474	U.S.A. Pennsylvania
Lepraria vouauxii		xxx	NY505	*Lendemer 16686*	NY-1106451	U.S.A. Pennsylvania
Lepraria vouauxii		xxx	NY504	*Lendemer 16800*	NY-1106808	U.S.A. Pennsylvania
Lepraria vouauxii		xxx	NY571	*Lendemer 18816*	NY-1133339	U.S.A. North Carolina
Lepraria vouauxii		xxx	NY1122	*Lendemer 25956*	NY-1199233	U.S.A. Arkansas
Lepraria vouauxii		xxx	NY1124	*Lendemer 26025*	NY-1218366	U.S.A. Arkansas
Lepraria friabilis		xxx	NY662	*Lendemer 20283*	NY-1152714	U.S.A. North Carolina
Lepraria friabilis		xxx	NY13	*Lendemer 9063*	NY-974484	U.S.A. Alabama
Lepraria friabilis		xxx	NY653	*Lendemer 21753*	NY-1104342	U.S.A. Georgia
Lepraria friabilis		xxx	NY659	*Lendemer 20269*	NY-1152728	U.S.A. North Carolina
Lepraria friabilis		xxx	NY711	*Lendemer 21369*	NY	U.S.A. Georgia
Lepraria friabilis		xxx	NY669	*Lendemer 21344*	NY-1150273	U.S.A. Georgia
Lepraria hodkinsoniana		xxx	NY552	*Lendemer 18478*	NY-1107055	U.S.A. Pennsylvania
Lepraria hodkinsoniana		xxx	NY671	*Lendemer 20482*	NY-1152860	U.S.A. Virginia
Lepraria rigidula		xxx	NY577	*Lendemer 19707*	NY-1107540	U.S.A. California
Lepraria humida		xxx	NY519	*Harris 55530*	NY-1103934	U.S.A. Maine
Lepraria humida		xxx	NY307	*Lendemer 12457*	NY-977485	U.S.A. Pennsylvania
Lepraria humida		xxx	NY1241	*Lendemer 22848*	NY-1181036	U.S.A. Maine

Lepraria pacifica	xxx	NY651	*Buck 53979*	NY-1028759	U.S.A. California
Lepraria pacifica	xxx	NY590	*Lendemer 19568*	NY-1133223	U.S.A. California
Lepraria pacifica	xxx	NY599	*Lendemer 19545*	NY-1133185	U.S.A. California
Lepraria pacifica	xxx	NY588	*Lendemer 19566*	NY-1133225	U.S.A. California
Lepraria pacifica	xxx	NY589	*Lendemer 19560*	NY-1133234	U.S.A. California
Lepraria pacifica	xxx	NY600	*Lendemer 19524*	NY-1133207	U.S.A. California
Lepraria pacifica	xxx	NY601	*Lendemer 19562*	NY-1133230	U.S.A. California
Lepraria pacifica	xxx	NY320	*McCune 29887*	NY-1079488	U.S.A. Oregon
Lepraria pacifica	xxx	NY744	*Fryday 9285*	MSC	U.S.A. California
Lepraria pacifica	xxx	NY738	*Fruday 9285*	MSC	U.S.A. California
Lepraria subalbicans	xxx	NY570	*Lendemer 19626*	NY-1133337	U.S.A. California
Lepraria subalbicans	xxx	NY1159	*McCune 30489*	OSC	U.S.A. Oregon
Lepraria subalbicans	xxx	NY634	*McCune 60040*	NY-1231528	U.S.A. Oregon
Lepraria elobata	xxx	NY674	*Harris 55713*	NY-1104177	U.S.A. Connecticut
Lepraria elobata	xxx	NY677	*Lendemer 9804*	NY-953120	U.S.A. New York
Lepraria elobata	xxx	NY29	*Lendemer 9804*	NY-953120	U.S.A. New York
Lepraria neglecta	xxx	NY607	*Lendemer 19691*	NY-1133071	U.S.A. California
Lepraria neglecta	xxx	NY603	*Lendemer 19735*	NY-1107514	U.S.A. California
Lepraria neglecta	xxx	NY586	*Lendemer 19632*	NY-1133336	U.S.A. California
Lepraria neglecta	xxx	NY602	*Lendemer 19684*	NY-1133078	U.S.A. California
Lepraria neglecta	xxx	NY525	*Buck 55113*	NY-1136449	U.S.A. Utah
Lepraria neglecta	xxx	NY604	*Lendemer 19735-A*	NY-1107513	U.S.A. California
Lepraria neglecta	xxx	NY605	*Lendemer 19703*	NY-1107543	U.S.A. California
Lepraria neglecta	xxx	NY582	*Lendemer 19616*	NY-1133144	U.S.A. California
Lepraria neglecta	xxx	NY587	*Kalb 36707*	NY-1107333	Austria, Osterreich
Lepraria neglecta	xxx	NY914	*Lendemer 22329*	NY-1228464	Canada, British Columbia
Lepraria neglecta	xxx	NY612	*Harris 55753*	NY-1104219	U.S.A. New York
Lepraria neglecta	xxx	NY334	*Rosentreter 16535*	NY-1079668	U.S.A. Idaho
Lepraria neglecta	xxx	NY465	*Lendemer 17220*	NY-1105782	U.S.A Pennsylvania
Lepraria neglecta	xxx	NY1115	*Beeching 10499*	NY-1218204	U.S.A. Georgia
Lepraria neglecta	xxx	NY321	*McCune 28484*	NY-1079493	U.S.A. Oregon
Lepraria neglecta	xxx	NY322	*McCune 28216*	NY-1079489	U.S.A. Oregon
Lepraria neglecta	xxx	NY581	*Lendemer 19584*	NY-1133173	U.S.A. California
Lepraria neglecta	xxx	NY1144	*Knudsen 13490*	NY-1219246	U.S.A. California
Lepraria rigidula	xxx	NY608	*Lendemer 19577*	NY-1133212	U.S.A. California
Lepraria eburnea	xxx	NY330	*Lay 08-00336*	NY-1079497	Canada, Ontario
Lepraria eburnea	xxx	NY335	*Harris 54768*	NY-978103	U.S.A. Maine
Lepraria eburnea	xxx	NY343	*Lendemer 14139-A*	NY-1050720	Canada, Ontario
Lepraria eburnea	xxx	NY340	*Harris 54409*	NY-1068432	U.S.A. New Hampshire
Lepraria eburnea	xxx	NY336	*Lendemer 14287A*	NY-1050853	Canada, Ontario
Lepraria eburnea	xxx	NY1176	*Lendemer 27960*	NY-1222637	Canada, New Brunswick
Lepraria eburnea	xxx	NY1174	*Lendemer 27959*	NY-1222638	Canada, New Brunswick
Lepraria lanata	xxx	NY1240	*Hollinger 2995*	NY-1228005	U.S.A. Tennessee

Lepraria lanata		xxx	NY922	*Lendemer 23306*	NY-1196711	U.S.A. Tennessee
Lepraria lanata		xxx	NY1239	*Hollinger 2975*	NY-1228004	U.S.A. Tennessee
Lepraria diffusa		xxx	NY1160	*McCune 30844*	NY-1220428	U.S.A. Oregon
Lepraria eburnea		xxx	NY583	*Lendemer 19624*	NY-1133136	U.S.A. California
Lepraria eburnea		xxx	NY585	*Lendemer 19655*	NY-1133107	U.S.A. California
Lepraria eburnea		xxx	NY337	*Knudsen 5122*	NY-1059028	U.S.A. California
Lepraria eburnea		xxx	NY737	*Fryday 9365*	MSC	U.S.A. California
Lepraria yunnaniana		xxx	NY1112	*Goffinet 10077*	NY-1218201	China, Yunnan
Lepraria neglecta		xxx	NY93	*McCune 29706*	NY-1046925	U.S.A. Oregon
Lepraria finkii		xxx	NY1252	*Lendemer 24774*	NY-1216407	U.S.A. Pennsylvania
Lepraria finkii		xxx	NY1250	*Lendemer 20133*	NY-1149825	U.S.A. North Carolina
Lepraria finkii		xxx	NY1253	*Harris 55990*	NY-1148817	U.S.A. South Carolina
Lepraria finkii		xxx	NY1249	*Lendemer 23542*	NY-1196483	U.S.A. North Carolina
Lepraria finkii		xxx	NY1245	*Lendemer 22479*	NY-1181295	U.S.A. Massachusetts
Lepraria finkii		xxx	NY1258	*Lendemer 28330*	NY-1223337	Canada, Quebec
Lepraria finkii		xxx	NY1243	*Lendemer 23042*	NY-1148370	U.S.A. Maine
Lepraria finkii		xxx	NY660	*Lendemer 20160*	NY-1152464	U.S.A. North Carolina
Lepraria finkii		xxx	NY1259	*Worthington 36143*	NY-1180757	U.S.A. New Mexico
Lepraria finkii		xxx	NY1246	*Lendemer 26640*	NY-1218503	U.S.A. Missouri
Lepraria finkii		xxx	NY1244	*Lendemer 20010*	NY-1133825	U.S.A. Maryland
Lepraria finkii		xxx	NY665	*Lendemer 20489*	NY-1152954	U.S.A. Virginia
Lepraria leprolomopsis		xxx	NY1166	*Clark N225Q7*	NY-1221446	U.S.A. West Virginia
Lepraria finkii		xxx	NY1247	*Lendemer 20051*	NY-1133785	U.S.A. New Jersey
Lepraria finkii		xxx	NY1178	*Lendemer 27913*	NY-1222126	Canada, New Brunswick
Lepraria finkii		xxx	NY1257	*Lendemer 28027*	NY-1222572	Canada, New Brunswick
Lepraria finkii		xxx	NY1260	*Lendemer 22302*	NY-1221317	Canada, British Columbia
Lepraria finkii		xxx	NY1242	*Lendemer 22728*	NY-1181154	U.S.A. Maine
Lepraria harrisiana		xxx	NY666	*Lendemer 21368*	NY-1180855	U.S.A. Georgia
Lepraria harrisiana		xxx	NY161	*Lendemer 15018*	NY-1069603	U.S.A. New Jersey
Lepraria harrisiana		xxx	NY663	*Lendemer 20503*	NY-1152941	U.S.A. Virginia
Lepraria harrisiana		xxx	NY1220	*Lendemer 29681*	NY-1228667	U.S.A. North Carolina
Lepraria harrisiana		xxx	NY667	*Lendemer 20437*	NY-1152904	U.S.A. Virginia
Lepraria harrisiana		xxx	NY661	*Lendemer 20191*	NY-1152438	U.S.A. North Carolina
Lepraria harrisiana		xxx	NY921	*Lendemer 23372*	NY-1196649	U.S.A. Tennessee
Lepraria caesiella		xxx	NY464	*Lendemer 17484*	NY-1105936	U.S.A. Pennsylvania
Stereocaulon nivale		xxx	NY593	*McCune 29712*	NY-1107460	U.S.A. Oregon
Leprocaulon terricola	xxx	xxx	NY657	*Knudsen 2658.2*	UCR	U.S.A. California
Leprocaulon terricola	xxx	xxx	NY355	*Knudsen 2658.2*	SBBG	U.S.A. California
Leprocaulon terricola	xxx	xxx	NY655	*Knudsen 9608*	NY-1205565	U.S.A. California
Leprocaulon santamonicae	xxx		NY1267	*Kocourková s.n.*	NY-1153049	U.S.A. California
Leprocaulon santamonicae	xxx		NY188	*Lendemer 11383*	NY-973316	U.S.A. California
Leprocaulon santamonicae	xxx	xxx	NY565	*Lendemer 19660*	NY-1133102	U.S.A. California
Leprocaulon santamonicae	xxx	xxx	NY1264	*Knudsen 12058*	NY-1152044	U.S.A. California

Leprocaulon quisquiliare	xxx		NY1262	*Buck 55927*	NY-1167639	Germany, Rheinland-Pfalz
Leprocaulon quisquiliare	xxx	xxx	NY1261	*Buck 55960*	NY-1167670	Germany, Rheinland-Pfalz
Leprocaulon knudsenii	xxx		NY62	*Lendemer 11476*	NY-973373	U.S.A. California
Leprocaulon knudsenii	xxx		NY734	*Lendemer 19641*	NY-1133122	U.S.A. California
Leprocaulon knudsenii	xxx	xxx	NY702	*Knudsen 9256*	NY-973446	U.S.A. California
Leprocaulon americana		xxx	NY1263	*Lendemer 15937A*	NY-1151618	Chile, BioBio Prov.
Leprocaulon americana	xxx	xxx	NY184	*Knudsen 9605*	NY-975859	U.S.A. California
Leprocaulon americana	xxx		NY185	*Lendemer 11445*	NY-973258	U.S.A. California
Leprocaulon adherens	xxx		NY351	*Lendemer 13456*	NY-1047428	U.S.A. Pennsylvania
Leprocaulon adherens	xxx		NY598	*Morse 16380*	NY-1107479	U.S.A. Kansas
Leprocaulon adherens	xxx		NY352	*Lendemer 12341*	NY-977224	U.S.A. Pennsylvania
Leprocaulon adherens	xxx		NY350	*Lendemer 13546*	NY-1049507	U.S.A. Pennsylvania
Leprocaulon adherens	xxx		NY354	*Buck 53439*	NY-1023918	U.S.A. Pennsylvania
Leprocaulon adherens	xxx		NY179	*Lendemer 9760*	NY-953153	U.S.A. New York
Leprocaulon texta	xxx		NY57	*Lendemer 11500*	NY-973353	U.S.A. California
Lecanora phryganitis		xxx	NY885	*Howe s.n.*		U.S.A. California
Lecanora phryganitis		xxx	NY887	*Howe s.n.*		U.S.A. California
Lecanora phryganitis		xxx	NY886	*Howe s.n.*		U.S.A. California
Lecanora phryganitis		xxx	NY884	*Howe s.n.*		U.S.A. California
Lepraria sp. (usnic+zeorin)		xxx	NY755	*Lendemer 14816*	NY-1068735	U.S.A. California
Lecanora thysanophora		xxx	NY193	*Lendemer 13179*	NY-1049633	U.S.A. Pennsylvania
Lepraria sp. (usnic+zeorin)		xxx	NY719	*Lendemer 16832*	NY-1106777	U.S.A. Pennsylvania
Lepraria sp. (usnic+zeorin)		xxx	NY518	*Lendemer 16832*	NY-1106777	U.S.A. Pennsylvania
Lecanora thysanophora		xxx	NY516	*Lendemer 16933*	NY-1106528	U.S.A. Pennsylvania
Lecanora sp. (usnic+zeorin)		xxx	NY725	*Lendemer 19499*	NY-1107078	U.S.A. Pennsylvania
Lecanora nothocaesiella		xxx	NY20	*Lendemer 9786*	NY-1198821	U.S.A. New York

Table 10. Isolate numbers and associated voucher data for newly generated sequences used in this study. [Note that at the time of deposition GenBank Numbers were not available.]

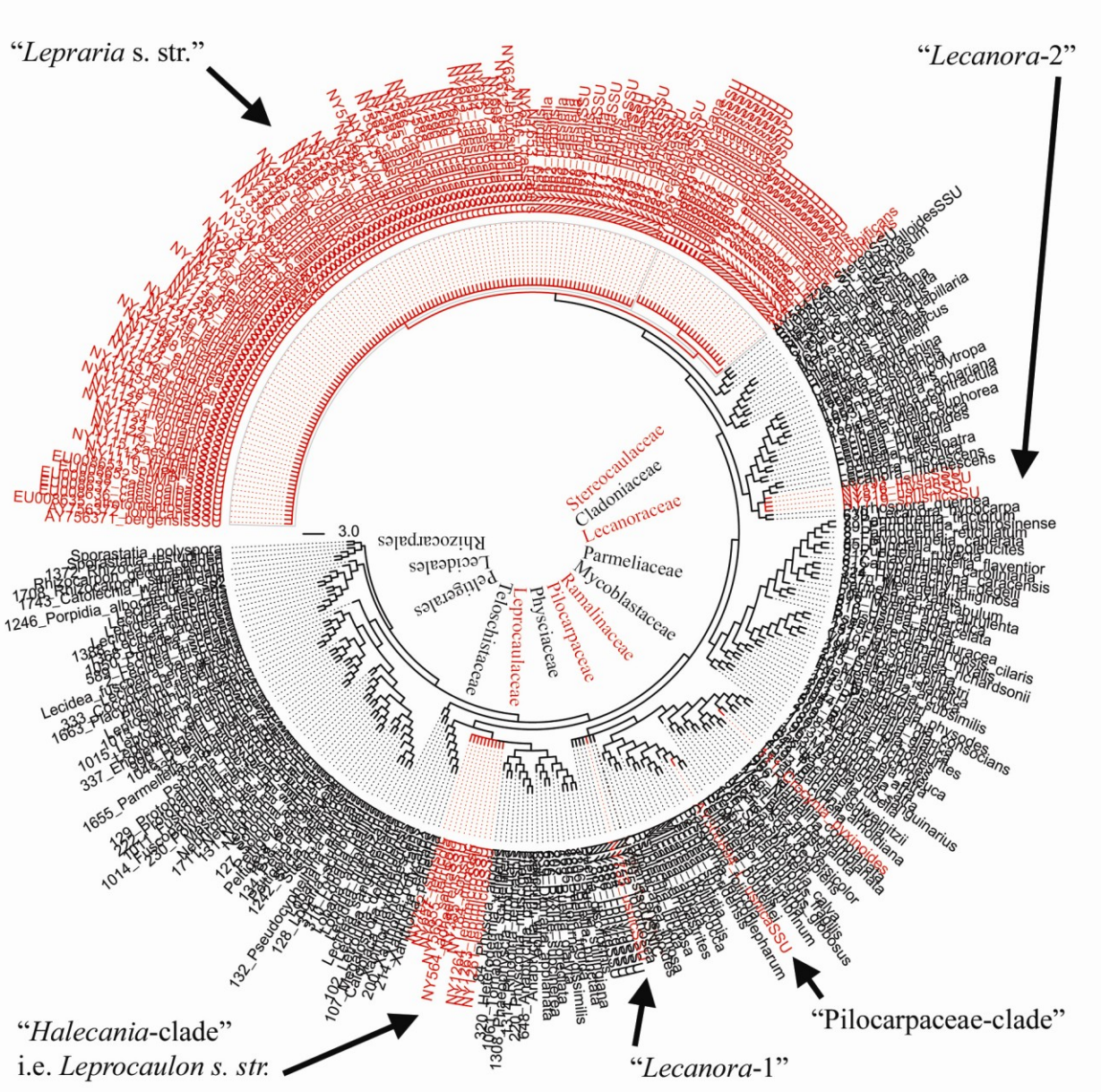

Figure 49, the dispersal of *Lepraria* s.l. into four units in four different families of lichen forming ascomycetes. Polar display of newly generated sequences of *Lepraria* s.l. mapped to the topology of the phylogeny of the Lecanoromycetidae published by Schmull et al. (2011). All taxa with a "leprarioid" growth form are highlighted in red (including *Crocynia pyxinoides* in the Ramalinaceae, whose placement has been confirmed previously by the discovery of fertile populations). The four groups of sequences selected for further detailed analyses are as indicated.

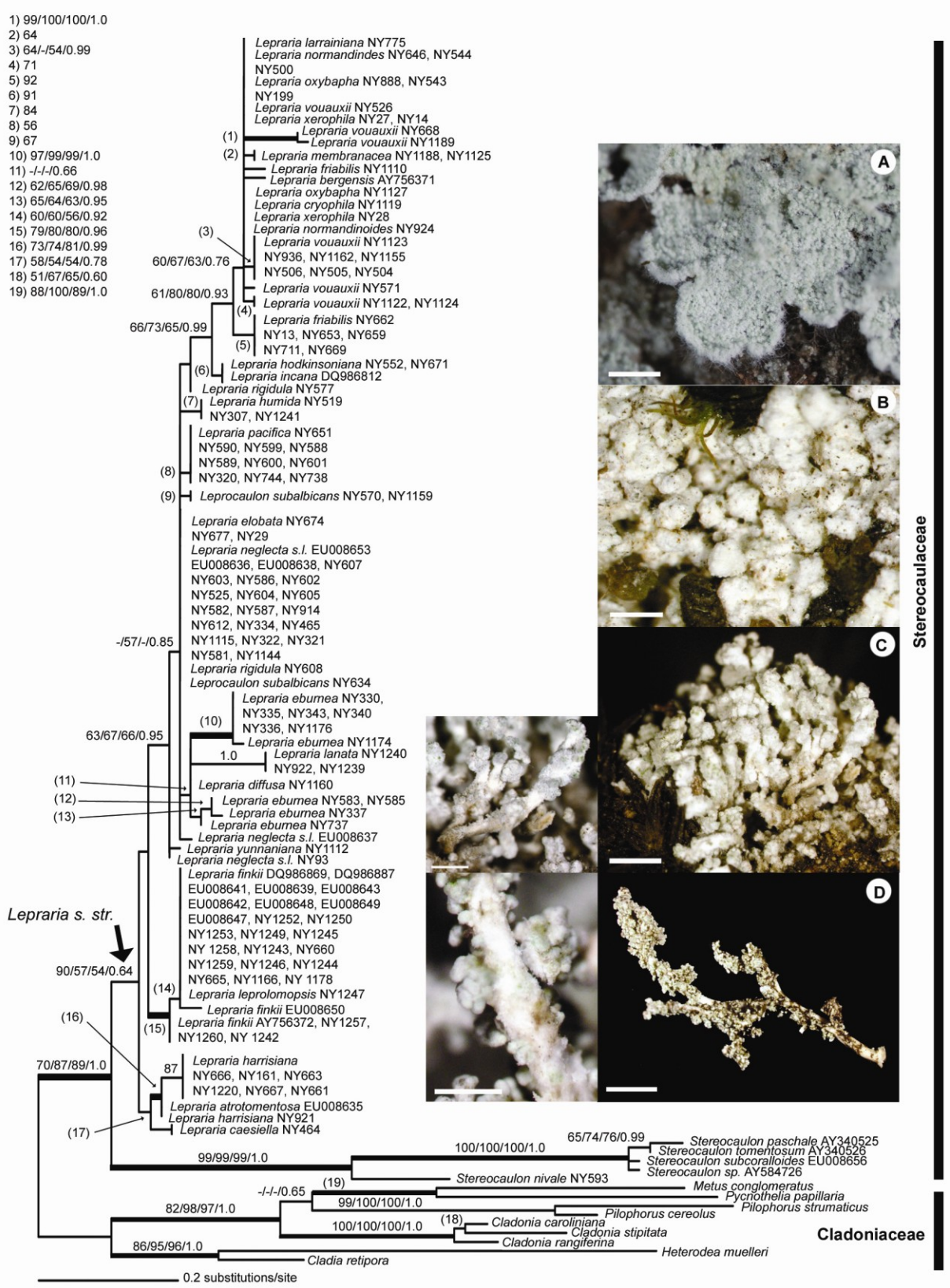
1) 99/100/100/1.0
2) 64
3) 64/-/54/0.99
4) 71
5) 92
6) 91
7) 84
8) 56
9) 67
10) 97/99/99/1.0
11) -/-/-/0.66
12) 62/65/69/0.98
13) 65/64/63/0.95
14) 60/60/56/0.92
15) 79/80/80/0.96
16) 73/74/81/0.99
17) 58/54/54/0.78
18) 51/67/65/0.60
19) 88/100/89/1.0
Lepraria larrainiana NY775
Lepraria normandindes NY646, NY544
NY500
Lepraria oxybapha NY888, NY543
NY199
Lepraria vouauxii NY526
Lepraria xerophila NY27, NY14
(1)
Lepraria vouauxii NY668
Lepraria vouauxii NY1189
(2)
Lepraria membranacea NY1188, NY1125
Lepraria friabilis NY1110
Lepraria bergensis AY756371
Lepraria oxybapha NY1127
Lepraria cryophila NY1119
Lepraria xerophila NY28
Lepraria normandinoides NY924
(3)
60/67/63/0.76
Lepraria vouauxii NY1123
NY936, NY1162, NY1155
NY506, NY505, NY504
61/80/80/0.93
Lepraria vouauxii NY571
(4)
Lepraria vouauxii NY1122, NY1124
66/73/65/0.99
Lepraria friabilis NY662
NY13, NY653, NY659
(5)
NY711, NY669
(6)
Lepraria hodkinsoniana NY552, NY671
Lepraria incana DQ986812
Lepraria rigidula NY577
(7)
Lepraria humida NY519
NY307, NY1241
Lepraria pacifica NY651
NY590, NY599, NY588
NY589, NY600, NY601
(8)
NY320, NY744, NY738
(9)
Leprocaulon subalbicans NY570, NY1159
Lepraria elobata NY674
NY677, NY29
Lepraria neglecta s.l. EU008653
EU008636, EU008638, NY607
NY603, NY586, NY602
NY525, NY604, NY605
NY582, NY587, NY914
NY612, NY334, NY465
-/57/-/0.85
NY1115, NY322, NY321
NY581, NY1144
Lepraria rigidula NY608
Leprocaulon subalbicans NY634
Lepraria eburnea NY330,
NY335, NY343, NY340
(10)
63/67/66/0.95
NY336, NY1176
Lepraria eburnea NY1174
1.0
Lepraria lanata NY1240
NY922, NY1239
(11)
Lepraria diffusa NY1160
(12)
Lepraria eburnea NY583, NY585
(13)
Lepraria eburnea NY337
Lepraria eburnea NY737
Lepraria neglecta s.l. EU008637
Lepraria yunnaniana NY1112
Lepraria neglecta s.l. NY93
Lepraria finkii DQ986869, DQ986887
EU008641, EU008639, EU008643
EU008642, EU008648, EU008649
EU008647, NY1252, NY1250
NY1253, NY1249, NY1245
NY 1258, NY1243, NY660
NY1259, NY1246, NY1244
NY665, NY1166, NY 1178
Lepraria s. str.
90/57/54/0.64
(14)
Lepraria leprolomopsis NY1247
Lepraria finkii EU008650
(16)
(15)
Lepraria finkii AY756372, NY1257,
NY1260, NY 1242
70/87/89/1.0
87
Lepraria harrisiana
NY666, NY161, NY663
NY1220, NY667, NY661
Lepraria atrotomentosa EU008635
Lepraria harrisiana NY921
(17)
Lepraria caesiella NY464
65/74/76/0.99
Stereocaulon paschale AY340525
100/100/100/1.0
Stereocaulon tomentosum AY340526
Stereocaulon subcoralloides EU008656
99/99/99/1.0
Stereocaulon sp. AY584726
Stereocaulon nivale NY593
(19)
Metus conglomeratus
-/-/-/0.65
Pycnothelia papillaria
99/100/100/1.0
Pilophorus strumaticus
82/98/97/1.0
Pilophorus cereolus
100/100/100/1.0
(18)
Cladonia caroliniana
Cladonia stipitata
Cladonia rangiferina
86/95/96/1.0
Heterodea muelleri
Cladia retipora
0.2 substitutions/site
A
B
C
D
Stereocaulaceae
Cladoniaceae

Figure 50 (previous page). Maximum likelihood (ML) phylogeny of *Lepraria* s. str., Stereocaulaceae and Cladoniaceae (outgroup) inferred from mtSSU sequence data. The topology shown here is derived from ML analyses of the full dataset. Support values are based on ML analyses of the full dataset as well as maximum parsimony (MP), ML, and Bayesian Inference (BI) analyses of the reduced dataset: MP-BP[reduced]/ML-BP[reduced]/ML-BP[full]/BI-PP[reduced]. A single value indicates support from the ML analyses of the full dataset for a clade of identical sequences that was collapsed in the reduced datset. Numbers in parentheses refer to the list of support values in the upper left corner of the figure. Thickened branches indicate support of $\geq$ 70% MP-BP and ML-BP as well as $\geq$ 0.95 BI-PP. Morphological variation in the Stereocaulaceae is illustrated in A–D. A, crustose placodioid thallus of *Lepraria oxybapha* (scale = 0.5 mm; *Lendemer 27101*). B, crustose aggregate thallus of *L. gelida* (scale = 0.5 mm; *Tønsberg 31049*). C, fruticose thallus (right, scale = 1.0 mm) with ecorticate granules (left, scale = 0.5 mm) in *L. subalbicans* (*McCune 30040*). D, fruticose thallus (right, scale = 2.0 mm) with corticated phyllocladia (left, scale = 0.5 mm) in *Stereocaulon glaucescens* (*Lendemer 27320*).

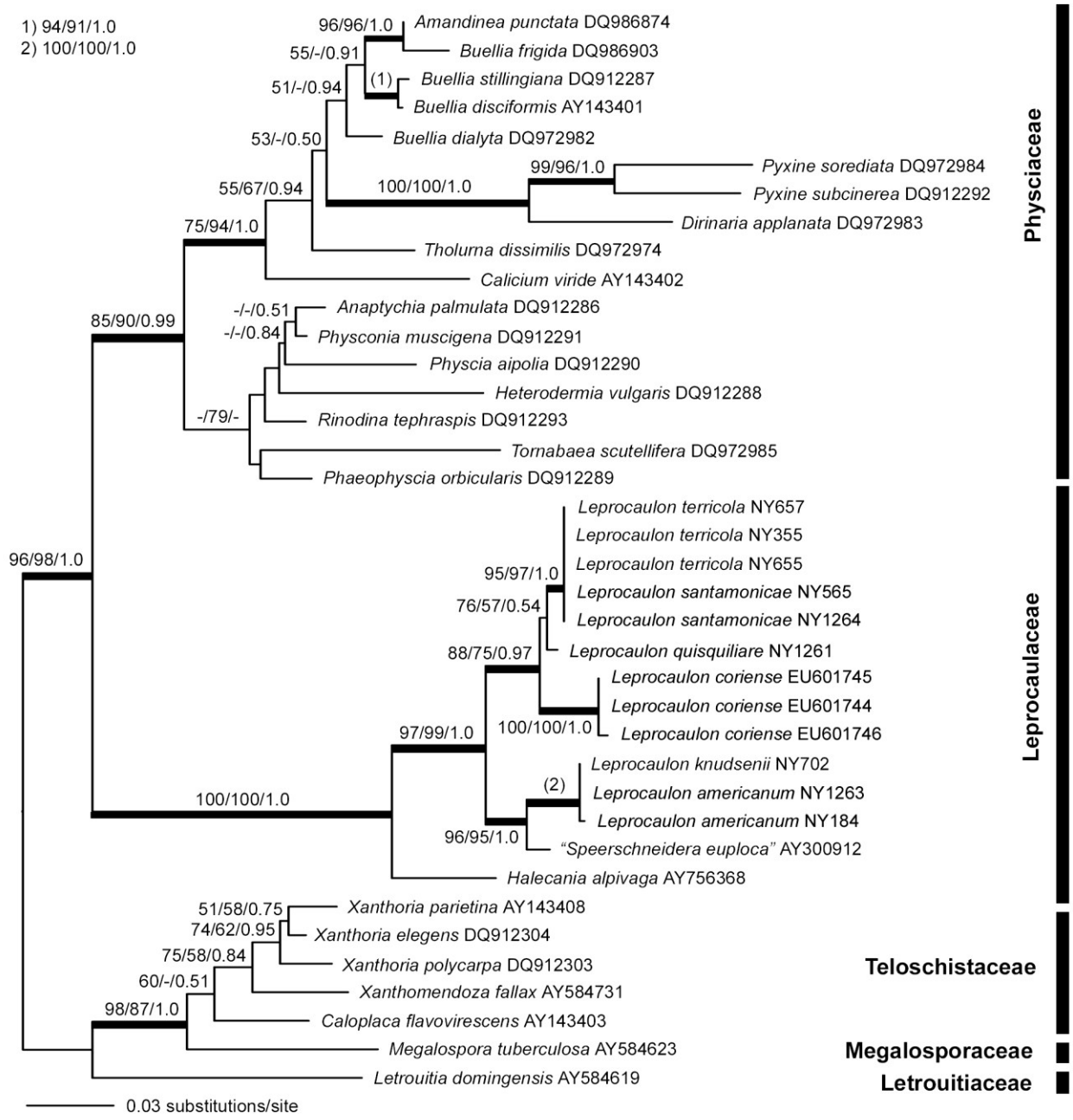

Figure 51. Maximum likelihood (ML) phylogeny of the "*Halecania*-clade" and other members of the Teloschistales inferred from mtSSU sequence data. Members of the Teloschistiinae were selected as the outgroup based on prior studies that have shown the *Halecania*-clade to be sister to the Physciaceae (Nelsen et al. 2008). Support values are displayed as: MP-BP/ML-BP/BI-PP. Numbers in parentheses refer to the list of support values in the upper left corner of the figure. Thickened branches indicate support of $\geq$ 70% MP-BP and ML-BP as well as $\geq$ 0.95 BI-PP. Accepted family level names are indicated on the left. Note that *Speerschneidera euploca* is included in quotes because the sequence may represent a contaminant or chimera.

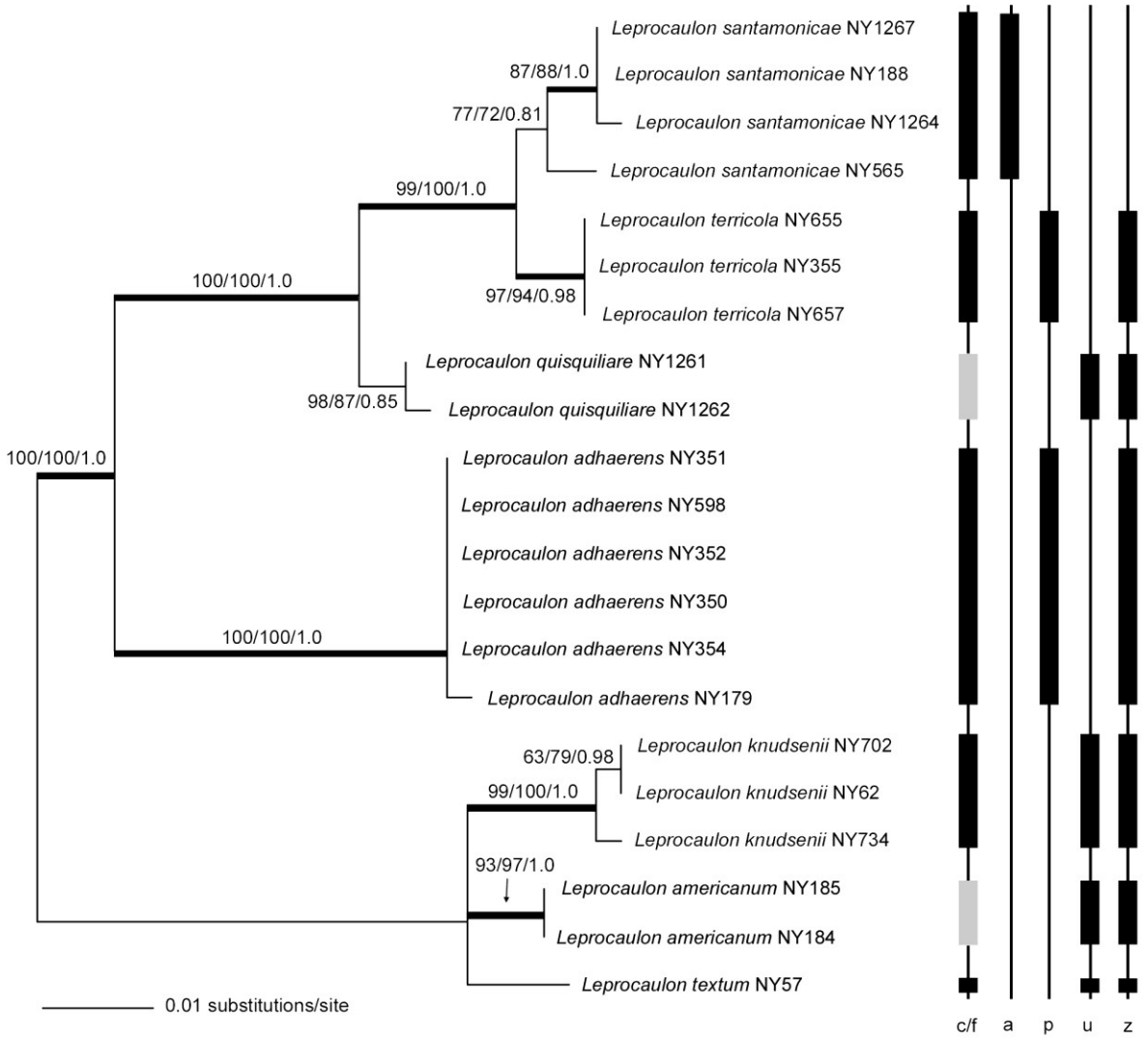

Figure 52. Maximum likelihood (ML) phylogeny of the "*Halecania*-clade" (i.e., Leprocaulaceae) inferred from nrITS sequence data. The tree is displayed with midpoint rooting. Support values are displayed as: MP-BP/ML-BP/BI-PP. Thickened branches indicate support of ≥ 70% MP-BP and ML-BP as well as ≥ 0.95 BI-PP. Note that *Halecania alpivaga* and *Speerschneidera euploca* are not included because nrITS sequence data were not available in GenBank at the time of this study. Growth form and major secondary metabolites of the species within this group are indicated as thickened lines to the right (a = argopsin, p = pannarin, u = usnic acid, z = zeorin; black(c) = crustose, gray(f) = fruticose).

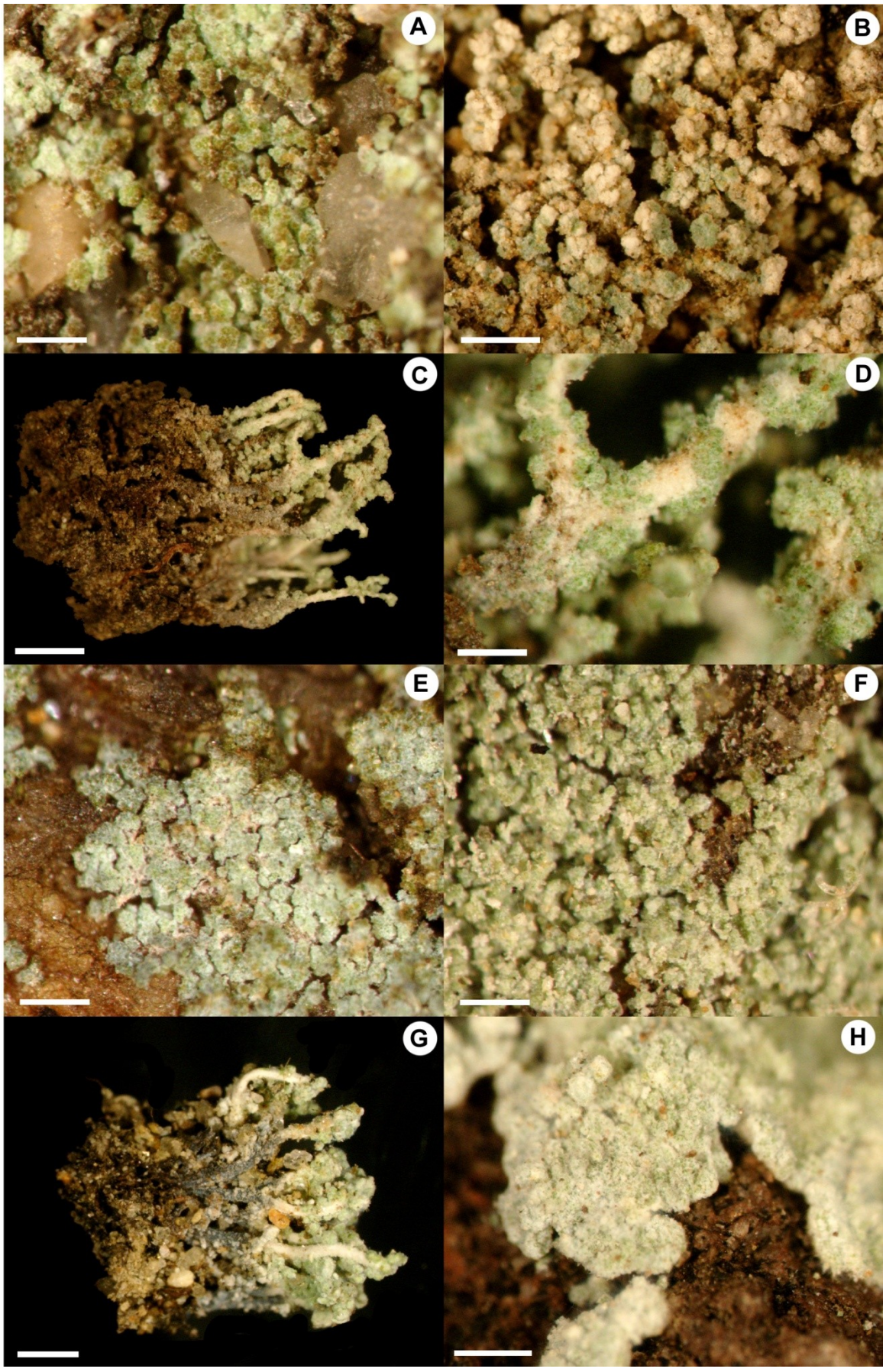
A
B
C
D
E
F
G
H

Figure 53 (previous page), morphological variation sterile asexually reproducing members of the newly established family Leprocaulaceae (order from A–H as in the phylogeny presented in figure 4). A, *Leprocaulon santamonicae* (scale = 0.25 mm; *Kocourková s.n.*). B, *L. terricola* (scale = 0.5 mm; *Knudsen 9608*). C–D, *L. quisquiliare* (scale = 1.0 & 0.25 mm respectively; *Buck 55960*). E, *L. adhaerens* (scale = 0.25 mm; *Beeching 11071*). F, *L. knudsenii* (scale = 0.25 mm; *Knudsen 9256*). G, *L. americanum* (scale = 1.0 mm; *Knudsen 9715*). H, *L. textum* (scale = 0.5 mm; *Lendemer 11500*).

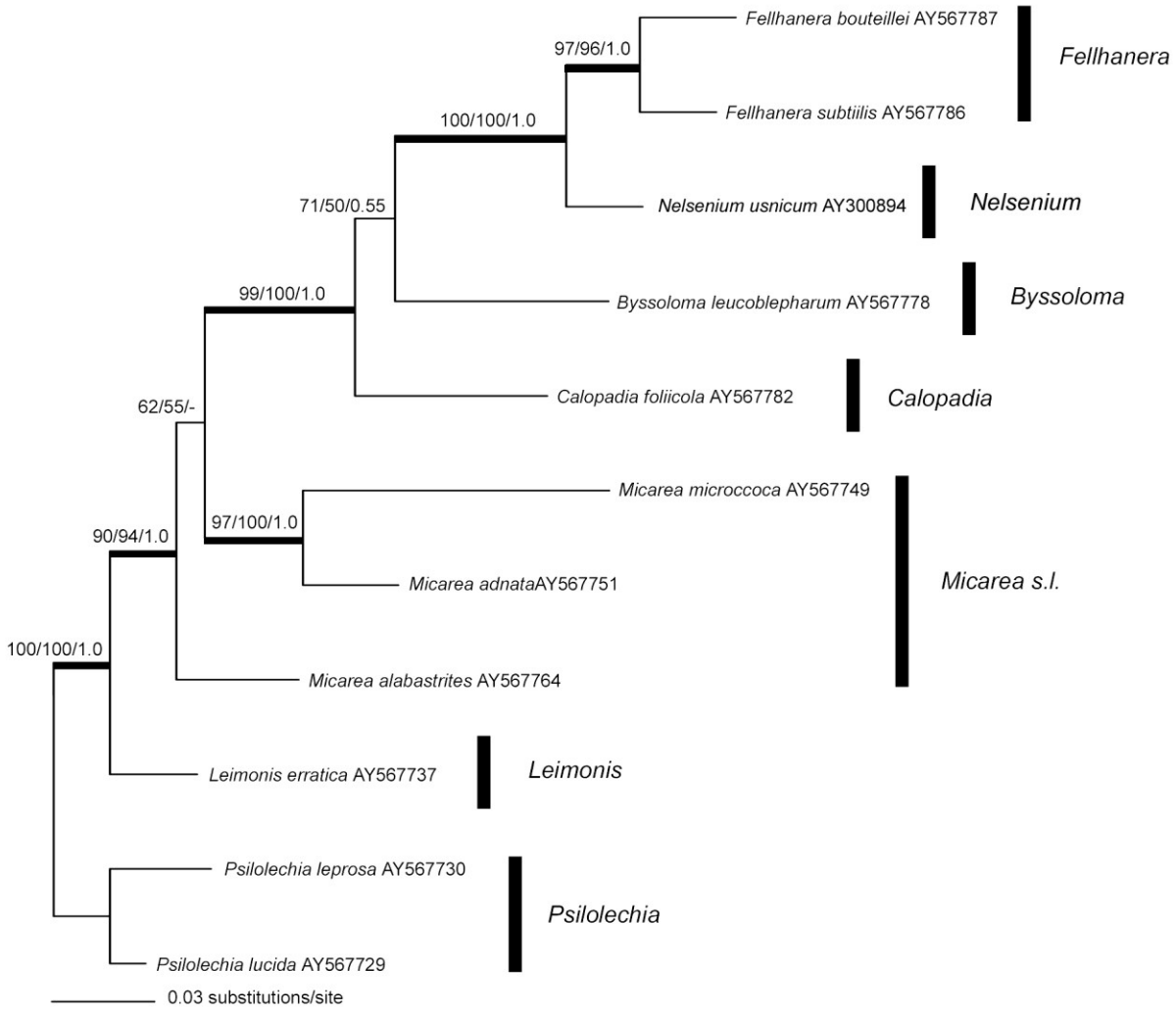

Figure 54. Maximum likelihood (ML) phylogeny of the Pilocarpaceae inferred from mtSSU sequence data and rooted with *Psilolechia* following Schmull et al. (2011). Support values are displayed as: MP-BP/ML-BP/BI-PP. Thickened branches indicate support of ≥ 70% MP-BP and ML-BP as well as ≥ 0.95 BI-PP. Genera are indicated with thickened bars to the right. *Nelsenium* is here described as a new genus to accommodate *Lepraria usnica* Sipman.

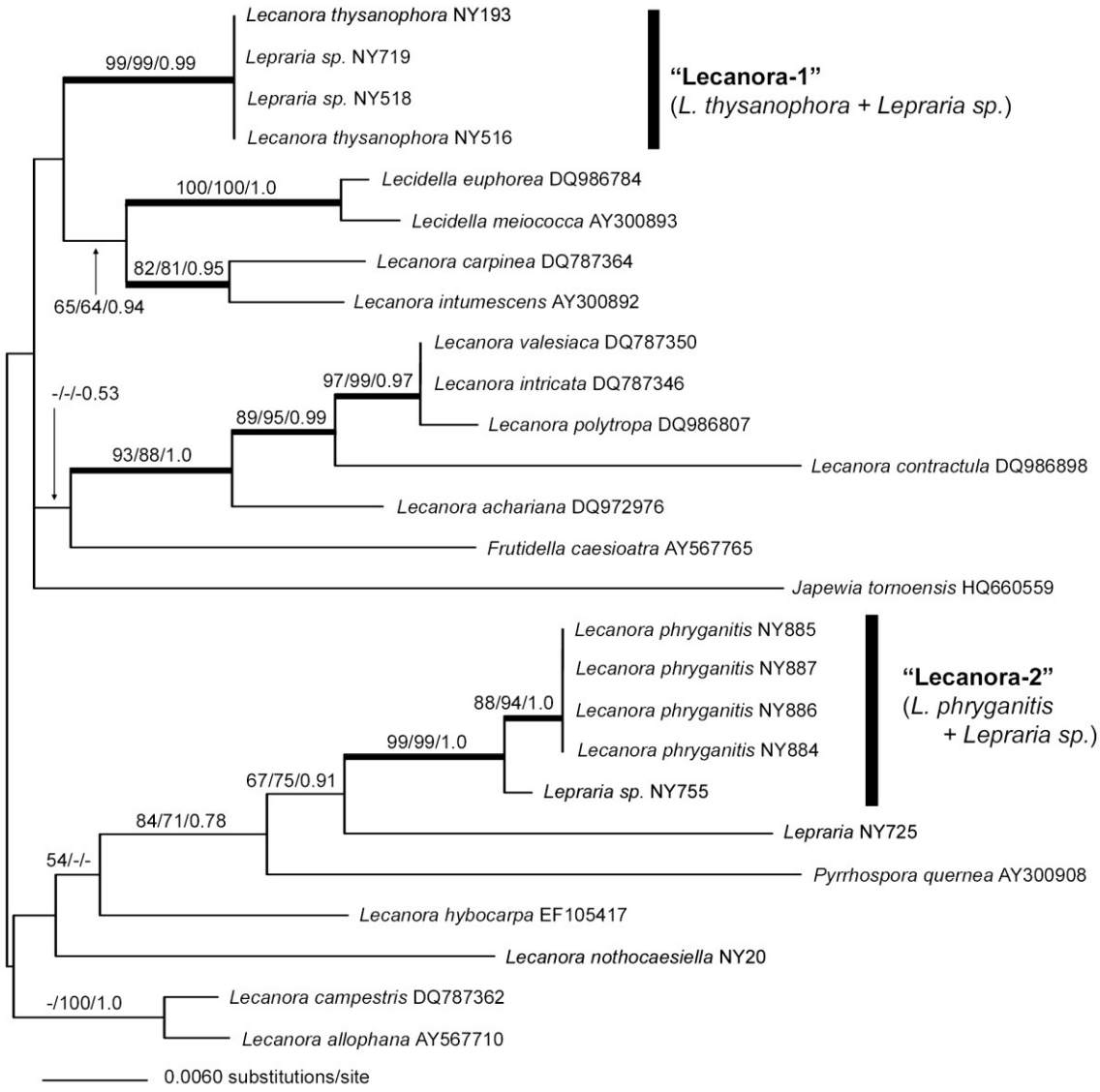

Figure 55. Maximum likelihood (ML) phylogeny of sequences in the "*Lecanoria*-1" and "*Lecanora*-2" groups, with other reference sequences of members of the Lecanoraceae inferred from mtSSU sequence data. The tree is displayed with midpoint rooting. Support values are displayed as: MP-BP/ML-BP/BI-PP. Thickened branches indicate support of $\geq$ 70% MP-BP and ML-BP as well as $\geq$ 0.95 BI-PP.

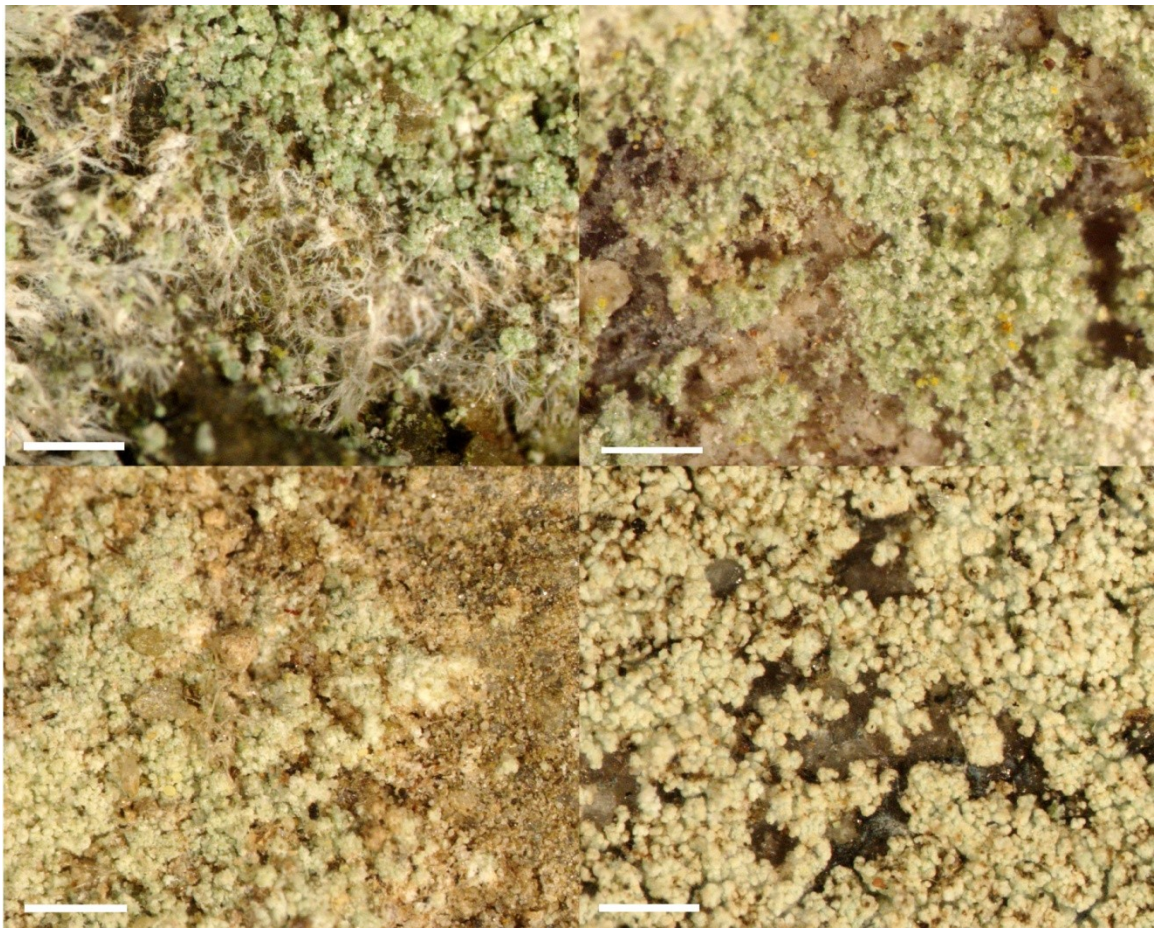

Figure 56, morphological variation of leprarioid Lecanoraceae included in figure 7. A, *Lecanora thysanophora* (*Lendemer 13179*). B, *Lepraria* sp. (*Lendemer 14816*). C, *Lepraria* sp. (*Lendemer 16832*). D, *Lepraria* sp. (*Lendemer 19499*). All scales = 0.5 mm.

CHAPTER XI - A MONOGRAPH OF THE CRUSTOSE MEMBERS OF THE GENUS *LEPRARIA* ACH. S. STR. (STEREOCAULACEAE, LICHENIZED ASCOMYCETES) IN NORTH AMERICA NORTH OF MEXICO

James C. Lendemer

Abstract. – A monograph of the crustose members of the genus *Lepraria* s. str. present in North America north of Mexico is provided. A key to all the crustose members of *Lepraria* s.l. present in the study area is included, and full descriptions and species accounts are provided for the twenty-six species belonging to the genus in a strict sense. Additionally, color illustrations and distribution maps of all the species treated are provided. A new thallus morphotype, the *lanata*-type, is defined to describe the thallus of *L. lanata*. *Lepraria leprolomopsis* is reported for the first time from North America (Alabama and West Virginia, U.S.A.). *Lepraria santosii* is placed in synonymy with *L. lobificans* and the name *L. finkii* is taken up for the common species previously referred to as *L. lobificans*.

Introduction

Lichens are a remarkable group of fungi that have evolved a semi-parasitic lifestyle involving the capture and propagation of an alga (photobiont). Lichens are also a polyphyletic group including representatives of multiple clades within the diverse fungal domain that have evolved the same unique lifestyle. The rampant and repeated evolution of taxa throughout these clades that reproduce asexually through fragmentation, and a corresponding suppression of sexual reproduction, is without a doubt one of the most intriguing aspects of these organisms. From a pragmatic standpoint it is somewhat intuitive that organisms dependant on the capture of

another organism would evolve a method of reproduction that assures the co-dispersal of both together. The widespread nature of this phenomenon in lichen forming fungi is nonetheless perplexing considering the potential long-term effects of sterility and clonal reproduction.

Unfortunately, the above phenomenon is also one of the least explored areas of lichen biology. This is largely due to that fact that the taxa that reproduce asexually via lichenized diaspores are among the most poorly understood and chronically understudied group of lichens. Although many factors contributed to the present situation, the most salient is the fact that the higher level taxonomic framework for lichens is based largely on characters of the sexual fruiting bodies, structures that are typically lacking in the majority of species that reproduce asexually.

The genus *Lepraria* Ach. s.l. comprises a group of approximately sixty asexually reproducing species that have continued to evolve and speciate despite an apparent absence of sexual reproduction. In light of the unresolved higher-level taxonomy and poor understanding of species delineation in *Lepraria*, the genus serves as a microcosm for both the problems currently hindering study of asexually reproducing lichens, and their potential solutions. Recognizing that a better understanding of *Lepraria* could elucidate a path for progress in other asexually reproducing taxa, I undertook a revision of this group in North America north of Mexico as the topic of my dissertation. The culmination of this study is the present publication, a monograph of the genus *Lepraria* s. str. in the aforementioned region.

Circumscription of the study group (i.e., what is and is not *Lepraria* s. str.?)

This monograph is an attempt to synthesize more than five years of research on *Lepraria* s.l. in North America, and abroad. When I began this study, *Lepraria* s.l. was a broadly circumscribed, evolutionarily heterogeneous assemblage of species united by an apparent lack of sexual reproduction and the presence of a crustose leprose thallus (Lendemer 2010, 2011).

Though there was evidence that the genus was polyphyletic (Ekman & Tønsberg 2002; Nelsen et al. 2008) no alternative scheme had been proposed. A monophyletic core group representing *Lepraria* s. str. had been proposed by Ekman and Tønsberg (2002) based on an analysis of nuITS sequence data, however, additional data were needed to confirm and elucidate this circumscription. Further, studies were also required to determine the phylogenetic affinities of the taxa that had been classified in *Lepraria* s.l. but that data indicated did not belong to *Lepraria* s. str. Thus, it was necessary to assemble a molecular dataset with broad taxon sampling, and conduct extensive molecular phylogenetic analyses, before proceeding with a revision of *Lepraria* s. str. A detailed account of the results of this work has been published by Lendemer and Hodkinson (in rev.), however, it is also summarized here to provide a context for the coverage of this monograph.

Lendemer and Hodkinson (in rev.) conducted a series of molecular phylogenetic analyses of sequence data from the mtSSU region of a taxonomically broad sampling of *Lepraria* s.l. as well as other groups that were thought to have affinities to the genus (i.e., *Leprocaulon* Nyl.). These analyses inferred a large monophyletic group of species embedded in a clade containing sequences of the type of *Lepraria*, *L. incana* (L.) Ach. This clade corresponds to *Lepraria* s. str. and was recovered as sister to the genus *Stereocaulon* Ach., with strong support as a member of the family Stereocaulaceae. Interestingly, sequences of species of *Leprocaulon* lacking usnic acid were also recovered as members of the *Lepraria* s. str. clade. As such the concept of *Lepraria* was expanded to include all the members of *Leprocaulon* except for the type species *L. microscopicum* (Vill.) Games *ex* D. Hawksw. (= *L. quisquiliare* (Leers) M. Choisy). In this new circumscription *Lepraria* s. str. comprises sterile members of the Stereocaulaceae with crustose leprose and crustose fruticose thalli.

The same series of analyses that led to the transfer of the majority of species of *Leprocaulon* to *Lepraria* also resulted in the dispersal to other genera in other families of all the studied members of *Lepraria* s.l. that produce argopsin, pannarin, or usnic acid. Ironically, many of these species were transferred to *Leprocaulon* because molecular phylogenetic analyses recovered their representative mtSSU sequences in a strongly supported clade with sequences of the type species of that genus (a relationship supported by morphological and chemical characters as well as biogeography). The same analyses inferred placement of *Leprocaulon* as sister to *Halecania* H. Mayrhofer in a previously unrecognized family within the Teloschistales. We also recovered several usnic acid-producing members of *Lepraria* s.l. in other families, namely the Lecanoraceae and Pilocarpaceae.

Based on the results summarized above, the present monograph has been restricted to the crustose species of *Lepraria* s.str. occurring in North America north of Mexico. This treatment specifically excludes the species remaining in *Lepraria* s.l. whose chemical profiles indicate they belong elsewhere in other families (i.e., all species with isousnic acid, usnic acid, and xanthones). A brief listing of these taxa is presented in the section detailing excluded species that immediately follows the species accounts below. Until molecular data are generated for these taxa they cannot be placed elsewhere unequivocally. The transfer of these species to another genus would be no more informative than retaining them in *Lepraria* and potentially result in superfluous nomenclatural acts. While the retention of these taxa in *Lepraria* s.l. is not an ideal solution because it maintains a polyphyletic circumscription of the genus, it is nonetheless the most parsimonious solution to a vexing problem. The fruticose members of *Lepraria* s.str. that were previously treated in *Leprocaulon* are also not treated here because they were not originally

intended to form part of this study. These taxa are poorly understood and require substantial additional study.

Materials and Methods

Field studies. – The foundation of the present revision is the one and a half years of person time that I spent in the field, observing species throughout their ranges in North America. This involved extensive fieldwork throughout the Appalachian Mountains (dozens of trips), six trips to southern California, four trips to the Canadian Maritimes (Newfoundland and New Brunswick),

two trips to the Coastal Plain of the southeastern United States, two trips to central California (coast and Sierra Nevada Mountains), two trips to the Great Lakes Region (Ontario and Quebec), and one trip each to northern Arizona, New England (Maine), the Ozark Ecoregion, the Pacific Northwest (British Columbia), the western boreal forests (Yukon). Supplementary field studies were also carried out in temperate regions of Chile and in Tasmania, Australia. Also, not included in the tally above are the numerous collecting trips prior to beginning my dissertation studies.

Large scale field studies are the corner stone of any solid taxonomic revision (Ahti 2001, Tehler 1983), and after more than five years of study I am convinced they are essential to understanding *Lepraria*. During my fieldwork in North America I conducted intensive collecting trips in multiple limited geographic areas of as many major biogeographic provinces as possible. This sampling scheme resulted in observations and collections with high sample size on a small scale, but roughly even coverage at a larger scale. Thus, the morphological and ecological variability of a given species could be assessed at both the population level and throughout its range. This is particularly relevant in *Lepraria* because it became clear over time that most

species are ecologically and morphologically consistent throughout their range, but aberrant thalli or populations can occur when the species occupy certain habitats. For instance typically saxicolous species can occur on bark or lignum in habitats with extremely high constant humidity. Similarly, species with aggregate thalli occurring in such habitats can develop abnormally thick prothalli that give the appearance of a hypothallus. The converse is also true, when species with placodioid thalli occur in unusually dry habitats, the hypothallus can be poorly developed and the morphology difficult to interpret. Clearly these phenomena are no different from when a plant or animal grows in an unusual habitat and produces an aberrant form. In other groups of organisms these cases simply considered to represent biological reality and are not used to justify the conclusion that the taxa are devoid of morphological characters. The research conducted for the present treatment supports this approach and strongly refutes the notion that *Lepraria*, let alone other groups of lichens, lacks non-molecular characters that can be used to distinguish monophyletic taxa.

Collection and preparation of voucher specimens. – During the fieldwork outlined above an effort was made to intensively collect *Lepraria* at every site that was visited (a total of >460 sites were visited in North America; figure 57 herein). This involved collecting representatives of all thalli that appeared different (in color, texture, and ecology) in the field. In order to decrease the potential for mixed collections, each collection consisted of a single thallus, or multiple thalli that were growing together side-by-side. Although this scheme often resulted in repeated sampling of the same taxon at a given site (e.g., many collections of *L. finkii*) it also led to the collection of many species that appear to be rare. Such large scale replicate sampling, repeated across many sites, is pivotal to adequately assessing *Lepraria* diversity in a given region. This is

particularly clear when one considers that the four most common species were represented in this study by 1177 (*L. finkii*), 692 (*L. neglecta*), 329 (*L. caesiella*), and 243 (*L. normandinoides*) specimens. Whereas all but five of the remaining twenty two species were represented by between ten and one hundred collections, and eight of those were represented by between ten and fifty specimens.

An additional focus of my fieldwork was to collect ample voucher specimens for use in chemical, morphological, and molecular studies. The ideal voucher specimen of most lichens comprises enough material to fit comfortably inside a standard 4 × 6" herbarium packet (Brodo et al. 2011). However, for *Lepraria* a specimen of this size can be difficult to identify unless it is from a region where the *Lepraria* biota is already well documented. Thus, during my field studies I typically collected enough material to fit on a 5 × 8" paper card. When collecting specimens I attempted to take whole thalli when possible, and to always include large intact pieces of the thallus showing the transition from the edge to the center. Fresh specimens were also always carefully packed to avoid damage or alteration to the morphology from crushing during transport from the field. Only in extreme conditions was it deemed acceptable to scrape the thallus from the substrate. When colleagues have expressed an interest in collecting *Lepraria* I have described the procedures outlined above in an effort to maximize the future utility of any resulting specimens for the scientific community.

Specimens examined. – As in previous contributions on *Lepraria* s.l. by the author, this study is based primarily on material in the herbarium of The New York Botanical Garden (NY), supplemented by material received on loan from the following herbaria: CANL, COLO, CONN, DUKE, FH, GZU, H, KANU, MIN, MSC, SBBG, UCR, and UTEP. This includes >1000

specimens collected by the author during his extensive field studies of *Lepraria* throughout North America. To date these total 1.5 years in the field and span the period August, 2007 to August, 2011. Through these field studies the author has been able to gather firsthand observations of ecology and morphology for all the species known to occur in North America. All the data associated with the voucher specimens examined for this study have also been databased at NY. The data for specimens deposited in that institution are accessible via the NYBG Virtual Herbarium (http://sciweb.nybg.org/science2/hcol/lena/index.asp). The data for vouchers from other institution are not searchable via the same online portal; however these are available from the author upon request. It is important to note that the locality data for the majority of specimens examined, both those at NY and those from other institutions, were also georeferenced as part of this study.

Chemical methods. – The chemistry of each specimen was studied as outlined by Lendemer et al. (2008). Specifically this involved analyses of a subsample with Thin Layer Chromatography at least one time, though often more than once. One of the most common problems encountered by those attempting to identify *Lepraria* is that subsamples used for chemical analyses consist of mixtures. Care must be taken to use only the smallest sample needed, and to remove this sample from an area where it appears only a single thallus is present. When one encounters a "novel" chemical profile that appears to combine those observed for one or more species the results should be assumed to be due to contamination. Even with repeated sampling from the same, and multiple, collections these results should be treated with a high degree of skepticism. The description of new taxa based on these data alone should also be considered highly suspect.

All specimens were examined initially using solvent systems A and/or C following Culberson and Kristinsson (1970) to determine which chemical profile was present (see Appendix A for a summary table of the chemical profiles of each species treated here). For many species additional analyses with other solvent systems and known chemical standards were required to determine where specific substances were present or absent. Such additional analyses were typically conducted following the peanut butter jar methodology outlined by Lendemer (2011a). Specifically these supplementary analyses were as follows:

1) Solvent A was consistently used to confirm the presence of, and discriminate between, dibenzofurans. Reference specimens of *Lepraria alpina* (= *L. neglecta* s.l.), *L. diffusa*, *L. membranacea*, and *L. vouauxii* were used as standards for these analyses.

2) Solvent C was used to reexamine samples that initially showed the presence of one or more of the following three fatty acids: nephrosteranic acid, roccellic/angardianic acid, and rangiformic/jackinic acid. Pallidic acid was always assumed to be present as an accessory to zeorin in *Lepraria caesiella* and *L. harrisiana*, because such samples of these species were not reanalyzed.

3) Solvent G (Tønsberg 1992) was consistently used to separate fumarprotocetraric acid and protocetraric acid. Although these substances can be determined with some degree of certainty using solvent C, in cases where it was necessary to determine exactly which one was present (i.e., *Lepraria normandinoides* vs. *L. oxybapha*) a subsample was also run in solvent G for confirmation.

4) Although xanthones are not present in members of *Lepraria* s. str., two species that produce these substances are retained in *Lepraria* s.l. here. Xanthones were detected using the methodology outlined in extensive detail by Lendemer (2010).

It should be noted that spot tests (see Brodo et al. (2001)) were rarely employed in this study because they often produce inconclusive results in *Lepraria*. The reason for this is that secondary compounds appear to be produced in highly variable concentrations in *Lepraria*, both in the same thallus and across different populations. If one does use spot tests, I recommend that they be conducted on an acetone extract of the thallus spotted onto filter paper. This method is used widely in the identification of the genera *Alectoria* and *Bryoria* (Brodo & Hawksworth 1977) for the same reasons it should be employed in *Lepraria*. Using this methodology, the keys published by Lendemer (2010, 2010b) one should arrive at an identification with some degree of certainty. However TLC is always required to confirm identification.

Morphological methods. – Morphological studies were carried out using the methodology outlined in detail by Lendemer et al. (2008) with the SEM methods as corrected by Lendemer and Elix (2010). Descriptive morphological terminology follows Lendemer (2011). A summary table of the thallus type present in each species treated here is presented in Appendix B.

Measurements of granule diameter, hyphal width, and photobiont cell diameter are given as the average (xbar) ± 1 standard deviation (sd) and bounded by the extreme observed values, or [smallest observed]-(xbar-1sd)-xbar-(xbar+1sd)-[largest observed]. The number of observations (*n*) is also provided. All measurements were based on fresh material (collected within the last 1–2 years) to minimize the potential impacts of long-term storage on the delicate morphological

structures involved. In the cases of *Lepraria leprolomopsis* and *L. xerophila* measurements of the above structures are expressed as simple ranges because of the paucity of fresh material that was examined. The reader should refer to Appendix C for a tabular summary and graphical representations of the size ranges of granule diameter, hyphal width, and photobiont cell diameter in the species treated here.

Taxonomic Treatment

Key to the species of *Lepraria* s.l. with crustose leprose thalli

The key provided below is divided into *Lepraria* s.l. and *Lepraria* s. str., only the members of the latter group are treated here (for further discussion see Lendemer & Hodkinson in rev.). This key assumes that TLC data are available for the specimen being identified. Users that wish to utilize spot tests should combine the keys published by Lendemer (2010, 2010b) with the accounts published here.

1. Thallus with argopsin, isousnic acid, pannarin, or usnic acid…2 (all members of *Lepraria* s.l.)…2

2. Argopsin present; southern California…*Leprocaulon santamonicae*

2. Argopsin absent; distribution various…3

3. Pannarin present…4

4. Granules small, <0.1 mm in diameter; widespread in North America…*Leprocaulon adhaerens*

4. Granules large, >0.1 mm in diameter; rare, restricted to soil communities in coastal southern California…*Leprocaulon terricola*

3. Pannarin absent…5

5. Isousnic acid and obtusatic acid present; rare and restricted to the Appalachian Mountains…*Lepraria moroziana*

5. Isousnic acid and obtusatic acid absent; distribution various…6

6. Usnic acid and barbatic acid present, zeorin absent; rare and restricted to the Ozark Ecoregion…*Lepraria barbatica*

6. Usnic acid and zeorin present, barbatic acid absent; distribution various…7

7. Xanthones present…8

8. Thallus placodioid-type; usnic acid, zeorin, thiophaninic acid, and arthothelin present…*Lepraria disjuncta*

8. Thallus aggregate-type; usnic acid, zeorin, and 5,7-dichloro-3-*O*-methylnorlichexanthone present…*Lepraria xanthonica*

7. Xanthones absent…9

9. Thallus placodioid-type; rare and restricted to southern California…*Leprocaulon textum*

9. Thallus aggregate-type; throughout mountainous regions of California, more widespread(?)…*Leprocaulon knudsenii*[9]

1. Thallus without argopsin, isousnic acid, pannarin, or usnic acid…10

10. Thamnolic acid or squamatic acid present; northern to central Florida and adjacent Georgia…11

[9] Note that following the key in Lendemer (2010) specimens would key out here under the name *Lepraria ecorticata* (J.R. Laundon) Kukwa. As has been discussed by Lendemer and Hodkinson (in rev.) this species should be tentatively excluded from North America. The populations from California were described as a new species, *Leprocaulon knudsenii*. The species of *Lepraria* s.l. that have aggregate thalli and produce usnic acid are poorly understood and desperately in need of a detailed taxonomic revision.

11. Thamnolic acid present…*Lepraria aurescens*

11. Squamatic acid present…*Lepraria squamatica*

10. Thamnolic acid and squamatic acid both absent; distribution various…12

12. Alectorialic acid present…13

13. Thallus placodioid-type; granules ecorticate; in sheltered microhabitats…*Lepraria eburnea*

13. Thallus aggregate-type; granules pseudocorticate; in exposed microhabitats…*Lepraria neglecta* s. str.

12. Alectorialic acid absent…14

14. Stictic acid or norstictic acid present…15

15. Norstictic acid present; stictic acid absent…*Lepraria neglecta* s.l.

15. Stictic acid present; norstictic acid absent…16

16. Thallus placodioid-type…17

17. Zeorin present; ubiquitous in most regions of North America…*Lepraria finkii*

17. Zeorin absent; rare in the central/southern Appalachian Mountains…*Lepraria leprolomopsis*

16. Thallus aggregate-type...18

18. Granules ecorticate; in sheltered microhabitats…*Lepraria elobata* s. str.

18. Granules pseudocorticate; in exposed microhabitats…*Lepraria neglecta* s.l.

14. Stictic acid and norstictic acid both absent…19

19. Salazinic acid present…*Lepraria elobata* s.l.

19. Salazinic acid absent…20

20. Divaricatic acid present…21

21. Zeorin absent; thallus placodioid-type; nordivaricatic acid present in high concentrations; on non-calcareous rocks; eastern and central North America...*Lepraria cryophila*

21. Zeorin present; thallus aggregate-type; nordivaricatic acid absent or present in very low concentrations; on hardwoods or conifers; distribution various…22

22. Eastern North America; nordivaricatic acid absent…*Lepraria hodkinsoniana*

22. Western North America; nordivaricatic acid present in low concentrations…*Lepraria pacifica*

20. Divaricatic acid absent…23

23. Fumarprotocetraric acid or protocetraric acid present…24

24. Granules forming massive spherical aggregations >0.1 in diameter; protocetraric acid and roccellic/angardianic acid present; rare and restricted to high elevations of the southern Appalachian Mountains…*Lepraria lanata*

24. Granules not as above; chemistry and distribution various…25

25. Thallus aggregate-type; fumarprotocetraric acid present…26

26. Atranorin present; granules pseudocorticate; in exposed microhabitats…*Lepraria neglecta* s.l.

26. Atranorin typically absent; granules ecorticate; in sheltered microhabitats…27

27. Roccellic/angardianic acid present; Pacific Northwest…*Lepraria torii*

27. Roccellic/angardianic acid absent; southeastern Coastal Plain…*Lepraria friabilis*

25. Thallus placodioid-type; fumarprotocetraric acid or protocetraric acid present…28

28. Thallus *finkii*-type, margins not rounded with a distinct lip; roccellic/angardianic acid absent; Pacific Northwest…*Lepraria nivalis*

28. Thallus *normandinoides*-type; margins rounded, with a distinct lip; roccellic/angardianic acid present…29

29. Protocetraric acid present…*Lepraria normandinoides*

29. Fumarprotocetraric acid present….*Lepraria oxybapha*

24. Fumarprotocetraric acid and protocetraric acid both absent…30

30. Psoromic acid present…*Lepraria neglecta* s.l.

30. Psoromic acid absent…31

31. Zeorin present…32

32. Thallus placodioid-type…*Lepraria harrisiana*

32. Thallus aggregate-type…*Lepraria caesiella*

31. Zeorin absent…33

33. Dibenzofurans present…34

34. Thallus aggregate type.35

35. Granules ecorticate; in sheltered microhabitats; atranorin absent, oxypannaric acid-2-methylester present…*Lepraria diffusa*

35. Granules pseudocorticate; in exposed habitats; atranorin and porphyrilic acid present…*Lepraria neglecta* s.l.

34. Thallus placodioid type…36

36. Pannaric acid present…*Lepraria membranacea*

36. Pannaric 6-methylester or norascomatic acid present…37

37. Norascomatic acid present…*Lepraria xerophila* (rare chemotype)

37. Pannaric 6-methylester present…38

38. Coastal habitats in southern California; thallus *xerophila*-type, margins rounded and with a raised lip…*Lepraria xerophila*

38. Not in the above habitats; thallus *finkii*-type, margins discrete, but not rounded and with a raised lip…*Lepraria vouauxii*

33. Dibenzofurans absent…39

39. Nephrosteranic acid present; granules frequently with projecting prothalline hyphae persisting to maturity…*Lepraria rigidula*

39. Nephrosteranic acid absent; granules typically not retaining prothalline hyphae into maturity…40

40. Roccellic/angardianic acid and rangiformic/jackinic acid both absent…*Lepraria neglecta* s.l.

40. Roccellic/angardianic acid or rangiformic/jackinic acid present…41

41. Thallus placodioid-type, with rounded margins and a raised lip; roccellic/angardianic acid present…*Lepraria normandinoides*

41. Thallus aggregate type, morphology not as above; roccellic/angardianic acid present or absent…42

42. Roccellic/angardianic acid present in addition to rangiformic/jackinic acid…*Lepraria jackii*

42. Roccellic/angardianic acid absent, rangiformic/jackinic acid present…*Lepraria humida*

Accounts of the species

Lepraria aurescens **Orange & Wolseley,** Lichenologist 37: 247. 2005. TYPE: **THAILAND. CHIANG MAI PROV.:** Dio Suthep, transect Wat Palad, alt. 680 m, dry evergreen forest, 25.xi.1991, on bark, *P.A. Wolseley & B. Aguirre-Hudson 5001* (BM [n.v.], holotype).

Figures 58 and 59.

Description. – **Thallus** crustose, leprose, placodioid, continuous and typically distinctly bi-layered, initially forming small discrete thalli which expand marginally, overlap and become confluent to form a thick crust (0.2–0.4 mm thick), light gray-blue in color; **hyphae** hyaline, [1.6]–(2.3)–2.8–(3.4)–[4.8] µm (*n*=64) in width, septate, secund, obscured by a thick layer of POL+ crystals that do not dissolve in KOH; **prothallus** disappearing with age; **hypothallus** a thin, often poorly developed layer of loosely intertwined hyphae surrounding and subtending the granules, extending outward from the edge of the thallus; **rhizohyphae** present, pale to dirty brownish; **granules** globose, [26]–(36)–45–(54) –[72] µm (*n*=126) in diameter, ecorticate, well organized and discrete, often forming compound units of multiple granules; **photobiont** green, coccoid, cells globose, [6]–(7.5)–9.2–(10.8)–[14.6] µm (*n*=135) in diameter.

Chemistry. – Thamnolic acid. Spot tests: K+ lemon yellow, C-, KC-, P+ orange, UV-.

Ecology and distribution. – *Lepraria aurescens* is essentially restricted to tropical and subtropical Florida in North America (figure 3). The species was originally described from Thailand and may be widespread in tropical regions of the world (Orange & Wolseley 2005). In

North America the species typically occurs on conifers (*Chamaecyparis* and *Taxodium*) and trunks of old Sabal Palms (*Sabal palmetto* L.). Rarely the species occurs on the bark of hardwood trees (*Quercus*).

Conservation status. – Despite its geographically restricted distribution in North America, this species is not likely currently threatened.

Discussion. – *Lepraria aurescens* is not likely to be confused with any other North American species of *Lepraria* because none is known to produce thamnolic acid as a secondary metabolite. Confusion with *L. squamatica* Elix is possible, as both species have thin placodioid thalli, are partially sympatric, and can occur as mixed populations. Both species also have similar spot test reactions with P because *L. squamatica* produces both baeomycesic and protocetraric acids in addition to squamatic acid. The best way to distinguish the species is to examine material with a long wave UV light, the squamatic acid produced in *L. squamatica* will fluoresce bright white while the thalli of *L. aurescens* will not fluoresce at all. *Lepraria harrisiana* is also morphologically similar to *L. aurescens*, although the geographic distribution of that species is largely allopatric and it produces atranorin, zeorin, and pallidic acid rather than thamnolic acid.

Forms of *L. friabilis* in which the aggregate thalli form a continuous crust could also be confused with *L. aurescens*. It differs in being restricted to the bark of conifers and in producing only fumarprotocetraric acid.

While reviewing *Lepraria* specimens for this revision, the author occasionally encountered specimens of *Cladonia ravenelii* Tuck. that had been misidentified because the squamulose thallus in that species had nearly completely dissolved into a sorediate crust. Since

C. ravenelii produces thamnolic acid, such specimens could be misidentified as *L. aurescens*. If one keys a specimen as *L. aurescens* from outside of the geographic region reported here, they should carefully confirm that it does not represent *C. ravenelii*. Typically, if the collection is ample, a diligent search will reveal the presence of some partially intact squamules. The placodioid thallus of *L. aurescens*, which possesses a thin hypothallus, is also fundamentally different from the sorediate crust formed in *C. ravenelii*.

Selected specimens Examined (13 total seen from North America). – **U.S.A. FLORIDA.** BAKER CO.: along CR 127 at Moccasin Creek, ~2 mi NNW of FL 2 at Baxter, 26.xi.1996, on *Taxodium*, *R.C. Harris 39321* (NY). FLAGLER CO.: along CR 304 at Sweetwater Creek, 6.i.1996, on *Taxodium*, *R.C. Harris 37406* (NY). HAMILTON CO.: Bee Haven Bay, N of CR 6, 15.xii.1993, on *Taxodium*, *W.R. Buck 24955* (NY). HERNANDO CO.: Withlacoochee State Forest, Richloam Wildlife Management Area, 4.xii.1992, on *Taxodium*, *R.C. Harris 29783* (NY). LAKE CO.: Seminole State Forest, along Atula Rd. from Cassia Trail head S of Brantley Branch Rd., 8.iii.2009, on *Quercus*, *J.C. Lendemer 15843-A & W.R. Buck* (NY). LEVY CO.: Goethe State Forest, Sand Slough, 4.xii.1996, at base of *Taxodium*, *R.C. Harris 39672* (NY). MADISON CO.: E of CR 150 at Little Aucilla River, 14.xii.1993, on *Taxodium*, *R.C. Harris 32443* (NY). MARION CO.: Ocala National Forest, 2.2 mi N of SR 40 on SR 19, Mormon Branch, 2.i.1996, on *Chamaecyparis*, *R.C. Harris 37201* (NY). ST. JOHNS CO.: South Ponte Vedra Beach, Guana Tolomato Matanzas National Estuarine Research Reserve, 28.xi.2008, on *Quercus*, *W.R. Buck 54370* (NY). VOLUSIA CO.: Blue Spring State Park, 23.iii.1998, on lower side of slanting burnt *Sabal*, *R.C. Harris 41493* (NY). WAKULLA CO.: Apalachicola National Forest, along FS Rd. 3.9 at Lost Creek, 4.xii.1988, on *Taxodium*, *R.C. Harris 23389*.

Lepraria caesiella **R.C. Harris *in* Lendemer,** Opuscula Philolichenum 2: 51. 2005. TYPE: **U.S.A. PENNSYLVANIA.** MONROE CO.: Delaware Water Gap National Recreation Area, Community Drive Wetlands, ca. 1 mi S of Shoemakers, ca. 2 mi S of Bushkill, 24.iv.2004, on large dead birch (*Betula*), *Lendemer et al. 2304 = Lich. East. N. Amer. Exs. IV: 172* (NY!, holotype; ASU!, B!, BG!, DOV!, FH!, GZU!, H!, MIN!, NDA!, S!, TU!, isotypes).

Figures 60 and 61.

Description. – **Thallus** crustose, leprose, aggregated, discontinuous and not stratified, initially composed of isolated granules that divide to form aggregations, eventually merging to form a thin crust (<0.1 mm thick), gray-blue in color; **hyphae** hyaline, [1.8]–(2.4)–3.1–(3.7)–[5] µm (*n*=42) in width, septate, secund, obscured by a thick layer of POL+ crystals that dissolve in KOH and recrystallize as thin bent needles; **prothallus** persistent; **hypothallus** absent; **rhizohyphae** absent; **granules** globose, [24]–(35)–47–(59)–[76] µm (*n*=96) in diameter, ecorticate, well organized and discrete, remaining distinct and not forming compound units; **photobiont** green, coccoid, cells globose, [6]–(7.9)–9.8–(11.8)–[15.6] µm (*n*=96) in width.

Chemistry. – Atranorin, zeorin, pallidic acid. Spot tests: K+ yellow, C-, KC-, P+ weak yellow, UV-.

Ecology and distribution. – *Lepraria caesiella* is common and widely distributed throughout temperate and boreal northeastern North America, extending southward in the

Appalachian Mountains (fig. 61). Disjunct saxicolous populations also occur in the Ozark Ecoregion. The species occurs on a wide diversity of corticolous substrates, including both conifers and hardwoods. Though the majority of the corticolous collections examined are from hardwoods, particularly oaks (*Quercus*), saxicolous populations are also often encountered on non-calcareous rocks. These saxicolous populations are ecologically distinct from other sympatric *Lepraria* species with aggregate thalli because they occur on dry, protected rock faces that are sheltered from rain but still exposed to some light. Most other species occur either on rock faces fully exposed to the elements, or in shaded overhangs that are completely protected and always shaded.

Conservation status. – This species is common and widely distributed in temperate eastern North America and does not likely warrant conservation consideration. It is often found in second or third growth forests in areas where the lichen biota has been significantly negatively impacted by past anthropogenic disturbance.

Discussion. – *Lepraria caesiella* is chemically comparable to *L. harrisiana* in producing atranorin, zeorin, and pallidic acid, but that species differs morphologically in having a *cryophila*-type placodioid thallus. Although previously considered conspecific, the two entities are strongly supported as distinct in molecular phylogenetic analyses of nuITS sequence data (Lendemer in press). Of the six other species of *Lepraria* with aggregate thalli in North America, only *L. elobata* and *L. neglecta* s.l. are likely to be confused with *L. caesiella*. The former species produces salazinic acid or stictic acid in addition to atranorin to zeorin, and does not produce pallidic acid. The latter species is extremely variable chemically (see the entry for that

species) and only the rare chemotype with zeorin and stictic acid could be confused with *L. caesiella*. That chemotype of *L. neglecta* differs chemically from *L. caesiella* in producing stictic acid, and morphologically from *L. caesiella* in having granules that produce a distinct pseudocortex at maturity. *Lepraria humida* Slav.-Bay. & Orange is a saxicolous species of shaded overhangs that was described from Europe and is widespread in the Appalachian Mountains of eastern North America. That species produces rangiformic/jackinic acid and does not produce zeorin. It is also much rarer than *L. caesiella*.

The lichen that has most often been confused with *Lepraria caesiella* is actually *Lecanora nothocaesiella* Lendemer & R.C. Harris. That species is a sorediate member of the *Lecanora subfusca* group with *allophana*-type apothecia. The species differs from *L. caesiella* in lacking pallidic acid and in not having a truly leprose thallus. Rather, the thallus in *L. nothocaesiella* only appears superficially leprose because it produces diffuse eroding soralia that are immersed in the substrate. It also typically produces a conspicuous, shiny, film-like prothallus with darkened areas near the growing edge of the thallus. Species of *Lepraria* never produce this type of prothallus because the hyphae of the thallus rest on, rather than penetrate and grow through, the substrate (Lendemer 2011).

Selected specimens examined (329 total seen from North America). – **CANADA. NEW BRUNSWICK.** CHARLOTTE CO.: Pennfield Parish, W of Jake Lee Rd., 1.v.2011, on *Betula*, *J.C. Lendemer 28023-A* (NY). **ONTARIO.** BRUCE CO.: Bruce Peninsula National Park, 19.ix.2008, on dead *Picea*, *R.C. Harris 54884* (NY). LANARK CO.: 0–0.2 mi N of Peneshula Rd., 0.6 mi W of jct. w/ Cedar Cove Rd., 21.v.2011, on *Pinus*, *J.C. Lendemer 28196 & R.E. Lee* (NY). **QUEBEC.** GATINEAU CO.: Gatineau Park, near Ramsey Lake, 30.vii.1978, on *Tsuga*,

T. Ahti 34648 & I.M. Brodo (H, NY). LES COLLINES DE L'OUTAOUAIS CO.: 6.1 km NW of Chelsea, 7.vi.2009, on stump, *I.M. Brodo 32422 & C. Freebury* (NY). PONTIAC MNP.: Gatineau Park, Church Hill, 22.v.2011, on *Tsuga*, *J.C. Lendemer 28332 & C. Freebury* (NY). **U.S.A. ARKANSAS.** FRANKLIN CO.: Ozark National Forest, Boston Mountains Ranger District, Shores Lake, 17.x.2005, on sandstone, *J.C. Lendemer 5605* (NY). **CONNECTICUT.** LITCHFIELD CO.: Town of Norfolk, Holleran Swamp Preserve, 20.ix.2003, on *Acer*, *R.C. Harris 48065* (NY). WINDHAM CO.: Town of Westford, Yale Myers Forest, Boston Hollow, 19.ix.2009, on *Tsuga*, *R.C. Harris 55660* (NY). **DELAWARE.** SUSSEX CO.: Ellendale State Forest, 25.x.1989, on *Ilex*, *R.C. Harris 24913* (NY). **KENTUCKY.** PERRY CO.: Daniel Boone National Forest, Old Field Branch of Leatherwood Creek, 6.x.2001, on *Tsuga*, *R.C. Harris 44971* (NY). **MAINE.** YORK CO.: Town of Saco, Ferry Beach State Park, 15.ix.2002, on *Betula*, *R.C. Harris 46335* (NY). **MARYLAND.** CECIL CO.: Elk Neck State Forest, Black Hill Tract, 30.xi.2009, on *Quercus*, *J.C. Lendemer 19967* (NY). **MASSACHUSETTS.** BERKSHIRE CO.: Town of Hancock, Pittsfield State Forest, 4.vi.2010, on *Acer*, *J.C. Lendemer 22492* (NY). PLYMOUTH CO.: Town of Carver, Myles Standish State Forest, 31.vii.2010, on *Picea*, *W.R. Buck 56448* (NY). **MICHIGAN.** JACKSON CO.: E of Updike Rd., ~.4 mi N of I-94, 25.vi.1979, on *Quercus*, *R.C. Harris 13098* (NY). ONTONAGON CO.: Ottawa National Forest, Lathrop Creek, 10.viii.2003, on *Tsuga*, *C.M. Wetmore 89965* (MIN, NY). **NEW JERSEY.** ATLANTIC CO.: Wharton State Forest, N of Pleasant Mills Cemetery, 24.x.2004, on *Sassafras*, *J.C. Lendemer 3355* (NY). BURLINGTON CO.: Wharton State Forest, Batsto Natural Area, 9.x.2004, on *Quercus*, *J.C. Lendemer 3181* (NY). CAMDEN CO.: Wharton State Forest, 0–0.5 mi W of CR 536 (Chew Rd.), 6.ii.2009, on *Quercus*, *J.C. Lendemer 15487* (NY). CUMBERLAND CO.: Peaslee Wildlife Management Area, 2.ii.2009, on *Quercus*, *J.C.*

Lendemer 15095 (NY). GLOUCESTER CO.: Winslow Wildlife Management Area, 6.ii.2009, on *Pinus*, *J.C. Lendemer 15441* (NY). HUNTERDON CO.: Mitchell property, 4.xi.1992, on *Crataegus*, *R.C. Harris 28993* (NY). MONMOUTH CO.: Allaire State Park, 5.ii.2009, on *Quercus*, *J.C. Lendemer 15417* (NY). OCEAN CO.: Manahawkin Wildlife Management Area, 2.xii.2009, on *Quercus*, *J.C. Lendemer 20067* (NY). SALEM CO.: Parvin State Park, 26.xii.2008, on *Quercus*, *J.C. Lendemer et al. 15012* (NY). WARREN CO.: Jenny Jump State Forest, 11.ix.1992, on *Tsuga*, *R.C. Harris 27924* (NY). **NEW YORK.** ESSEX CO.: SW side of Chapel Pond, 19.ix.2004, on *Acer*, *J.C. Lendemer 3093* (NY). JEFFERSON CO.: Wellesley Island State Park, 20.ix.1983, on *Tsuga*, *R.C. Harris 16751* (NY). SUFFOLK CO.: 1.5 km W of Promised Land, 22.iv.1984, on *Pinus*, *R.C. Harris 17967* (NY). ST. LAWRENCE CO.: J.F. Dubuar Forest Trail, 6.ix.1981, on dead *Picea*, *R.C. Harris 13971* (NY). ULSTER CO.: along Esopus Creek, 13.ix.1980, on *Pinus*, *R.C. Harris 13460* (NY). WESTCHESTER CO.: Mianus Gorge, 14.viii.1976, on bark, *C.R. Prince 76-48* (NY). **NORTH CAROLINA.** GRAHAM CO.: Nantahala National Forest, Hooper Bald, 1.x.1997, on *Betula*, *R.C. Harris 41064* (NY). SWAIN CO.: Great Smoky Mountains National Park, terminus of Lakeshore Drive to jct. of Lakeshore Trail and White Oak Branch Trail, 29.vi.2010, on *Pinus*, *J.C. Lendemer et al. 23421* (NY). TRANSYLVANIA CO.: Gorges State Park, 12.viii.2005, on *Betula*, *J.C. Lendemer 4958 & E. Tripp* (NY). **OHIO.** GALLIA CO.: Wayne National Forest, Symmes Creek Natural Area, 19.v.2006, on *Quercus*, *R.C. Harris 52648* (NY). SCIOTO CO.: Shawnee National Forest, above Turkey Creek Lake, 18.v.2006, on *Acer*, *J.C. Lendemer 7525* (NY). **PENNSYLVANIA.** CLARION CO.: Cook Forest State Park, 6.ix.2010, on *Tsuga*, *J.C. Lendemer 24542* (NY). ELK CO.: Moshannon State Forest, Barr Hollow, 31.viii.2010, on rock, *J.C. Lendemer 23974* (NY). FRANKLIN CO.: Michaux State Forest, N-slopes of Rocky Mountain, 1.vi.2009, on *Quercus*,

J.C. Lendemer 18206 (NY). MONROE CO.: Tannersville Bog TNC Preserve, 24.xi.2009, on *Tsuga*, *J.C. Lendemer 19940-A & V. Doyle* (NY). NORTHUMBERLAND CO.: Wildlife Preserve, ~2.5 mi W of Alaska, 20.v.2009, on *Quercus*, *R.C. Harris 55331* (NY). PHILADELPHIA CO.: Fairmount Park, Forbidden Drive, 3.iii.2007, on *Acer*, *J.C. Lendemer 8603 & A. Moroz* (NY). SULLIVAN CO.: Ricketts Glen State Park, 19.ix.2010, on *Tsuga*, *J.C. Lendemer 25661 & J. Kunsman* (NY). TIOGA CO.: Tioga State Forest, Asaph Wild Area, 11.v.2009, on *Tsuga*, *J.C. Lendemer 16435 & D. Atha* (NY). UNION CO.: Bald Eagle State Park, Joyce Kilmer Natural Area, 14.ix.2010, on *Tsuga*, *J.C. Lendemer 25411* (NY). YORK CO.: Apollo County Park, 9.xiii.2009, on *Betula*, *J.C. Lendemer 19429* (NY). **RHODE ISLAND.** WASHINGTON CO.: Marion Eppley Wildlife Sanctuary, 16.ix.2006, on *Nyssa*, *R.C. Harris 53142* (NY). **TENNESSEE.** BLOUNT CO.: Great Smoky Mountains National Park, White Oak Sinks, 13.x.2010, on *Tsuga*, *J.C. Lendemer et al. 26884* (NY). COCKE CO.: Great Smoky Mountains National Park, Gabes Mountain Trail, 5.viii.2009, on *Tsuga*, *J.C. Lendemer 19034 & E. Tripp* (NY). **VERMONT.** ADDISON CO.: Green Mountain National Forest, Bristol Cliffs Wilderness Area, 20.x.2010, on *Betula*, *J.C. Lendemer 27367 & M. Sundue* (NY). CALEDONIA CO.: Town of Wheelock, Wheelock Farm, 22.x.2010, on *Abies*, *J.C. Lendemer 27547 & M. Sundue* (NY). CHITTENDEN CO.: Town of Huntington, Lower Gorge Preserve, 17.x.2010, on *Tsuga*, *J.C. Lendemer 27089 & M. Sundue* (NY). ESSEX CO.: Town of Averill, Quimby Country resort property, 19.v.2008, on *Betula*, *R.C. Harris 54586* (NY). ORLEANS CO.: Town of Craftsbury, Craftsbury Outdoor Center, 16.v.2005, on *Abies*, *R.C. Harris 51478* (NY). **VIRGINIA.** GILES CO.: Pond Drain, 7.x.1995, on *Betula*, *R.C. Harris 36700* (NY). PATRICK CO.: Blue Ridge Parkway, Rock Castle Gorge Loop Trail, 6.x.1995, on *Quercus*, *R.C. Harris 36648* (NY). **WEST VIRGINIA.** PRESTON CO.: 2 mi N of Terra Alta,

287.vi.1963, on trees, *C.F. Reed 62858* (NY). POCAHONTAS CO.: Watoga State Park, 30.ix.2000, on *Tsuga*, *R.C. Harris 43932* (NY). TUCKER CO.: Monongahela National Forest, Olsen's Bog, 22.iv.2001, on *Betula*, *R.C. Harris 44871* (NY). **WISCONSIN.** BAYFIELD CO.: Chequamegon National Forest, Rainbow Lake, 29.v.2004, on *Tsuga*, *C.M. Wetmore 90460* (MIN, NY). ONEIDA CO.: Town of Minocqua, Patterson Hemlocks State Natural Area, 29.iv.2002, on *Tsuga*, *R.C. Harris 45977* (NY).

Lepraria cryophila **Lendemer,** Brittonia 62: 275. 2010. TYPE: **U.S.A. NORTH CAROLINA.** HAYWOOD CO.: Great Smoky Mountains National Park, vicinity of Baxter Creek, trail to summit of Mt. Sterling (2 miles to summit), 1370–1760 m, spruce-fir-birch forest with sparse hemlock, on vertical rock face, 26.iv.2008, *J.C. Lendemer et al. 11961* (NY!, holotype).

Figures 62–64.

Description. – **Thallus** crustose, leprose, placodioid, continuous and distinctly bi-layered, initially forming small discrete thalli which expand marginally, overlap and become confluent to form a thick crust (0.2–0.7 mm thick), off white to light blue-white in color; **hyphae** hyaline, [1.4]–(2.2)–2.8–(3.4)–[5] µm (*n*=120) in width, septate, secund, obscured by a thick layer of crystals that dissolve in KOH; **prothallus** disappearing with age; **hypothallus** a well-developed layer of loosely intertwined hyphae surrounding and subtending the granules, extending outward from the edge of the thallus; **rhizohyphae** present, abundant, pale to dirty brownish; **granules** globose, [18]–(24)–30–(37)–[52] µm (*n*=120) in diameter, ecorticate, well organized and

discrete, often forming compound units of multiple granules; **photobiont** green, coccoid, cells globose, [5]–(6.3)–8.0–(9.6)–[12.5] μm (*n*=120) in diameter.

Chemistry. – Divaricatic acid, nordivaricatic acid. Spot tests: K-, C+ pink-red, KC+ pink-red, P-, UV+ blue-white.

Ecology and distribution. – *Lepraria cryophila* is a species that typically occurs on non-calcareous rocks in high humidity microhabitats throughout eastern North America and the Ozark Ecoregion (figure 64). Occasional populations have been found on the bases of trees directly adjacent to typical thalli occurring on rock or in habitats with unusually high humidity.

Conservation status. – Although *Lepraria cryophila* is common throughout eastern North America, with disjunct occurrences in the Ozarks, it may warrant consideration in management practices in some regions. There is also a strong potential for this species to be used as an indicator of other, rarer species that are less conspicuous. The habitats occupied by *L. cryophila* often host populations of rare or disjunct lichens and can be easily damaged by anthropogenic influences (Lendemer et al. 2009).

Discussion. – *Lepraria cryophila* is a distinctive saxicolous species of eastern North America that can readily by recognized by its chemistry (divaricatic acid and nordivaricatic acid in high concentrations) and *cryophila*-type placodioid thallus. Only two other species of North American *Lepraria* produce divaricatic acid, *L. hodkinsoniana* and *L. pacifica*. Both of these differ from *L. cryophila* in having aggregate thalli, and in the production of zeorin as well as the

presence of only traces of nordivaricatic acid. *Lepraria finkii* is the species most likely to be confused with *L. cryophila* on account of its placodioid thallus and frequent occurrence in similar habitats. That species differs chemically from *L. cryophila* in producing atranorin, zeorin, and stictic acid, as well as morphologically in having a *finkii*-type thallus with sparse rhizohyphae, adanate margins, and a dense hypothallus. Thalli in populations of *L. cryophila* from the Ozark Ecoregion (figure 63) often appear thinner and more poorly developed than those from the southern Appalachians (figure 62). This difference is likely due to the drier conditions of the Ozarks compared to the southern Appalachians.

Selected specimens examined (96 total seen from North America). – **CANADA. NEW BRUNSWICK.** CHARLOTTE CO.: Saint Patrick Parish, Caughey-Taylor Nature Preserve, 2.v.2011, on rock, in overhang, *J.C. Lendemer 28060* (NY). **ONTARIO:** Algonquin Park, Track and Tower Trail area, 28.iii.2011, on shaded rock, *C. Lewis 565* (NY). **U.S.A. ALABAMA.** MARION CO.: North Fork Creek, on W side of US 43, 4.x.1999, on sandstone, *W.R. Buck 36427* (NY). **ARKANSAS.** MADISON CO.: Kings River Falls Natural Area, 5.x.2010, on rock in overhang, *J.C. Lendemer et al. 25918* (NY). **GEORGIA.** RABUN CO.: Chattahoochee National Forest, Rabun Bald, 20.ix.1996, on rock, under an overhang, *R.C. Harris 38933* (NY). **ILLINOIS.** JACKSON CO.: Shawnee National Forest, Pomona Natural Bridge, 13.x.1993, on *Liriodendron*, *R.C. Harris 31293* (NY). **MAINE.** KNOX CO.: Vinalhaven, Starboard Rock Sanctuary, 15.vi.2010, on rock, in overhang, *J.C. Lendemer 23235* (NY). WASHINGTON CO.: Town of Beals, Great Wass Island, Great Wass Island TNC Preserve, 7.vi.2010, on rock, in overhang, *J.C. Lendemer 22588* (NY). **MISSOURI.** Don Robinson Property, Club Moss Hollow, 24.iii.2006, on sandstone ledge, *J.C. Lendemer et al. 5978* (NY). **NEW HAMPSHIRE.**

COUNTY UNKNOWN: White Mountains, x.1843, *E. Tuckerman s.n. = Reliq. Tuck. 56p.p.* (NY). **NEW JERSEY.** BERGEN CO.: Palisades, sine date, *C.F. Austin s.n.* (NY). **NEW YORK.** BRONX CO.: New York Botanical Garden, 4.v.1980, on *Tsuga*, *R.C. Harris 13284* (NY). GREENE CO.: Town of Hunter, Catskill Forest Preserve, Stony Clove Notch on W side of NY 214, 13.ix.2008, on moss, in overhang, *J.C. Lendemer 14089* (NY). ULSTER CO.: Town of Shandaken, Catskill Forest Preserve, Slide Mountain Wilderness Area, W slope of Slide Mountain, 13.ix.2008, on rock in overhang, *J.C. Lendemer 14023* (NY). **NORTH CAROLINA.** BUNCOMBE CO.: Craggy Dome Ridge, 14.vi.1986, on protected rock face, *K. Heiman s.n.* (NY). **PENNSYLVANIA.** CENTRE CO.: Bald Eagle State Forest, Stony Run Rd., 0.5 mi N of jct w/ Negro Hollow Rd., 14.ix.2010, on rock, in overhang, *J.C. Lendemer 25545* (NY). ELK CO.: Elk State Forest, E shore of Chase Run, 1.ix.2010, on rock, in overhang, *J.C. Lendemer 24258* (NY). WAYNE CO.: Lacawac Sanctuary, 25.vii.2009, on rock, in overhang, *J.C. Lendemer 18443* (NY). **VERMONT.** ADDISON CO.: Town of Bristol, Green Mountain National Forest, Bristol Cliffs Wilderness, 20.x.2010, on humus, in overhang, *J.C. Lendemer 27392 & M. Sundue* (NY). ORLEANS CO.: Town of Westfield, Long Trail State Forest, Hazen Notch, 21.x.2010, on rock, in overhang, *J.C. Lendemer 27450 & M. Sundue* (NY). **WEST VIRGINIA.** FAYETTE CO.: New River Gorge, vi.2010, on sandstone, *P. Clark N220Q7* (NY). **WISCONSIN.** BURNETT CO.: Upper St. Croix National Scenic Riverway, Governor Knowles State Forest, Sandrock Cliffs Park, 2.xi.1984, on sandstone, *J.P. Schuster 2207* (MIN, NY).

Lepraria diffusa **(J.R. Laundon) Kukwa,** Ann. Bot. Fenn. 39: 226. 2002.

Leproloma diffusum J.R. Laundon, Lichenologist 21: 16. 1989. TYPE: **FINLAND:** lääni Oulo, Paltamo, Melalahti, Viilonkallio, 11.x.1959, ad rupeum dolomitcam, *A.J. Huuskonen & L. Heikkinen s.n. = Lichenotheca Fennica No. 1293* (BM!, holotype).

Leproloma diffusum var. *chrysodetoides* J.R. Laundon, Lichenologist 21: 18. 1989. TYPE: **SWEDEN. VÄRMLAND PROV.:** Burtjärn Par., on the western slope of the hill between Trettondetjärnen and Svarttjärnen, 920 m, 8.xi.1977, over mosses on perpendicular rock (greenstone) in a clearing, previously very likely in shaded situation, *L.E. Muhr 598 = Lich. Sel. Exs. Ups. No. 61* (BM [n.v.], holotype; ASU!, BM! isotype).

Lepraria diffusa var. *chrysodetoides* (J.R. Laundon) Kukwa, Ann. Bot. Fenn. 39: 226. 2002.

Figures 65–67.

Description. – **Thallus** crustose, leprose, aggregated, discontinuous and not stratified to continuous, initially composed of isolated granules that divide to form aggregations, eventually merging to form a crust of variable thickness (0.1–0.3 mm), yellow-green in color turning dull yellowish in the herbarium; **hyphae** hyaline, [1.4]–(1.9)–2.4–(2.9)–[3.3] µm (*n*=32) in width, septate, secund, obscured by a thick layer of crystals that do not dissolve in KOH; **prothallus** persistent; **hypothallus** absent; **rhizohyphae** absent; **granules** globose, [26]–(40)–62–(84)–[132] µm (*n*=32) in diameter, ecorticate, well organized and discrete; **photobiont** green, coccoid, cells globose, [6]–(7.2)–9.2–(11.1)–[13.2] µm (*n*=32) in diameter.

Chemistry. – Pannaric acid (tr.), oxypannaric acid-2-methylester, unknown dibenzofuran (remaining at origin). Spot tests: K- or K+ dark red to magenta in pigmented portions, C-, KC-, P+ orange, UV-.

Ecology and distribution. – *Lepraria diffusa* is a rare species that is widely distributed throughout the Northern Hemisphere (Laundon 1989). In North America the species appears to be restricted to the Pacific Northwest (figure 67), however, this may be due to the scarcity of collections available from the Canadian Maritime provinces. The species occurs on the bark of hardwood trees as well as on humus, bryophytes, and rocks in sheltered microhabitats.

Conservation status. – At this time I prefer to consider this taxon "data deficient" and in need of further study. It appears to be rare based on the available material, however, more intensive collection efforts are needed to document its geographic range and ecology.

Discussion. – Thalli of *Lepraria diffusa* typically have the same yellowish hue as other dibenzofuran-producing species. Some populations appear to have orange-pigmented granules in patches across in the thallus (see figures 65 and 66). These were treated as *L. diffusa* var. *chrysodetoides*, an infraspecific entity within *L. diffusa*, by some authors (Laundon 1989, Kukwa 2002) until Kukwa (2006a) formally merged them based on the conclusion that the pigments were actually high concentrations of dibenzofurans. This synonymy is followed here. A similar phenomenon involving "pigmented" populations occurs in *L. vouauxii* (see the entry for that species for further discussion). Reliable identification of *L. diffusa*, and all other dibenzofuran-

producing species of *Lepraria*, requires study with TLC using known standards from reliably determined specimens.

Selected specimens examined (9 total seen from North America; "pigmented" form as indicated). – **CANADA. BRITISH COLUMBIA.** CHOWICHAN VALLEY REGIONAL DISTRICT: Vancouver Island, between Riverbottom Rd. and Cowchian River, 25.v.2010, on *Acer*, *J.C. Lendemer 22344 & A. Moroz* (NY). QUEEN CHARLOTTE ISLANDS: Graham Island, 4 mi N of Skidegate Mission, 9.vii.1971, on *Alnus, I.M. Brodo 17949* (NY). **ENGLAND:** Stafford, Wetton, 8.vii.1964, on dry-stone wall, *G.A. Matthews 64/7* (NY). **U.S.A. ALASKA:** Washington Bay, Kuiu Island, 20.viii.1947, on moss, *W.J. Eyerdam 440* (NY), 2.x.1949, on sea cliff, *W.J. Eyerdam 809* (NY). **OREGON.** DOUGLAS CO.: Panther Leap, above Umpqua River, iii.2010, on rock outcrops, *B. McCune 30488* (NY, pigment present). **WASHINGTON.** SNOHOMISH CO.: Mt. Pilchuck, Gold Basin, xii.1947, on ground, *E. Larrison 1040* (NY).

***Lepraria eburnea* J.R. Laundon,** Lichenologist 24: 331. 1992. TYPE: **ENGLAND:** London Borough of Hammersmith and Fulham (V.C. 21, Middlesex), All Saints Fulham churchyard, 51/243759, east side of churchyard, 30.vi.1987, in slight shade on moss, brick, and mortar on vertical surface of brick wall, *J.R. Laundon 3185* (BM [n.v.], holotype).

Lepraria frigida J.R. Laundon, Lichenologist 24: 332. 1992. TYPE: **SCOTLAND:** Tayside Region (V.C. 90, Forfar), Glenisla, Caenlochan Glen, 15.v.1964, on calcareous rocks, *P.W. James s.n.* (BM [n.v.], holotype).

Figures 68 and 69.

Description. – **Thallus** crustose, leprose, placodioid, without crisped margins, continuous and distinctly bi-layered, initially forming small discrete thalli which expand marginally, overlap, and become confluent to form a thick crust (0.2–0.5 mm thick), dirty white to light yellowish-white; **hyphae** hyaline, [1.6]–(2.3)–2.9–(3.6)–[4.5] µm (*n*=100), septate, secund, obscured by a layer of POL+ crystals that do not dissolve in KOH; prothallus disappearing with age; **hypothallus** a variably developed layer of densely intertwined hyphae surrounding and subtending the granules, sometimes outward from the edge of thallus; **rhizohyphae** uncommon, not pigmented; **granules** globose, [22]–(33)–51–(68)–[114] µm (*n*=97), ecorticate, well organized and discrete, often forming compound units, sometimes separating from the hypothallus and then producing abundant projecting hyphae; **photobiont** green, coccoid, cells globose, [5.4]–(7.4)–9.3–(11.4)–[14.4] µm (*n*=97).

Chemistry. – Alectorialic acid, ± barbatolic acid, ± protocetraric acid. Spot tests: K-, C-, KC+ red, P+ yellow or orange-red, UV-.

Laundon (1992) described populations that produce protocetraric acid in additional to alectorialic acid as *L. frigida*. Later he considered the taxa to be synonymous when further study revealed that protocetraric acid was sometimes produced in low concentrations that could not be detected with TLC, and that the types of both names produced protocetraric acid. The positive reaction of alectorialic acid with a KC spot test is difficult to observe on the thallus. This test is best performed on an acetone extract of the thallus spotted on filter paper.

Ecology and distribution. – *Lepraria eburnea* primarily has a northern boreal distribution in North America (figure 69). It is particularly common in the Great Lakes Region and Canadian Maritime provinces, where it often grows on the bases of trees in swamps (especially *Thuja*). Although apparently absent from the central and southern Appalachian Mountains, the species is known from scattered localities in the central and southwestern United States where it occurs on shaded soil, especially of road banks. In western North America the species occurs in the Pacific Northwest with scattered disjunct occurrences extending southward in mountainous regions.

Conservation status. – In eastern North America *Lepraria eburnea* does not likely have significant conservation needs, as it is locally common where it occurs. The species typically occurs in swamps and other types of forested wetland habitats which are, generally speaking, more protected than other habitat types. The disjunct populations of *L. eburnea* in the central and southwestern United States may warrant some degree of conservation consideration. Long term monitoring of these populations would be particularly useful to evaluate the potential impacts of climate change, especially increased temperatures and decreased availability of moisture.

Discussion. – *Lepraria eburnea* is a distinctive species on account of its *finkii*-type placodioid thallus that produces alectorialic acid (figure 68). In most regions it is likely to be confused with *L. finkii*, which has the same ecology and whose geographic distribution is entirely sympatric. *Lepraria finkii* differs in producing atranorin, zeorin, and stictic acid and the thalli typically have a distinctive greenish-blue hue when fresh whereas those of *L. eburnea* are typically yellowish-white. In the Pacific Northwest, where *L. nivalis* occurs, confusion with that

species is also possible. *Lepraria nivalis* does not produce alectorialic acid however (see that species for further details).

Selected specimens examined (82 total seen from North America). – **CANADA. BRITISH COLUMBIA.** DEWDNEY-ALOUETTE DIST.: Golden Ears Provincial Park, 10.v.2009, on rock, *W.R. Buck 54701* (NY). GREATER VANCOUVER REGIONAL DIST.: Lynn Headwaters Regional Park, E side of Lynn Creek, 24.v.2010, on *Populus*, *J.C. Lendemer 22312 & A. Moroz* (NY). QUEEN CHARLOTTE ISLANDS: Haida Gwaii, Graham Island, along the road between Rennell Sounds and the main road junction, 15.vii.2010, on organic matter in deep rock crevice, *J. Harpel 48560 & S. Joya* (NY). **NEW BRUNSWICK.** CHARLOTTE CO.: Saint James Parish, Grand Falls Flowage, 20.iv.2011, on *Fraxinus*, *J.C. Lendemer 27913* (NY). **NEWFOUNDLAND & LABRADOR:** NEWFOUNDLAND: Placentia East Distr., Cataracts Provincial Park, 5.viii.1978, on mosses, *T. Ahti 34863* (H). **NOVA SCOTIA.** COLCHESTER CO.: Five Islands Provincial Park, N of Bentley Branch Rd., 16.v.2004, on *Betula, R.C. Harris 49881* (NY). **ONTARIO.** BRUCE CO.: Bruce Peninsula National Park, Halfway Log Dump on Georgian Bay, 19.ix.2008, on *Thuja*, *R.C. Harris 54913* (NY). LANARK CO.: N of Peneshula Rd. 0.6 mi W of int. w/ Cedar Creek Rd., 21.v.2011, on *Thuja*, *J.C. Lendemer 28296 & R.E. Lee* (NY). OTTAWA-CARLTON CO.: City of Ottawa, Stony Swamp, 19.v.2011, on *Thuja*, *J.C. Lendemer 28108 & I.M. Brodo* (NY). **CHILE. BÍO BÍO PROV.:** Alto Bío Bío, RN Ralco, vicinity of Laguna la Mula, 11.iv.2009, on bryophytes in sheltered overhang, *J.C. Lendemer et al. 16110* (NY). **ENGLAND:** Mid-West York, Kettlewell, Crab Gill, 6.v.1961, in moss, *S. Phillips s.n.* (NY). **U.S.A. ALASKA:** Washington Bay, Kuiu Island, 15.vii.1947, on moss, *W.J. Eyerdam 408* (NY). **CALIFORNIA.** MARIPOSA CO.:

Yosemite National Park, vicinity of base of Bridal veil Falls, 22.ix.2009, on *Quercus* base, *J.C. Lendemer 19725* (NY). NEVADA CO.: near Weaver Falls, 5 mi NE of Graniteville, 29.ix.2004, on dry basalt cliffs, *L. Ahart 11419* (SBBG). PLUMAS CO.: 1 mi SW of Blue Nose Mountain, 7.ix.2000, on shale, *L. Ahart 13242* (SBBG). RIVERSIDE CO.: San Bernardino National Forest, San Jacinto Mountains, vicinity of Boxwood Spring (Halfway Spring), 11.i.2008, on wood, *J.C. Lendemer 11487 & K. Knudsen* (NY). SAN DIEGO CO.: Cleveland National Forest, Palomar Mountain, shaded drainage near Will Valley, 1.ii.2006, on rock, *K. Knudsen 5122 & J.C. Lendemer* (NY, UCR). **COLORADO.** BOULDER CO.: Upper Gregory Canyon, ~1 mi W of Boulder, 15.viii.1974, on bryophytes, *G. Kunkel L-602* (COLO). **KANSAS.** RAWLINS CO.: ~7.5 mi S, 11.5 mi E of Atwood, 12.vi.2007, over *Molendoa* on seep on bluff, *C.A. Morse 15411 & E.M. Smith* (KANU, NY). **MAINE. HANCOCK CO.:** T10SD, Maine Public Reserve Land, S slopes of Tunk Mountain, 10.vi.2010, on *Acer*, *J.C. Lendemer 23046* (NY). WASHINGTON CO.: Town of Cutler, Coastal Public Lands, Loop Trail via Black Point Cove Cutoff, 8.vi.2010, on *Thuja*, *J.C. Lendemer 22734* (NY). **MICHIGAN.** CHEBOYGAN CO.: Mill Creek SW from U.S. Hwy 23, 1.viii.1974, on humus soil, *R.C. Harris 9290* (NY). **MINNESOTA.** BELTRAMI CO.: ~3 mi N of Kelliher, 8.viii.1975, on *Thuja*, *W.R. Buck B387* (NY). **NEBRASKA.** SIOUX CO.: Fort Robinson State Park, 2 mi NW of Fort Robinson, 28.v.1988, on soil, *R.S. Egan 13376* (NY). **NEW HAMPSHIRE.** COOS CO.: Town of Stewartstown, Hurlbert Swamp Preserve, 17.v.2008, on base of *Thuja*, *R.C. Harris 54449* (NY). **OREGON.** JOSEPHINE CO.: Siskiyou Mountains, 0.3 mi SSW of Chalet, xii.2001, on moss/plant debris, *A. Mikulin 4-LIPK* (NY, OSC). LAKE CO.: Glass Butte, 0.8 mi S of Hwy 20, 16.x.2009, on soil, *H.T. Root 1784b* (NY, OSC). **SOUTH DAKOTA.** PENNINGTON CO.: 3 mi SW of Rochford, 21.vii.1960, on soil, *C.M. Wetmore 7878* (MSC). **VERMONT.** CALEDONIA CO.: Town of Wheelock, Wheelock

Farm, 22.x.2010, on *Thuja*, *J.C. Lendemer 27569 & M. Sundue* (NY). **WASHINGTON.** SNOHOMISH CO.: Mount Baker-Snoqualmie National Forest, near South Fork Stillaguamish River, iii.2009, on phyllite outcrops, *B. McCune 29942* (NY).

Lepraria elobata **Tønsberg,** Sommerfeltia 14: 197. 1992. TYPE: **NORWAY:** Vest-Agder, Kristiansand, Hamresand, 5 m, 28.ix.1991, on *Pinus sylvestris*, *T. Tønsberg 17404* (BG!, holotype).

Lepraria salazinica Tønsberg, Opuscula Philolichenum 4: 52. 2007. TYPE: **U.S.A. NORTH CAROLINA.** SWAIN CO.: Great Smoky Mountains National Park, Balsam Mountain, along Balsam Mountain Rd., 4 miles (along the road) from the gate (near the end of Heintooga Rd.), 1420–1450 m, 20.vi.2002, saxicolous under overhang, *T. Tønsberg 30941* (BG!, holotype).

Figures 70–72.

Description. – **Thallus** crustose, leprose, aggregated, discontinuous and not stratified, initially composed of isolated granules that divide to form aggregations, eventually merging to form a thin crust (<0.1 mm thick), greenish-gray in color; **hyphae** hyaline, [1.4]–(2.3)–2.9–(3.6)–[4.8] µm (*n*=96) in width, septate, secund, obscured by a thick layer of POL+ crystals that dissolve in KOH; **prothallus** persistent; **hypothallus** absent; **rhizohyphae** absent; **granules** globose, [18]–(27)–35–(42)–[62] µm (*n*=155) in diameter, ecorticate, well organized and

discrete, remaining distinct and not forming compound units; **photobiont** green, coccoid, cells globose, [4.8]–(7.0)–9.0–(11.0)–[16.8] µm (*n*=96) in width.

Chemistry. – Chemotype 1: atranorin, zeorin, stictic acid agg.; spot tests: K+ yellow turning dirty brownish, C-, KC-, P+ orange, UV-. Chemotype 2: atranorin, zeorin (in low concentrations, occasionally absent), roccellic/angardianic acid (occasionally in low concentrations), salazinic acid; spot tests: K+ yellow turning red, C-, KC-, P+ yellow, UV-.

Ecology and distribution. – In the expanded circumscription proposed by Lendemer (in rev.C) *Lepraria elobata* is an infrequent but widely distributed species throughout boreal/northern North America, extending southward at high elevations of the major continental mountain ranges (i.e., the Sierra Nevada and Peninsular Ranges; Rocky Mountains, and Appalachian Mountains). The salazinic acid chemotype is restricted to eastern North America and apparently allopatric with the stictic acid chemotype (figure 72). The species occurs on non-calcareous rocks in sheltered microhabitats, on soil and organic substrates in similar habitats, and on the bases of trees in humid habitats especially along the margins of conifer swamps.

Conservation status. – *Lepraria elobata* is widespread and, overall, does not likely warrant conservation consideration. The chemotype with salazinic acid is rare and geographically restricted, thus it could be considered in future conservation and management plans.

Discussion. – *Lepraria elobata* is characterized by its aggregate thallus and secondary chemistry (figures 70A–B and 71). Populations of *L. neglecta* s.l. with zeorin and stictic acid could be confused with *L. elobata*, however, that taxon has a thallus in which the granules develop a thick pseudocortex and appear compact. *Lepraria caesiella*, which is sympatric with *L. elobata* in some regions, also produces zeorin and atranorin, but it lacks salazinic acid and stictic acid, producing pallidic acid instead. Historically, *L. elobata* has been confused with *L. finkii* (Hue) R.C. Harris (i.e., *L. lobificans* auct.) because both species produce atranorin, zeorin, and stictic acid. The two species are morphologically distinct as *L. finkii* has a thick placodioid thallus of the *finkii*-type whereas *L. elobata* has an aggregate thallus of the *caesiella*-type (Lendemer 2011).

Selected specimens examined (36 total seen from North America; chemotype 1). – **CANADA. NEW BRUNSWICK.** CHARLOTTE CO.: Grand Falls Flowage, 30.iv.2011, on *Abies* base, *J.C. Lendemer 27871* (NY). **NEWFOUNDLAND & LABRADOR.** NEWFOUNDLAND: Lookout Mountain, 19.vii.2006, on conifer bark, *J.C. Lendemer 8516* (NY). **NORTHWEST TERR.:** Daring Lake, 26.viii.2011, on sandy soil, *C. Lewis 747* (CANL, NY). **ONTARIO.** OTTAWA-CARLTON CO.: City of Ottawa, Stony Swamp, 19.v.2011, on *Populus* stump, *J.C. Lendemer 28116 & I.M. Brodo* (NY). SUDBURY DIST.: Wakamai Provincial Park, 55 km SE of Chapleau, 18.vii.2011, on rock, *I.M. Brodo 33000 & F. Brodo* (CANL). **QUEBEC.** PONTIAC MNP.: Gatineau Park, Church Hill, 22.v.2011, on *Pinus* base, *J.C. Lendemer 28349-A & C. Freebury* (NY). **U.S.A. CALIFORNIA.** MARIPOSA CO.: Yosemite National Park, Mount Hoffman marble outcrops, 21.ix.2009, on soil, *J.C. Lendemer 19670* (NY). RIVERSIDE CO.: San Bernardino National Forest, San Jacinto Mountains, Fuller

Ridge, 2.x.2008, on granite in overhang, *J.C. Lendemer 14612 & K. Knudsen* (NY). **IDAHO.** BONNER CO.: Sandpoint, 8.v.2005, on conifer bark, *D.T. Perlmutter s.n.* (NCU [n.v.], NY). **NEW YORK.** GREENE CO.: Catskill Mountains, E end of Mink Hollow, 8.x.2007, on rock in overhang, *J.C. Lendemer 9804 & A. Moroz* (NY). **UTAH.** UNITAH CO.: Ashley National Forest, vicinity of Leidy Peak, 31.vii.2009, on *Selaginella*, *W.R. Buck 55092* (NY). **VERMONT.** CALEDONIA CO.: Town of Wheelock, Wheelock Farm, 22.x.2010, on *Abies*, *J.C. Lendemer 27625 & M. Sundue* (NY).

Specimens examined (5 total seen from North America; chemotype 2). – **U.S.A. CONNECTICUT.** WINDHAM CO.: Town of Westford, Yale Myers Forest, 19.ix.2009, on vertical rock face, *R.C. Harris 55666* (NY). **MARYLAND.** CECIL CO.: S/E shore of Octoraro Creek, 26.v.2009, on rock in overhang, *J.C. Lendemer 17830* (NY). **NEW JERSEY.** SUSSEX CO.: Delaware Water Gap, 26.iv.1975, on exposed rock, *C.R. Prince 75-32* (NY). **NORTH CAROLINA.** HAYWOOD CO.: Great Smoky Mountains National Park, E slopes above Caldwell Fork, 4.viii.2009, on rock in overhang, *J.C. Lendemer 18957 & E.A. Tripp* (NY). **PENNSYLVANIA.** PIKE CO.: Delaware Water Gap National Recreation Area, 23.iv.2004, on rock, *R.C. Harris 49483* (NY).

***Lepraria finkii* (B. de Lesd.) R.C. Harris,** Evansia 2: 45. 1985.

Crocynia finkii B. de Lesd., Bull. Soc. Bot. France 71: 334. 1924. TYPE: **U.S.A. IOWA.** FAYETTE CO.: Fayette, 7.vii.1894, on moss and limestone, *B. Fink s.n. = Decades N. American Lich. #161* and = *Lich. Boreal-Americani #93* (NY! [barcode 1196926], lectotype [selected by Harris 1985]; COLO!, NY! [barcode 1196927], isolectotypes).

Figures 73 and 74.

Description. – **Thallus** crustose, leprose, placodioid, without crisped margins, continuous and distinctly bi-layered, initially forming small discrete thalli which expand marginally, overlap, and become confluent to form a thick crust (0.2–0.8(–1.0) mm thick), greenish-blue; **hyphae** hyaline, [2.4]–(2.6)–3.1–(3.6)–[4.8] µm (*n*=107), septate, secund, obscured by a layer of POL+ crystals that do not dissolve in KOH; prothallus disappearing with age; **hypothallus** a variably developed layer of densely intertwined hyphae surrounding and subtending the granules, sometimes outward from the edge of thallus; **rhizohyphae** uncommon, not pigmented; **granules** globose, [24]–(33)–48–(63)–[104] µm (*n*=166), ecorticate, well organized and discrete, rarely forming compound units, sometimes separating from the hypothallus and then producing abundant projecting hyphae; **photobiont** green, coccoid, cells globose, [4.8]–(6.8)–9.1–(11.3)–[15.6] µm (*n*=97).

Chemistry. – Atranorin, zeorin, ± roccellic/angardianic acid (trace), norstictic acid (trace), stictic acid aggregate, ± two unidentified spots (UV+ dull yellowish-white in long wave UV before charring) above and below stictic acid in Solvent C. Spot tests: K+ dull yellow-brown, C-, KC+ dull yellow-brown, P+ orange, UV-.

The two unidentified spots mentioned above may represent dibenzofurans, however, if so, then they are not the same as any of the dibenzofurans currently reported from *Lepraria*. These spots are present in the vast majority of specimens examined and are not restricted to those from any given substrate.

Ecology and distribution. – *Lepraria finkii* is the most common and widely distributed species of the genus that occurs in North America. It is ubiquitous throughout eastern North America, except in the Arctic and tropical regions (figure 74). In western North America it is less frequent in northern and temperate areas, but nonetheless widespread. Scattered disjunct populations occur in mountainous regions of the arid Southwest and western interior.

The species shows no specific ecological requirements except the need for shade and humidity typical of its congeners. Indeed, these two factors likely account for its absence, or rarity, in the arid regions of the western and southwestern United States. The ability of this species to tolerate disturbance and pollution is also remarkable, as it often can be found on old shaded stone walls in highly urbanized areas (it occurs in every borough of New York City). Interestingly, *L. finkii* is the only North American member of *Lepraria* s. str. that occurs on calcareous rocks.

Conservation status. – *Lepraria finkii* is not considered to warrant any conservation concerns at this time.

Discussion. – Most readers will know *Lepraria finkii* as *L. lobificans*, because that is the name that has been used for this taxon since Laundon (1992) placed the two names in synonymy. Those who remember a time before 1992 will, however, already be familiar with *L. finkii*. As has been discussed elsewhere (Lendemer 2010), examination of the type specimen of *L. lobificans* has confirmed that in fact it is conspecific with *L. santosii* and not *L. finkii*. Thus I here formally

place *L. santosii* in synonymy with *L. lobificans*[10] and take up the name *L. finkii* for the common species previously referred to as *L. lobificans*.

Lepraria finkii is paradoxically one of the most easily recognizable and easily confused species of *Lepraria*. The combination of a *finkii*-type placodioid thallus and production of atranorin, zeorin, and stictic acid are distinctive. Nonetheless, the species is sympatric with nearly every other species known from the continent, and reliance on spot tests alone for identification can potentially lead to confusion.

Only three species are chemically similar to *Lepraria finkii* in producing atranorin and stictic acid. *Lepraria elobata* produces these substances as well as zeorin, however, it does not produce the two unknowns mentioned above and it has an aggregate rather than placodioid thallus. The stictic acid-producing chemotype of *L. neglecta* is chemically similar to *L. finkii*, and indeed some populations even produce zeorin, however, that species has an aggregate thallus in which the granules develop a distinct pseudocortex. *Lepraria leprolomopsis* is most likely to be confused with *L. finkii* as it is morphologically identical and entirely sympatric in North America. The only distinguishing feature of *L. leprolomopsis* is that it lacks zeorin, and in some cases produces abundant anthraquinones on the underside of the thallus. Further studies with molecular data are needed to elucidate whether *L. leprolomopsis* is a distinct taxon or merely a chemical variant of *L. finkii*. It should be noted that the report of *L. finkii* (sub. *L. lobificans*)

[10]*Lepraria lobificans* Nyl., Flora 56: 196. 1873. TYPE: **FRANCE:** Pyrénées-Orientales, Collioure, 4.vii.1872, [on soil], *W. Nylander s.n.* (H-NYL-10864!, lectotype [selected by Laundon 1992]; H-NYL-10865!, isolectotype).

Leproloma lobificans (Nyl.) Boistel, Nouv. Fl. Lich. 2: 318. 1903.

Lepraria santosii Argüello & A. Crespo *in* Crespo et al., Lichenologist 38: 218. 2006, syn. nov. TYPE: **SPAIN. CANARY ISLANDS:** Tenerife, Las Mercedes, acantilado del borde de la carretera, 28.iii.2005, on soil, *A. Crespo s.n.* (MAF [n.v.], holotype; GZU!, isotype).

See figure 75 for a comparison of the lectotype of *L. lobificans* and an isotype of *L. santosii*.

from Fiji by Lumbsch et al. (2011) requires confirmation as those authors did not mention the occurrence of zeorin in the material they examined.

Selected specimens examined (1177 total seen from North America). – **CANADA. BRITISH COLUMBIA.** GREATER VANCOUVER REGIONAL DIST.: City of Vancouver, University of British Columbia Campus, W of int of W Mall and NW Marine Dr., 23.v.2010, on *Acer*, *J.C. Lendemer 22302 & A. Moroz* (NY). QUEEN CHARLOTTE ISLANDS: Skidegate Inlet, Balch Island (North), at entrance to Maude Channel, 14.vii.1967, on rock, *I.M. Brodo 11529 & M. Shchepanek* (NY). SQUAMISH-LILLOOET DIST.: Murrin Provincial Park, around Browning Lake, 11.v.2000, on bryophytes on rock face, *W.R. Buck 54792* (NY). **MANITOBA:** SE of Flin Flon, Little Spruce Lake Rd., 24.v.2004, on mossy rock face, *T. Ahti et al. 62914* (H, NY). **NEW BRUNSWICK.** CHARLOTTE CO.: Pennfield Parish, W of Jake Lee Rd., 1.v.2011, on *Acer*, *J.C. Lendemer 27955* (NY); Saint Patrick Parish, Caughey-Taylor Nature Preserve, E of NB127, 2.v.2011, on *Thuja* base, *J.C. Lendemer 28094* (NY). **NEWFOUNDLAND AND LABRADOR.** NEWFOUNDLAND: Island of Newfoundland, Avalon Peninsula, Hawke Hills, 7.ix.2007, on rocks and soil, in overhang, *J.C. Lendemer 9957* (NY); Island of Newfoundland, Northern Peninsula, Ha-Ha Mountain, 20.viii.2007, on soil, in overhang, *J.C. Lendemer 10546 & A. Moroz* (NY); White Bay South Region, Sop's Arm W, Main River Forest Access Rd., 6.ix.2011, on rock, *T. Ahti 70738i* (H, NY). **NOVA SCOTIA.** CUMBERLAND CO.: Cape Chignecto Provincial Park, along trail from Red Rocks to McGahey Brook, 15.v.2004, on soil, *R.C. Harris 49806* (NY). LUNENBURG CO.: Upper Lattare, Beck's Lake, 28.xii.2008, on *Acer*, *F. Anderson s.n.* (NY). QUEENS CO.: Thomas H. Raddall Provincial Park, 7.v.1999, on rock, *R.C. Harris 42935* (NY). SHELBURNE CO.: along Hwy 302 just S of Upper Ohio, at Philip

Lake, 9.v.1999, on *Betula*, *R.C. Harris 43077* (NY). **ONTARIO.** BRUCE CO.: Bruce Peninsula National Park, just SW of parking area for Halfway Log Dump, 20.ix.2008, on log, *J.C. Lendemer 14291* (NY). LANARK CO.: N of Peneshula Rd. 0.6 mi W of int w/ Cedar Cove Rd., 21.v.2011, on *Thuja*, *J.C. Lendemer 28290 & R.E. Lee* (NY). PETERBOURGH CO.: Jermyn-Indian River, 10 km E of Peterborough, 2.iii.2011, on *Acer*, *C. Lewis 515* (NY). SUDBURY DIST.: Hallum Twp., on S side of Hwy 17, 5.6 km W of Webbwood, 19.iv.2011, on rock, *C. Lewis 599* (NY). **QUEBEC.** KAMOURASKA CO.: Sainte-Anne de la Pocatière, 27.iv.1935, on soil, *E. LaPage 131* (NY). PONTIAC CO.: Gatineau Park, 0.3–0.5 mi E of parking area on Chemin Eardley Masham, 22.v.2011, on *Tsuga*, *J.C. Lendemer 28330 & C. Freebury* (NY). **U.S.A. ALABAMA.** COVINGTON CO.: Conecuh National Forest, Solon Dixon Forestry Education Center, Blue Springs, 14.iv.2007, on *Ulmus*, *R.C. Harris 53496* (NY). ESCAMBIA CO.: Escambia Creek Wildlife Management Area, N of Conway Rd., 13.iv.2007, on *Persea*, *R.C. Harris 53361* (NY). JACKSON CO.: Pisgah, Jones Cove below Pisgah Civitan Park, 2.x.1999, on rock, *R.C. Harris 43323* (NY). LAWRENCE CO.: near Prairie Grove Glades, 3.i.2011, on *Juniperus*, *E. Tripp 1321* (NY). WINSTON CO.: Dismals Wonder Garden, 26.ix.1992, on rock, *R.C. Harris 28480* (NY). **ALASKA:** Washington Bay, Kuiu Island, 5.ix.1947, on large spruce, *W.J. Eyerdam 470* (NY). **ARIZONA.** COCOINO CO.: 1.5 mi up West Fork of Oak Creek Canyon, 13.v.1960, on sandstone, *S.A. Johnsen & T.N. Johnsen 892* (COLO). **ARKANSAS.** CLEBURNE CO.: N portion of North Unit of Big Creek Natural Area, 9.x.2010, on *Juniperus*, *J.C. Lendemer et al. 26363* (NY). FAULKNER CO.: Cove Creek Natural Area, 7.x.2010, on *Juniperus*, *J.C. Lendemer 26253* (NY). MADISON CO.: Sweden Creek Falls Natural Area, 5.x.2010, on mosses over rock, *J.C. Lendemer et al. 25942* (NY). NEWTON CO.: Ozark National Forest, E of FSR 1463, 2.6 mi N of AR 21, 28.iii.2006, on

Fagus, *R.C. Harris 52296* (NY). PERRY CO.: Ouachita National Forest, vicinity of Goat Bluff along N side of South Fourche LaFarve River, 6.x.2010, on *Quercus*, *J.C. Lendemer et al. 26044* (NY). **CALIFORNIA.** MARIN CO.: Point Reyes National Seashore, Laguna Trail on way to beach camp, 9.vii.2008, on sandstone, *K. Knudsen 9876* (NY). MARIPOSA CO.: Yosemite National Park, bottom of Tuolumne Grove, 19.ix.2009, on *Abies*, *J.C. Lendemer 19564* (NY). MONTEREY CO.: Santa Lucia Mountains, Lime Kiln State Park, 11.ix.2007, on clay, *K. Knudsen 10274.1* (NY). SANTA BARBARA CO.: Channel Islands, Santa Cruz Island, S of Canada Cervida along truck trail, 16.vi.2007, on *Adenostoma*, *K. Knudsen et al. 8572* (NY). SONOMA CO.: McMurray Ranch Winery, 12.vii.2008, on moss and old bark of *Umbellularia, K. Knudsen et al. 9916* (NY, UCR). **COLORADO.** GRAND CO.: Rocky Mountains National Park, 4.5 mi NE of Grand Lake, North Inlet, 20.vi.1959, on rock, *J. Douglass & M. Douglass s.n.* (COLO). **CONNECTICUT.** FAIRFIELD CO.: Town of Redding, Highstead Arboretum, 9.vi.2005, on *Quercus*, *R.C. Harris 51567* (NY). LITCHFIELD CO.: Town of Caanan, Great Mountain Forest, Sam Yankee Woodlot, 20.ix.2003, on *Quercus*, *R.C. Harris 48027* (NY). WINDHAM CO.: Town of Westford, Yale Myers Forest, 19.ix.2009, on rock in overhang, *R.C. Harris 55661* (NY). **DELAWARE.** NEW CASTLE CO.: N of Walter S. Carpenter Jr. State Park, 19.i.1991, on trees, *C.F. Reed 133826* (NY). SUSSEX CO.: Ellendale State Forest, 25.x.1989, on *Liquidambar*, *R.C. Harris 24909* (NY). **FLORIDA.** ALACHUA CO.: Paynes Prairie State Preserve, vicinity of Alachua Sink, 1.xii.1992, on *Quercus*, *R.C. Harris 29463* (NY). CITRUS CO.: Withlacoochee State Forest, along Florida Trail N to Brush Sink from CR 480, 6.xii.1993, on rock, *R.C. Harris 31820* (NY). DUVAL CO.: Jacksonville Beach, NE corner of int. of N 20th St. and 2nd Ave. N., 9.iii.2009, on log, *J.C. Lendemer 15897 & W.R. Buck* (NY). GULF CO.: N of Lake Grove Rd. (SR22), 1.5 mi E of SR71 at Wewahitchka, 11.xii.1993, on

Taxodium, *R.C. Harris 32191* (NY). HERNARDNO CO.: E of Brooksville, 6.viii.1964, on *Quercus*, *B.J. Moore 2065* (DUKE, NY). JACKSON CO.: Black Spring, ~1 mi W of FL167, 3.xii.1988, on *Carpinus*, *R.C. Harris 23338* (NY). **GEORGIA.** BAKER CO.: Ichauway Plantation/Jones Ecological Research Center, E shore of Ichauway-Notchaway Creek, 16.iv.2007, on *Quercus*, *J.C. Lendemer et al. 9507* (NY). COFFEE CO.: Broxton Rocks Ecological Preserve, Ricketson Tract, 4.ii.1995, on soil, under overhang, *R.C. Harris 38876* (NY). EARLY CO.: Williams Bluff Preserve, ~3 mi W of Cedar Springs, 15.iv.2007, on sandstone, *J.C. Lendemer et al. 9323* (NY). HEARD CO.: Camp Meeting Rock Preserve, 1.x.1999, over mosses, *W.R. Buck 36272* (NY). McINTOSH CO.: Sapelo Island, Sapelo Island Wildlife Management Area, West Perimeter Rd. 1 mi S of Kenan Fields, 10.xii.2009, on *Quercus*, *J.C. Lendemer 20797* (NY). TOWNS CO.: Chattahoochee National Forest, Hightower Gap to Rich Knob, 11.xi.2007, on *Acer*, *J.C. Lendemer et al. 10937* (NY). **ILLINOIS.** COLES CO.: Lakeview Park, ¼ mi E of Charleston, 4.ix.1986, on bark, *T. Motley 40* (NY). JACKSON CO.: Shawnee National Forest, Pomona Natural Bridge, 15.x.1993, on rock, *R.C. Harris 31274* (NY). SHELBY CO.: Hidden Springs State Forest, ~14 mi W of Neoga, 28.iv.1988, on bark, *R.C. Harris 21812* (NY). UNION CO.: Shawnee National Forest, Panther Den, 17.x.1993, on rock, *R.C. Harris 31434* (NY). **INDIANA.** OWEN CO.: Owen-Putnam State Forest, 3.2 mi W of Carp, 28.iv.1988, on bark, *R.C. Harris 21829* (NY). PUTNAM CO.: along bank of Big Walnut, 1 mi E of Bainbridge, 30.iv.1945, on soil, *K.A. Wagner 392* (NY). **IOWA.** BOONE CO.: Ledges State Park, ~4 mi S of Boone, 9.vii.2007, on sandstone, *M.K. Advaita 6478* (NY). FAYETTE CO.: Fayette, vii.1894, on calcareous rock and mosses, *B. Fink s.n.* (NY). **KANSAS.** CHAUTAUQUA CO.: 2.25 mi S of Niotaze, along Birch Creek, 18.ix.2007, on *Betula*, *C.A. Morse 15698* (NY). CHEROKEE CO.: Spring River Wildlife Area, N of NE 100th St.,

13.iv.2004, on sandstone, *W.R. Buck 46376* (NY). DOUGLAS CO.: "Baldwin Woods," 3.iv.1954, on rock, *C.L. Kramer 346* (NY). ELLSWORTH CO.: Kanopolis State Park, 4 mi S, 2 mi E Carneiro, 27.iv.2008, on bluffs, *C.A. Morse 12699 & C.C. Freeman* (KANU, NY). **KENTUCKY.** BULLITT CO.: Bernheim Arboretum and Research Forest, near S end of Rice Orchard Ridge Rd., 24.x.2002, on mossy limestone, *D. Ladd 24038* (NY). EDMONSON CO.: Mammoth Cave National Par, Cedar Sink area, viii.1979, on soil, *J.P. Dey 12597* (NY). KNOTT CO.: 2 mi E of Hindman, 10.xi.1968, on soil, *C.F. Reed 80207* (NY). MASON CO.: Rt. 10, 2 mi W of Plumville, 9.xi.1980, on bark, *C.F. Reed 119875* (NY). PERRY CO.: Daniel Boone National Forest, Old Field Branch of Leatherwood Creek, 6.x.2001, on soil, *R.C. Harris 44962* (NY). **LOUISIANA.** EAST BATON ROUGE PARISH: Burden Research Plantation, southeast Baton Rouge, 8.iii.1977, on bark, *S.C. Tucker 16903* (NY). ORLEANS PARISH: New Orleans, near Industrial Canal, 14.iii.1939, on *Taxodium*, *F. Pennebaker s.n.* (NY). **MAINE.** HANCOCK CO.: Town of Aurora, N-facing slopes above Middle Branch of the Union River, 11.vi.2010, on *Thuja*, *J.C. Lendemer 23225* (NY). KNOX CO.: Rockport, Cramer Park on N-facing slope of Goose River, 6.vi.2010, on limestone, *B. Shaw 9744* (NY). WALDO CO.: Town of Lincolnville, Ducktrap River Preserve, ~4 mi NE of Lincolnville Center on ME 52, 28.v.2009, on tree trunk, *W.R. Buck 54871* (NY). WASHINGTON CO.: T18 ED BPP, Maine Public Reserve Lands, S end of Rocky Lake, 9.vi.2010, on *Betula*, *J.C. Lendemer 22900* (NY). YORK CO.: Town of Alfred, Massabesic Experimental Forest, Southern Unit, 14.ix.2002, on face of erratic boulder, *R.C. Harris 46252* (NY). **MARYLAND.** ALLEGHANY CO.: Fort Hill, just NE of jct US220 and Potomac River, 24.iv.1989, on rock, *R.C. Harris 24621* (NY). ANNE ARUNDEL CO.: Furnace Branch, 15.x.1907, on *Acer*, *C.C. Plitt s.n.* (NY). CECIL CO.: New Valley, along Basin Run, Liberty Grove Rd., 1.xii.2009, on *Fraxinus*, *J.C. Lendemer 20010* (NY). MONTGOMERY CO.:

Great Falls State Park, Bear Island, 25.iv.1993, on rock, *T. Ahti 51502 & D.H. Nicholson* (H, NY). **MASSACHUSETTS.** BERKSHIRE CO.: Town of Pittsfield, Pittsfield State Forest, E of Lulu Cascade, 4.vi.2010, on *Acer*, *J.C. Lendemer 22479* (NY). FRANKLIN CO.: Town of Rowe, just off Tunnel Road, 3.8 mi NW of Zoar on River Rd., 19.ix.1998, on mosses, *R.C. Harris 42280* (NY). MIDDLESEX CO.: Cambridge, sine date, *E. Tuckerman s.n.* (NY). **MICHIGAN.** JACKSON CO.: NW corner of intersection of I-94 and Race Rd., NE of Jackson, 15.vi.1979, on *Quercus*, *R.C. Harris 13082* (NY). WASHTENAW CO.: W of Merrill Rd., ~0.3 mi N of Barker Rd., W of Whitmore Lake, 10.vii.1979, on *Quercus*, *R.C. Harris 13131* (NY). **MINNESOTA.** BLUE EARTH CO.: Mankato, 23.vi.1899, on shaded rock, *J.R. Gardner 40* (NY). COOK CO.: Grand Portage Island, 17.vi.1897, on earth, *B. Fink 1* (NY). RAMSEY CO.: Mississippi River National River, Battle Creek Park in St. Paul, 16.vi.2008, on rock, *C.M. Wetmore 97019* (MIN, NY). **MISSISSIPPI.** ITAWAMBA CO.: Donivan Slough, Mile 283.3 along Natchez Trace Parkway, 28.ix.1992, on *Taxodium*, *R.C. Harris 28636* (NY). JASPER CO.: Bienville National Forest, along CR 506 at Little Tallahala Creek, 30.ix.1992, on *Quercus, R.C. Harris 28818* (NY). SCOTT CO.: Bienville National Forest, Bienville Pines Scenic Area, just SE of Forest, 29.ix.1992, on *Acer*, *R.C. Harris 28751* (NY). WINSTON CO.: Tombigee National Forest, Choctaw Wildlife Area, near int of FSR 954 & FSR 955S, 28.ix.1992, on *Carpinus*, *R.C. Harris 28672* (NY). **MISSOURI.** BOLLINGER CO.: Dark Cypress Swamp Conservation Area, 22.x.2001, on *Platanus*, *R.C. Harris 45212* (NY). IRON CO.: Pilot Knob National Wildlife Refuge, ~1 mi N of Ironton, 30.iii.2006, on soil, *R.C. Harris 52436* (NY). LINCOLN CO.: Cuiver River State Park, Big Sugar Creek Wild Area, 23.v.2003, on *Quercus*, *R.C. Harris 47830* (NY). MARIES CO.: Clifty Creek Natural Area, ~5.5 air mi ENE of Dixon, 4.x.2010, on *Quercus*, *J.C. Lendemer 25763 & D. Ladd* (NY). ST. FRANCOIS CO.: Buck Creek

Winery, just NE of Buck Mountain Conservation Area, 9.x.2010, on *Juniperus*, *J.C. Lendemer 26640 & D. Ladd* (NY). WAYNE CO.: Sam A. Baker State Park, Shut-Ins Trail, 15.x.2003, on rock, *W.R. Buck 45375* (NY). **NEBRASKA.** KEYA PAHA CO.: 3 mi N Riverview, 16.vi.1948, on bark, *W. Kiener 23706* (NY). NEMAHA CO.: Peru, 6.x.1899, on soil, *J.L. Sheldon s.n.* (NY). **NEW JERSEY.** ATLANTIC CO.: Tuckahoe Wildlife Management Area, gravel road .05 mi E of jct w/ Griscom Mill Rd., 2.xii.2009, on *Quercus*, *J.C. Lendemer 20043* (NY). CAPE MAY CO.: Peaslee Wildlife Management Area, ~3.75 mi W of jct NJ 49 & CR 548 (Weatherby Rd.), 2.ii.2009, on concrete, *J.C. Lendemer 15187* (NY). GLOUCESTER CO.: Glassboro Wildlife Management Area, ~1.5 mi S of jct NJ 322 and CR 655 (Fries Mill Rd.), on *Quercus*, *J.C. Lendemer 15305* (NY). MONMOUTH CO.: Allaire State Park, S corner between Squankum Rd. and Hospital Rd., 5.ii.2009, on *Quercus*, *J.C. Lendemer 15430* (NY). OCEAN CO.: Manahawkin Wildlife Management Area, S of Stafford Ave., 2.xii.2009, on *Quercus*, *J.C. Lendemer 20051* (NY). **NEW MEXICO.** EDDY CO.: Guadalupe Mountains, near end of FSR 540 in uppermost branch of Big Canyon, 7.vii.2010, on shaded limestone, *R.D. Worthington 36143* (NY, UTEP). SOCORRO CO.: Gallinas Canyon, Los Negas [sic], 3.vii.1934, on rock, *R.A. Studhalter 51815* (NY). **NEW YORK.** DUTCHESS CO.: Dover Plains, Nellie Hill Preserve, 27.v.2007, on base of *Cornus, J.C. Lendemer 12057 & W.R. Buck* (NY). ESSEX CO.: SW side of Chapel Pond, 18.ix.2004, on shaded, vertical rock face, *J.C. Lendemer et al. 2969* (NY). GREENE CO.: Town of Hunter, Catskill Forest Preserve, Stony Clove Notch, 13.ix.2008, on rock, in overhang, *J.C. Lendemer 14091* (NY). HERKIMER CO.: Little Falls, Moss Island, 14.vi.2011, on rock, *R.C. Harris 56825* (NY). ONTARIO CO.: 3.4 mi N, 0.6 mi E Springwater, 25.xii.2003, on rock, *C.A. Morse 9976* (NY). ROCKLAND CO.: Bear Mountains State Park, Iona Island Bird Sanctuary, 18.vii.2010, on moss, in overhang, *J.C. Lendemer 23686 & A. Moroz* (NY). **NORTH**

CAROLINA. CAMDEN CO.: Dismal Swamp State Park, S side of Corapeake Ditch, 10.x.2009, on *Acer*, *J.C. Lendemer 20133 & S. Williams* (NY). DARE CO.: Nags Head Woods Ecological Preserve, 29.ix.1993, on *Quercus*, *R.C. Harris 31024* (NY). JONES CO.: Croatan National Forest, Island Creek Walk, 17.iii.2003, on marl, *W.R. Buck 43735* (NY). HAYWOOD CO.: Great Smoky Mountains National Park, areas around Caldwell Fork, 4.viii.2009, on moss, in overhang, *J.C. Lendemer 18977 & E.A. Tripp* (NY). ORANGE CO.: Mason Farm Biological Reserve, Plot 5, 1.iii.2007, on *Acer rubrum*, *G.B. Perlmutter et al. 833* (NY). SWAIN CO.: Great Smoky Mountains National Park, Beauregard Ridge, 14.x.2010, on soil, *J.C. Lendemer et al. 26977* (NY). WILKES CO.: Stone Mountain State Park, ~8 mi SSW of Sparta, 23.ix.1993, on *Quercus*, *R.C. Harris 30801* (NY). **OHIO.** ADAMS CO.: Chaparral Prairie State Nature Preserve, 22.v.2006, on *Fraxinus*, *J.C. Lendemer et al. 7266* (NY). GALLIA CO.: Wayne National Forest, above Symmes Creek, 19.v.2006, on sandstone, *J.C. Lendemer et al. 7428* (NY). PIKE CO.: Strait Creek Prairie Preserve, 20.v.2006, on bryophytes, *W.R. Buck 50275* (NY). SCIOTO CO.: Shawnee State Forest, at S jct of FR 3 & FR 6, 21.v.2006, on wood, *R.C. Harris 52750* (NY). **OKLAHOMA.** CHEROKEE CO.: 11 mi N of Hulbert, along Clear Creek, vi.2010, on *Quercus*, *V.P. Doyle 1078* (NY). MUSKOGEE CO.: Gruber Wildlife Management Area, along OK 10, 15.iv.2004, on rock, *R.C. Harris 49045* (NY). SEQUOYAH CO.: Sallisaw-Brushy Lake State Park, ~6 mi N of Sallisaw, 1.xi.2000, on rock, *W.R. Buck 38666* (NY). **OREGON.** COOS CO.: South Slough National Estuarine Research Reserve, 12 km SW of town of Coos Bay, ix.2008, on bark, *B. McCune 29774* (NY, OSC). LINCOLN CO.: Oregon Coast Trail just S of Cape Perpetua, 18.viii.2009, on soil bank, *B. McCune 30200* (NY). **PENNSYLVANIA.** CENTRE CO.: Bald Eagle State Forest, Stover Gap, 14.ix.2010, on *Quercus*, *J.C. Lendemer 25524* (NY). JEFFERSON CO.: Clear Creek State Park, Phyllis Run

0.25–0.75 mi N of confluence w/ Clear Creek, 8.ix.2010, on *Acer*, *J.C. Lendemer 24774* (NY). MONROE CO.: Tannersville Bog TNC Preserve, 24.xi.2009, on *Acer*, *J.C. Lendemer 19874 & V.P. Doyle* (NY). SULLIVAN CO.: Ricketts Glen State Park, S slope above Big Run, 19.ix.2010, on rock, in overhang, *J.C. Lendemer 25655 & J. Kunsman* (NY). UNION CO.: Bald Eagle State Forest, N-facing slopes above Spruce Run, 13.ix.2010, on *Quercus*, *J.C. Lendemer 25379* (NY). **SOUTH CAROLINA.** AIKEN CO.: The Hitchcock Woods, just SW of city of Aiken, along Tea Cottage Path from entrance on Dibble Rd., 14.iii.2010, on *Ilex*, *J.C. Lendemer 22048* (NY). EDGEFIELD CO.: Sumter National Forest, along FSR 634, 1.3 mi W of SC 52, 17.iii.1997, on *Carpinus*, *R.C. Harris 40283* (NY). McCORMICK CO.: Sumter National Forest, Stevens Creek Heritage Preserve, 11.iii.2010, on *Carya*, *R.C. Harris 55882* (NY). PICKENS CO.: along Eastatoe Creek, ~2.5 mi SW of town of Rocky Bottom, 27.ix.1989, on soil, *R.C. Harris 24782* (NY). **SOUTH DAKOTA.** CUSTER CO.: south of Jewel Cave National Monument, 0.8 mi S of US 16 in Hell Canyon, 6.vii.1960, on rock, *C.M. Wetmore 6924* (MSC). FALL RIVER CO.: TNC Whitney Preserve, Alabaugh Canyon, 3.vii.2007, on *Junipers*, *M.K. Advaita 6443* (NY). LAWRENCE CO.: Town of Whitewood (9 mi NE of Lead), 10.viii.1961, on rock, *C.M. Wetmore 11186* (MSC). PENNINGTON CO.: 3.3 mi W of Canyon Lake on Rim Rock Drive, 31.vii.1960, on rock, *C.M. Wetmore 8626* (NY). **TENNESSEE.** BLOUNT CO.: Great Smoky Mountains National Park, White Oak Sinks, manhole to blowhole, 13.x.2010, on limestone, *J.C. Lendemer et al. 1462* (NY). MONROE CO.: Cherokee National Forest, Bald River Falls Quad., along Laurel Creek between SR 165 and FSR 35, 6.viii.1994, on soil, *R.C. Harris 32888* (NY). POLK CO.: Cherokee National Forest, along FSR 62 at Sheeds Creek, 5.x.1998, on *Liriodendron*, *R.C. Harris 42486* (NY). SEVIER CO.: Great Smoky Mountains National Park, summit of the Chimney Tops, 28.vi.2010, on rock, in overhang, *J.C. Lendemer et*

al. 23336 (NY). **UTAH.** UINTAH CO.: Ashley National Forest, along FSR 104 (Paradise Park Rd.), vicinity of Weasel Point, 30.vii.2009, on bryophytes, *W.R. Buck 55066* (NY). **VERMONT.** ADDISON CO.: Town of Bristol, Green Mountain National Forest, Bristol Cliffs Wilderness Area, 20.x.2010, on humus, in overhang, *J.C. Lendemer 27391 & M. Sundue* (NY). CHITTENDEN CO.: City of Burlington, Lone Rock Point, 17.x.2010, on *Thuja*, *J.C. Lendemer et al. 27037* (NY). ESSEX CO.: Town of Victory, Victory State Forest, Umpire Mountain, 16.v.2008, on rock, *R.C. Harris 54384* (NY). LAMOILLE CO.: Town of Eden, Babcock Nature Preserve of Johnson State College, 14.v.2005, on *Betula*, *R.C. Harris 51378* (NY). **VIRGINIA.** CHESAPEAKE CITY: Dismal Swamp National Wildlife Refuge, E of Laurel Ditch, 13.xii.2009, on *Magnolia*, *J.C. Lendemer et al. 20562* (NY). GRAYSON CO.: Jefferson National Forest, ~0.5 mi SW of VA 16 on VA 741/Homestead Rd., 5.iv.2008, on *Acer*, *R.C. Harris 54046* (NY). SMYTH CO.: Jefferson National Forest, Whitetop Mountain, 5.iv.2008, on rock, *R.C. Harris 54110* (NY). SUFFOLK CITY: Dismal Swamp National Wildlife Refuge, Jericho Ditch, 12.xii.2009, on *Acer*, *J.C. Lendemer 20489 & B.P. Hodkinson* (NY). **WEST VIRGINIA.** GRANT CO.: Greenland Gap, along CR3-3, 21.ix.2001, on bryophytes, *R.C. Harris 44847* (NY). PENDLETON CO.: Monongahela National Forest, along Smoke Hole Rd. (CR 2), 2.x.2000, on rock, *R.C. Harris 44077* (NY). POCAHONTAS CO.: Watoga State Park, Pyle Mountain vic. picnic area at north entrance, 21.x.2007, on *Carya*, *J.C. Lendemer 9836 & A. Moroz* (NY). TUCKER CO.: Monongahela National Forest, Olsen's Bog, 22.ix.2001, on *Betula*, *R.C. Harris 44870* (NY). **WISCONSIN.** LINCOLN CO.: Town of Rock Falls, along E shore of Wisconsin River at Grandfather Dam, 27.iv.2002, on soil, *R.C. Harris 45876* (NY). ONEIDA CO.: Town of Minocqua, Patters Hemlocks State Natural Area, 29.iv.2002, on *Betula*, *R.C. Harris 46004* (NY). RICHLAND CO.: Bosstown, 24.ix.1958, on rock, *T. Ahti 5008* (H, NY).

SAUK CO.: Baraboo Hills, Hemlock Draw Preserve, 4.ix.1998, on sandstone, *R.C. Harris 42204* (NY). VILAS CO.: Town of Plum Lake, Northern Highland State Forest, N of Co. Hwy. K just E of Old K Rd., 26.iv.2002, on *Betula*, *R.C. Harris 45832* (NY).

Lepraria friabilis **Lendemer, K. Knudsen & Elix,** Opuscula Philolichenum 5: 64. 2008. TYPE: **U.S.A. ALABAMA.** BALDWIN CO.: Splinter Hill Bog Preserve, south of CR 47, 0.8 mi NW of Dyas Creek, 1.3 mi WNW of I-65, 250 ft., *Sarracenia* bog with adjacent hardwood swamp and bottomlands along stream, 12.iv.2007, on *Pinus*, *J.C. Lendemer et al. 9063* (NY!, holotype; B!, CANB!, UCR!, UGDA!, hb. Lendemer!, isotypes).

Figures 76 and 77.

Description. – **Thallus** crustose, leprose, aggregated, discontinuous and not stratified, initially composed of isolated granules that divide to form aggregations, eventually merging to form a thin crust (<0.1 mm thick), gray-blue in color; **hyphae** hyaline, [1.5]–(2.2)–2.8–(3.4)–[4.3] µm (*n*=96) in width, septate, secund, obscured by a thick layer of POL+ crystals that dissolve in KOH; **prothallus** persistent; **hypothallus** absent; **rhizohyphae** absent; **granules** globose, [24]–(32)–42–(52)–[66] µm (*n*=128) in diameter, ecorticate, well organized and discrete, remaining distinct and not forming compound units; **photobiont** green, coccoid, cells globose, [4.8]–(6.9)–8.9–(11.0)–[15.6] µm (*n*=90) in width.

Chemistry. – Fumarprotocetraric acid, protocetraric acid, ± atranorin. Spot tests: K-, C-, KC-, P+ orange-red, UV-.

When *Lepraria friabilis* was described it was considered to include only populations with fumarprotocetraric acid as the major substance (Lendemer et al. 2008). Subsequent study led to the discovery of several additional populations in eastern North America that produced atranorin. Inclusion of one such population in molecular phylogenetic analyses that included a diverse sampling of *L. friabilis* did not reject the inclusion of this chemotype in the species (Lendemer in press). It should be noted that *L. friabilis* often occurs in mixed populations with *L. harrisiana*, and TLC results that show the presence of zeorin and/or fatty acids should be evaluated in this light. Lendemer et al. (2008) also noted the existence of a second chemotype that produced only fumarprotocetraric acid and was restricted to southern California. Subsequent study has revealed the vouchers of this chemotype represent a sterile sorediate crust unrelated to *Lepraria* s.str.

Ecology and distribution. – *Lepraria friabilis* is widespread throughout the subtropical regions of the Coastal Plain of southeastern North America (figure 76). The species is common in freshwater wetlands, particularly swamps, where it typically occurs on the bark of conifers. This taxon is likely more widespread than the available collections indicate and further studies are needed to fully elucidate its geographic distribution.

Conservation status. – This species is locally abundant in, and endemic to, freshwater wetlands throughout the subtropical portions of the Coastal Plain in southeastern North America. Its apparent restriction to these habitats, which are under mounting threat from a myriad of forces including climate change and anthropogenic habitat alteration, indicates that the conservation needs of this species require further study.

Discussion. – There are relatively few species of *Lepraria* sympatric with *L. friabilis* and as such there is likely little chance for confusion. The species often occurs with *L. finkii* and *L. harrisiana*, sometimes as mixed populations. Both of those species have placodioid rather than aggregate thalli, and thus only populations of *L. friabilis* where the aggregate thalli have fused to form a continuous crust are likely to be misidentified. Those species are also chemically distinct in producing atranorin and zeorin (with accessory pallidic acid in *L. harrisiana* and stictic acid in *L. finkii*). As has been noted above in the chemistry section, TLC analyses of *L. friabilis* specimens that reveal the presence of zeorin or fatty acids are almost certainly the result of admixtures of *L. harrisiana*. *Lepraria aurescens* and *L. squamatica* could also be confused with *L. friabilis* if one does not notice the different thallus type (placodioid in those species). However, *L. aurescens* produces thamnolic acid and *L. squamatica* produces squamatic acid (note, the latter also produces protocetraric acid as an accessory to squamatic acid).

When Lendemer et al. (2008) described *Lepraria friabilis* they questioned whether the species was sympatric with *L. caesiella*, which they considered to be morphologically similar in producing an aggregate thallus. Subsequent study of *L. caesiella* revealed that the populations that were sympatric with *L. friabilis* actually represented a new species, *L. harrisiana*, which has a placodioid rather than aggregate thallus (Lendemer in press.). Surprisingly, no other *Lepraria* species with aggregate thalli that are sympatric with *L. friabilis*. Corticolous populations of the fumarprotocetraric acid producing-chemotype of *L. neglecta* would be the most likely to cause confusion, however, that the granules in that species produce a well-developed pseudocortex and thus appear compact (vs. ecorticate and fuzzy in *L. friabilis*).

Selected specimens examined (35 total seen from North America; specimens with atranorin are indicated with +at). – **U.S.A. ALABAMA.** BALDWIN CO.: Perdido River Wildlife Management Area, 2.5 mi N of CR 112, 11.iv.2007, on *Chamaecyparis*, *R.C. Harris 53245* (NY). **ARKANSAS.** FAULKNER CO.: Cove Creek Natural Area, 7.x.2010, on *Pinus*, *J.C. Lendemer et al. 26202* (NY). JEFFERSON CO.: Pine Bluff Arsenal, along S side of Arkansas River, 1.xii.1999, on *Taxodium*, *D. Ladd 21852 & M. Pederson* (NY), *D. Ladd 22032 & M. Pederson* (NY, at+). PERRY CO.: Ouachita National Forest, vicinity of Goat Bluff along N side of South Fourche LaFave River, 6.x.2010, on *Pinus*, *J.C. Lendemer et al. 26011* (NY, at+). **FLORIDA.** ESCAMBIA CO.: along CR 99A at Brushy Creek, 4.3 mi W of SR 97 at Walnut Hill, 8.xii.1993, on *Taxodium, R.C. Harris 31922* (NY). WAKULLA CO.: Apalachicola National Forest, Bradwell Bay Wilderness, along Apalachicola Trail at Sopchoppy River, 29.xi.1988, on bark, *R.C. Harris 22994* (NY). **GEORGIA.** APPLING CO.: Moody Forest Natural Area, 0–0.5 mi E of head of River Trail on Miller Landing Rd., 17.xii.2009, on *Pinus*, *J.C. Lendemer 21079-A* (NY). BURKE CO.: Boggy Gut Creek tract, N of GA 56 Spur (River Rd.), 15.iii.2010, on *Pinus*, *J.C. Lendemer 22248-A* (NY). CANDLER CO.: Charles Harold TNC Preserve, 0–0.25 mi N of Salem Church Rd., 22.xii.2009, on *Pinus*, *J.C. Lendemer et al. 21784* (NY). McINTOSH CO.: Sapelo Island, Sapelo Island Wildlife Management Area, Dogpatch Rd. ¾ mi E of Lumber Landing, 15.xii.2009, on *Pinus*, *J.C. Lendemer 20653* (NY). TATTNALL CO.: Big Hammock Natural Area, 0.25 mi W of parking area on CR 441, 20.xii.2009, on *Taxodium*, *J.C. Lendemer 21344 & S.Q. Beeching* (NY). **MARYLAND.** WORCESTER CO.: Pocomoke State Forest, along Corbin Branch where it enters the Pocomoke River, 19.iv.2006, on *Taxodium*, *J.C. Lendemer et al. 6415* (NY). **MISSISSIPPI.** SHARKEY CO.: Delta National Forest, near Lost Lake, 23.vi.1978, on *Taxodium*, *G.T. Johnson 7104* (NY, at+). **NORTH**

CAROLINA. BLADEN CO.: N of Elwell Ferry Rd., ~0.5 mi W of Elwell Ferry, 15.vi.2002, on *Taxodium*, *R.C. Harris 46078* (NY). CRAVEN CO.: Croatan National Forest, N of Havelock, W of SR 70, W of Flanners Beach Campground, 13.xii.2004, on *Pinus*, *J.C. Lendemer 4217 & E.A. Tripp* (NY). GATES CO.: Merchants Millpond State Park, Coleman Trail 0–1 mi SE of Boat Launch Parking Area, 11.xii.2009, on *Fagus*, *J.C. Lendemer 20283 & F. Williams* (NY). ORANGE CO.: Mason Farm Biological Reserve, Big Oak Woods, 26.vii.2007, on *Pinus*, *G.B. Perlmutter 1100* (NY). WAKE CO.: Umstead State Park, Sycamore Lake area, 14.i.2007, on *Pinus*, *G.B. Perlmutter 794 & J.C. Lendemer* (NCU, NY). **SOUTH CAROLINA.** EDGEFIELD CO.: Sumter National Forest, along FSR 634, 1.3 mi W of SC 52, 17.iii.1997, on *Pinus*, *R.C. Harris 40277* (NY).

***Lepraria harrisiana* Lendemer,** Castanea. TYPE: **U.S.A. NORTH CAROLINA.** CAMDEN CO.: Dismal Swamp State Park, S side of Kim Saunders Ditch, at 5 mi marker, Atlantic white cedar (*Chamaecyparis thyoides*) stand with blow-downs and mixed hardwoods (*Magnolia, Acer, Nyssa*), 10.xii.2009, on *Chamaecyparis*, *J.C. Lendemer 20191 & S. Williams* (NY!, holotype).

Figures 78 and 79.

Description. – **Thallus** crustose, leprose, placodioid, continuous and typically distinctly bi-layered, initially forming small discrete thalli which expand marginally, overlap and become confluent to form a thick crust (0.2–0.4 mm thick), light greenish-blue in color, often with a slightly yellowish hue as the thallus ages in the herbarium; **hyphae** hyaline, [1.2]–(2.2)–2.9–(3.5)–[4.8] µm (*n*=68) in width, septate, secund, obscured by a thick layer of POL+ crystals that dissolve in KOH; **prothallus** disappearing with age; **hypothallus** a thin, often poorly developed

layer of loosely intertwined hyphae surrounding and subtending the granules, extending outward from the edge of the thallus; **rhizohyphae** present, pale to dirty brownish; **granules** globose, [19]–(27)–38–(48) –[72] µm (*n*=124) in diameter, ecorticate, well organized and discrete, often forming compound units of multiple granules; **photobiont** green, coccoid, cells globose, [6]–(7.8)–9.6–(11.4)–[14.4] µm (*n*=97) in diameter.

Chemistry. – Atranorin, zeorin, pallidic acid. Spot tests: K+ yellow, C-, KC-, P+ weak yellow, UV-.

Ecology and distribution. – *Lepraria harrisiana* is widespread in eastern North America (figure 79) where it occurs in high humidity habitats, particularly those associated with bodies of water. It is widely distributed in conifer swamps and riparian forests throughout both the Gulf and Atlantic coastal plains of southeastern North America, occurring from Massachusetts to Georgia and westward to at least Alabama. Disjunct populations also occur at high elevations of the southern Appalachian Mountains, riparian conifer forests along the edges of the Ozarks and in the Ouachita Mountains, oceanic forests of the Canadian Maritimes, and conifer swamps of the Great Lakes Region. As geographically disparate as this distribution may appear, it is not illogical considering the recent biogeographic patterns outlined by Lendemer et al. (in press.) for other lichens that occur in the southern Appalachian Mountains. Interestingly, saxicolous populations of *L. harrisiana* have not been encountered and the majority of the collections are from coniferous hosts (*Picea*, *Pinus*, *Thuja*, especially *Chamaecyparis*). Whereas the chemically similar *L. caesiella* rarely occurs on conifers, and rather is most often found on the bark of hardwoods and on non-calcareous rocks.

Conservation status. – The status of *Lepraria harrisiana* is somewhat similar to that of *L. friabilis*, although *L. harrisiana* is more widespread. The populations of *L. harrisiana* in the southeastern Coastal Plain are more-or-less restricted to freshwater wetlands, and thus threatened by the ecosystem-wide changes predicted to occur in those habitats as a result of global climate change. The populations in the Appalachian Mountains mostly occur at higher elevations, and the species is particularly abundant in the endemic spruce-fir forests of the southern Appalachians. These habitats are also under significant pressure from a diverse array of forces (Lendemer et al. in press.). The species also occurs on conifers in the Canadian Maritimes and Great Lakes regions. These populations are likely the least threatened of the species, but could be negatively impacted by forest management policies that cull too many trees and alter long-term moisture regimes.

Discussion. – *Lepraria harrisiana* was initially included within a broadly circumscribed *L. caesiella* until molecular phylogenetic analyses of nuITS sequence data confirmed that the significant differences in thallus morphology warranted taxonomic recognition (Lendemer in press). Both species have the same chemical profile but express the two fundamentally different types of leprarioid thalli outlined by Lendemer (2001). The thallus in *L. harrisiana* is placodioid and of the *cryophila*-type (i.e., with a hypothallus composed of loosely intertwined hyphae) whereas that of *L. caesiella* is aggregate and of the *caesiella*-type.

Morphologically *Lepraria harrisiana* is most similar to *L. aurescens* and *L. squamatica* Elix both of which also occur in southeastern North America. The two species are easily distinguished chemically by the production of thamnolic acid in *L. aurescens* and squamatic acid

in *L. squamatica.* Harris (*in* Lendemer 2005) compared *L. harrisiana* to *L. rigidula*. That species has an aggregate thallus and differs in producing nephrosteranic acid rather than zeorin and pallidic acid.

Selected specimens examined (93 total seen from North America). – **CANADA. NEW BRUNSWICK.** CHARLOTTE CO.: Grand Falls Flowage on St. Croix River, 20.iv.2011, on *Tsuga*, *J.C. Lendemer 27854* (NY), on *Betula*, *J.C. Lendemer 27786* (NY). **ONTARIO.** BRUCE CO.: Bruce Peninsula National Park, 20.ix.2008, on *Pinus*, *J.C. Lendemer 14283* (NY). FRONTENAC CO.: Arcol Road, 24.ii.2011, on *Pinus*, *C. Lewis 508* (NY). NIPISSING DIST.: Algonqiun Provincial Park, SE corner of Carl Wilson Lake, 31.vii.2011, on stump, *I.M. Brodo et al. 33035* (CANL). OTTAWA-CARLTON CO.: City of Ottawa, Stony Swamp, 19.v.2011, on *Thuja*, *J.C. Lendemer 28152 & I.M. Brodo* (NY). **U.S.A. ALABAMA.** CLAY CO.: Cheaha State Park, 24.ix.1992, on *Pinus*, *R.C. Harris 28298* (NY). COVINGTON CO.: Conecuh National Forest, Solon Dixon Forestry Education Center, 14.iv.2007, on *Pinus*, *J.C. Lendemer et al. 9430* (NY). MARSHALL CO.: Lake Guntersville State Park, 13.viii.2005, on *Pinus*, *J.C. Lendemer 4872* (NY). **ARKANSAS.** FAULKNER CO.: Cove Creek Natural Area, 7.x.2010, on *Pinus*, *J.C. Lendemer et al. 26201* (NY). GARLAND CO.: Ouachita National Forest, W side of North Fork Ouachita River, 6.x.2010, on *Pinus*, *J.C. Lendemer et al. 26116* (NY). **CONNECTICUT.** WINDHAM CO.: Town of Windham, Windham Bog, 20.ix.2009, on *Chamaecyparis*, *R.C. Harris 55730* (NY). **FLORIDA.** ESCAMBIA CO.: along CR 99a at Brushy Creek, 8.xii.1993, on *Taxodium*, *R.C. Harris 31907* (NY). SANTA ROSA CO.: Blackwater River State Forest, 9.xii.1993, on *Taxodium*, *R.C. Harris 32004* (NY). **GEORGIA.** DAWSON CO.: Amicalola Falls State Park, 21.xii.2009, on *Pinus*, *M.F. Hodges s.n.* (NY).

ROCKDALE CO.: Panola Mountain State Park, 17.iv.2007, on *Pinus*, *J.C. Lendemer et al. 9002* (NY). TATNALL CO.: Big Hammock Natural Area, 20.xii.2009, on *Taxodium*, *J.C. Lendemer 21368 & S.Q. Beeching* (NY). **ILLINOIS.** ALEXANDER CO.: Shawnee National Forest, Grapevine Trail Picnic and Camping Area, 6.xi.2004, on *Pinus*, *R.C. Harris 50521* (NY). **KENTUCKY.** BULLITT CO.: Bernheim Arboreum and Research Forest, 28.iii.2002, on *Pinus*, *D. Ladd 23610 & M. Ladd* (NY). **MAINE.** HANCOCK CO.: T.10 SD, N of ME 182, 27.vii.2006, on *Tsuga*, *R.C. Harris 53071* (NY). WASHINGTON CO.: Town of Cutler, Cutler Coastal Public Lands, 8.vi.2010, on conifer, *J.C. Lendemer 22755* (NY). **MARYLAND.** CAROLINE CO.: Idylwild State Wildlife Management Area, 18.iv.2006, on *Acer*, *J.C. Lendemer 6226 & W. Knapp* (NY). WICOMICO CO.: Sharptown Dunes, 19.iv.2006, on *Quercus*, *J.C. Lendemer et al 6291* (NY). **MISSISSIPPI.** SCOTT CO.: Bienville National Forest, Bienville Pines Scenic Area, 29.ix.1992, on *Pinus*, *R.C. Harris 28713* (NY). **MISSOURI.** CARTER CO.: Mark Twain National Forest, vicinity of Big Barren Creek Natural Area, 9.x.2010, on *Pinus*, *J.C. Lendemer 26576 & D. Ladd* (NY). **NEW JERSEY.** ATLANTIC CO.: Wharton State Forest, Batsto Natural Area, 15.iii.2000, on *Chamaecyparis*, *R.C. Harris 43787* (NY). BURLINGTON CO.: Wharton State Forest, W shore of Batsto River, 13.xi.2004, on *Pinus*, *J.C. Lendemer 3522 & R.F. Lendemer* (NY). CAMDEN CO.: Wharton State Forest, 0–0.5 mi W of CR 536 (Chew Rd.), 6.ii.2009, on *Pinus*, *J.C. Lendemer 15477* (NY). CAPE MAY CO.: Peaslee Wildlife Management Area, 2.ii.2009, on *Chamaecyparis*, *J.C. Lendemer 15208* (NY). CUMBERLAND CO.: Peaslee Wildlife Management Area, 2.ii.2009, on log, *J.C. Lendemer 15116* (NY). GLOUCESTER CO.: Glassboro Wildlife Management Area, 4.ii.2009, on *Quercus*, *J.C. Lendemer 15302* (NY). OCEAN CO.: Manchester Wildlife Management Area, 5.ii.2009, on *Gaylussacia*, *J.C. Lendemer 15366* (NY). SALEM CO.: Parvin State Park,

26.xii.2008, on *Pinus*, *J.C. Lendemer et al. 15018* (NY). **NEW YORK.** SUFFOLK CO.: Cranberry Bog Nature Preserve, 20.ix.1986, on *Chamaecyparis*, *R.C. Harris 19438-B* (NY). ST. LAWRENCE CO.: Twin Lakes, 6.ix.1981, on *Tsuga*, *R.C. Harris 13900* (NY). **NORTH CAROLINA.** ORANGE CO.: Johnston Mill Nature Preserve, 7.iii.2009, on *Pinus*, *G.B. Perlmutter et al. 1690* (NY). MACON CO.: Sunset Rocks, Highlands, 9.vi.1981, on *Pinus*, *R.C. Harris 13658* (NY). SURRY CO.: Pilot Mountain State Park, 26.viii.2006, on *Pinus*, *G.B. Perlmutter 587 & J. Pearson* (NY). TRANSYLVANIA CO.: Gorges State Park, 9.viii.2005, on *Pinus*, *J.C. Lendemer 4617 & E.A. Tripp* (NY). WILKES CO.: Stone Mountain State Park, 23.ix.1993, on *Pinus*, *R.C. Harris 30793* (NY). **PENNSYLVANIA.** WAYNE CO.: Dripping Cliffs TNC Preserve, 2.vii.2008, on *Tsuga*, *J.C. Lendemer 12373* (NY). **SOUTH CAROLINA.** AIKEN CO.: The Hitchcock Woods, 14.iii.2010, on *Pinus*, *J.C. Lendemer 22069* (NY). McCORMICK CO.: Sumter National Forest, Stevens Creek Heritage Preserve, 11.iii.2010, on *Pinus*, *R.C. Harris 55848* (NY). **TENNESSEE.** BLOUNT CO.: GSMNP, Rich Mountain Gap at Ace Gap Trail, 30.vi.2010, on *Pinus*, *J.C. Lendemer et al. 23601* (NY). COCKE CO.: GSMNP, Gabes Mountain Trail, 5.viii.2009, on *Tsuga*, *E.A. Trip 656 & J.C. Lendemer* (NY). SEVIER CO.: GSMNP, Chimney Tops Trail, 28.vi.2010, on *Betula*, *J.C. Lendemer et al. 23372* (NY). **VERMONT.** ESSEX CO.: Town of Averill, Quimby Country resort property, 19.v.2008, on *Abies*, *R.C. Harris 54600* (NY). **VIRGINIA.** CHESAPEAKE CITY: Dismal Swamp National Wildlife Refuge, E of Laurel Ditch, 13.xii.2009, on *Pinus*, *J.C. Lendemer et al. 20572* (NY). SUFFOLK CO.: Dismal Swamp National Wildlife Refuge, vicinity of canal bridge over Jerico Ditch, 12.xii.2009, on *Fagus*, *J.C. Lendemer 20437 & B.P. Hodkinson* (NY). **WISCONSIN.** ONEIDA CO.: Town of Woodruff, Northern Highland State Forest, 28.iv.2002, on *Tsuga*, *R.C. Harris 45954* (NY).

Lepraria hodkinsoniana **Lendemer,** Mycologia 103: 1224. [verify page number, surely not in issue 6] 2011. TYPE: **U.S.A. NEW JERSEY.** ATLANTIC CO.: Wharton State Forest, E of Nesco, NW of Batsto, N of Pleasant Mills Cemetery, 3–10 m, mixed *Pinus rigida-Quercus* forest with sparse *Betula*, on *Quercus*, 24 Oct 2004, *J.C. Lendemer 3358 = Lich. East. N. Amer. Exs. IV: 185* (NY! [barcode-1232372], holotype; ASU!, B!, BG!, CANB!, CHR!, DOV!, FH!, GZU!, H!, M!, MIN!, NY! [barcode-1069104], S!, TNS!, TU!, UPS!, isotypes).

Description. – **Thallus** crustose, leprose, aggregated, discontinuous and not stratified, initially composed of isolated granules that divide to form aggregations, eventually merging to form a thin crust (<0.1 mm thick), greenish-blue in color; **hyphae** hyaline, [1.7]–(2.3)–3–(3.8)–[7] µm (*n*=180) in width, septate, secund, obscured by a thick layer of crystals that dissolve in KOH; **prothallus** persistent; **hypothallus** indistinct, poorly developed or absent; **rhizohyphae** absent; **granules** globose, [15]–(25)–33–41–[60] µm (*n*=180) in diameter, ecorticate initially and eventually forming a poorly developed pseudocortex, well organized and discrete; **photobiont** green, coccoid, cells globose, [5]–(6.8)–8.8–(10.9)–[16] µm (*n*=180) in diameter.

Figures 80 and 81.

Chemistry. – Divaricatic acid, zeorin, ± atranorin, ± traces of nordivaricatic acid. Spot tests: K-, C-, KC- or very weakly KC+ pink, P-, UV+ blue-white.

Ecology and distribution. – *Lepraria hodkinsoniana* is restricted to eastern North America (figure 81) where it occurs primarily on the bark of hardwoods, especially *Acer* and *Quercus*. Occasionally the species has been found on conifers, and very rarely on non-calcareous rocks in habitats with high humidity. The species is most common in the Mid-Atlantic Coastal Plain, where it is frequently associated with *L. caesiella* in mesic upland hardwood forests. It is however widely distributed with populations throughout the Appalachian Mountains, the Ozark Ecoregion, and the Great Lakes Region. In many respects the distribution of this species mirrors that of *Punctelia caseana* Lendemer & Hodkinson (see Lendemer & Hodkinson 2010).

Conservation status. – Although this species is infrequent throughout its geographic range, it nonetheless does not appear to have any imminent threats.

Discussion. – *Lepraria hodkinsoniana* is morphologically and chemically identical to *L. incana*, another corticolous species with divaricatic acid and zeorin that is ubiquitous in central and northern Europe. In phylogenetic analyses conducted by Lendemer (2011b) sequences derived from eastern North American populations of *L. incana* s.l. were recovered in a strongly supported clade sister to that comprised of sequences from European populations of *L. incana*. These monophyletic groups were strongly correlated with biogeography and as divergent as other species of lichen-forming fungi. Thus two geographically defined semi-cryptic species were recognized and the North American populations formally described as *L. hodkinsoniana*.

This species is easily recognized because it is the only member of the genus in eastern North America with an aggregate thallus that produces divaricatic acid. *Lepraria cryophila* is chemically similar in that it also produces divaricatic acid, however, it additionally produces

high concentrations of nordivaricatic acid and lacks zeorin. That species also occurs primarily on rocks and has a thick placodioid thallus.

Selected additional specimens examined (56 total seen from North America; atranorin absent unless indicated). – **CANADA. ONTARIO.** LANARK CO.: N of Peneshula Rd. 0.6 mi W of int. w/ Cedar Cove Rd., 21.v.2011, on *Picea*, *J.C. Lendemer 28250 & R.E. Lee* (NY, at+). **U.S.A. GEORGIA.** McINTOSH CO.: Sapelo Island, Sapelo Island Wildlife Management Area, Dixie Highway between West Perimeter Rd., 15.xii.2009, on *Quercus*, *J.C. Lendemer 20608* (NY). **ILLINOIS.** ALEXANDER CO.: Shawnee National Forest, Grapevine Trail Picnic and Camping Area, 6.xi.2004, on bark, *R.C. Harris 50531* (NY, at+). JACKSON CO.: Shawnee National Forest, along W side of Cedar Lake, 6.xi.2004, on *Sassafras*, *R.C. Harris 50446* (NY). UNION CO.: Shawnee National Forest, N side of Hamburg Hill, 6.xi.2004, on *Liriodendron*, *R.C. Harris 50464* (NY). **MAINE.** WASHINGTON CO.: Town of Steuben, Dyer Neck, Eagle Hill, 26.vii.2006, on *Betula*, *R.C. Harris 52984* (NY, at+). **MARYLAND.** CALVERT CO.: Battle Creek Cypress Swamp, 4.vi.1977, on *Taxodium*, *E.G. Worthley s.n.* (NY). CAROLINE CO.: Idylwild State Wildlife Management Area, ~1 mi SW of Smithville, 18.iv.2006, on *Acer*, *J.C. Lendemer 6203 & W. Knapp* (NY). CECIL CO.: Elk Neck State Forest, Black Hill Tract, 30.xi.2009, on *Quercus*, *J.C. Lendemer 19963* (NY, at+). KENT CO.: Millington State Wildlife Management Area, along McKays Corner Rd. (Maryland Line Rd.), 18.iv.2006, on *Acer*, *J.C. Lendemer 6184 & W. Knapp* (NY). **MICHIGAN.** CHEBOYGAN CO.: Reese's Bog, W of Roberts Rd., 13.vii.2002, on bark, *W.R. Buck 41995* (NY, at+). GOGEBIC CO.: Ottawa National Forest, Bobcat Lake, 2 mi SW of Marenisco, 3.viii.2003, on *Tsuga*, *C.M. Wetmore 89388* (NY). **MINNESOTA.** HUBBARD CO.: Itasca State Park, just N of Twin Lakes along Lakes Drive,

8.viii.1975, on bark, *W.R. Buck B377* (NY). **NEW JERSEY.** CAMDEN CO.: Winslow Wildlife Management Area, ~3 mi S of jct 73/561[spur] and East Piney Hollow Rd., 4.ii.2009, on *Quercus*, *J.C. Lendemer 15361* (NY, at+). CUMBERLAND CO.: Peaslee Wildlife Management Area, ~2.4 mi S of jct NJ 49 and CR 646 (Cumberland Rd.), 2.ii.2009, on *Quercus*, *J.C. Lendemer 15170* (NY). GLOUCESTER CO.: Glassboro Wildlife Management Area, ~1.5 mi S of jct NJ 322 and CR 655 (Fries Mill Rd.), 4.ii.2009, on *Acer*, *J.C. Lendemer 15301* (NY, at+). OCEAN CO.: Manchester Wildlife Management Area, along N shore of Old Hurricane Brook 0–1 mi W of CR 359 (Hornerstown-Whiting Rd.), 5.ii.2009, on *Quercus*, *J.C. Lendemer 15373* (NY, at+). **NEW YORK.** BRONX CO.: New York Botanical Garden, 1.i.2001, on *Quercus*, *A. Amtoft 320* (NY). CLINTON CO.: Town of Mooers, trail to The Gulf Unique Area, 18.x.1996, on *Acer*, *R.C. Harris 39058* (NY). **PENNSYLVANIA.** CUMBERLAND CO.: Michaux State Forest, Big Pond Rd. 0.5 mi S of int. w/ Hogshead Rd., 2.vi.2009, on *Quercus*, *J.C. Lendemer 18304* (NY, at+). FOREST CO.: Cook Forest State Park, Jacks Hollow Rd. at jct w/ River Rd., 6.ix.2010, on *Acer*, *J.C. Lendemer 24535* (NY, at+). MONROE CO.: Tannersville Bog TNC Preserve, North Woods Trail, 26.vii.2009, on dead *Acer*, *J.C. Lendemer 18478* (NY). SULLIVAN CO.: Ricketts Glen State Park, S slopes above Big Run, 19.ix.2010, on rock, in overhang, *J.C. Lendemer 25670 & J. Kunsman* (NY). UNION CO.: Bald Eagle State Forest, Weikert Run Rd. 3 mi E of jct w/ Hoofnagle Rd., 15.ix.2010, on *Acer*, *J.C. Lendemer 25622* (NY). YORK CO.: York County Bridge No. 6, Mill Creek, W of confluence with Susquehanna River, 27.v.2009, on *Juglans*, *J.C. Lendemer 17937* (NY). **RHODE ISLAND.** WASHINGTON CO.: border of Towns of Exeter and South Kingstown, Marion Epply Wildlife Sanctuary, 16.ix.2006, on rotten tree, *R.C. Harris 53144* (NY). **SOUTH CAROLINA.** AIKEN CO.: The Hitchcock Woods, just SW of city of Aiken, along Tea Cottage Path from entrance on Dibble

Rd., 14.iii.2010, on *Nyssa*, *J.C. Lendemer 22149* (NY). EDGEFIELD CO.: Sumter National Forest, along FSR 634, 1.3 mi W of SC 52, 17.iii.1997, on *Betula*, *R.C. Harris 40249* (NY). **TENNESSEE.** BLOUNT CO.: Great Smoky Mountains National Park, Foothills Parkway tract, bluffs above the NW shore of the Little River, 22.vi.2011, on *Tsuga*, *J.C. Lendemer et al. 29476* (NY). **VIRGINIA.** SUFFOLK CITY: Dismal Swamp National Wildlife Refuge, vicinity of canal bridge over Jericho Ditch, 12.xii.2009, on *Ilex*, *J.C. Lendemer 20482 & B.P. Hodkinson* (NY).

Lepraria humida **Slav.-Bayr. & Orange,** Lichenologist 38: 505. 2006. TYPE: **WALES:** Merioneth (V.C. 48), Rhyd-y-sarn, south of Clogwyn y Geifr, grid ref. 23/684.421, 90 m, *Quercus petraea* woodland, 25.1.2005, on shaded, rain-sheltered siliceous rock face, *A. Orange 15754* (PRA [n.v.], holotype; BG, NMW, isotypes [both n.v.]).

Figures 82 and 83.

Description. – **Thallus** crustose, leprose, aggregated, discontinuous and not stratified, initially composed of isolated granules that divide to form aggregations, the aggregations often remaining distinct and separated, sometimes merging to form a thin crust (<0.2 mm thick), white-gray in color; **hyphae** hyaline, [1.4]–(1.9)–2.6–(3.3)–[3.7] µm (*n*=43) in width, septate, secund, obscured by a thick layer of POL+ crystals that dissolve in KOH; **prothallus** persistent; **hypothallus** absent; **rhizohyphae** absent; **granules** globose, [18]–(27)–36–(46)–[72] µm (*n*=93) in diameter, ecorticate, well organized and discrete, remaining distinct and not forming

compound units; **photobiont** green, coccoid, cells globose, [5.7]–(6.9)–8.8–(10.7)–[13.8] µm (*n*=103) in width.

Chemistry. – Atranorin, rangiformic/jackinic acid, norrangiformic/norjackinic acid (occasionally in low concentrations). Spot tests: K+ yellow, C-, KC-, P+ weak yellow, UV-.

It should be noted that neither stictic acid nor anthraquinones were detected in any of the specimens of *Lepraria humida* reviewed for this study. These substances were considered minor accessories that were not detected in all the specimens revised by Slavíková-Bayerová and Orange (2006).

Ecology and distribution. – *Lepraria humida* is not uncommon in the central and northern Appalachian Mountains of eastern North America (figure 82). Interestingly, it is not known to occur outside of this region in North America, however this may be due to collector bias that often negatively impacts sterile crustose lichens (Lendemer 2011b). All the populations encountered to date were found on non-calcareous rocks in shaded rock overhangs or sheltered vertical rock faces.

Conservation status. – The North American populations of *Lepraria humida* may warrant conservation consideration because they all occur in protected rock overhangs, a habitat easily impacted by changes in small scale moisture regimes (Lendemer et al. 2009). As is the case for *L. cryophila*, this species may be useful as an indicator of the presence of rarer lichens that are less conspicuous.

Discussion. – Somewhat surprisingly *Lepraria humida* is a very distinctive species that is only likely to be confused with *L. caesiella* or *L. jackii* in the field. *Lepraria caesiella* differs in producing zeorin and pallidic acid rather than jackinic acid and norjackinic acid. It also has a more temperate distribution and wider ecological amplitude (Lendemer in rev.B). *Lepraria jackii* is morphologically identical to *L. humida*, essentially differing only in the production of roccellic/angardianic acid in addition to jackinic acid and norjackinic acid. Populations of *L. neglecta* that produce only atranorin and fatty acids could be confused with *L. humida*, however in that species the granules have a distinct compact appearance due to the production of a thick pseudocortex. *Lepraria rigidula* (Hue) J.R. Laundon could also be confused with *L. humida*, however it produces nephrosteranic acid rather than jackinic acid and norjackinic acid. It also has a thicker placodioid thallus and granules that produce conspicuous projecting hyphae that persist into maturity (Lendemer 2011). This is the first report of *L. humida* for North America.

Selected specimens examined (17 total seen from North America). – **CANADA. NEW BRUNSWICK.** CHARLOTTE CO.: Caughey-Taylor Nature Preserve, 2.v.2011, on rock in overhang, *J.C. Lendemer 28090-A* (NY). **NEWFOUNDLAND AND LABRADOR.** NEWFOUNDLAND: Island of Newfoundland, Avalon Peninsula, Butterpot Provincial Park, E side of Big Otter Pond, 9.ix.2007, on rock, in overhang, *J.C. Lendemer 10157* (NY). **ONTARIO.** NIPISSING DIST.: Algonquin Provincial Park, E shore of Carl Wilson Lake, 31.vii.2011, on rock, in overhang, *I.M. Brodo et al. 33067* (NY). **U.S.A. MAINE.** KNOX CO.: Camden Hills State Park, 29.v.2009, on rock in overhang, *R.C. Harris 55530* (NY). **NEW YORK.** ESSEX CO.: Chapel Pond, 19.ix.2004, on rock, *J.C. Lendemer et al. 3133* (NY). GREENE CO.: Town of Hunter, Stony Clove Notch, 13.ix.2008, on rock in overhang, *J.C.*

Lendemer 14107 (NY). **PENNSYLVANIA.** LACKAWANNA CO.: State Game Lands No. 300, N end of Moosic Mountain, 3.vii.2008, on rock in overhang, *J.C. Lendemer 12457* (NY). **VERMONT.** ORLEANS CO.: Willoughby State Forest, 18.x.2010, on rock in overhang, *J.C. Lendemer 27118 & M. Sundue* (NY). **VIRGINIA.** WARREN CO.: Shenandoah National Park, North Marshall, 13.iii.2005, on rock face, *J.C. Lendemer 4413* (NY).

Lepraria jackii **Tønsberg,** Sommerfeltia 14: 200. 1992. TYPE: **NORWAY. NORD-TRØNDELAG.** Grong, W of Abrahammyra, 800–1000 m, 16.ix.1981, on *Picea abies*, *T. Tønsberg 6176* (BG!, holotype).

Figures 84 and 85.

Description. – **Thallus** crustose, leprose, aggregated, discontinuous and not stratified, initially composed of isolated granules that divide to form aggregations, eventually merging to form a thin crust (<0.1 mm thick), greenish-gray in color; **hyphae** hyaline, [1.4]–(2.3)–2.9–(3.4)–[4.2] µm (*n*=96) in width, septate, secund, obscured by a thick layer of POL+ crystals that dissolve in KOH; **prothallus** persistent; **hypothallus** absent; **rhizohyphae** absent; **granules** globose, [14]–(23)–30–(38)–[54] µm (*n*=125) in diameter, ecorticate, well organized and discrete, remaining distinct and not forming compound units; **photobiont** green, coccoid, cells globose, [4.8]–(6.7)–8.9–(11.1)–[14.4] µm (*n*=101) in width.

Chemistry. – Chemotype 1 (not present in North America; corresponds to the holotype): atranorin, roccellic/angardianic acid, jackinic/rangiformic acid, stictic acid; spot tests: K+

yellow, KC-, C-, P+ orange, UV-. Chemotype 2 (present in North America): atranorin, roccellic/angardianic acid, jackinic/rangiformic acid; spot tests: K+ yellow, KC-, C-, P+ weak yellow, UV-.

Ecology and distribution. – In North America *Lepraria jackii* occurs throughout the Great Lakes region and Canadian Maritime provinces, extending southward in the Appalachian Mountains (figure 85). The species typically occurs on the bases of conifers, especially spruce (*Picea*), and more rarely on sheltered rocks or humus.

Conservation status. – *Lepraria jackii* is infrequent in North America, however this may be an artifact of collector bias. The taxon should be classified as "data deficient" pending further study of its northern distribution. The populations in the central and southern Appalachians may warrant conservation consideration because they are restricted to the threatened spruce-fir forests (Lendemer et al. in press.).

Discussion. – *Lepraria jackii* is actually a very distinct taxon on account of its aggregate thallus, granules that lack projecting hyphae at maturity, and production of roccellic/angardianic acid and rangiformic/jackinic acid, in addition to atranorin. Corticolous populations are unlikely to be confused with any other species, except for the fatty acid producing chemotypes of *L. neglecta* s.l. That taxon typically occurs in exposed habitats (whereas *L. jackii* occurs in protected habitats) and the granules appear compact due to the presence of a well-developed pseudocortex. Saxicolous populations can be confused with *L. humida* and TLC is required to

elucidate the presence of roccellic/angardianic acid in *L. jackii* (that substance is absent in *L. humida*).

Selected specimens examined (16 total seen from North America). – **CANADA. NEW BRUNSWICK.** CHARLOTTE CO.: Caughey-Taylor Nature Preserve, 2.v.2011, on *Picea* base, *J.C. Lendemer 28050* (NY). **ONTARIO.** NIPISSING DIST.: Lake Temagami area, Kokoko Bay, cove N of Birch Lake, 14.vii.2009, on *Pinus*, *I.M. Brodo 32442 & F. Brodo* (CANL). **U.S.A. MAINE.** WASHINGTON CO.: Town of Beals, Great Wass Island, 7.vi.2010, on *Picea* base, *J.C. Lendemer 22610* (NY). **MASSACHUSETTS.** BERKSHIRE CO.: Town of Williamstown, Mt. Greylock State Reservation, 6.v.1995, on bark, *R.C. Harris 36493* (NY). **NORTH CAROLINA.** MITCHELL CO.: Pisgah National Forest, trail to Roan High Bluff, 25.ix.1993, on *Abies*, *R.C. Harris 30927* (NY). **TENNESSEE.** SEVIER CO.: Great Smoky Mountains National Park, Chimney Tops Trail, 28.vi.2010, on soil at base of *Betula lenta*, *J.C. Lendemer et al. 23345* (NY). **VERMONT.** CALEDONIA CO.: Town of Wheelock, Wheelock Farm, 22.x.2010, on stump, *J.C. Lendemer 27518 & M. Sundue* (NY).

***Lepraria lanata* Tønsberg,** Opuscula Philolichenum 4: 51. 2007. TYPE: **U.S.A. TENNESSEE.** SEVIER CO.: Great Smoky Mountains National Park, along the Boulevard Trail, 1870 m, on shaded, overhanging, schistose rock wall, 8.ix.2005, *T. Tønsberg 36347* (BG [n.v.], holotype; NY!, isotype).

Figures 86 and 87.

Description. – **Thallus** crustose, leprose, aggregated, discontinuous and not stratified, initially composed of isolated granules that divide to form aggregations, eventually merging to form a thick crust (<0.1–1.0 mm thick), gray-blue in color; **hyphae** hyaline or darkly pigmented, [2.6]–(3.7)–5.7–(7.4)–[9.6] µm (*n*=32) in width, septate, secund, obscured by a thick layer of POL+ crystals that dissolve in KOH; **prothallus** persistent, extending outward from the granules, eventually causing them to become entangled a loose network of hyphae and form large spherical masses; **hypothallus** absent; **rhizohyphae** absent; **granules** globose, [28]–(40)–57–(74)–[102] µm (*n*=32) in diameter, ecorticate, well organized and discrete, immediately forming large compound units surrounded and entwined in prothalline hyphae; **photobiont** green, coccoid, cells globose, [6]–(7.4)–8.6–(9.8)–[11] µm (*n*=32) in width.

Chemistry. – Roccellic/angardianic acid, protocetraric acid. Spot tests: K-, C-, KC-, P+ orange-red, UV-.

Ecology and distribution. – *Lepraria lanata* is a rare species that is restricted to the higher elevations (>4,000 ft.) of the southern Appalachian Mountains in eastern North America (figure 87). It occurs on soil, humus, and other organic matter in sheltered habitats, such as shaded rock faces and overhangs. All but one population (whose substrate is unknown) were found associated with rocks of the Anakeesta Formation in Great Smoky Mountains National Park. While it is unlikely that this relationship is obligatory, it may indicate *L. lanata* shows a preference for a chemically similar rock types.

Conservation status. – This species should be considered a conservation priority. All the known populations are from high elevations in the southern Appalachian Mountains, specifically in Great Smoky Mountains National Park. (Note: a single historical record is known from Grandfather Mountain in Avery Co., NC.) Although the species occurs within a federally protected biological reserve, it occurs in habitats that are under considerable threats from acid fog and climate change. How these forces will impact *Lepraria lanata*, and numerous other species, remains unknown. Many of the populations of this species are also small, and do not form the large colonies typical of most other *Lepraria* species. Additional study is needed to determine whether *L. lanata* occurs at high elevation localities elsewhere in the southern Appalachians. While one typically could attribute the absence of such collections to collector bias, this is not likely the case for *L. lanata*.

Discussion. – *Lepraria lanata* is an extremely distinctive species that can easily be identified with the naked eye in the field. The morphology of the thallus of *L. lanata* appears to be unique within the genus and does not fit well within any of the types defined by Lendemer (2011). Thus, a new type, the *lanata*-type is proposed here. Following the morphological scheme of Lendemer (2011) the *lanata*-type thallus is a unique type of aggregate thallus in which the granules become connected by the persistent prothalline hyphae to form massive spherical aggregations that resemble large granules (> 0.1 mm). Although based on a limited sampling, the granules in *L. lanata* appear to be larger than any other species of *Lepraria* studied to date. This is also the case for the prothalline hyphae (see figure 86).

The aggregations of granules in *Lepraria lanata*, which are larger than 0.1 mm in diameter, are among the largest known in the genus. The size of these structures, which resemble

fuzzy cotton balls, coupled with the production of protocetraric acid and roccellic/angardianic acid are diagnostic. No other species of *Lepraria* could possibly be confused with *L. lanata*.

Selected specimens examined (8 total seen from North America). – **U.S.A. NORTH CAROLINA.** SWAIN CO.: Great Smoky Mountains National Park, Appalachian Trail between Laurel Top and Pecks Corner, 3.vi.2011, on siliceous rock, *J. Hollinger 2962* (NY). **TENNESSEE.** SEVIER CO.: Great Smoky Mountains National Park, Ramsey Cascades, 6.v.1973, on shaded bluff face, *P.R. Fantz s.n.* (NY); Great Smoky Mountains National Park, Mt. LeConte near Alum Cave, 29.xi.2009, on steep rock face, *J. Hollinger 20091129.12* (NY); Great Smoky Mountains National Park, summit of the Chimney Tops, 28.vi.2010, on humus in overhang, *J.C. Lendemer et al. 23306* (NY); Great Smoky Mountains National Park, Boulevard Trail, 2.vi.2011, on siliceous rock, *J. Hollinger 2975* (NY).

Lepraria lecanorica **Tønsberg,** Lichen Flora of the Greater Sonoran Desert Region 2: 326. 2004. TYPE: **U.S.A. ARIZONA.** COCONINO CO.: Coconino National Forest, SSW of Flagstaff, uphill from Hwy 89, 1675–1710 m, dry W-facing slope with *Pseudotsuga menziesii* and *Pinus*, 18.iv.2001, corticolous on shaded base of trunk of *Quercus*, *T. Tønsberg 28991* (BG!, holotype).

Figures 88–90.

Description. – **Thallus** crustose, leprose, aggregated, discontinuous and not stratified, initially composed of isolated granules that divide to form aggregations, eventually merging to

form a thin crust (0.05–0.2 mm thick), blue-white in color; **hyphae** hyaline, [1.5]–(2.1)–2.6–(3.1)–[3.6] µm (*n*=32) in width, septate, secund, obscured by a thick layer of POL+ crystals that not dissolve in KOH; **prothallus** persistent; **hypothallus** absent; **rhizohyphae** absent; **granules** globose, [42]–(51)–81–(111)–[156] µm (*n*=32) in diameter, ecorticate, developing a pseudocortex with age, well organized and discrete, remaining distinct and not forming compound units; **photobiont** green, coccoid, cells globose, [6]–(7.3)–10–(12.7)–[15.9] µm (*n*=32) in width.

Chemistry. – Atranorin, lecanoric acid. Spot tests: K+ yellowish, C+ pink/red, KC+ pink/red, P-, UV-.

Ecology and distribution. – This species was originally described from mountainous regions of the Sonoran Desert in northern Arizona (Tønsberg 2004) and subsequently reported from South America by Flakus and Kukwa (2007). Only a handful of North American populations have been discovered (figure 90), however this is almost certainly due to the limited collection efforts focused on sterile crustose lichens in the greater Sonoran Desert region. The type collection was found growing on a tree in a humid ravine (Lendemer unpublished data). An effort was made to locate additional material during a collecting trip in 2010. This search yielded a single population that was found growing on humus in a sheltered rock overhang where it was mixed with thalli of the fatty acid producing chemotype of *Lepraria neglecta* s.l.

Conservation status. – *Lepraria lecanorica* is rare in North America, however, further study of its geographic distribution is needed before its status can be evaluated.

Discussion. – *Lepraria lecanorica* represents a chemically distinctive taxon with a *caesioalba*-type aggregate thallus (figures 88 and 89). Further study with molecular methods is needed to determine whether this taxon is distinct, or represents another chemical variant within *L. neglecta* s.l. The only species likely to be confused with *L. lecanorica* is *L. neglecta* s.l.

Additional specimen examined (2 total seen from North America). – **U.S.A. ARIZONA.** YAVAPAI CO.: Coconino National Forest, W-facing slopes of Doe Mountain, 7.iii.2010, on soil, on cliff, *J.C. Lendemer 21817-A & A. Moroz* (NY).

Lepraria leprolomopsis **Diederich & Sérus. *in* Aptroot et al.,** Biblioth. Lichenol. 64: 76. 1997. TYPE: **PAPUA NEW GUINEA. SIMBU PROV.:** Mt. Wilhelm area, c. 16 km on new road under construction from Gembogl to Goroka, 2800 m, mossy mountain forest, 9.viii.1992, epiphyte, *H. Sipman 3591* (B [n.v.], holotype; LG [n.v.], hb. Diederich!, isotypes).

Figures 91, 92, and 94.

Description – Thallus crustose, leprose, continuous and distinctly bi-layered, whitish-blue to bluish-green in color; hypothallus a well-developed layer of loosely intertwined hyphae surrounding and subtending the granules, projecting outward from the edge of the thallus, lowermost layers variably pigmented with an orange-brown pigment that intensifies in KOH and then turns purplish; hyphae anchoring the granules to each other, (3.0-)3.5–4.0 µm wide, hyaline, obscurely septate, frequently branching at the septa, thick-walled, obscured by a thick layer of

crystals that dissolve in KOH; rhizohyphae pale to orange-brown-pigmented depending on proximity to the thallus margin, extending from the hypothallus to anchor the thallus to the substrate; granules small, (25-)30–40(-45) µm in diameter, with a poorly developed pseudocortex of one to several layers of gelatinized hyphae, globose, well organized and discrete, with a core of 1 to ± 10 aggregated algal cells, regularly dividing and regularly forming compound units of many individual granules; photobiont green, coccoid, cells globose, 8–13 µm in diameter.

Chemistry - Atranorin, unidentified pigment (?), norstictic acid complex (trace), stictic acid complex (major). Spot tests: unpigmented portions, K+ yellowish, C-, KC-, P+ orange, UV-; pigmented portions, K+ dark red turning purplish, C-, KC-, P-, UV-.

Ecology and distribution. – This species is known from two localities in the Appalachian Mountains of eastern North America (figure 94). It was first discovered in a remarkable series of deep sandstone channels with many high humidity microhabitats in northwestern Alabama at the foothills of the Appalachian Mountains. It should be noted that the disjunct population of *Crocynia pyxinoides* Nyl. mapped in northwestern Alabama by Brodo et al. (2001) refers to this record. The second occurrence was found in the New River Gorge in eastern West Virginia. Although it would be easy to explain this unusual distribution as reflecting collector bias, the number of collections of the morphologically similar species *L. finkii* likely indicate that *L. leprolomopsis* is rare.

Conservation status. – This the status of this species in North America requires further study.

Discussion. – *Lepraria leprolomopsis* most closely resembles *L. cryophila* in thallus morphology, particularly in having a thallus with marginal "lobes," a well-developed hypothallus, and an underside with pale (rather than black) rhizohyphae. Despite their morphological similarity the species can be readily distinguished from *L. cryophila* by the production of atranorin and the stictic acid complex (P+ orange, C-, UV-) rather than divaricatic acid and nordivaricatic acid (P-, C+ red, UV+ blue-white). *Lepraria leprolomopsis* can further be separated from *L. cryophila* by the tendency of its thalli to grow along the plane of the substrate, resulting in flat margins. Thalli of *L. cryophila* often have strongly ascending margins that resemble shelves when viewed from the side.

Only five other species of *Lepraria* are known to produce atranorin and stictic acid: *L. elobata*, *L. finkii*, *L. lobificans*, *L. multiacida* Aptroot, and a chemotype of *L. neglecta*. Of these species, only *L. finkii* is known to occur in the same region as *L. leprolomopsis*. *Lepraria elobata* differs from the species by the production of zeorin and in having an aggregate thallus. *Lepraria finkii* is morphologically similar but typically lacks rhizohyphae and additionally produces zeorin in the thallus. *Lepraria lobificans* is chemically comparable but additionally produces roccellic/angardianic acid and has a pseudocorticate placodioid thallus that resembles *L. xerophila*. *Lepraria multiacida* is a tropical species that is also morphologically similar to *L. leprolomopsis* but differs in the additional production of zeorin and dibenzofurans. *Lepraria neglecta* is a chemically variable species comprised of numerous chemotypes, one of which is chemically comparable to *L. leprolomopsis* but differs in the production of additional

roccellic/angardianic acid. That species has an aggregate thallus with granules that are distinctly pseudocorticate and compact, at least in later stages of development.

Crocynia pyxinoides is another species that is likely to be confused with *Lepraria leprolomopsis* because it has a thick byssoid thallus that is typically sterile and produces atranorin and stictic acid (figures 92 and 93A). The two taxa are not presently known to be sympatric, *C. pyxinoides* is a corticolous species of the southern Coastal Plain while *L. leprolomopsis* is saxicolous and known only from two localities in the Appalachian Mountains (figure 94). They are also easily distinguished by thallus morphology, *C. pyxinoides* has a byssoid thallus with a black hypothallus, and elongate lobes that resemble a foliose lichen whereas *L. leprolomopsis* has a leprarioid thallus with a white hypothallus, pale rhizohyphae, and lacks elongate lobes. Although both *C. pyxinoides* and *L. leprolomopsis* are chemically similar in producing both atranorin and stictic acid as their major secondary metabolites, the species appear to differ in the production of additional substances in the stictic acid complex. While *L. leprolomopsis* produces numerous substances in the stictic acid complex in high concentrations that can be detected with TLC, as well as trace amounts of norstictic and connorstictic acids, the specimens of *C. pyxinoides* examined produced only stictic acid in high enough concentration to be detected with TLC.

Additional specimens examined (4 total seen from North America). – **U.S.A. ALABAMA.** FRANKLIN CO.: Dismals Wonder Garden, just N of Hodges, 100–120 m, dry sandstone glade above, deeply cut, narrow sandstone ravines along small stream below, on rock, 26.ix.1992, *J.G. Guccion 1663* (NY), *1664* (hb. Guccion), *R.C. Harris 28456* (NY). **WEST VIRGINIA.** FAYETTE CO.: New River Gorge, vii.2001, on sandstone, *P. Clark N225Q7* (NY).

***Lepraria membranacea* (Dicks.) Vain.,** Acta Soc. Fauna Flora Fenn. 49(2): 265. 1921.

Lichen membranaceus Dicks., Fasc. Pl. Crypt. Brit. 2: 21. 1790. TYPE: **SCOTLAND:** on humus over mountain rocks, *J. Dickson* (BM [n.v.], holotype).

Leproloma membranaceum (Dicks.) Vain., Term. Füz. 22: 293. 1899.

Crocynia membranacea (Dicks.) Zahlbr., Cat. Lich. Univers. 2: 575. 1923 [1924].

Figures 95 and 96.

Description. – **Thallus** crustose, leprose, placodioid, without crisped margins, continuous and distinctly bi-layered, initially forming small discrete thalli which expand marginally, overlap, and become confluent to form a thick crust (0.2–0.4 mm thick), yellowish-white; **hyphae** hyaline, [1.4]–(2.0)–3.2–(3.6)–[4.8] µm (*n*=97), septate, secund, obscured by a layer of POL+ crystals that dissolve in KOH; prothallus disappearing with age; **hypothallus** a variably developed layer of densely intertwined hyphae surrounding and subtending the granules, sometimes outward from the edge of thallus; **rhizohyphae** uncommon, not pigmented; **granules** globose, [24]–(27)–41–(54)–[90] µm (*n*=90), ecorticate, well organized and discrete, often forming compound units, sometimes separating from the hypothallus and then producing abundant projecting hyphae; **photobiont** green, coccoid, cells globose, [6]–(7.1)–9.4–(11.8)–[15.6] µm (*n*=96).

Chemistry. – Pannaric acid, roccellic/angardianic acid, ± atranorin. Spot tests: K+ yellowish, C-, KC-, P- orange-red, UV-.

Ecology and distribution. – *Lepraria membranacea* is an uncommon species that is widespread in boreal eastern North America, with disjunct populations occurring in the central Appalachian Mountains (central and eastern Pennsylvania) (figure 96). The species is ecologically variable, although in the boreal forests it typically occurs on the bases of *Thuja* in cedar swamps. Populations have also been found growing on the bark of hardwoods and on sheltered non-calcareous rocks in humid microhabitats.

Based on my limited field studies in boreal eastern North America it appears that *Lepraria membranacea* is the least frequent species of the genus occurring in *Thuja* swamps. As such, the paucity of vouchers may be to due collector bias. Indeed, the ubiquity of *L. finkii* in such habitats can make it difficult to locate rare species without intensive sampling of what appear to be different entities in the field.

Conservation status. – Based on the available data *Lepraria membranacea* appears to be a rare species in North America, potentially worthy of conservation measures. Populations occurring in cedar swamps in the core northern geographic range of the species should be considered in management plans of those wetland habitats, as is the case of other similar rare lichen species. Further study of this taxon is needed, however, to better estimate its frequency and abundance in its core range.

Discussion. – In the field *Lepraria membranacea* is most likely to be confused with *L. vouauxii* because both species have *finkii*-type placodioid thalli and distinctive yellowish colored thalli due to the presence of abundant dibenzofurans. *Lepraria vouauxii* is much more common

than *L. membranacea* and is widely distributed in eastern North America. The two species can only be distinguished with certainly using TLC. *Lepraria membranacea* is also likely to be confused with other common sympatric species with *finkii*-type placodioid thalli, namely *L. finkii* and *L. eburnea*. The former species typically has a greenish-blue thallus and differs chemically in the production of atranorin, zeorin, and stictic acid. The latter species typically has a distinctive off-white thallus with a yellowish hue (not nearly as apparent as in the species that produce dibenzofurans), and differs chemically in the production of alectorialic acid rather than dibenzofurans.

Selected specimens examined (17 total seen from North America). – **CANADA. ONTARIO.** NIPPISING DIST.: Algonquin Provincial Park, below Barron Canyon walking trail, 8.viii.2010, on *Thuja*, *C. Lewis 450* (NY). **QUEBEC.** GATINEAU CITY: Gatineau Park, Trail 05, 0–0.5 mi N of parking area behind Asticou Center, 22.v.2011, on *Quercus, J.C. Lendemer 28306-A & C. Freebury* (NY). KAMOURASKA CO.: Sainte-Anne de la Pocatière, 13.iv.1935, on hemlock, *E. Lepage 131* (NY). **GERMANY:** Niederbayern, Kreis Regen, 22.x.1997, on *Acer*, *K. Kalb s.n.* (NY) **SPAIN. ASTURIAS:** Villanueva, ~8 km SW of La Florida, 4.viii.1987, on rock, *W.R. Buck 15003* (NY). **U.S.A. NEW YORK.** CLINTON CO.: Town of Altona, Altona Flat Rock area in Miner Experimental Forest, 19.x.1996, on rock, in overhang, *R.C. Harris 39138* (NY); Town of Peru, Valcour Island in Lake Champlain, Bluff Point, 18.v.2007, on *Thuja*, *R.C. Harris 53541* (NY). **PENNSYLVANIA.** CENTRE CO.: Bald Eagle State Forest, Stony Run Rd., 0.5 mi N of jct w/ Negro Hollow Rd., 14.ix.2010, on *Quercus*, *J.C. Lendemer 25547* (NY). MONROE CO.: Delaware Water Gap National Recreation Area, Mount Minsi, 3.v.2006, on shaded sandstone, *J.C. Lendemer 6803 & N. Howe* (NY).

***Lepraria neglecta* (Nyl.) Erichsen,** Flechtenfl. Nordwestdeustchl. 394. 1957.

Lecidea neglecta Nyl., Not. Sällsk. Fauna Fl. Fenn. Förh., N. Ser. 1: 233. 1859. TYPE: **FINLAND:** Uusimaa, near Helsinki, 1858, on small moss cushions covering granitic rocks, *W. Nylander s.n.* (H [n.v.], lectotype [selected by Laundon 1992]).

Crocynia neglecta (Nyl.) Hue, Bull. Soc. Bot. France 81: 376. 1924.

Crocynia alpina B. de Lesd., Bull. Soc. Bot. France 61: 85. 1914 . TYPE: **U.S.A. COLORADO.** LARIMER CO.: Rocky Mountain National Park, Toll Pass, 12050 ft, alpine tundra, in snow accumulation sites with good drainage, 21.viii.1979, *Willard s.n. = Lich. Exs. COLO 609* (UPS [n.v.], neotype [selected by Baruffo et al. 2006]; DUKE!, NY!, isoneotypes).

Lepraria alpina (B. de Lesd.) Tretiach & Baruffo *in* Baruffo et al., Nova Hedwigia 83: 390. 2006.

Lepraria alpina var. *zeorinica* L. Saag, Mycotaxon 102: 68. 2007. TYPE: **GREENLAND:** Qingertivaq, 550 m, 30.vii.1970, on mosses and soil (humus), *E. Hansen ESH-70.0032* (C [n.v.], holotype; TU [n.v.], isotype).

Lepraria zeorinica (L.) Kukwa, Mycotaxon 108: 361. 2009.

Lepraria angardiana Øvstedal, Nova Hedwigia 37: 687. 1983. TYPE: **ANTARCTICA:** Dronning Maud Land, Sverdrupfjella, Sørhausane, 31.xii.1970, on soil, *Angard s.n.* (BG [n.v.], holotype).

Leproloma angardianum (Øvstedal) J.R. Laundon, Lichenologist 21: 19. 1989.

Lepraria borealis Loht. & Tønsberg *in* Lohtander, Ann. Bot. Fenn. 31: 224. 1994. TYPE: **NORWAY. NORD-TRØNDELAG:** Namsskogan, Børgefjell Nat. Park, Namskroken, N bank of river Namsen E of Mattisflya, 340 m, 23.viii.1975, muscicolous on rock, *T. Tønsberg 1519* (BG!, holotype; H!, isotype).

Lepraria cacuminum sensu Lohtander, Ann. Bot. Fenn. 32: 52. 1995.
Leproloma cacuminum sensu Laundon, Lichenologist 24: 345. 1992.

Crocynia caesioalba B. de Lesd., Bull. Soc. Bot. France 61: 84. 1914. TYPE: **FRANCE:** Seine-et-Marne, Forêt de Fontainebleau, Jul 1913, very common on mosses and lichens on sandstone rocks, *Bouly de Lesdain* s.n. (E [n.v.], holotype; BM, GL, PC [all n.v.], isotypes).
Leproloma caesioalba (B. de Lesd.) M. Choisy, Bull Mens. Soc. Linn. Lyon 19: 12. 1950.
Lepraria caesioalba (B. de Lesd.) J.R. Laundon, Lichenologist 24: 324. 1992.

Lepraria caesioalba var. *groenlandica* L. Saag, Mycotaxon 102: 73. 2007. TYPE: **GREENLAND:** Qaanaaq, Thule, 28.vi.1986, on soil (humus) and mosses, *E. Hansen ESH-86.0321* (C [n.v.], holotype;TU [n.v.], isotype).

Lepraria gelida Tønsberg & Zhurb., Graphis Scripta 18: 64. 2006. TYPE: **NORWAY. SVALBARD:** Spitzbergen, SSE of Wijdefjorden, E of Austfjorden, SE of Finnekroken, the

W-facing foothills of Finlandsveggen, 20–30 m, 5.vii.2002, on slightly sloping ground in *Dryas octopetala* heath, *T. Tønsberg 31049* (BG!, holotype).

Lepraria svalbardensis Tønsberg *in* Øvstedal et al., Sommerfeltia 33: 213. 2009. TYPE: **NORWAY. SVALBARD:** Spitsbergen, the W-facing foothill of Mt. Platåberget, uphill from the road between Longyearbyen and Bjørndalen, 70–75 m, 26.vi.2003, muscicolous in dry niche in scree, *T. Tønsberg 31851* (BG [n.v.], holotype; UDGA [n.v.], isotype).

Lepraria zonata Brodo, Bull. New York State Mus. Sci. Surv. 410: 263. 1968. TYPE: **U.S.A. NEW YORK.** SUFFOLK CO.: Wading River, 11.viii.1962, on vertical surface of partially shaded boulder, *I.M. Brodo 3869* (MSC!, holotype).

Figures 97 and 98.

Description. – **Thallus** crustose, leprose, aggregated, discontinuous and not stratified, initially composed of isolated granules that divide to form aggregations, eventually merging to form a thin crust (<0.1 mm thick), gray-blue to blue-white in color; **hyphae** hyaline, [1.8]–(2.3)–2.9–(3.4)–[4.8] µm (*n*=96) in width, septate, secund, obscured by a thick layer of POL+ crystals that dissolve in KOH; **prothallus** persistent; **hypothallus** absent; **rhizohyphae** absent; **granules** globose to subglobose, typically [30]–(42)–59–(77)–[102] µm (*n*=131) in diameter however some forms with *alpina*-type thalli develop large granules (>1.0 mm in diameter), initially ecorticate but quickly developing a pseudocortex of gelatinized hyphae, well organized and

discrete, remaining distinct and not forming compound units; **photobiont** green, coccoid, cells globose, [4.8]–(7.2)–9.4–(11.7)–[15.6] µm (*n*=96) in width.

Chemistry. – As has been summarized by Lendemer (in rev.C) the chemical variation within *Lepraria neglecta* s.l. is very complex (see also Appendix A). The following chemotypes are present in North America. Chemotype 1 (*L. neglecta* s. str.): roccellic/angardianic acid, alectorialic acid, ± atranorin (tr.); spot tests: K+ yellow, C-, KC+ red, P+ yellow, UV-. Chemotype 2 (*L. borealis* and an unnamed chemotype): atranorin, ± roccellic/angardianic acid, ± rangiformic/jackinic acid; spot tests: K+ yellow, C-, KC-, P+ weak yellowish, UV-. Chemotype 3 (*L. caesioalba* and an unnamed chemotype): atranorin, fumarprotocetraric acid, ± roccellic/angardianic acid, ± rangiformic/jackinic acid; spot tests: K+ yellow, C-, KC-, P+ orange-red, UV-. Chemotype 4 (unnamed chemotype): atranorin, norstictic acid agg.; spot tests: K+ yellow turning dirty red, C-, KC-, P+ orange, UV-. Chemotype 5 (*L. alpina*): atranorin, roccellic/angardianic acid, porphyrilic acid; spot tests: K+ yellow, C-, KC-, P+ weak yellowish, UV-. Chemotype 6 (unnamed chemotype): atranorin, psoromic acid, ± roccellic/angardianic acid, ± rangiformic/jackinic acid; spot tests: K+ yellow, C-, KC-, P+ yellow, UV-. Chemotype 7 (*L. svalbardensis* and other chemotypes): atranorin, stictic acid agg., ± zeorin, ± roccellic/angardianic acid, ± rangiformic/jackinic acid; spot tests: K+ yellow, C-, KC-, P+ orange, UV-.

Ecology and distribution. – *Lepraria neglecta* is common and widely distributed throughout northern temperate, boreal and arctic regions of North America, extending southward in mountain regions throughout the continent (figure 98). The species occurs on many substrates in

exposed habitats that lack calcareous influences, including soil, organic matter, bryophytes and non-calcareous rocks. Corticolous populations are also frequently encountered in humid habitats in open forests throughout eastern North America.

Discussion. – The chemical variation found within *Lepraria neglecta* is unparalleled in the genus *Lepraria* s.l. The different chemotypes of *L. neglecta* can potentially be confused with numerous other sympatric species of *Lepraria* that have aggregate thalli. All those species have ecorticate granules with a fluffy appearance rather than granules that develop a thick pseudocortex, appearing compact and hard (Lendemer 2011).

Selected specimens examined [237 total seen from North America; alectorialic acid chemotype, Chemotype 1]. – **CANADA. ONTARIO.** LANARK CO.: 0–0.2 mi N Peneshula Rd., 21.v.2011, on sheltered rock, *J.C. Lendemer 28209 & R.E. Lee* (NY). NIPISSING DIST.: Algonquin Provincial Park, E shore of Carl Wilson Lake, 31.vii.2011, on rock, *I.M. Brodo et al. 33055* (CANL). **NEW BRUNSWICK.** CHARLOTTE CO.: Caughey-Taylor Nature Preserve, 2.v.2011, on moss over rock, *R.C. Harris 56780* (NY). **NORTHWEST TERRITORIES:** MACKENZIE DIST.: Mackenzie Mts., Nahanni National Park, Tlogotsho Plateau, 11.vii.1977, on mosses over sandstone boulders, *T. Ahti 32694* (H, NY). **NOVA SCOTIA.** COLCHESTER CO.: Economy River Wilderness Area, vicinity of Economy Falls, 14.v.2004, on mosses over rock, *T. Ahti 62445a* (H). YARMOUTH CO.: Flintstone Rock, 8.v.1999, on *Parmelia saxatilis* over rock, *R.C. Harris 42975-A* (NY). **QUEBEC.** GATINEAU CO.: Gatineau Park, near Ramsey Lake, 30.vii.1978, *T. Ahti 34649 & I.M. Brodo* (H, NY). **U.S.A. ARKANSAS.** PERRY CO.: Ouachita National Forest, vicinity of Goat Bluff, 6.x.2010, on rock in overhang, *J.C.*

Lendemer et al. 26073 (NY). **CALIFORNIA.** MARIPOSA CO.: Yosemite National Park, S shore of Merced River, 20.ix.2009, on rock, *J.C. Lendemer 19635* (NY). **CONNECTICUT.** LITCHFIELD CO.: Town of Canaan, Great Mountain Forest, 20.ix.2003, on log, *R.C. Harris 48028* (NY). **FLORIDA.** WASHINGTON CO.: Rock Hill, 30.ix.1994, on rock, *R.C. Harris 35580* (NY). **GEORGIA.** FANNIN CO.: Cohutta Mountains, 3.vii.2009, on *Liriodendron*, *M. Hodges 4327* (NY). **ILLINOIS.** POPE CO.: Shawnee National Forest, Hayes Creek, 16.x.1993, *R.C. Harris 31370* (NY). **KANAS.** ELLSWORTH CO.: Kanopolis State Park, 27.iv.2006, on *Cladonia* over rock, *C.A. Morse 12732 & C.C. Freeman* (NY). GREENWOOD CO.: 1 mi N, 5 mi E of the town of Fall River, 30.iii.2003, on sandstone, *M.K. Advaita 3059* (KANU). **KENTUCKY.** ESTILL CO.: Daniel Boone National Forest, ~1.5 mi NE of Cottage Furnace Campground, 11.x.1995, on sandstone, *R.C. Harris 36962* (NY). **MARYLAND.** ANNE ARUNDEL CO.: Wishing Rock, 28.iii.1963, on rock, *C.F. Reed 55089* (NY). **MAINE.** WASHINGTON CO.: Town of Cutler, Cutler Coastal Public Lands, 8.vi.2010, on humus over rock, *J.C. Lendemer 22688* (NY). **MASSACHUSETTS.** BERKSHIRE CO.: Town of Pittsfield, Pittsfield State Forest, 4.vi.2010, on rock, *J.C. Lendemer 22466* (NY). **MISSOURI.** IRON CO.: Ketcherside Mountain Conservation Area, 26.iii.2006, on rhyolite, *J.C. Lendemer et al. 6105* (NY). **NEW HAMPSHIRE.** COOS CO.: Mt. Washington, 31.viii.1933, on rock, *G.P. Anderson s.n.* (NY). **NEW JERSEY.** WARREN CO.: Jenny Jump State Forest, 11.ix.1992, on rock, *W.R. Buck 21544* (NY). **NEW YORK.** HERKIMER CO.: Little Falls, Moss Island, 14.vi.2011, on rock, *R.C. Harris 56805* (NY). **NORTH CAROLINA.** SWAIN CO.: Great Smoky Mountains National Park, along Balsam Mountain Road, 6.viii.2009, on rock in overhang, *J.C. Lendemer 19149 & E.A. Tripp* (NY). **OHIO.** PORTAGE CO.: West Branch State Park, 10.iii.1990, on sandstone, *S.J. Mazzer 37099* (NY). **OKLAHOMA.** OSAGE CO.: Woolaroc Wildlife Preserve,

start of nature trails area, 6.iv.2007, on sandstone, *C.A. Morse 14610 & D. Ladd* (KANU). **PENNSYLVANIA.** CARBON CO.: State Game Lands No. 129, 19.v.2009, on *Acer*, *J.C. Lendemer 17453 & R.C. Harris* (NY). **RHODE ISLAND.** PROVIDENCE CO.: Town of Lincoln, Lime Rock Nature Preserve, 17.ix.2006, on rock, *R.C. Harris 53209* (NY). **TENNESSEE.** CARTER CO.: Roan Mountain, Carver's Gap, 6.x.1985, on mosses over rock, *R.C. Harris 18342* (NY). **VERMONT.** ORLEANS CO.: Town of Westmore, Willoughby State Forest, 18.x.2010, on *Betula*, *J.C. Lendemer 27114 & M. Sundue* (NY). **VIRGINIA.** WISE CO.: Jefferson National Forest, High Knob, 13.ix.1991, on *Quercus*, *R.C. Harris 26973* (NY). **WEST VIRGINIA.** POCAHONTAS CO.: Monongahela National Forest, vic. Blue Knob, 20.x.2007, on *Acer*, *J.C. Lendemer 9937 & A. Moroz* (NY).

Selected specimens examined [85 total seen from North America; depside deficient chemotype, Chemotype 2]. – **CANADA. ONTARIO.** BRUCE CO.: Bruce Alvar Nature Reserve, 26.ix.2010, on glacial erratic, *R.C. Harris 56539* (NY). **U.S.A. ARIZONA.** YAVAPAI CO.: Coconino National Forest, W-facing slopes of Doe Mountain, 7.iii.2010, on soil in crevice, *J.C. Lendemer 21817 & A. Moroz* (NY). **CALIFORNIA.** RIVERSIDE CO.: San Bernardino National Forest, San Jacinto Mountains, vicinity of Boxwood Spring, 11.i.2008, on rock, *J.C. Lendemer 11488 & K. Knudsen* (NY). **NEW YORK.** ESSEX CO.: Whiteface Mtn., 28.ix.1989, on rock, *C.C. Bratt 6645* (NY). **NORTH CAROLINA.** JACKSON CO.: Nantahala National Forest, Chattooga Wild and Scenic River/Ellicott Rock Wilderness, 18.ix.2006, on granite, *J.C. Lendemer et al. 7882* (NY). **OREGON.** UNION CO.: just W of Starkey Experimental Forest, 21.v.2006, on *Grimmia*, *B. McCune 28216* (NY, OSC).

Selected specimens examined [227 total seen from North America; fumarprotocetraric chemotype, Chemotype 3]. – **CANADA. BRITISH COLUMBIA:** Moresby Island, Takakia Lake, 4.vii.1967, on mosses over rock, *I.M. Brodo 10932 & M.J. Shchepanek* (NY). **U.S.A. ARKANSAS.** IZARD CO.: Calico Rock, 26.iv.1988, on rock, *R.C. Harris 21679* (NY). **CALIFORNIA.** RIVERSIDE CO.: Santa Jacinto Mountains, above Pacific Crest Trail, 8.v.2005, on granite, *K. Knudsen 2838 & J.C. Lendemer* (NY, UCR). TRINITY CO.: 3 mi SW of Douglas City, 21.iv.1965, on rock, *W.A. Weber s.n.* (NY). **CONNECTICUT.** FAIRFIELD CO.: Town of Redding, Highstead Arboretum, 9.vi.2005, on *Quercus*, *R.C. Harris 51565* (NY). **GEORGIA.** WALKER CO.: Chattahoochee National Forest, Johns Mountain Overlook, 23.ix.1992, on bark, *R.C. Harris 28239* (NY). **IDAHO.** BONNER CO.: Clark Fork, 9.vii.1981, on moss, *C.C. Bratt 2678* (NY, SBBG). **ILLINOIS.** JOHNSON CO.: Shawnee National Forest, Simpson Township's Barrens, 16.x.1993, on rock, *R.C. Harris 31330* (NY). **KENTUCKY.** ESTILL CO.: Daniel Boone National Forest, ~1.5 mi NW of Cottage Furnace Campground, 11.x.1995, on rock, *R.C. Harris 36884* (NY). **MAINE.** OXFORD CO.: East Buckfield, 18.v.1933, on *Ptilidium*, *J.C. Parlin 11738* (NY). **MARYLAND.** GARRETT CO.: High Bog, 24.ix.1989, on rock, *R.C. Harris 24579* (NY). **MASSACHUSETTS.** BRISTOL CO.: Norton, 2.xii.1936, on conglomerate, *M. Rice s.n.* (NY). **MISSOURI.** MONTGOMERY CO.: Graham Cove State Park, 27.x.2010, on rock, *R.C. Harris 45740* (NY). **NEW JERSEY.** BERGEN CO.: Ramapo, 8.xi.1942, on rock, *W.H. Wiegmann s.n.* (NY). **NEW YORK.** ROCKLAND CO.: Bear Mountain State Park, Iona Island Bird Sanctuary, 18.vii.2010, on *Quercus*, *J.C. Lendemer 23666 & A. Moroz* (NY). **OREGON.** CROOK CO.: Biak Training Center, v.2009, on soil and moss, *B. McCune 29967* (NY). **PENNSYLVANIA.** CENTRE CO.: Bald Eagle State Forest, above Winkleblech Vista, 14.ix.2010, on *Quercus*, *J.C. Lendemer 25481* (NY). **SOUTH CAROLINA.**

ABBEVILLE CO.: Sumter National Forest, Parsons Mountain, 17.iii.1997, on rock, *R.C. Harris 40320* (NY). **TENNESSEE.** SEVIER CO.: Gatlinburg, vi.1935, on rock, *A.J. Sharp s.n.* (NY). **UTAH.** UINTAH CO.: Ashley National Forest, along FS 104, 30.vii.2009, on rock, *W.R. Buck 55078* (NY). **VERMONT.** ADDISON CO.: Green Mountain National Forest, Bristol Cliffs Wilderness Area, 20.x.2010, on rock, *J.C. Lendemer 27394 & M. Sundue* (NY). **VIRGINIA.** PAGE CO.: Hawksbill Mountain, 8.vi.1936, on rock, *G.P. Anderson s.n.* (NY). **WEST VIRGINIA.** TUCKER CO.: Monongahela National Forest, Olsen's Bog, 22.iv.2001, on *Magnolia*, *R.C. Harris 44869* (NY).

Specimens examined [2 total seen from North America; norstictic chemotype, Chemotype 4]. – **U.S.A. GEORGIA.** LUMPKIN CO.: Desoto Falls, 9.vii.2010, on rock, *S.Q. Beeching 9893* (NY). **OREGON.** WHEELER CO.: Blue Mountains, Shelton Wayside Campground, v.2008, on rhyolite, *B. McCune 29620* (NY).

Selected specimens examined [34 total seen from North America; porphyrilic chemotype, Chemotype 5]. – **U.S.A. COLORADO.** CHAFFEE CO.: San Isabel National Forest, Cottonwood Pass, 18.viii.1983, on soil, *T.H. Nash 22283* (NY). **IDAHO.** VALLEY CO.: 4 mi E of McCall, 5.ix.2008, on soil, *R. Rosentreter 16535* (NY). **OREGON.** WALLOWA CO.: Wallowa Mountains, Eagle Cap Wilderness, 7–9.ix.2005, on soil, *B. McCune 27983* (NY). **UTAH.** UINTAH CO.: Ashley National Forest, vicinity of Leidy Peak, 31.vii.2009, on humus, *W.R. Buck 55129B-831* (NY).

Selected specimens examined [51 total seen from North America; psoromic chemotype, Chemotype 6]. – **CANADA. ONTARIO.** NIPISSING DIST.: Algonquin Park, 10.viii.2010, on rock, *I.M. Brodo 32719* (CANL). **U.S.A. CALIFORNIA.** SAN BERNARDINO CO.: Joshua Tree National Park, Wonderland of Rocks, 31.xii.2010, on rock, *K. Knudsen 13490* (NY, UCR). **CONNECTICUT.** MIDDLESEX CO.: Town of Middletown, 10.vi.1961, on rock, *D. Wiley 27* (COLO). **MASSACHUSETTS.** WORCESTER CO.: Gardner, 12.x.1955, on bark, *V. Ahmadjian L97* (NY). **NEW YORK.** ORANGE CO.: Harriman State Park, 19.iv.1998, on *Fraxinus*, *R.C. Harris 42142* (NY). **NORTH CAROLINA.** JACKSON CO.: Nantahala National Forest, Chattooga Wild and Scenic River/Elliott Rock Wilderness, 18.ix.2006, on rock, *J.C. Lendemer et al. 7881* (NY). **OREGON.** LAKE CO.: Glass Butte, 13.x.2009, on soil, *H. Root 1781b* (NY). **PENNSYLVANIA.** ELK CO.: State Game Lands No. 28, 9.ix.2010, on rock, *J.C. Lendemer 25110* (NY). **UTAH.** UNITAH CO.: Ashley National Forest, along FS 104, 30.vii.2009, on soil in rock crevice, *W.R. Buck 55065* (NY). **WASHINGTON.** SKAGIT CO.: summit of Haystack Mt., ix.2008, on alpine soil, *B. McCune 28879* (NY, OSC).

Selected specimens examined [49 total seen from North America; stictic chemotype, Chemotype 7]. – **CANADA. BRITISH COLUMBIA:** Whistler Mountain, 22.viii.1994, on humus, *T. Ahti 52038* (H, NY). **U.S.A. CALIFORNIA.** MARIPOSA CO.: Yosemite National Park, S shore of Merced River, 20.ix.2009, on *Quercus*, *J.C. Lendemer 19632* (NY). TUOLUMNE CO.: Yosemite National Park, Mammoth Peak Overlook, 21.ix.2009, on soil, *J.C. Lendemer 19691* (NY, zeorin present). **GEORGIA.** RABUN CO.: Tallulah Gorge State Park, 11.xi.2010, on rock, *S.Q. Beeching 10955* (NY). **IDAHO.** BUTTE CO.: Craters of the Moon National Monument, near Sentinel Butte, 4.vi.1964, on rock, *E.P. Menning 3* (COLO).

OREGON. UMATILLA CO.: Pikes Peak, 21.x.2006, on *Grimmia*, *B. McCune 28484* (NY, OSC). **WASHINGTON.** PIERCE CO.: Mt. Rainier, 13.vii.1957, on ground, *W.J. Eyerdam 1834* (NY, SBBG).

Lepraria nivalis **J.R. Laundon,** Lichenologist 24: 327. 1992. TYPE: **FRANCE:** Alpes-Maritimes, Nice, vieux chemin de Gairaut, vii.1945, sur les murs de soutènement et sur les talus, *M. Bouly de Lesdain s.n.* (MARSSJ [n.v.], holotype).

Figures 99 and 100.

Description. – **Thallus** crustose, leprose, placodioid, without crisped margins, continuous and distinctly bi-layered, initially forming small discrete thalli which expand marginally, overlap, and become confluent to form a thick crust (0.2–0.4 mm thick), blue-white in color; **hyphae** hyaline, [1.4]–(1.9)–2.4–(3.0)–[4.2] µm (*n*=65), septate, secund, obscured by a layer of POL+ crystals that do not dissolve in KOH; prothallus disappearing with age; **hypothallus** a variably developed layer of densely intertwined hyphae surrounding and subtending the granules, sometimes outward from the edge of thallus; **rhizohyphae** uncommon, not pigmented; **granules** globose, [19]–(29)–43–(58)–[74] µm (*n*=65), ecorticate, well organized and discrete, rarely forming compound units, sometimes separating from the hypothallus and then producing abundant projecting hyphae; **photobiont** green, coccoid, cells globose, [6]–(6.8)–8.7–(10.6)–[13.2] µm (*n*=65).

Chemistry (of North American specimens examined). – Protocetraric acid, ± atranorin. Spot tests: K+ yellowish, C-, KC-, P+ orange-red, UV-.

Ecology and distribution. – In North America *Lepraria nivalis* appears to be restricted to the humid coastal regions of the Pacific Northwest (figure 100) where it occurs on the bark of hardwood trees as well as over bryophytes and rocks in sheltered situations. Populations have also been reported from coniferous substrates, however, no such vouchers were reviewed for this study.

Conservation status. – This taxon should be considered "data deficient" until more detailed studies are carried out in the Pacific Northwest to elucidate its range and abundance.

Discussion. – As has been discussed by Lendemer and Harris (2007), *Lepraria nivalis* was broadly circumscribed by Laundon (1992) to include elements that were heterogeneous chemically, ecologically, and morphologically. Based on the protologue, and on reference material from Great Britain, *L. nivalis* should be restricted to material with a *finkii*-type thallus and lacking roccellic/angardianic acid. *Lepraria nivalis* was widely misapplied in eastern North America to the taxon that is now referred to as *L. normandinoides*. That species differs from *L. nivalis* in the production of roccellic/angardianic acid and in having a *normandinoides*-type placodioid thallus with crisped margins and dark rhizohyphae.

In North America only two species are likely to be morphologically confused with *Lepraria nivalis*, namely *L. finkii* and *L. torii*. Both species have *finkii*-type placodioid thallus but differ chemically from *L. nivalis* in producing atranorin, zeorin, and stictic acid (*L. finkii*) or

roccellic/angardianic acid and fumarprotocetraric acid (*L. torii*). *Lepraria finkii* is not common in the Pacific Northwest, and thus is less likely to be confused with *L. nivalis* than *L. torii* is. *Lepraria torii* is ubiquitous in coastal forests of the Pacific Northwest (see the entry for that taxon for further discussion).

In the present study *Lepraria nivalis* is considered to be a rare species with a *finkii*-type placodioid thallus that produces protocetraric acid, often with accessory atranorin. The morphologically and ecologically identical sympatric populations that lack atranorin are tentatively retained in *L. nivalis* pending further study with molecular data. Additionally, it should be noted that no North American populations of *L. nivalis* producing fumarprotocetraric acid as the major substance were encountered for this study. Should such populations be found, they should also be included in a revision of the aforementioned populations that lack atranorin. Although the above chemical differences may seem minor, this is particularly salient considering the complex situation that the molecular phylogenetic analyses of the chemotypes of *L. normandinoides* have revealed (Lendemer in rev.A). In that study, the fumarprotocetraric acid and protocetraric acid chemotypes were found to represent well supported monophyletic entities, worthy of recognition as distinct species, while the depside deficient chemotype was found to represent a reversion within the protocetraric acid chemotype.

It should also be noted that the present delineation of *Lepraria nivalis* still requires confirmation with molecular data despite the fact that sequences have been included in molecular phylogenetic analyses for multiple published studies (Ekman & Tønsberg 2002; Lendemer in rev.A; Tretiach et al. 2009). This is due to the fact that there are neither molecular data available for the populations of *L. nivalis* from the type region (France) nor for the chemotype represented by the type (atranorin and fumarprotocetraric acid). The original nuITS sequence (GenBank

accession #AF517895) generated for this taxon by Ekman and Tønsberg (2002) is derived from a voucher[11] collected in Alaska that produces atranorin and protocetraric acid (T. Tønsberg pers. comm.). This sequence has been assumed to be representative of *L. nivalis* s.str. and thus used for reference in subsequent studies (Lendemer in rev.A; Tretiach et al. 2009). Both of these subsequent studies recovered a sequence (GenBank accession #FJ184309) derived from a voucher from Europe (Solvenia) in a strongly supported clade with the original sequence generated from the Alaskan population.

Specimens examined (8 total seen from North America; the presence of atranorin is indicated by "at+"). – **CANADA. BRITISH COLUMBIA.** QUEEN CHARLOTTE ISLANDS: Maude Island, in Skidegate Inlet, 12.vii.1967, on shaded mossy bank, *I.M. Brodo 11268 & M.J. Shchepanek* (NY, at+). CAPITAL REGIONAL DIST.: Vancouver Island, Lizard Lake Loop, Lizard Lake, 25.v.2010, on *Alnus*, *J.C. Lendemer 22375 & A. Moroz* (NY). DEWDNEY-ALOUETTE DIST.: Golden Ears Provincial Park, along Lower Falls Trail from parking area, 10.v.2009, on *Populus*, *W.R. Buck 54713* (NY). **U.S.A. WASHINGTON.** SNOHOMISH CO.: near Skyhomish, 2.vi.1937, on Douglas fir, *W.J. Eyerdam 1826* (NY, SBBG, at+).

Lepraria normandinoides **Lendemer & R.C. Harris,** Opuscula Philolichenum 4: 45. 2007. TYPE: **U.S.A. NORTH CAROLINA.** TRANSYLVANIA CO.: Nantahala National Forest, Shower Falls, along Cold Mountain Road, south slope of Panthertail Mountain, Lake Toxaway Quad., 3500 ft., small roadside waterfall over gneiss, 29.v.2006, on gneiss in overhang, *J.C.*

[11] Voucher data for GenBank AF517895 – **U.S.A. ALASKA:** City and Borough of Sitka, Baranof Island SW, E of NE tip of Redoubt Lake, S of inlet stream, 56°55.251'N, 135°07.555'W, 10–15 m, 11.ix.1999, corticolous on trunk of *Chamaecyparis nootkatensis* in a muskeg, *T. Tønsberg 27804* (NY). – TLC (performed by Tønsberg): atranorin, protocetraric acid.

Lendemer et al. 7001 = Lich. East. N. Amer. Exs. V: 221 (NY!, holotype; ASU!, B!, BG!, CANB!, FH!, GZU!, H!, M!, MIN!, S!, TNS!, TU!, isotypes).

Figures 101 and 102.

Description. – **Thallus** crustose, leprose, placodioid with crisped margins, continuous and distinctly bi-layered, initially forming small discrete thalli which expand marginally, overlap, and become confluent to form a thick crust (0.2–0.5 mm thick), bone white to light blue-white; **hyphae** hyaline or pigmented, [1.8]–(2.3)–2.9–(3.7)–[4.8] µm (*n*=58), septate, secund, obscured by a layer of POL+ crystals that do not dissolve in KOH; prothallus disappearing with age; **hypothallus** a variably developed layer of loosely intertwined hyphae surrounding and subtending the granules, extending outward from the edge of thallus; **rhizohyphae** present, typically abundant, pigmented brown; **granules** globose, [19]–(24)–38–(53)–[84] µm (*n*=52), ecorticate, well organized and discrete, not forming compound units, sometimes separating from the hypothallus and then producing abundant projecting hyphae; **photobiont** green, coccoid, cells globose, [6]–(7.9)–9.8–(11.6)–[13.2] µm (*n*=72).

Chemistry. – Atranorin, roccellic/angardianic acid, ± protocetraric acid. Spot tests: K+ yellow, C-, KC-, P+/- orange/red, UV-.

Ecology and distribution. – *Lepraria normandinoides* is a North American endemic that is widely distributed throughout temperate eastern North America with rare disjunct populations extending westward into the greater Sonoran Desert region and the Black Hills of South Dakota

(figure 102). The species occurs in high humidity low-light habitats on sheltered non-calcareous rock faces and overhangs as well as the bark of hardwoods and conifers. Acid deficient populations of *L. normandinoides* are rare and so far known only from the northern/boreal portions of its geographic range.

Conservation status. – *Lepraria normandinoides* is a North American endemic that is common throughout its core range as outlined above. This species does not likely represent a conservation priority.

Discussion. – *Lepraria normandinoides* is a common and conspicuous species that is widespread in eastern North America. Due to its appearance, as well as the thickness of the thallus which facilities easy collection with minimal substrate, the species is somewhat less impacted by collector bias when compared to other sympatric species with less conspicuous thalli. Historically the species was confused with *L. lobificans* auct. (i.e., *L. finkii*) until Laundon (1989) restricted the use of that name to material producing atranorin and stictic acids. Laundon (1989) then described material with atranorin and protocetraric or fumarprotocetraric acids that has been referred to *L. lobificans* as a new taxon, *L. nivalis*. *Lepraria normandinoides* was included within Laundon's broad concept of *L. nivalis* until Lendemer and Harris (2007) recognized it was chemically, ecologically, and morphologically distinct.

In the field the only species likely to be confused with *Lepraria normandinoides* is *L. oxybapha*, which was previously included within a broad circumscription of that species (Lendemer in rev.A). Molecular phylogenetic analyses, however, showed that the populations of *L. normandinoides* that produce fumarprotocetraric acid form a strongly supported monophyletic

group sister to *L. normandinoides* s. str. As such *L. normandinoides* was restricted to only the populations that produce protocetraric acid or are acid deficient.

Other species of *Lepraria* with placodioid thalli might be confused with *L. normandinoides* are *L. eburnea*, *L. finkii*, *L. nivalis*, *L. torii*, and *L. vouauxii*. However, none of those species have thalli that develop a marginal lip and all have different chemical profiles (atranorin, zeorin, stictic acid aggregate in *L. finkii*; protocetraric acid with or without atranorin in *L. nivalis*; roccellic/angardianic acid and fumarprotocetraric acid in *L. torii*; and dibenzofurans of the pannaric acid aggregate in *L. vouauxii*). *Lepraria lanata*, a species endemic to the high elevations of the southern Appalachians, is chemically similar to *L. normandinoides* but differs markedly in having massive granules (>100 μm in diameter) which resemble small cotton balls.

Selected specimens examined (243 total seen from North America; "*" denotes depside deficient collections). – **CANADA. ONTARIO.** ALGOMA DIST.: Lake Superior Provincial Park, Agawa Rock Pictographs trail, 14.vii.2011, on rock, *I.M. Brodo 32945 & F. Brodo* (CANL). **U.S.A. ALABAMA.** CLAY CO.: Talladega National Forest, Chinnabee Silent Trail, 24.ix.1992, on rock, *R.C. Harris 28390* (NY). DeKALB CO.: DeSoto State Park, 4.x.1998, on rock, *W.R. Buck 34741* (NY). JACKSON CO.: Buck's Pocket State Park, 3.x.1998, on rock, *R.C. Harris 42378* (NY). **ARKANSAS.** FRANKLIN CO.: Ozark National Forest, Ozark Highlands National Recreational Trail, 8.vi.2000, on rock, *W.R. Buck 37275* (NY). NEWTON CO.: Ozark National Forest, E of FSR 1463, 28.iii.2006, on sandstone, *J.C. Lendemer et al. 6557* (NY, PH). **CONNECTICUT.** WINDHAM CO.: Town of Ashford, Yale Forest, along Boston Hollow Rd. ~0.6 mi NE of jct w/ Eastford Rd., 24.vii.2002, on rock, *R.C. Harris 46097* (NY). **GEORGIA.** COFFEE CO.: Broxton Rocks Ecological Preserve, 9.ii.2003, on *Magnolia*, *R.C. Harris 47050*

(NY). COLUMBIA CO.: Heggie's Rock TNC Preserve, 8.xi.2008, on rock, *G.B. Perlmutter et al. 1639* (NY). RABUN CO.: Lake Burton Wildlife Management Area, 17.ix.2006, on rock, *J.C. Lendemer et al. 7657* (NY). ROCKDALE CO.: Panola Mountain State Park, 17.iv.2007, on granite, *J.C. Lendemer et al. 8984* (NY). **ILLINOIS.** POPE CO.: Shawnee National Forest, Burden Falls, 26.v.1997, on rock, *L.R. Phillippe 28777* (NY). RANDOLPH CO.: Piney Creek Ravine Nature Preserve, 7.xi.2004, on sandstone, *R.C. Harris 50566* (NY). **KANSAS.** CHAUTAUQUA CO.: 0.5 mi E, 0.25 mi S of Chautauqua, private land along Turkey Creek to Devil's Canyon, 21.ii.2003, on *Quercus*, *M.K. Advaita 2142* (KANU). **KENTUCKY.** BATH CO.: Daniel Boone National Forest, Tater Knob, 10.x.1995, on *Nyssa*, *R.C. Harris 36882* (NY). LETCHER CO.: Bad Branch Nature Preserve, 14.ix.1991, on rock, *R.C. Harris 27023* (NY). **MAINE.** HANCOCK CO.: T10SD, slope of Tunk Mountain, 8.vii.2008, on rock, *R.C. Harris 54743* (NY); T10SD, Maine Public Reserve Lands, 0–0.25 mi N of ME82 directly across from Tunk Lake Access, 10.vi.2010, on rock, *J.C. Lendemer 23028* (NY*); Little Deer Isle, 8.xi.2005, on rock, *T. Harris 194* (NY*). OXFORD CO.: Berry Ledge, Paris, 29.ix.1931, on rock, *J.C. Parlin 9897* (NY). WASHINGTON CO.: T10SD, Donnell Pond Public Reserve Lands, Black Mountain, 10.vi.2010, on *Betula*, *J.C. Lendemer 29957* (NY). **MARYLAND.** CECIL CO.: New Valley, along Basin Run, 1.xii.2009, on rock, *J.C. Lendemer 20017* (NY). BALTIMORE CO.: Gunpowder Falls, 18.ix.1090, on rock, *C.C. Plitt 328* (NY). WASHINGTON CO.: Sideling Creek, 4.vi.1990, on rock, *C.F. Reed 136003-A* (NY). **MASSACHUSETTS.** BERKSHIRE CO.: Bartholomew's Cobble, 28.iii.1981, on rock, *R.C. Harris 13540* (NY). **MINNESOTA.** ST. LOUIS CO.: Voyageurs National Park, E side of Browns Bay, 13.vii.1979, on rock, *C.M. Wetmore 39989* (NY*). **MISSISSIPPI.** TISHOMINGO CO.: Tishomingo State Park, 27.ix.1992, on rock, *R.C. Harris 28584* (NY). **MISSOURI.** IRON CO.: E slope of Royal Gorge,

27.iv.1988, on rock, *R.C. Harris 21781* (NY). MADISON CO.: Amidon Memorial Conservation Area, 21.x.2001, on rock, *R.C. Harris 45058* (NY). NEWTON CO.: Wildcat Glade Natural Area, 6.xi.2002, on rock, *W.R. Buck 42997* (NY). **NEW JERSEY.** BERGEN CO.: Palisades, *sine date*, on rock, *C.F. Austin s.n.* (NY). MORRIS CO.: Ledgewood, 10.viii.1914, on rock, *C. Barbour 3* (NY). **NEW MEXICO.** SIERRA CO.: Black Range, along North Percha Creek, 8.vii.1995, on rock, *R.D. Worthington* (NY, UTEP). **NEW YORK.** ESSEX CO.: Town of Keene, Chapel Pond, 19.ix.2004, on rock, *R.C. Harris 50039* (NY). JEFFERSON CO.: Wellesley Island State Park, 20.ix.1983, on mosses over rock, *R.C. Harris 16746* (NY). ROCKLAND CO.: Bear Mountain State Park, Iona Island Bird Sanctuary, 18.vii.2010, on rock, *J.C. Lendemer 23681 & A. Moroz* (NY). ULSTER CO.: Ice Caves Mountain National Landmark, 14.v.1994, on rock, *R.C. Harris 32840* (NY). WESTCHESTER CO.: Croton Dam Rd., 0.5 mi S of NY129, 27.vii.1975, on *Carya*, *C.R. Prince 75-65* (NY). **NORTH CAROLINA.** CLAY CO.: Nantahala National Forest, Southern Nantahala Wilderness, 17.ix.2006, on *Acer*, *J.C. Lendemer et al. 7736* (NY). JACKSON CO.: Bad Creek Trail to Ellicott's Rock, 28.ix.1989, on *Quercus*, *R.C. Harris 24866* (NY). MACON CO.: Nantahala National Forest, McDowell Mt., 12.viii.1994, on rock, *R.C. Harris 33149* (NY). SWAIN CO.: Great Smoky Mountains National Park, Straight Fork Rd., 6.viii.2009, on rock, *J.C. Lendemer 19159 & E.A. Tripp* (NY). TRANSYLVANIA CO.: Gorges State Park, 9.viii.2005, on *Quercus*, *J.C. Lendemer 4586 & E.A. Tripp* (NY). YANCEY CO.: NE slope of Mt. Mitchell and Commissary Ridge in Black Mountains, along Maple Camp Ridge trail, 1972, on rock, *J.P. Dey 1367* (NY). **OHIO.** GALLIA CO.: Wayne National Forest, above Symmes Creek, 19.v.2006, on sandstone, *J.C. Lendemer et al. 7437* (NY). SCIOTO CO.: Shawnee State Forest, along Pond Lick Run, 21.v.2005, on sandstone, *J.C. Lendemer 7161* (NY). **OKLAHOMA.** CHEROKEE

CO.: J.T. Nickel Family Nature and Wildlife Preserve, 30.x.2000, on rock, *W.R. Buck 38448* (NY). LOVE CO.: Lake Murray State Park, 24.iv.2004, on sandstone, *S. Strawn & S. Strawn 92* (NY). **PENNSYLVANIA.** CARBON CO.: Hickory Run State Park, 19.v.2009, on rock, *J.C. Lendemer 17573 & R.C. Harris* (NY). HUNTINGDON CO.: Trough Creek State Park, 22.iv.2008, on rock, *W.R. Buck 53550* (NY). MONROE CO.: Tannersville Bog TNC Preserve, 24.xi.2009, on rock, *J.C. Lendemer 19552 & V. Doyle* (NY). TIOGA CO.: Tioga State Forest, E shore of Gleason Hollow Run, 14.v.2009, on rock, *J.C. Lendemer 16925* (NY). WYOMING CO.: Bowmans Creek Ledges, 20.vii.2008, on rock, *J.C. Lendemer 13510* (NY). YORK CO.: Apollo County Park, 9.viii.2009, on rock, *J.C. Lendemer 19455* (NY). **RHODE ISLAND.** PROVIDENCE CO.: Town of Lincoln, Lime Rock Nature Preserve, 17.ix.2006, on rock, *R.C. Harris 53210* (NY). **SOUTH CAROLINA.** GREENVILLE CO.: Caesars Head State Park, 14.iii.1997, on base of tree, *R.C. Harris 40035* (NY). **SOUTH DAKOTA.** MINNEHAHA CO.: Palisades State Park, 10.v.2004, on quartzite, *M.K. Advaita 3491* (NY). **TENNESSEE.** BLOUNT CO.: Great Smoky Mountains National Park, Rich Mountain Gap, 30.vi.2010, on *Quercus*, *J.C. Lendemer et al. 23584* (NY). COCKE CO.: Great Smoky Mountains National Park, Gabes Mountain Trail, 5.viii.2009, on *Quercus*, *J.C. Lendemer 19078 & E.A. Tripp* (NY). MONROE CO.: Cherokee National Forest, Citico Creek Wilderness, 6.viii.1994, on *Acer*, *R.C. Harris 32915* (NY). SCOTT CO.: Big South Fork National River and Recreation Area, 7.viii.1994, on rock, *R.C. Harris 32967* (NY). **VIRGINIA.** GRAYSON CO.: Jefferson National Forest, Comers Creek, 5.iv.2008, on hardwood, *G.B. Perlmutter 1291* (NY). PATRICK CO.: Blue Ridge Parkway, Rock Castle Gorge Loop Trail, 6.x.1995, on rock, *R.C. Harris 36656* (NY). **WEST VIRGINIA.** POCAHONTAS CO.: Monongahela National Forest, upper slopes of

Spruce Mountain, 20.x.2007, on *Acer*, *J.C. Lendemer 9936 & A. Moroz* (NY). WYOMING CO.: near New Richmond, 30.v.1967, on rock, *C.F. Reed 74818* (NY).

Lepraria oxybapha **Lendemer,** J. Torrey Bot. Soc. TYPE: **U.S.A. PENNSYLVANIA.** SULLIVAN CO.: Ricketts Glen State Park, S slopes above Big Run, 1 mi W of jct w/ PA 487, 2067 ft., *Tsuga/Betula/Acer* forest with cold air talus slope and large sheer cliffs, 19.ix.2010, on rock in overhang, *J.C. Lendemer 25637 & J. Kunsman* (NY!, holotype).

Figures 103 and 104.

Description. – **Thallus** crustose, leprose, placodioid with crisped margins, continuous and distinctly bi-layered, initially forming small discrete thalli which expand marginally, overlap, and become confluent to form a thick crust (0.2–0.5 mm thick), bone white to light blue-white in color; **hyphae** hyaline or pigmented, [2.1]–(2.6)–3.3–(4.0)–[4.8] µm (*n*=39), septate, secund, obscured by a layer of POL+ crystals that do not dissolve in KOH; prothallus disappearing with age; **hypothallus** a variably developed layer of loosely intertwined hyphae surrounding and subtending the granules, extending outward from the edge of thallus; **rhizohyphae** present, typically abundant, pigmented brown; **granules** globose, [24]–(35)–48–(61)–[80] µm (*n*=45), ecorticate, well organized and discrete, not forming compound units, sometimes separating from the hypothallus and then producing abundant projecting hyphae; **photobiont** green, coccoid, cells globose, [5]–(7.7)–9.6–(11.5)–[13.2] µm (*n*=39).

Etymology. – The epithet “oxybapha” refers to the saucer shaped “lobes” produced at the margins of the thallus.

Chemistry. – Atranorin, roccellic/angardianic acid, fumarprotocetraric acid, and traces of protocetraric acid. Spot tests: K+ yellow, C-, KC-, P+ orange/red, UV-.

Ecology and distribution. – *Lepraria oxybapha* is a North American endemic whose range primarily correlates to an Appalachian-Ozarks distribution (Brodo et al. 2001; figure 104 herein). Although it is widespread throughout those regions it is most frequent in the northern/central Appalachians, becoming rarer further south and in the Ozarks. Rare disjunct populations occur westward in the Great Plains and the Greater Sonoran Desert Region. The species is restricted to high humidity low-light habitats where it occurs on the bark of conifers and hardwoods as well as sheltered non-calcareous rock faces and overhangs.

Conservation status. – This species is a North American endemic that is common in its core range, as outlined in the preceding section. The taxon does not likely require conservation measures at present.

Discussion. – *Lepraria oxybapha* is a distinctive North American species that can be recognized by its thalli, which develop a marginal lip, brown rhizohyphae on the lower surface, occurrence on trees and non-calcareous substrates, and the presence of fumarprotocetraric acid in addition to atranorin and roccellic/angardianic acid. *Lepraria normandinoides* is morphologically identical but differs chemically in the presence or absence of protocetraric acid instead of fumarprotocetraric acid as a major substance. *Lepraria torii*, a species whose distribution is primarily bi-coastal in boreal North America, with occasional disjunct occurrences throughout

the boreal forests, also produces roccellic/angardianic acid and fumarprotocetraric acids and thus could be confused with *L. oxybapha*. That species is rarely sympatric with *L. oxybapha*, typically does not produce atranorin, and has a distinctive green thallus that lacks a marginal lip.

Selected specimens examined (110 total seen from North America). – **U.S.A. ARKANSAS.** STONE CO.: Ozark National Forest, City Rock Bluff Special Interest Area, 18.iv.2005, on sandstone, *R.C. Harris 51247* (NY). **GEORGIA.** COFFEE CO.: Broxton Rocks Ecological Preserve, 16–17.xii.1993, on rock, *R.C. Harris 32693* (NY). TOWNS CO.: Southern Nantahala Wilderness, Chattahoochee National Forest, Hightower Gap to Rich Knob, 11.xi.2007, on rock, *J.C. Lendemer et al. 10935* (NY). **KANSAS.** ELLSWORTH CO.: 2.25 mi S, 10.5 mi E of Kanopolis, Kanopolis Reservoir, 12.x.2003, on soil, *T. Hildebrand & D. Hildebrand s.n.* (KANU). **KENTUCKY.** LETCHER CO.: Bad Branch Nature Preserve, 14.ix.1991, on rock, *R.C. Harris 27016* (NY). **LOUISIANA.** NATCHITOCHES PARISH: Kisatchie National Forest, Longleaf Trail Vista, 27.iii.1982, on sandstone, *J. Pruski 2472* (NY). **MAINE.** HANCOCK CO.: T28MD, SE slopes of Lead Mountain, 11.vi.2010, on *Fagus*, *J.C. Lendemer 23199* (NY). **MASSACHUSETTS.** BERKSHIRE CO.: Town of Pittsfield, Pittsfield State Forest, 4.vi.2010, on *Betula*, *J.C. Lendemer 22459* (NY). **MISSOURI.** SHANNON CO.: Ozark National Scenic Riverways, Rocky Falls, 22.ix.1990, on rock, *R.C. Harris 25576* (NY). **NEW YORK.** GREENE CO.: Town of Hunter, Stony Clove Notch, 13.ix.2008, on *Betula*, *J.C. Lendemer 14083-A* (NY). ULSTER CO.: Town of Shandaken, Slide Mountain Wilderness Area, 13.ix.2008, on *Betula*, *J.C. Lendemer 14011* (NY). **NORTH CAROLINA.** CLAY CO.: Nantahala National Forest, Buck Creek, 10.xi.2007, on *Betula*, *J.C. Lendemer et al. 10453* (NY). HAYWOOD CO.: Great Smoky Mountain National Park, around Caldwell Fork, 4.viii.2009, on

dead tree, *E.A. Tripp 631 & J.C. Lendemer* (NY). MADISON CO.: 3 mi S of Hot Springs, 27.viii.1968, on rock, *W.L. Culberson 14276* (NY). **OKLAHOMA.** GREER CO.: Quartz Mountain Arts & Conference Center and Nature Park, 10.iv.2007, on moss over rock, *C.A. Morse 15061 & D. Ladd* (KANU, NY). OTTAWA CO.: N of E0060 Rd. on W side of Spring River, 31.x.2000, on rock, *R.C. Harris 44436* (NY). **PENNSYLVANIA.** BRADFORD CO.: State Game Lands No. 36, 18.v.2009, on *Betula*, *J.C. Lendemer 17349 & R.C. Harris* (NY). MONROE CO.: Tobyhanna State Park, 27.vii.2009, on *Acer*, *J.C. Lendemer 18627* (NY). TIOGA CO.: Tioga State Forest, slopes above jct US 15/I-99, 12.v.2009, on rock, *J.C. Lendemer 16495 & D. Atha* (NY). WAYNE CO.: Lacawac Sanctuary, 25.vii.2009, on *Betula*, *J.C. Lendemer 18451* (NY). WYOMING CO.: State Game Lands No. 57, Henry Lott Rd., 21.vii.2008, on rock, *J.C. Lendemer 13626* (NY). YORK CO.: W-shore of Susquehanna River, 0.5 mi S of jct River Rd. & PA 372, 28.v.2009, on *Betula*, *J.C. Lendemer 17978* (NY). **TENNESSEE.** BLOUNT CO.: Great Smoky Mountains National Park, Gabes Mountain Trail, 5.viii.2009, on rock, *J.C. Lendemer 19059 & E.A. Tripp* (NY). SEVIER CO.: Great Smoky Mountains National Park, Chimney Tops Trail, 28.vi.2010, on *Liriodendron*, *R.C. Harris 56182* (NY). **TEXAS.** LLANO CO.: sine loc., 30.i.1931, on granite, *E. Whitehouse 48* (NY). **VERMONT.** ESSEX CO.: Town of Victory, Victory State Forest, Umpire Mountain, 16.v.2008, on rock, *R.C. Harris 54350* (NY). ORLEANS CO.: Town of Westfield, Green Mountains, Hazen's Notch, 13.v.2005, on rock, *R.C. Harris 51347* (NY). **VIRGINIA.** GILES CO.: Mountain Lake Biological Station, 7.x.1995, on *Betula*, *R.C. Harris 36718* (NY). SMYTH CO.: Jefferson National Forest, Whitetop Mountain, 5.iv.2008, on *Sorbus*, *R.C. Harris 54112* (NY).

Lepraria pacifica **Lendemer,** Mycologia 103: 1226. 2011. TYPE: **U.S.A. CALIFORNIA.** MARIPOSA CO.: Yosemite National Park, S side of Merced River, downstream of Phono Bridge, W end of Yosemite Valley, 1172 m, mesic north-facing talus slope, with mixed conifer forest, on *Calocedrus*, 22 Sept 2009, *J.C. Lendemer 19765* (NY!, holotype).

Figures 105 and 106.

Description. – **Thallus** crustose, leprose, aggregated, discontinuous and not stratified to continuous and weakly stratified, initially composed of isolated granules that divide to form aggregations, eventually merging to form a crust of variable thickness (0.1–0.4(–0.7) mm), greenish-blue in color; **hyphae** hyaline, [1.7]–(2.2)–2.8–(3.3)–[4] µm (*n*=120) in width, septate, secund, obscured by a thick layer of crystals that dissolve in KOH; **prothallus** persistent and readily visible; **hypothallus** typically indistinct, poorly developed or absent; **rhizohyphae** absent; **granules** globose, [16]–(24)–32–(40)–[60] µm (*n*=120) in diameter, ecorticate initially and eventually forming a poorly developed pseudocortex, well organized and discrete; **photobiont** green, coccoid, cells globose, [5]–(6.1)–7.5–(8.9)–[13] µm (*n*=120) in diameter.

Etymology. – The epithet "pacifica" refers to the geographic distribution of the species, which is restricted to mountain ranges more-or-less near the Pacific Coast in western North America.

Chemistry. – *Lepraria pacifica* is characterized by the presence of divaricatic and zeorin with nordivaricatic acid as an accessory in low, but highly variable, concentrations. The C+ and

KC+ spot test reactions vary in intensity depending on the concentration of the latter substance. Comparative TLC of *L. crassissima*, *L. cryophila*, and *L. pacifica* revealed nordivaricatic acid to be present in consistently higher concentrations in the former two species than the latter one. Anthraquinone pigments were also found to be present in populations of *L. pacifica* from an isolated redwood forest in the Santa Lucia Mountains of central California. This population was not studied with molecular methods and has since been extirpated by a wildfire (Lendemer & Knudsen 2010).

TLC: Divaricatic acid, zeorin, traces of nordivaricatic acid, ± an unidentified anthraquinone pigment.

Spot tests: K- (or K+ purple when pigment is present), C± pink, KC+ pink, P-, UV+ blue-white.

Ecology and distribution. – The vast majority of collections of *Lepraria pacifica* were found on conifer taxa endemic to western North America (figure 106). It is the dominant *Lepraria* species in the redwood forests of the Coastal Range and the Sierra Nevada Mountains in central and northern California, becoming less frequent farther north in coastal British Columbia where it is replaced by the ecologically similar *L. torii*.

Conservation status. – *Lepraria pacifica* is a North American endemic whose core geographic distribution is western North America. Although it is common in some areas, many of the forests where *L. pacifica* occurs are threatened by disruptions to the fire regime and by changes in overall forest composition and dynamics. The species is not likely in need of

conservation measures at present, but should be considered in community management plans as they are developed and implemented.

Discussion. – *Lepraria pacifica* was described by Lendemer (2011b) to accommodate the populations of *Lepraria* in western North America that produce divaricatic acid and zeorin. These populations had previously been referred to *L. incana*, however, molecular phylogenetic analyses performed in the above study recovered sequences of them as distinct from that taxon. In western North America only *L. torii* could potentially be confused with *L. pacifica*, as both species have greenish colored aggregate thalli of variable thickness. *Lepraria torii* differs from *L. pacifica* in the production of roccellic/angardianic acid and fumarprotocetraric acid. *Lepraria pacifica* is most similar to the allopatric species *L. hodkinsoniana*. Both species produce divaricatic acid and zeorin, however, nordivaricatic acid is typically present as a minor assessor in *L. pacifica* while it is not detectable in *L. hodkinsoniana* with TLC.

Selected additional specimens examined (51 total seen from North America; specimens with anthraquinones as indicated). – **CANADA. BRITISH COLUMBIA.** CAPITAL REGIONAL DIST.: Vancouver Island, Lizard Lake Loop, Lizard Lake, 26.v.2010, on conifer, *J.C. Lendemer 22391 & A. Moroz* (NY). GREATER COWICHAN VALLEY REGIONAL DIST.: Vancouver Island, between Riverbottom Rd. and Cowichan River, 25.v.2010, on log, *J.C. Lendemer 22358 & A. Moroz* (NY). GREATER VANCOUVER REGIONAL DIST.: Vancouver, Pacific Spirit Regional Park, Spanish Bank Creek area, 9.v.2009, on *Acer*, *W.R. Buck 54625* (NY). SQUAMISH-LILLOOET REGIONAL DIST.: Stawamus Chief Provincial Park, along Lower Falls Trail, 11.v.2009, on rock, *W.R. Buck 54722* (NY). QUEEN CHARLOTTE

ISLANDS: Kunghit Island, Rose Harbour, 4.vii.1971, on *Thuja*, *I.M. Brodo 17682* (NY); Graham Island, along the road to Rennell Sound, 15.vii.2010, on red cedar, *J.A. Harpel 48499 & S. Joya* (NY). **U.S.A. CALIFORNIA.** MARIPOSA CO.: Yosemite National Park, 1 km E of Hogdon Meadow on Old Big Oak Flat Rd. where it crosses Hazel Green Creek, 19.ix.2009, on *Calocedrus*, *J.C. Lendemer 19525* (NY); Yosemite National Park, bottom of Tuolumne Grove, 19.ix.2009, on burnt log, *J.C. Lendemer 19579-A* (NY). MONTEREY CO.: Santa Lucia Mountains, Lime Kiln State Park, 16.viii.2008, on redwood, *K. Knudsen 10159* (NY, UCR; anthraq+); Point Lobos State Reserve, Allan Memorial Grove, 14.v.2009, on *Cupressus*, *K. Knudsen 11125* (NY, UCR). SANTA CRUZ CO.: The Forest of Nisene Marks State Park, along trail from parking area at Porter Family Picnic Area, 13.vii.2008, on *Sequoia*, *W.R. Buck 23923* (NY). **OREGON.** COOS CO.: South Slough National Estuarine Research Reserve, 12 km SW of town of Coos Bay, ix.2008, on *Chamaecyparis*, *B. McCune 29769* (NY, OSC). LINN CO.: W side of Clear Lake, S of Clear Lake Resort, i.2009, on wood, *B. McCune 29887* (NY, OSC). **WASHINGTON.** THURSTON CO.: between Bald Hill and Bald Hill Lake, 20 km SE of town of Yelm, iii.2010, on *Thuja*, *B. McCune 30504* (NY, OSC).

***Lepraria rigidula* (B. de Lesd.) Tønsberg,** Sommerfeltia 14: 205. 1992.

Crocynia rigidula B. de Lesd. *in* Hue, Bull. Soc. Bot. France 71: 331. 1924. TYPE: **SCOTLAND:** Perth, Pitlochry, byside of R. Tummel, vi.1914, *J. McAndrew s.n.* (E [n.v.], holotype).

Figures 107 and 108.

Description. – **Thallus** crustose, leprose, aggregated, discontinuous and not stratified, initially composed of isolated granules that divide to form aggregations, eventually merging to form a thin crust (<0.1 mm thick), off-white or gray-blue in color; **hyphae** hyaline, [2.1]–(2.5)–3.1–(3.6)–[5.2] µm (*n*=64) in width, septate, secund, obscured by a thick layer of POL+ crystals that dissolve in KOH; **prothallus** persistent; **hypothallus** absent; **rhizohyphae** absent; **granules** globose, [24]–(30)–43–(55)–[78] µm (*n*=96) in diameter, ecorticate, well organized and discrete, remaining distinct and not forming compound units, often with projecting prothalline hyphae persisting into maturity; **photobiont** green, coccoid, cells globose, [6]–(7.7)–9.7–(11.8)–[13.3] µm (*n*=64) in width.

Chemistry. – Atranorin, nephrosteranic acid. Spot tests: K+ yellowish, C-, KC-, P-, UV-.

Ecology and distribution. – *Lepraria rigidula* is rare but widespread in boreal and arctic regions of North America (figure 108). Rare disjunct populations extend its geographic distribution southwards in mountainous regions of both the East and West. The species typically occurs on bryophytes and humus in sheltered microhabitats. Rarely it grows directly on non-calcareous rocks or on the bark of trees (though often at the base).

Conservation status. – This species is infrequent, however, considering its wide geographic distribution and ecological amplitude it is unlikely in need of conservation measures. The rare disjunct populations in more southerly mountainous regions should be monitored to determine the potential impact of climate change.

Discussion. – *Lepraria rigidula* is a distinctive species with an aggregate thallus and granules which the projecting prothalline hyphae tend to persist into maturity (figure 107D–F). These features, coupled with the presence of nephrosteranic acid, serve to distinguish the species from all its North American congeners. *Lepraria humida* and *L. jackii* are the two species most likely to be confused with *L. rigidula* because they are sympatric, have aggregate thalli, ecorticate granules, and produce fatty acids as well as atranorin. *Lepraria humida* typically occurs directly on rocks, rather than on organic matter and bryophytes, and differs in producing rangiformic/jackinic acid rather than nephrosteranic acid. *Lepraria jackii* is ecologically similar to *L. rigidula*, however, it produces roccellic/angardianic acid and rangiformic/jackinic acid rather than nephrosteranic acid. Both *L. humida* and *L. jackii* also do not typically have granules in which the prothalline hyphae persist to maturity.

It should be noted that *Lepraria rigidula* was mentioned as having a hypothallus in the morphological scheme presented by Lendemer (2011). This was, however, based on a misinterpretation of the thallus, and further study showed that in fact the species has an aggregate thallus. In some ways, *L. rigidula* is morphologically similar to *L. lanata* in that the prothalline hyphae extend from the granules and cause them to adhere to each other and the substrate. In *L. lanata* this phenomenon causes the granules to collect and form massive globose aggregations that resemble tiny cotton calls. However, in *L. rigidula* this occurs within individual granules.

Selected specimens examined (31 total seen from North America). – **CANADA. NORTHWEST TERRITOERIES:** MACKENZIE DIST.: Mackenzie Mountains, Nahanni National Park, small lake 6 km W of Hole-in-the-Wall Lake, 13.vii.1977, on humus, *T. Ahti 32869c* (H, NY). **QUEBEC.** KAMOURASKA CO.: Sainte-Anne de la Pocatière, 27.v.1935, on

N side of rock, *E. Lepage 5* (NY). **CHILE. BÍO BÍO PROV.:** Alto Bío Bío, along road 1.6 km S of Chenqueco, 12.iv.2009, on *Nothofagus*, *J.C. Lendemer et al. 16150* (NY). **U.S.A. CALIFORNIA.** MARIPOSA CO.: Yosemite National Park, vicinity of base of Bridal Veil Falls, 22.ix.2009, on *Calocedrus*, *J.C. Lendemer 19707* (NY). RIVERSIDE CO.: San Bernardino National Forest, San Jacinto Mountains, North Fork of the San Jacinto River, 6.x.2008, on mosses, in overhang, *J.C. Lendemer 14812 & K. Knudsen* (NY). **COLORADO.** BOULDER CO.: Rocky Mountain National Park, Wild Basin, vicinity of Calypso Cascades, 27.vi.1962, on bryophytes, *R.A. Anderson 2110* (COLO). **MAINE.** WASHINGTON CO.: Town of Cutler, Cutler Coastal Public Lands, Loop Trail via Black Point Cove Cutoff, 8.vi.2010, on moss, *J.C. Lendemer 22691* (NY). **MONTANA.** LAKE CO.: Lower S Woodward Creek, 12.vii.1977, on moss, *B. McCune 7531* (NY). **NEW HAMPSHIRE.** CHESHIRE CO.: Mount Monadnock State Park, White Arrow Trail on S slope of Mt. Monadnock, 5.iv.1992, on *Betula*, *T. Ahti et al. 51123* (NY). **NEW YORK.** ULSTER CO.: along trail to top of Slide Mountain from Winisook, 14.ix.1980, on moss, *R.C. Harris 13480* (NY). **OREGON.** BENTON CO.: 1840 NE Seavy Ave., just NE of Corvallis, xii.2010, on wooden arbor, *B. McCune 30967* (NY). **VIRGINIA.** GRAYSON CO.: Grayson Highlands State Park, Wilburn Ridge, 12.ix.1991, on *Picea*, *R.C. Harris 26869* (NY).

Lepraria squamatica **Elix,** Australasian Lichenology 58: 20. 2006. **AUSTRALIA. NORTHERN TERRITORY:** Tabletop Range, Litchfield National Park, 25 km SW of Batchelor, burnt *Eucalyptus* woodland, with a dense understory of *Grevillea*, *Owenia*, and *Acacia*, 2.vii.1991, on charred wood, *J.A. Elix et al. 27513* (CANB [n.v.], holotype).

Figures 109 and 110.

Description. – **Thallus** crustose, leprose, placodioid, continuous and typically distinctly bi-layered, initially forming small discrete thalli which expand marginally, overlap and become confluent to form a thick crust (0.2–0.3 mm thick), light gray-blue in color; **hyphae** hyaline, [1.5]–(2.2)–2.7–(3.2)–[4] µm (*n*=66) in width, septate, secund, obscured by a thick layer of POL+ crystals that dissolve in KOH; **prothallus** disappearing with age; **hypothallus** a thin, often poorly developed layer of loosely intertwined hyphae surrounding and subtending the granules, extending outward from the edge of the thallus; **rhizohyphae** present, pale to dirty brownish; **granules** globose, [22]–(29)–42–(54) –[80] µm (*n*=132) in diameter, ecorticate, well organized and discrete, not typically forming compound units of multiple granules; **photobiont** green, coccoid, cells globose, [5.6] –(6.9)–8.8–(10.7)–[14.4] µm (*n*=66) in diameter.

Chemistry. – Squamatic acid, baeomycesic acid, protocetraric acid, numerous additional other substances present as minor or trace accessories (Elix 2006). Spot tests: K+ dirty yellow, C-, KC-, P+ orange-red, UV+ blue-white.

Ecology and distribution. – *Lepraria squamatica* is a rare species of the Coastal Plain of southeastern North America, where it is distributed primarily in central and northern Florida, extending as far north as Cumberland Island in Georgia (figure 110). It typically occurs in humid habitats such as conifer swamps or riparian corridors on conifers (*Chamaecyparis*, *Pinus*, *Taxodium*) and more rarely on *Sabal* or on hardwoods (*Quercus*).

Conservation status. – *Lepraria squamatica* is rare in North America, and warrants the same treatment as *L. aurescens*.

Discussion. – *Lepraria squamatica* is morphologically and ecologically identical to *L. aurescens*, which differs in the production of thamnolic acid. Both species are entirely sympatric in North America and often occur as mixed populations, which can lead to confusing spot test and TLC results. The easiest way to make usable voucher specimens is to sort pieces of bark under long wave UV light because the squamatic acid in *L. squamatica* fluoresces. Interestingly the species is also morphologically quite similar to *L. harrisiana*, another species of humid habitats, especially conifer swamps, in the southeastern Coastal Plain. That species differs in producing atranorin, zeorin, and pallidic acid.

Selected specimens examined (16 total seen from North America). – **AUSTRALIA. NORTHERN TERRITORY:** Litchfield Park, 39 km SE of Batchelor, 4.vii.1991, on *Eucalyptus*, *H. Streimann 48244* (NY); Litchfield Park, below Florence Falls, 42 km SW of Batchelor, 6.viii.2005, on tree trunk, *J.A. Elix 37755* (NY). **U.S.A FLORIDA.** HIGHLANDS CO.: Highlands Hammock State Park, 30.iv.1964, on *Sabal*, *B. Moore 1059* (DUKE, NY). HILLSBOROUGH CO.: Hillsborough River State Park, 15.v.1964, on *Sabal*, *B. Moore 1342* (DUKE, NY). LAKE CO.: Seminole State Forest, along Atula Rd. from Cassia Trailhead, 8.iii.2009, on *Quercus*, *J.C. Lendemer 15843 & W.R. Buck* (NY). MARION CO.: Ocala National Forest, NE of Mill Dam Lake, 8.xii.1987, on *Quercus*, *R.C. Harris 21096* (NY). OKEECHOBEE CO.: Viking Property, ~1 mi NW of jct CR 700A and CR 724, 28.iii.1998, on *Eucalyptus*, *R.C. Harris 41879* (NY). ST. JOHNS CO.: South Ponte Vedra Beach, Guana Tolomato Matanzas

National Estuarine Research Reserve, 28.xi.2008, on *Pinus*, *W.R. Buck 54362* (NY). **GEORGIA.** CAMDEN CO.: Cumberland Island, Southcut Rd., 24.x.2004, on *Quercus*, *S.Q. Beeching s.n.* (NY).

Lepraria torii **Pérez-Ortega & T. Sprib.,** Graphis Scripta 21: 37. 2009. TYPE : **U.S.A ALASKA.** SKAGWAY BOROUGH : Chilkoot Trail, along trail between Canyon City and Pleasant Camp, 28.vii.2008, on *Tsuga*, *S. Pérez-Ortega 1061 & T. Spribille* (US [n.v.], holotype; NY!, isotype).

Figures 111 and 112.

Description. – **Thallus** crustose, leprose, aggregated, discontinuous and not stratified to continuous and weakly stratified, initially composed of isolated granules that divide to form aggregations, eventually merging to form a crust of variable thickness (0.1–0.3 mm), distinctly greenish in color; **hyphae** hyaline, [1.8]–(2.2)–2.7–(3.1)–[4.5] µm (*n*=64) in width, septate, secund, obscured by a thick layer of crystals that partially dissolve in KOH; **prothallus** persistent and often readily visible; **hypothallus** typically indistinct, poorly developed or absent; **rhizohyphae** absent; **granules** globose, [19]–(28)–37–(46)–[62] µm (*n*=133) in diameter, ecorticate initially and eventually forming a poorly developed pseudocortex, well organized and discrete; **photobiont** green, coccoid, cells globose, [5]–(6.2)–8.1–(9.9)–[13.2] µm (*n*=64) in diameter.

Chemistry. – Roccellic/angardianic acid, fumarprotocetraric acid, ± traces of protocetraric acid, ± atranorin (very rare). Spot tests: K-, C-, KC-, P+ orange-red, UV-.

Ecology and distribution. – As has been discussed by Lendemer (2010) *Lepraria torii* is a common species of the coastal rainforests of the Pacific Northwest, with infrequent disjunct occurrences in the Canadian Maritimes, Great Lakes, and northern Appalachians (figure 112). The species typically occurs on the bark of conifers, or more rarely on the bark of hardwoods in conifer-dominated habitats (such as *Thuja* swamps). In the Pacific Northwest, the species is ubiquitous and often the dominant member of some low-light high humidity rainforest communities.

Conservation status. – At the present time this species does not likely warrant conservation measures. However, excessive logging and other anthropogenic influences on native forests could have a native impact on the species. It is noteworthy that all the localities where the author has collected the species have been mature forests without recent disturbance.

Discussion. – Due to the production of fumarprotocetraric acid in *Lepraria torii*, the species is most likely to be confused with *L. nivalis*. That species differs in lacking roccellic/angardianic acid, typically producing atranorin, and in having a *finkii*-type placodioid thallus. It also often has a white or blue-white hue in the field, whereas the thallus of *L. torii* always has a distinctly greenish coloration, resembling *L. pacifica* or some forms of *L. finkii*. The thallus type of *L. torii* can also be difficult to interpret because the occurrence in extremely humid habitats seems to induce the development of a thick thallus. This phenomenon is similar

to that observed in another species of the Pacific Northwest, *L. pacifica* (Lendemer 2011a). Based on the specimens examined I consider *L. torii* to have an aggregate thallus that displays the same degree of morphological variability as *L. pacifica*.

Selected specimens examined (60 total seen from North America). – **CANADA. BRITISH COLUMBIA.** CAPITAL REGIONAL DIST.: Vancouver Island, Lizard Lake Loop, Lizard Lake, 26.v.2010, on wood, *J.C. Lendemer 22387 & A. Moroz* (NY). DEWDNEY-ALOUETTE DIST.: Golden Ears Provincial Park, along road from head of Menzies Trail, 10.v.2009, on *Populus*, *W.R. Buck 54678* (NY). GREATER VANCOUVER REGIONAL DIST.: Lynn Headwaters Regional Park, E side of Lyn Creek, ~4 km N of terminus of Lynn Creek Rd., 24.v.2010, on conifers, *J.C. Lendemer 22328 & A. Moroz* (NY). QUEEN CHARLOTTE ISLANDS: Haida Gwaii, Graham Island, along the road to Rennell Sound, 15.vii.2010, on red cedar, *J.A. Harpel 48502 & S. Joya* (NY). **NEWFOUNDLAND AND LABRADOR.** NEWFOUNDLAND: Island of Newfoundland, Avalon Peninsula, Hall's Gully, 9.7 km W of Trans-Canada Hwy on Fox Marsh Resource Rd., 8.ix.2007, on rotting log, *J.C. Lendemer 10138* (NY); White Bay South Region, Main River Reserve, Sop's Arm W, Main River Forest Access Rd., 6.ix.2011, on *Betula*, *T. Ahti 70738j* (H). **ONTARIO.** BRUCE CO.: Bruce Peninsula National Park, Shingle Marsh along road to Halfway Log Dump, 20.ix.2008, under log, *J.C. Lendemer 14331* (NY). **U.S.A. ALASKA:** Washington Bay, Kuiu Island, 15.vii.1947, on spruce, *W.J. Eyerdam 405* (NY). **OREGON.** LANE CO.: H.J. Andrews Experimental Forest, 5.viii.1971, on *Pseudotsuga*, *D. Tracy 33496* (COLO). **MAINE.** WASHINGTON CO.: Town of Cutler, Cutler Public Reserve Land, Coastal Trail, 4.vii.2008, on bryophytes over rock, *R.C. Harris 54657* (NY). **MICHIGAN.** CHEBOYGAN CO.: W of Hebron Mail Rd., 24.vii.1974, on

Thuja, *R.C. Harris 9204* (NY). **NEW HAMPSHIRE.** COOS CO.: Town of Stewartstown, Hurlbert Swamp Preserve, 17.v.2008, on rotten root of *Abies*, *R.C. Harris 54439* (NY). **VERMONT.** CALEDONIA CO.: Town of Wheelock, Wheelock Farm, 22.x.2010, on *Betula*, *J.C. Lendemer 27536 & M. Sundue* (NY). **WASHINGTON.** CLALLAM CO.: Olympic National Park, Soleduck Hot Springs, 7.viii.1967, on *Tsuga*, *I.M. Brodo 13240 & M. Shchepanek* (DUKE).

***Lepraria vouauxii* (Hue) R.C. Harris *in* Egan,** Bryologist 90: 163. 1987.

Crocynia vouauxii Hue, Bull. Soc. Bot. France 71: 392. 1924. TYPE: **FRANCE:** Meurthe-et-Moselle, Nancy, [Jarville]la-Malgrange, on bark and mosses on *Ulmus*, *L. Vouaux s.n. = Lich. Gall. Praec. Exs. #300* (BM [n.v.], lectotype [selected by Laundon 1989].

Leproloma vouauxii (Hue) J.R. Laundon, Lichenologist 21: 13. 1989.

Figures 113 and 114.

Description. – **Thallus** crustose, leprose, placodioid, without crisped margins, continuous and distinctly bi-layered, initially forming small discrete thalli which expand marginally, overlap, and become confluent to form a thick crust (0.2–0.4 mm thick), distinctly yellowish in color; **hyphae** hyaline, [1.4]–(2.1)–2.7–(3.3)–[4.2] µm (*n*=97), septate, secund, obscured by a layer of POL+ crystals that do not dissolve in KOH; prothallus disappearing with age; **hypothallus** a variably developed layer of densely intertwined hyphae surrounding and subtending the granules, sometimes outward from the edge of thallus; **rhizohyphae** uncommon, not pigmented; **granules** globose, [24]–(32)–47–(61)–[90] µm (*n*=97), ecorticate, well organized

and discrete, rarely forming compound units, sometimes separating from the hypothallus and then producing abundant projecting hyphae; **photobiont** green, coccoid, cells globose, [4.8]–(6.3)–8.9–(11.5)–[18] μm (*n*=97).

Chemistry. – Pannaric acid 6-methylester, pannaric acid (tr.), oxypannaric acid 6-methylester (minor), ± roccellic/angardianic acid, ± pigments (?), ± atranorin. Spot tests: K- or K+ red turning magenta in pigmented portions, C-, KC-, P+ orange, UV-.

Ecology and distribution. – *Lepraria vouauxii* is uncommon but widespread throughout temperate North America (figure 114). It occurs on the bark of hardwoods, on sheltered non-calcareous rocks, and on organic matter in protected microhabitats. Thalli with the same type of pigmentation that is present in *L. diffusa* var. *chrysodetoides* are apparently restricted to the Ozark Ecoregion and adjacent Ouachita Mountains.

Conservation status. – This species is rare throughout its range in North America, however, it is not likely in need of conservation measures at this time.

Discussion. – *Lepraria vouauxii* is most similar to *L. membranacea*, which is sympatric in the northern portions of the range of *L. vouauxii* and also has a *finkii*-type placodioid thallus. That species differs in producing pannaric acid as the major secondary metabolite rather than pannaric acid 6-metyhlester. *Lepraria diffusa*, another species that produces dibenzofurans, is restricted to the Pacific Northwest in North America and could be confused with depauperate forms of *L. vouauxii*. That species differs in having an aggregate thallus and in producing

oxypannaric acid-2-methylester as the major secondary metabolite. As has been discussed under *L. diffusa*, thalli of that species with orange coloration that has been interpreted as a type of pigment have been recognized as a distinct taxon, *L. diffusa* var. *chrysodetoides*. A similar phenomenon has been observed in *L. vouauxii*, however such populations have never been recognized formally. These populations appear to be restricted to the Ozark Ecoregion in North America.

Another species that is likely to be confused with *Lepraria vouauxii* is *L. finkii*. That species is ubiquitous in the habitats occupied by *L. vouauxii* and has a similar placodioid thallus. It differs in the production of atranorin, zeorin, and stictic acid.

Selected specimens examined (116 total seen from North America; "pigmented form" as indicated). – **CANADA. ALBERTA:** Whitehorse Creek, ~4 km S of Cadomin on Hwy 40, 1.viii.1995, on bryophytes, *W.R. Buck 27983* (NY). **NEWFOUNDLAND AND LABRADOR.** NEWFOUNDLAND: Island of Newfoundland, Avalon Peninsula, Cataracts Provincial Park, 9.ix.2007, on bryophytes, *W.R. Buck 52510* (NY). **ONTARIO.** FRONTENAC CO.: Hwy 7 – Salmon River, N of the Hamlet of Ardendale, 2.iii.2001, on rock, *D. Lewis 512* (NY). **U.S.A. ALABAMA.** MORGAN CO.: near Beaver Creek Swamp Wildlife Refuge, S of I-565 and N of Tennessee River, 2.i.2011, on tupelo, *E. Tripp 1304* (NY). WINSTON CO.: Bankhead National Forest, Sipsey Wilderness, along trail by Sipsey Fork, 25.ix.1992, on *Quercus*, *R.C. Harris 28414* (NY). **ALASKA:** Prince William Sound, Port Wells, 26.vii.1899, on humus, *W. Trelease & D. Saunders s.n.* (DUKE). **ARIZONA.** APACHE CO.: Apache National Forest, along Black River and FSR25, 5.vi.1998, on soil, *C. Bratt 10647* (NY, SBBG). **ARKANSAS.** GARLAND CO.: Ouachita National Forest, NE-facing slope along W side of North Fork Ouachita River,

~0.1 mi S of AR298, 6.x.2010, on soil in overhang, *J.C. Lendemer et al. 26102* (NY, pigmented form). MADISON CO.: Sweden Creek Falls Natural Area, W of CR9, 5.x.2010, on rock, in overhang, *J.C. Lendemer et al. 25955* (NY, pigmented form). NEWTON CO.: Boston Mountains, Ozark National Forest, Alum Cove Recreation Area, 24.iv.1988, on rock, *R.C. Harris 21473* (NY). PERRY CO.: Ouachita National Forest, vicinity of Goat Bluffs along north side of South Fourche LaFave River, 6.x.2010, on soil, in overhang, *J.C. Lendemer et al. 26025* (NY, pigmented form). **GEORGIA.** WHEELER CO.: along GA 46 at the Oconee River, 19.iii.1995, on rock, *R.C. Harris 36383* (NY). **ILLINOIS.** Shawnee National Forest, along W side of Cedar Lake, 6.xii.2004, on *Quercus*, *R.C. Harris 50456* (NY). **KANSAS.** CHAUTAUQUA CO.: 2.1 mi NE of Sedan, 4.iv.1954, on rock, *C.L. Kramer 301* (NY). JOHNSON CO.: Overland Park Arboretum and Botanical Garden, 1.5 mi N Aubry, 8.vi.2007, on rock, *C.A. Morse et al. 15306* (KANU). **KENTUCKY.** LETCHER CO.: Line Fork Caverns, edge of Kingdom Come State Park, 15.ix.1991, on rock, *R.C. Harris 27118* (NY). **MASSACHUSETTS.** BERKSHIRE CO.: Bartholomew's Cobble, Ashley Falls, 10.vi.1982, on rock, *R.C. Harris s.n.* (NY). FRANKLIN CO.: Town of Rowe, just off Tunnel Road, 3.8 mi NW of Zoar on River Rd., 19.ix.1998, on rock, *R.C. Harris 42267* (NY). **MISSOURI.** LAURENCE CO.: Fall Hollow Gorge along small tributary to Goose Creek, 27.iii.2006, on sandstone, *W.R. Buck 49831* (NY, pigmented form). MONTGOMERY CO.: Graham Cave State Park, 27.x.2001, on sandstone, *W.R. Buck 40609* (NY). **NEBRASKA.** SCOTTS BLUFF CO.: Wildcat Hills State Recreation Area, 10 mi S of Scottsbluff, 29.v.1983, on soil, *R.S. Egan 13473* (NY). **NEW YORK.** ULSTER CO.: Long Path, from Upper Cherrytown Road to Vernooy Kill Falls, 8.v.1993, on *Tsuga*, *R.C. Harris 30420* (NY). **NORTH CAROLINA.** CLAY CO.: Nantahala National Forest, Buck Creek Barren, 1–1.5 mi N of US 64 on Buck Creek Rd., 10.xi.2007, on

Quercus, *J.C. Lendemer et al. 10873* (NY). HAYWOOD CO.: Great Smoky Mountains National Park, E slopes above (E of) Caldwell Fork, 4.viii.2009, on *Acer*, *J.C. Lendemer 18816 & E. Tripp* (NY). SWAIN CO.: Great Smoky Mountains National Park, Beauregard Ridge, Noland Divide Trail 0–2 mi from parking area on Deep Creek Rd., 14.x.2010, on *Acer*, *J.C. Lendemer et al. 26899* (NY). YADKIN CO.: Lime Rock, 7.viii.1938, on rock, *P.O. Schallert 13439* (NY). **OKLAHOMA.** LATIMER CO.: Robber's Cave State Park, 20.vii.1962, on sandstone, *D. Keck 1684* (NY). **PENNSYLVANIA.** LUZERNE CO.: Ricketts Glen State Park, Adams Falls on Kitchen Creek, 19.ix.2010, on rock in overhang, *J.C. Lendemer 25737 & J. Kunsman* (NY). LYCOMING CO.: State Game Lands No. 133, E shore of Lycoming Creek, N-facing slopes of Batys Mountain, 12.v.2009, on moss in overhang, *J.C. Lendemer 16683 & D. Atha* (NY). SCHUYLKULL CO.: Weiser State Forest, E end of Manhantango Mountain, 20.v.2009, on *Quercus*, *R.C. Harris 55347* (NY). TIOGA CO.: Tioga State Forest, along Colton Rd./West Rim Rd. at jct w/ Spiningwheel [sic] Trail, 13.v.2009, on *Quercus*, *J.C. Lendemer 16880* (NY). **SOUTH DAKOTA.** FALL RIVER CO.: 9 mi N of Edgemont, 15.vii.1960, on rock, *C.M. Wetmore 7509* (MSC, NY). PENNINGTON CO.: Billover Creek (4 mi NW of Hermosa), 25.vi.1961, on rock, *C.M. Wetmore 10291* (MSC, NY). **TENNESSEE.** BLOUNT CO.: Great Smoky Mountains National Park, ~2 mi NE of Happy Valley, Abrams Creek, 30.vi.2010, on *Carpinus*, *R.C. Harris 56396* (NY). **UTAH.** UINTAH CO.: BLM (Vernal Office), Snake John Reed, N of US40, 1.viii.2009, on rock, *W.R. Buck 55225* (NY). **VIRGINIA.** SUFFOLK CO.: Dismal Swamp National Wildlife Refuge, Railroad Ditch ¾ mi E of entrance on Desert Rd., 12.xii.2009, on *Nyssa*, *J.C. Lendemer 20381 & B.P. Hodkinson* (NY). **WISCONSIN.** RICHLAND CO.: along Hwy I, 2 mi E of Hub City, 16.viii.1974, on sandstone, *M. Nee 13467* (NY). SAUK CO.: Baraboo Hills, Hemlock Draw Preserve, 4.ix.1998, on rock, *R.C. Harris*

42205 (NY). **WYOMING.** WESTON CO.: along US 85 at Soldier Creek (20 mi NNW of Newcastle), 12.viii.1961, on rock, *C.M. Wetmore 11238* (MSC, NY).

***Lepraria xerophila* Tønsberg,** Lichen Flora of the Greater Sonoran Desert Region 2: 328. 2004. TYPE: **MEXICO. BAJA CALIFORNIA:** along ridge interior from Punta Santo Tomás, 200 m, chaparral to desert transition, 30.xii.1995, on soil, *T.H. Nash 38224 = Lich. Exs. ASU #229* (ASU [n.v.], holotype; NY!, isotype).

Figures 115 and 116.

Description. – **Thallus** crustose, leprose, placodioid with crisped margins, continuous and distinctly bi-layered, initially beginning as singular isolated granules that open apically to form small discrete flat circular thalli which expand marginally, overlap, and become confluent to form a thick crust (0.3–1.0 mm thick), bone white or more commonly dirty brownish in color; **hyphae** hyaline or pigmented, 4–6 μm, septate, secund, obscured by a layer of POL+ crystals that do not dissolve in KOH; **prothallus** disappearing with age; **hypothallus** a variably developed layer of densely intertwined hyphae surrounding and subtending the granules, rarely extending outward from the edge of thallus; **rhizohyphae** absent; **granules** globose, 100–400 μm, initially ecorticate but soon developing a thick pseudocortex, well organized and discrete before becoming incorporated into the hypothallus, not forming compound units, sometimes separating from the hypothallus; **photobiont** green, coccoid, cells globose, 6–10 μm.

Chemistry. – Chemotype 1: atranorin, pannaric 6-methylester, roccellic/angardianic acid and/or rangiformic/jackinic acid; spot tests: K+ yellowish, C-, KC-, P+ yellowish, UV-. Chemotype 2: atranorin, norascomatic acid; spot tests: K+ yellowish, C-, KC-, P+ yellowish, UV-. Which chemotype corresponds to the type?

Ecology and distribution. – In North America *Lepraria xerophila* is restricted to soil communities in coastal habitats along the Pacific Ocean from southern California to southern Baja California in Mexico (figure 116).

Conservation status. – Based on the distribution map presented by Tønsberg (2004) this species appears to be common along the Pacific Coast in Baja California, Mexico. However, these collections are based on a sampling period of decades and it not possible to determine the current status of soil communities there. In the study area (i.e., the United States), the species is locally abundant in coastal soil communities. However, these communities have been heavily impacted by anthropogenic change and many of the endemic species in them have become rare (Knudsen 2009, 2010).

Discussion. – *Lepraria xerophila* belongs to a distinct group of morphologically defined species with *xerophila*-type placodioid thalli that occur on soil and organic matter in Mediterranean habitats throughout the world. No other members of this group occur in North America, and thus *L. xerophila* is not likely to be confused with any of its congeners in the study area with the exception of *L. vouauxii*. That species is chemically similar in the production of

pannaric acid 6-methylester, however, it differs in having a *finkii*-type placodioid thallus and in typically occurring in sheltered microhabitats rather than in sheltered soil communities.

Selected specimens examined (35 total seen from North America; chemotype 1). – **U.S.A. CALIFORNIA.** ORANGE CO.: Santa Ana Mountains, Weir Canyon, N/S of Windy Ridge Rd., 17.v.2006, on soil, *K. Knudsen 6111* (NY). SAN DIEGO CO.: Cabrillo National Monument, above 2nd parking lot, 13.i.1988, on soil, *C. Bray 5636 & B.D. Ryan* (NY). SANTA BARBARA CO.: Santa Rosa Island, Soledad Grove, SE of Main Rd., 19.x.2006, on soil, *K. Knudsen et al. 7778* (NY).

Specimen examined (chemotype 2). – **U.S.A. CALIFORNIA.** SANTA BARBARA CO.: Santa Rosa Island, lower Water Canyon, near road, 16.x.2006, on soil, *K. Knudsen 7624* (NY).

Excluded and doubtful taxa

This section outlines the disposition of the species that either do not belong to *Lepraria* s. str., or do not occur in North America. It will be noticed that some species excluded from *Lepraria* s. str. here are, nonetheless, retained in the genus. This is due to the fact that molecular data are needed to determine, or confirm, their higher level taxonomic placement (Hodkinson & Lendemer in rev.; Lendemer & Hodkinson in rev.). In the absence of well-supported results from molecular phylogenetic analyses of these data, I strongly advocate the retention of these species within *Lepraria* with the recognition that they belong to the genus in its historical (i.e., non-monophyletic) sense. The continued existence of a two-tiered taxonomy within *Lepraria* is clearly not ideal from the standpoint of evolutionary biology. It is nonetheless an efficient

pragmatic solution that allows the continued description of leprarioid species so that they can enter the taxonomic dialogue and be evaluated by other workers.

Lepraria adhaerens K. Knudsen, Lendemer & Elix

As per Lendemer & Hodkinson (in rev.) this species should be treated as *Leprocaulon adhaerens* (K. Knudsen, Lendemer & Elix) Lendemer & Hodkinson.

Lepraria barbatica Lendemer

This is a rare species with a leprarioid crustose thallus that clearly does not belong to *Lepraria* s. str. based on its production of usnic acid and barbatic its. Its true phylogenetic affinities remain unresolved at present. *Lepraria barbatica* was known only from the type specimen, however a second population was found during fieldwork in the Ozark Ecoregion, it is cited below.

Specimens examined. – **U.S.A. ARKANSAS.** CLEBURNE CO.: N portion of North Unit of Big Creek Natural Area, ~1.5 mi due E of Wilburn, 9.x.2010, on rock in overhang, *J.C. Lendemer 26372 & D. Ladd* (NY).

Lepraria crassissima (Hue) Lettau

As has been discussed in detail by Lendemer (2011a) this species is not present in North America.

Lepraria crassissima var. *isidiata* Llimona

This taxon is now recognized as a species, *L. isidiata* (Llimona) Llimona & A. Crespo, and occurs in Mediterranean regions of Europe. It belongs to the distinctive group of species with *xerophila*-type placodioid thalli that occur in such habitats. The species does not occur in North America and all previous reports represent identifications of other species (e.g., *L. nivalis*, *L. normandinoides*, or *L. oxybapha*).

Lepraria disjuncta Lendemer

This is an infrequent species that is widespread on calcareous rocks throughout eastern North America. Based on the production of usnic acid, zeorin, and xanthones in this species it clearly does not belong to *Lepraria* s. str. Its true phylogenetic position is uncertain at this time.

Lepraria incana (L.) Ach.

As has been discussed in detail by Lendemer (2011a) this species is not present in North America.

Lepraria lobificans Nyl.

The status of this name has been resolved in the section for *Lepraria finkii* above. The species is not present in North America.

Lepraria moroziana Lendemer

This is a rare species endemic to the spruce-fir forests of the Appalachian Mountains of eastern North America. It is not infrequent at high elevations of the southern Appalachian Mountains and occurs as far north as New Brunswick, Canada. Based on the production of

isousnic acid and obtusatic acid in this species it clearly is not a member of *Lepraria* s. str. The true phylogenetic position of this taxon is uncertain at this time.

Lepraria santamonicae K. Knudsen & Elix

As per Lendemer & Hodkinson (in rev.) this species should be treated as *Leprocaulon santamonicae* (K. Knudsen & Elix) Lendemer & Hodkinson.

Lepraria terricola Lendemer

As per Lendemer & Hodkinson (in rev.) this species should be treated as *Leprocaulon terricola* (Lendemer) Lendemer & Hodkinson.

Lepraria texta K. Knudsen, Elix & Lendemer

As per Lendemer & Hodkinson (in rev.) this species should be treated as *Leprocaulon texta* (K. Knudsen, Elix & Lendemer) Lendemer & Hodkinson.

Lepraria xanthonica Lendemer

This is an infrequent species that is widely distributed in eastern North America. It is characterized by the presence of usnic acid, zeorin, and 5,7-dichloro-3-*O*-methylnorlichexanthone and thus clearly does not belong to *Lepraria* s.str. The true phylogenetic affinities of this taxon remain unresolved at the present time.

Appendix A – Summary of chemical profiles of the North American species of *Lepraria* s.l.

		argopsin	atranorin	isousnic acid	pannarin	usnic acid	zeorin	alectorialic acid	baeomycesic acid	barbatic acid	divaricatic acid	fumarprotocetraric acid	lecanoric acid	nordivarricatic acid	norstictic acid	obtusatic acid	protocetraric acid	psoromic acid	salazinic acid	squamatic acid	stictic acid	thamnolic acid	nephrosteranic acid	pallidic acid	rangiformic/jackinic acid	roccellic/angardianic acid	norascomatic acid	oxypannaric acid-2-methylester	pannaric acid	pannaric acid-6-methylester	porphyrilic acid	arthothelin	thiophanninic acid
aurescens																						x											
caesiella			x				x																	x									
cryophila											x			x																			
diffusa																												x	tr.				
eburnea								x									tr.																
elobata	typical		x				x														x												
	"salazinica"		x				tr.												x							x							
finkii			x				x														x												
friabilis			(+/-)									x					tr.																
harrisiana			x				x																	x									
hodkinsoniana							x				x																						
humida			x																						x								
jackii			x																						x	x							
lanata																	x									x							
lecanorica			x										x																				
leprolomopsis			x																		x												
membranacea			(+/-)																							x			x				
neglecta	typical		tr.					x																		(+ / -)							
	"alpin		x																							(+					x		

	a"																								/-)								
	"boreal is"		x																					x								
	"caesioalba"		x									x												(+/-)	(+/-)							
	"gelida"							x																						x			
	"svalbardensis"		x																		x				(+/-)	?							
	unnamed		x														x								(+/-)	?							
	unnamed		x												x										(+/-)	?							
	unnamed		x																							x							
nivalis			(+/-)									?					x																
normandinoides			x																							x							
oxybapha			x									x														x							
pacifica							x				x			tr.																			
rigidula			x																				x										
squamatica								x								x			x														
torii											x															x							
vouauxii			(+/-)																						(+/-)	(+/-)				x			
xerophila	typical																								(+/-)	(+/-)				x			
	unnamed																								(+/-)	(+/-)	x						

Leprocaulon adhaerens					x		x																								
Lepraria barbatica						x				x																					
Lepraria disjuncta						x	x																							x	x
Leprocaulon knudsenii						x	x																								
Lepraria moroziana				x			(+/-)								x																
Leprocaulon santamonicae		x																													
Leprocaulon terricola					x		x																								
Leprocaulon texta						x	x																								

Table 11. Diagnostic secondary compounds present in the North American species of *Lepraria* s.l. with crustose thalli. Mustard colored cells with an "x" denote presence of a major substance. Brick colored cells with a "tr." denote substances consistently present as a trace or minor accessory. Light pink cells with a "+/-" denote substances that not consistently present. Question marks ("?") denote substances that may be present but were not detected in North American specimens examined.

Appendix B – Tabular summary of the thallus types present in each species of *Lepraria* s. str. treated here.

		primary thallus type	thallus subtype
Lepraria s. str.			
	aurescens	placodioid	cryophila-type
	caesiella	aggregate	caesiella-type
	cryophila	placodioid	cryophila-type
	diffusa	aggregate	caesiella-type
	eburnea	placodioid	finkii-type
	elobata	aggregate	caesiella-type
	finkii	placodioid	finkii-type
	friabilis	aggregate	caesiella-type
	harrisiana	placodioid	finkii-type
	hodkinsoniana	aggregate	caesiella-type
	humida	aggregate	caesiella-type
	jackii	aggregate	caesiella-type
	lanata	aggregate	lanta-type
	lecanorica	aggregate	caesioalba-type
	leprolomopsis	placodioid	finkii-type
	membranacea	placodioid	finkii-type
	neglecta	aggregate	caesioalba-type
	nivalis	placodioid	finkii-type
	normandinoides	placodioid	normandinoides-type
	oxybapha	placodioid	normandinoides-type
	pacifica	aggregate	caesiella-type
	rigidula	aggregate	caesiella-type
	squamatica	placodioid	cryophila-type
	torii	aggregate	caesiella-type
	vouauxii	placodioid	finkii-type
	xerophila	placodioid	xerophila-type

Table 12. Listing of thallus types of the North American species of *Lepraria* s. str. following the descriptive scheme outlined by Lendemer (2011).

Appendix C – Summary of measurements of morphological structures

	granules	hyphae	photobiont
aurescens	[26]–(36)–45–(54) –[72] µm (n=126)	[1.6]–(2.3)–2.8–(3.4)–[4.8] µm (n=64)	[6.0]–(7.5)–9.2–(10.8)–[14.6] µm (n=135)
caesiella	[24]-(35)-47-(59)-[76] µm (n=96)	[1.8]-(2.4)-3.1-(3.7)-[5.0] µm (n=42)	[6.0]-(7.9)-9.8-(11.8)-[15.6] µm (n=96)
cryophila	[18]-(24)-30-(37)-[52] µm (n=120)	[1.4]-(2.2)-2.8-(3.4)-[5.0] µm (n=120)	[5.0]-(6.3)-8.0-(9.6)-[12.5] µm (n=120)
diffusa	[26]-(40)-62-(84)-[132] µm (n=32)	[1.4]-(1.9)-2.4-(2.9)-[3.3] µm (n=32)	[6.0]-(7.2)-9.2-(11.1)-[13.2] µm (n=32)
eburnea	[22]-(33)-51-(68)-[114] µm (n=97)	[1.6]-(2.3)-2.9-(3.6)-[4.5] µm (n=100)	[5.4]-(7.4)-9.3-(11.4)-[14.4] µm (n=97)
elobata	[18]-(27)-35-(42)-[62] µm (n=155)	[1.4]-(2.3)-2.9-(3.6)-[4.8] µm (n=96)	[4.8]-(7.0)-9.0-(11.0)-[16.8] µm (n=96)
finkii	[24]-(33)-48-(63)-[104] µm (n=166)	[2.4]-(2.6)-3.1-(3.6)-[4.8] µm (n=107)	[4.8]-(6.8)-9.1-(11.3)-[15.6] µm (n=97)
friabilis	[24]-(32)-42-(52)-[66] µm (n=128)	[1.5]-(2.2)-2.8-(3.4)-[4.3] µm (n=96)	[4.8]-(6.9)-8.9-(11.0)-[15.6] µm (n=90)
harrisiana	[19]–(27)–38–(48) –[72] µm (n=124)	[1.2]–(2.2)–2.9–(3.5)–[4.8] µm (n=68)	[6.0]–(7.8)–9.6–(11.4)–[14.4] µm (n=97)
hodkinsoniana	[15]-(25)-33-41-[60] µm (n=180)	[1.7]-(2.3)-3.0-(3.8)-[7.0] µm (n=180)	[5.0]-(6.8)-8.8-(10.9)-[16.0] µm (n=180)
humida	[18]-(27)-36-(46)-[72] µm (n=93)	[1.4]-(1.9)-2.6-(3.3)-[3.7] µm (n=43)	[5.7]-(6.9)-8.8-(10.7)-[13.8] µm (n=103)
jackii	[14]-(23)-30-(38)-[54] µm (n=125)	[1.4]-(2.3)-2.9-(3.4)-[4.2] µm (n=96)	[4.8]-(6.7)-8.9-(11.1)-[14.4] µm (n=101)
lanata	[28]-(40)-57-(74)-[102] µm (n=32)	[2.6]-(3.7)-5.7-(7.4)-[9.6] µm (n=32)	[6.0]-(7.4)-8.6-(9.8)-[11.0] µm (n=32)
lecanorica	[42]-(51)-81-(111)-[156] µm (n=32)	[1.5]-(2.1)-2.6-(3.1)-[3.6] µm (n=32)	[6.0]-(7.3)-10.0-(12.7)-[15.9] µm (n=32)
leprolomopsis	(25)-30-40-(45) µm	(3.0)-3.5-4.0 µm	8.0-13.0 µm
membranacea	[24]-(27)-41-(54)-[90] µm (n=90)	[1.4]-(2.0)-3.2-(3.6)-[4.8] µm (n=97)	[6.0]-(7.1)-9.4-(11.8)-[15.6] µm (n=96)
neglecta	[30]-(42)-59-(77)-[102] µm (n=131)	[1.8]-(2.3)-2.9-(3.4)-[4.8] µm (n=96)	[4.8]-(7.2)-9.4-(11.7)-[15.6] µm (n=96)
nivalis	[19]-(29)-43-(58)-[74] µm (n=65)	[1.4]-(1.9)-2.4-(3.0)-[4.2] µm (n=65)	[6.0]-(6.8)-8.7-(10.6)-[13.2] µm (n=65)
normandinoides	[19]-(24)-38-(53)-[84] µm (n=52)	[1.8]-(2.3)-2.9-(3.7)-[4.8] µm (n=58)	[6.0]-(7.9)-9.8-(11.6)-[13.2] µm (n=72)
oxybapha	[24]-(35)-48-(61)-[80] µm (n=45)	[2.1]-(2.6)-3.3-(4.0)-[4.8] µm (n=39)	[5.0]-(7.7)-9.6-(11.5)-[13.2] µm (n=39)
pacifica	[16]-(24)-32-(40)-[60] µm (n=120)	[1.7]-(2.2)-2.8-(3.3)-[4.0] µm (n=120)	[5.0]-(6.1)-7.5-(8.9)-[13.0] µm (n=120)
rigidula	[24]-(30)-43-(55)-[78] µm (n=96)	[2.1]-(2.5)-3.1-(3.6)-[5.2] µm (n=64)	[6.0]-(7.7)-9.7-(11.8)-[13.3] µm (n=64)
squamatica	[22]–(29)–42–(54) –[80] µm (n=132)	[1.5]–(2.2)–2.7–(3.2)–[4.0] µm (n=66)	[5.6]–(6.9)–8.8–(10.7)–[14.4] µm (n=66)
torii	[19]-(28)-37-(46)-[62] µm (n=133)	[1.8]-(2.2)-2.7-(3.1)-[4.5] µm (n=64)	[5]-(6.2)-8.1-(9.9)-[13.2] µm (n=64)
vouauxii	[24]-(32)-47-(61)-[90] µm (n=97)	[1.4]-(2.1)-2.7-(3.3)-[4.2] µm (n=97)	[4.8]-(6.3)-8.9-(11.5)-[18] µm (n=97)
xerophila	100-400 µm	4-6 µm	6-10 µm

Table 13. Tabular comparison of granule size, hyphal width, and photobiont cell diameter in the species of *Lepraria* s. str. treated here. (All data are excerpted from the descriptions below).

[IN A PUBLICATION FIGURES 117–119 WOULD BE INSERTED HERE]

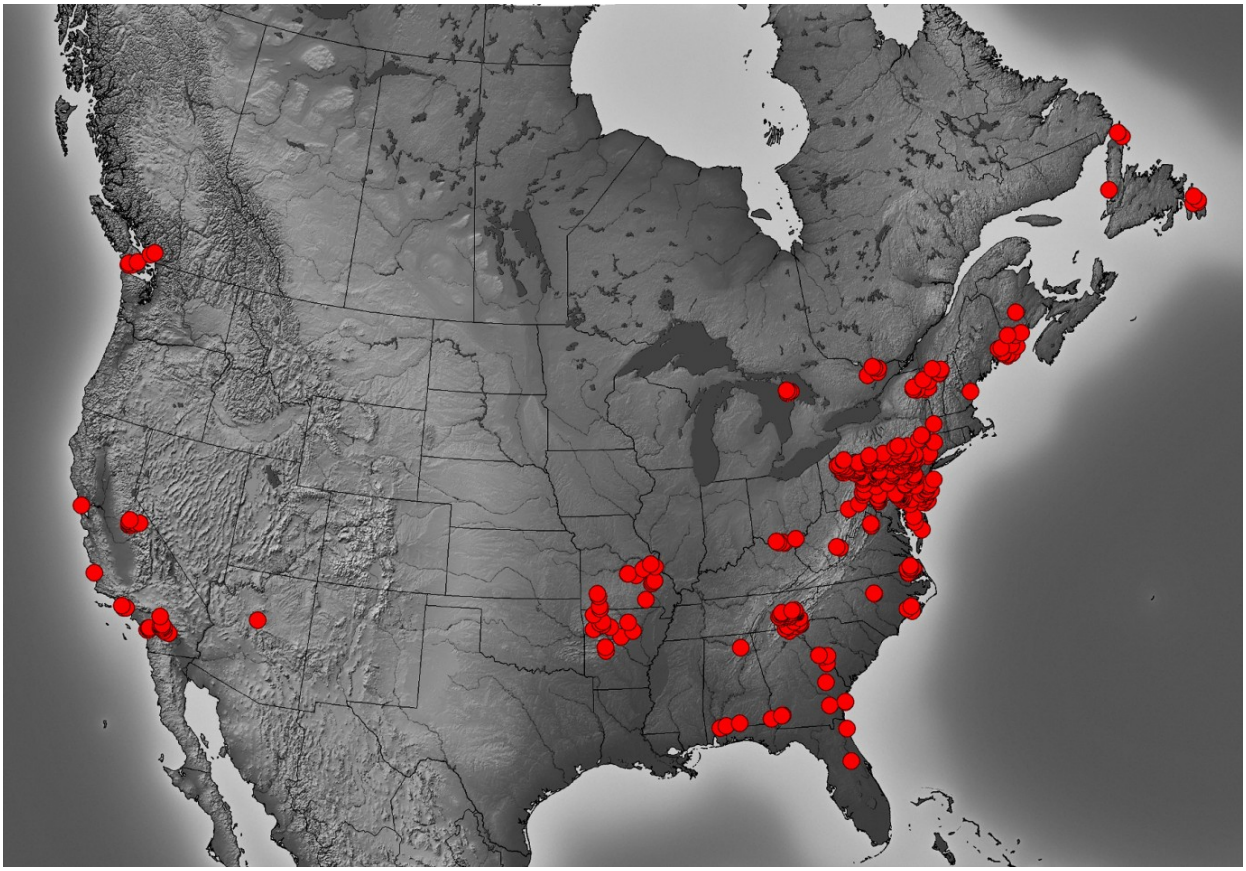

Figure 57, North American collection localities visited by the author as part of this study (note localities from the Yukon Territory of Canada are not shown here).

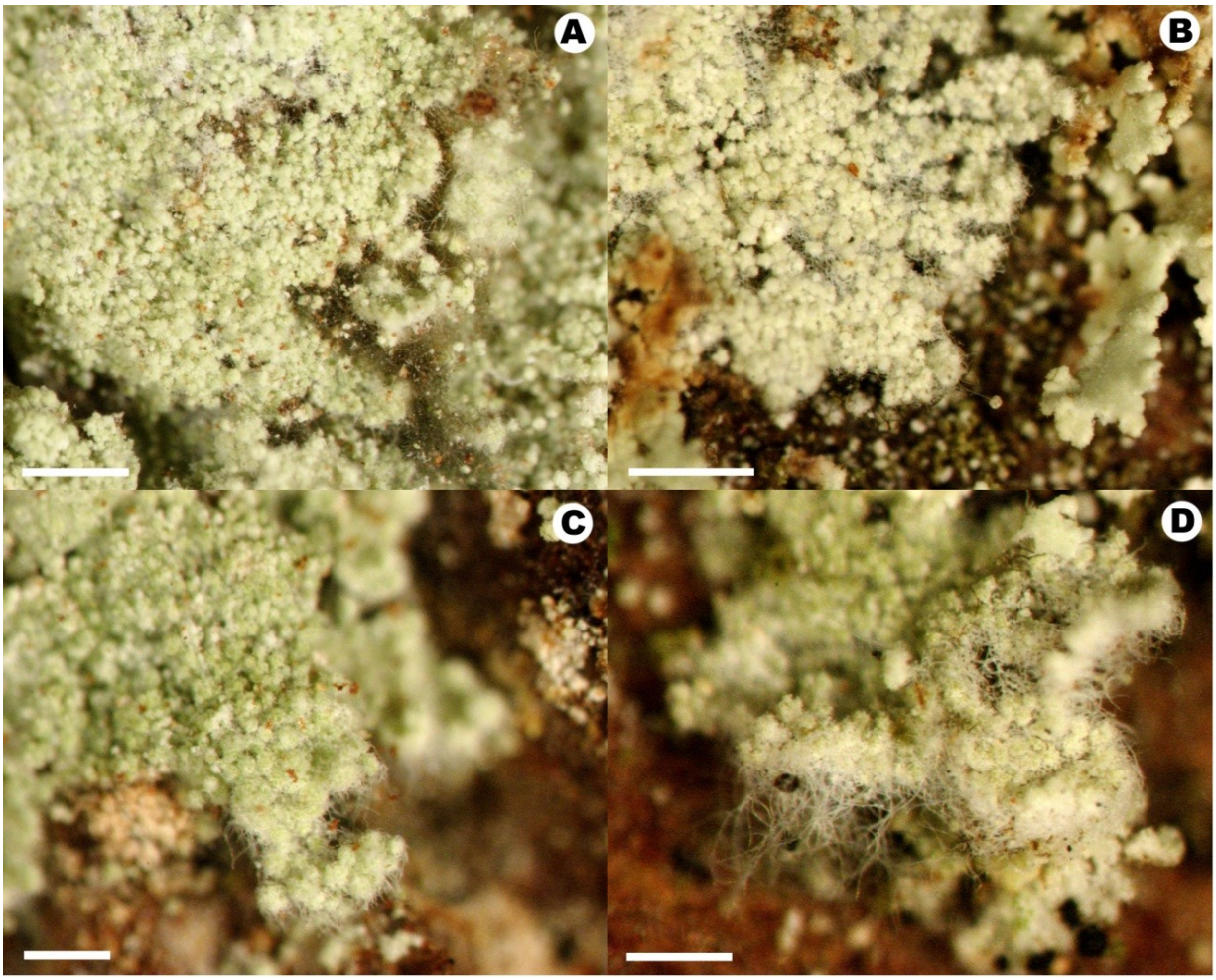

Figure 58, *Lepraria aurescens*. A, thallus surface, and edge with dark rhizohyphae (*Lendemer 15843A*, scale = 0.5 mm). B, thallus surface and edge with pale rhizohyphae (*Harris 39672*, scale = 0.5 mm). C, detail of thallus surface and edge (*Lendemer 15843A*, scale = 0.25 mm). D, detail of thallus with upturned margins and pale rhizohyphae (*Harris 39672*, scale = 0.25 mm).

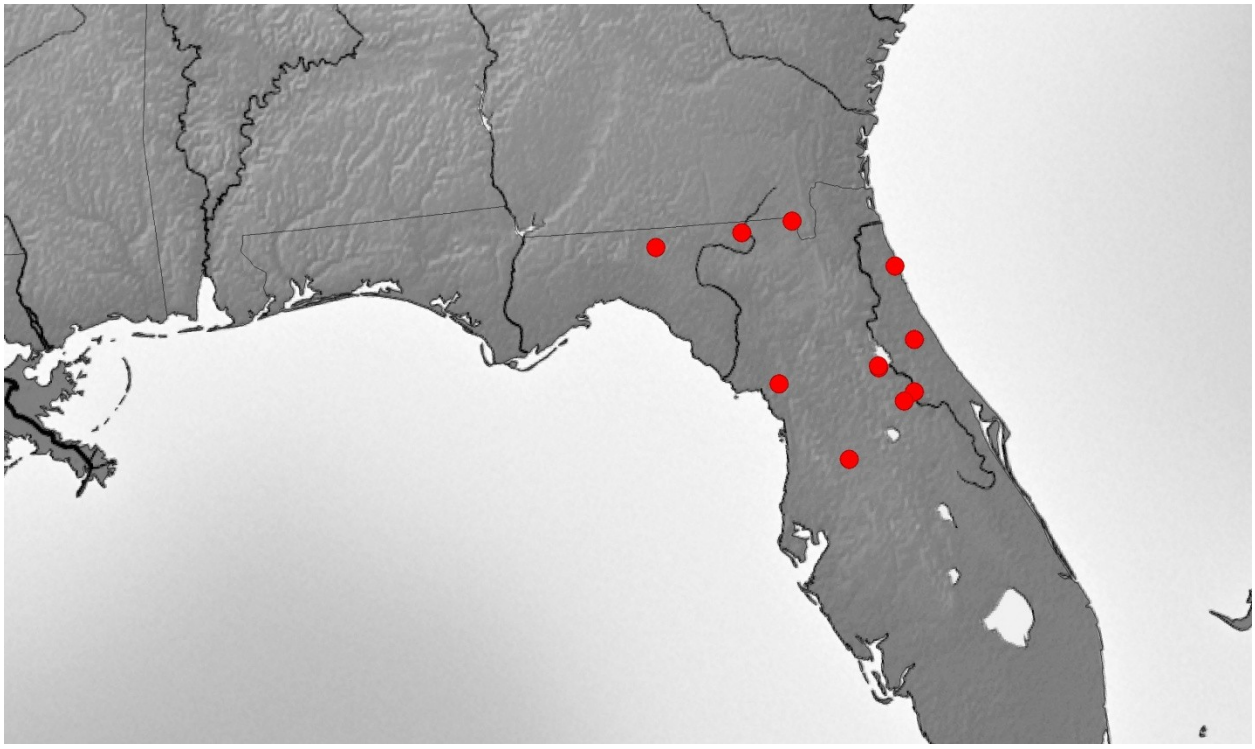

Figure 59, geographic distribution of *Lepraria aurescens* based on the specimens examined for this study.

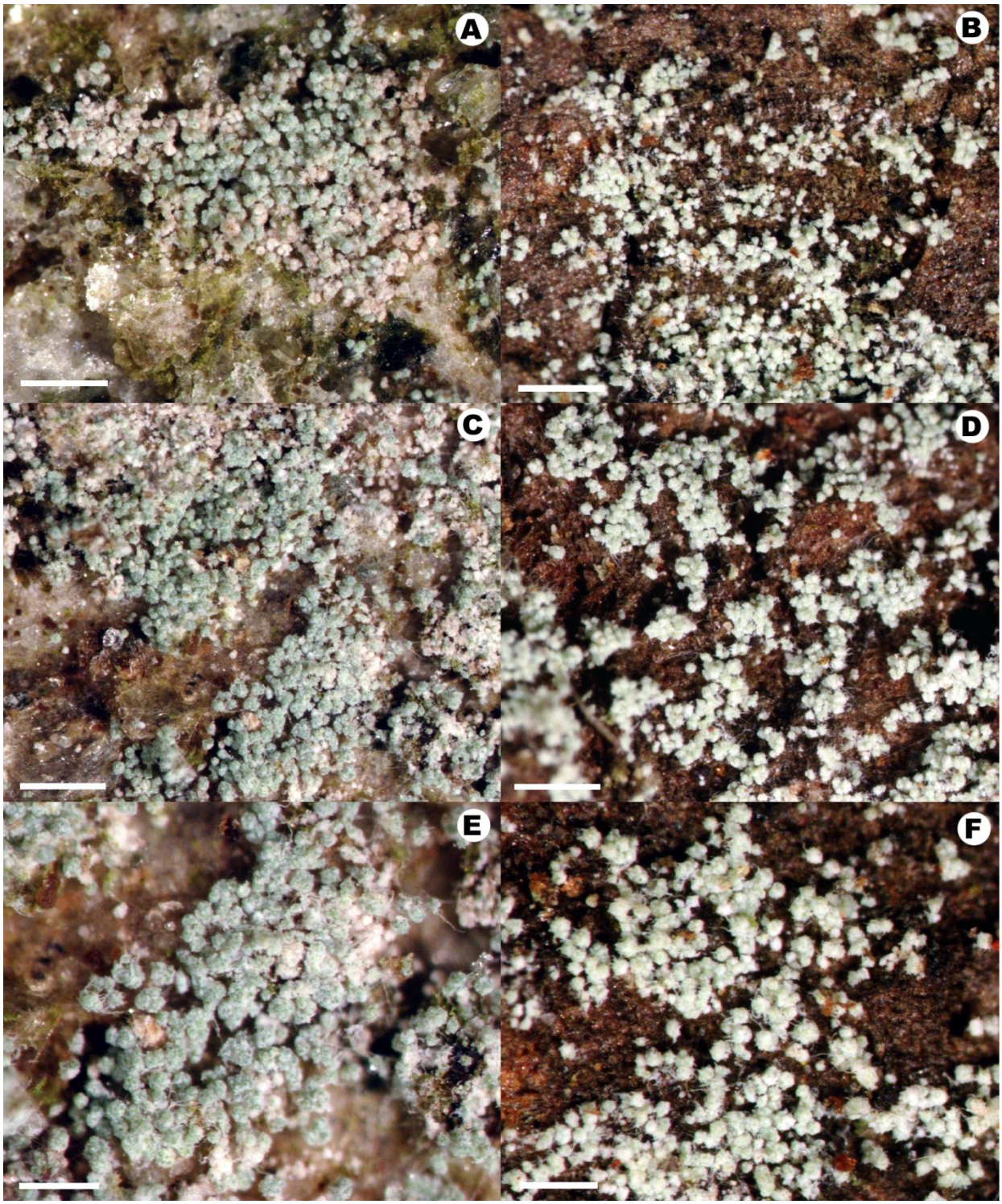

Figure 60, *Lepraria caesiella*, comparison of saxicolous population (all from *Lendemer 27454*) and corticolous population (all from *Lendemer 26684*). A–D, gross morphology of the thallus (scales = 0.5 mm). E–F, detail of the granules (scales = 0.25 mm).

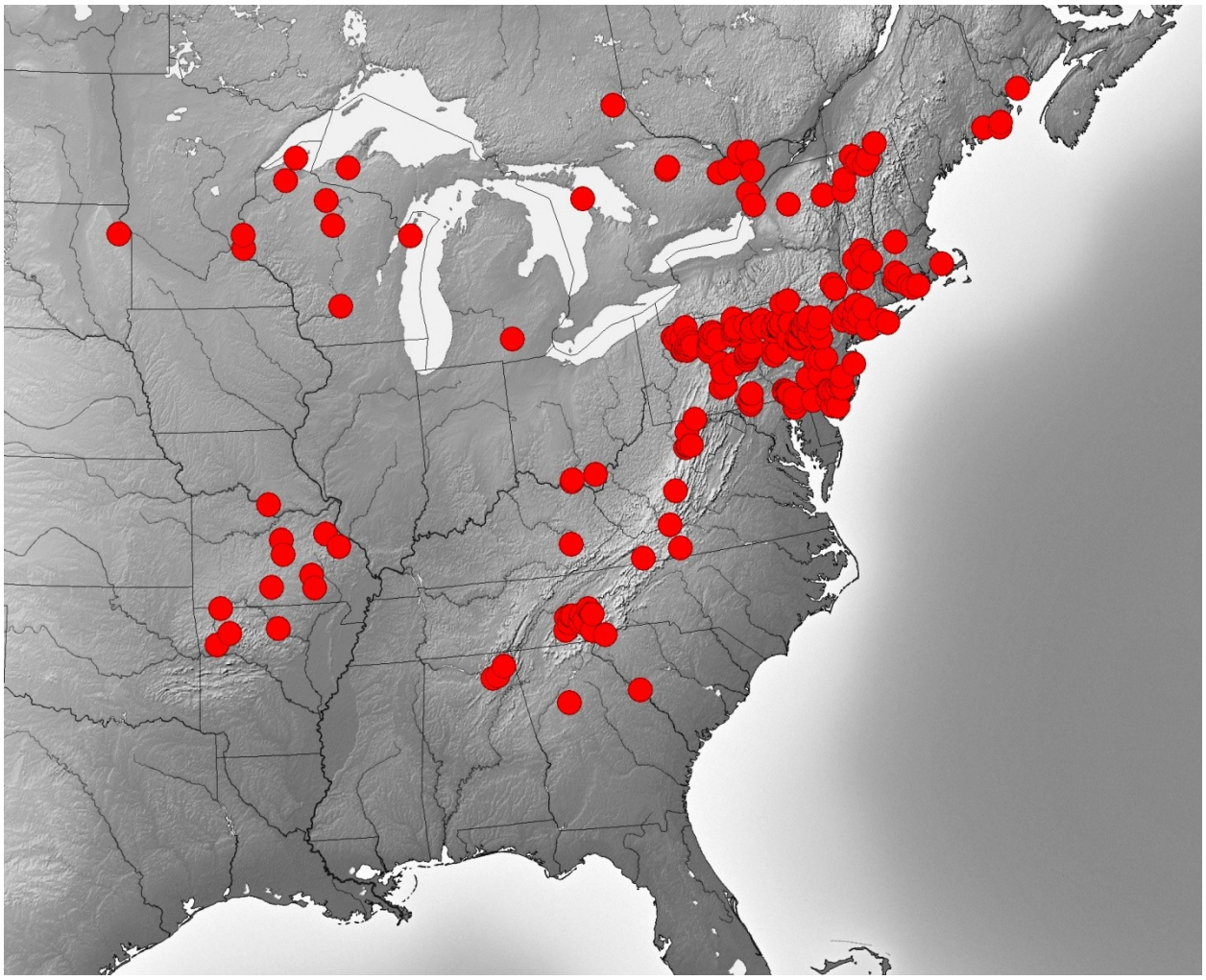

Figure 61, geographic distribution of *Lepraria caesiella* based on the specimens examined for this study.

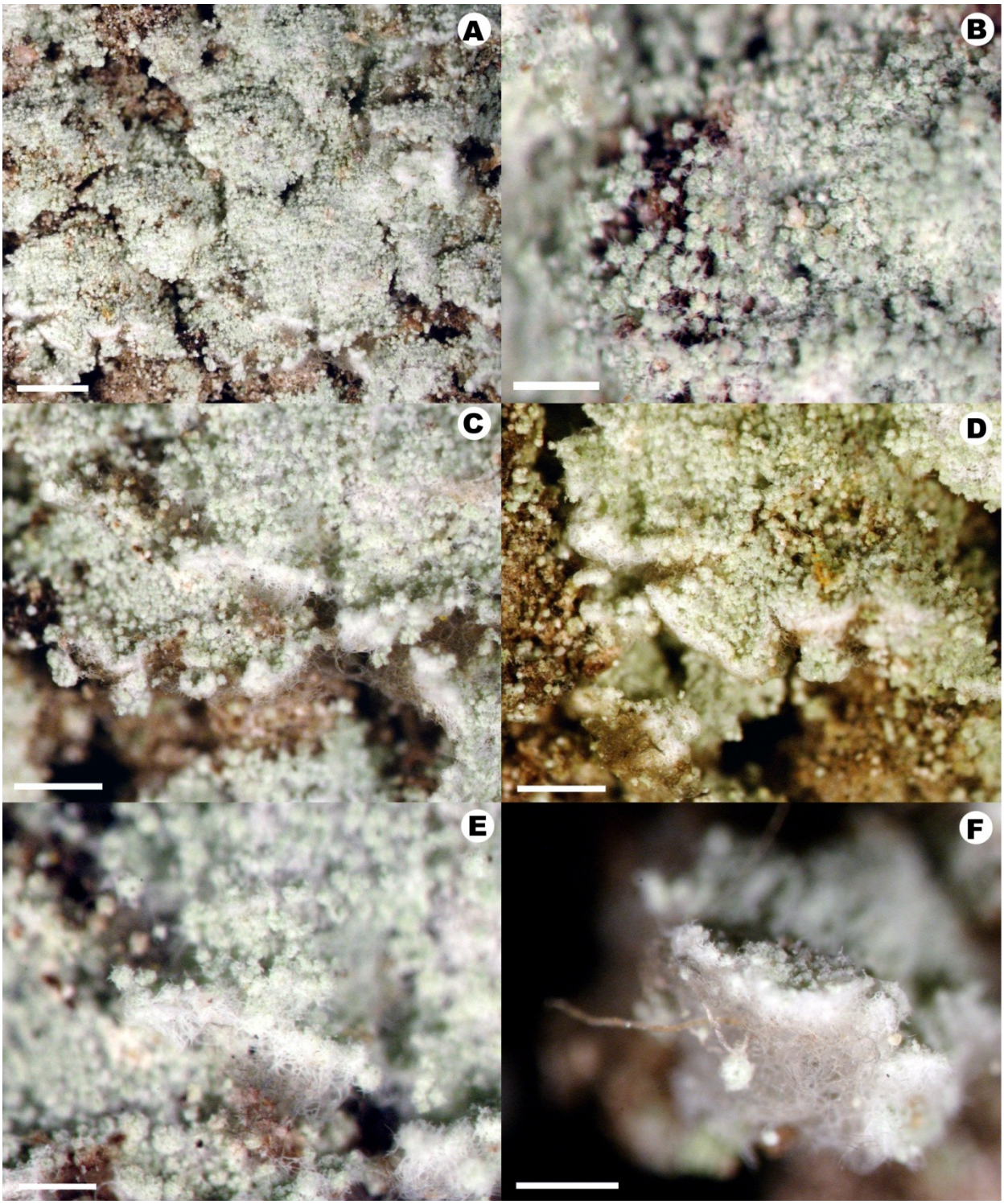

Figure 62, typical population of *Lepraria cryophila* from the Appalachian Mountains (all from *Lendemer 22721*). A, gross morphology of the thallus (scale = 1.0 mm). B, detail of the granules and thallus surface (scale = 0.25 mm). C–D, ascending thallus margins (scale = 0.5 mm). E–F, detail of ascending thallus margins, note the presence of pale rhizohyphae that begin to turn dirty brownish towards the tips (scales = 0.5 mm).

Figure 63, typical population of *Lepraria cryophila* from the Ozark Ecoregion (all from *Lendemer 25898*). A, gross morphology of thallus (scale = 0.5 mm). B, detail of the thallus surface (scale = 0.25 mm). C, detail of thallus margin (scale = 0.25 mm). D, aspect of ascending thallus margins (scale = 0.5 mm). E, young thallus with ascending margins (scale = 0.5 mm). F, detail of thallus margin, not the poorly developed pale rhizohyphae (scale = 0.25 mm).

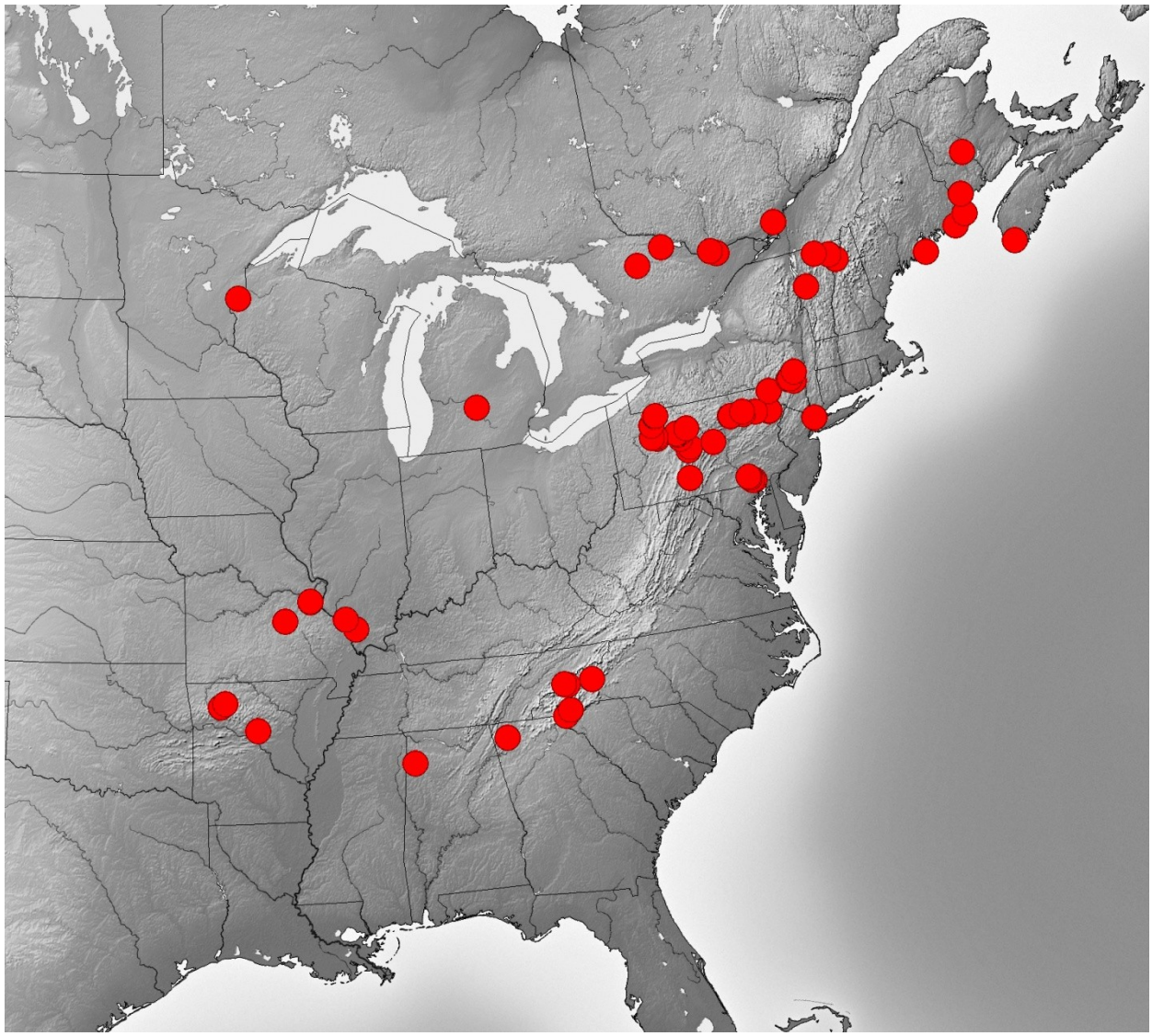

Figure 64, geographic distribution of *Lepraria cryophila* based on the specimens examined for this study.

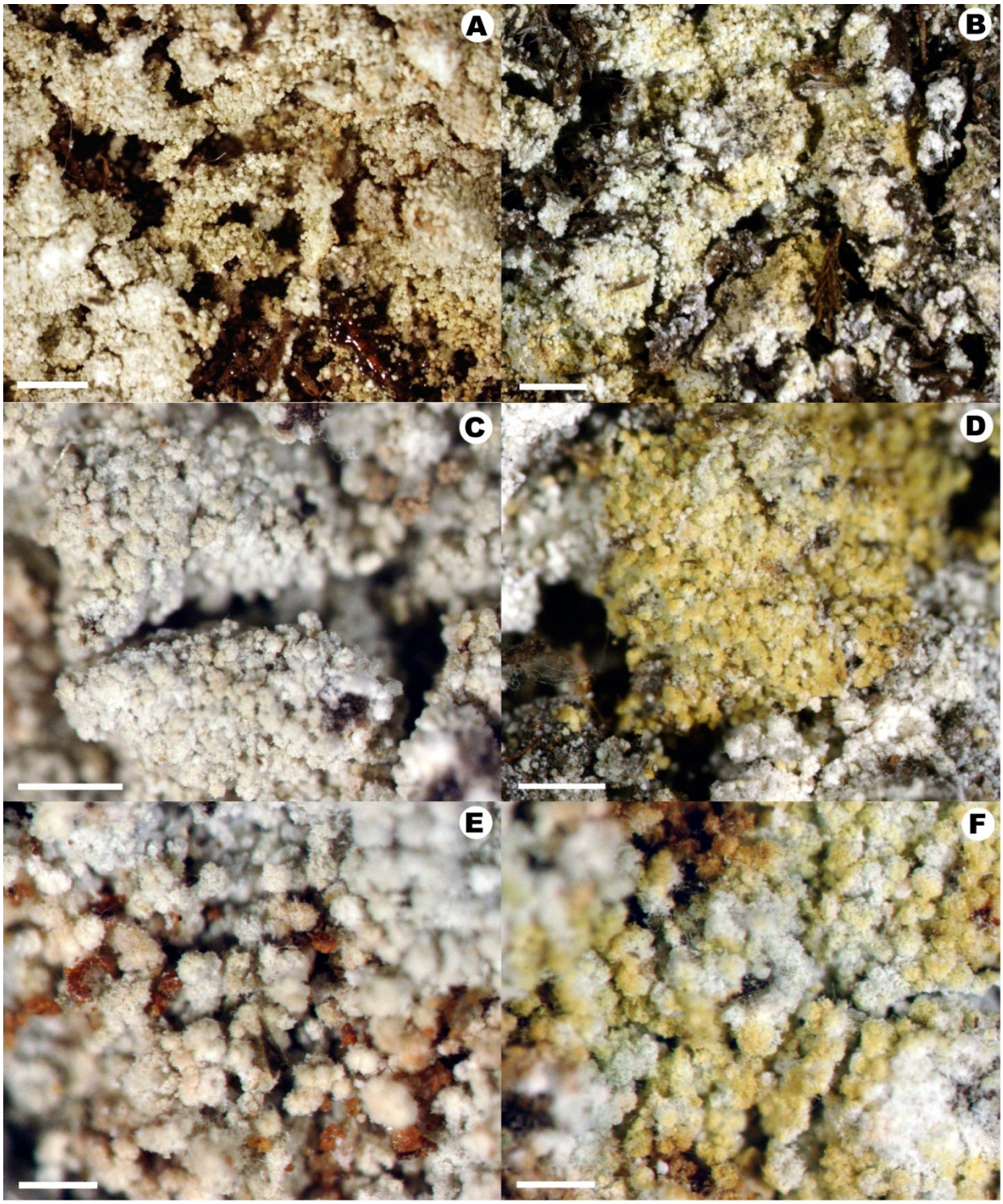

Figure 65, comparison of type specimens of *Lepraria diffusa* (left, from holotype) and *L. diffusa* var. *chrysodetoides* (right, from isotype in BM). A–B, gross morphology of the thallus (scales = 1.0 mm). C–D, thallus surface (scales = 0.5 mm). E–F, detail of the granules (scales = 0.25 mm).

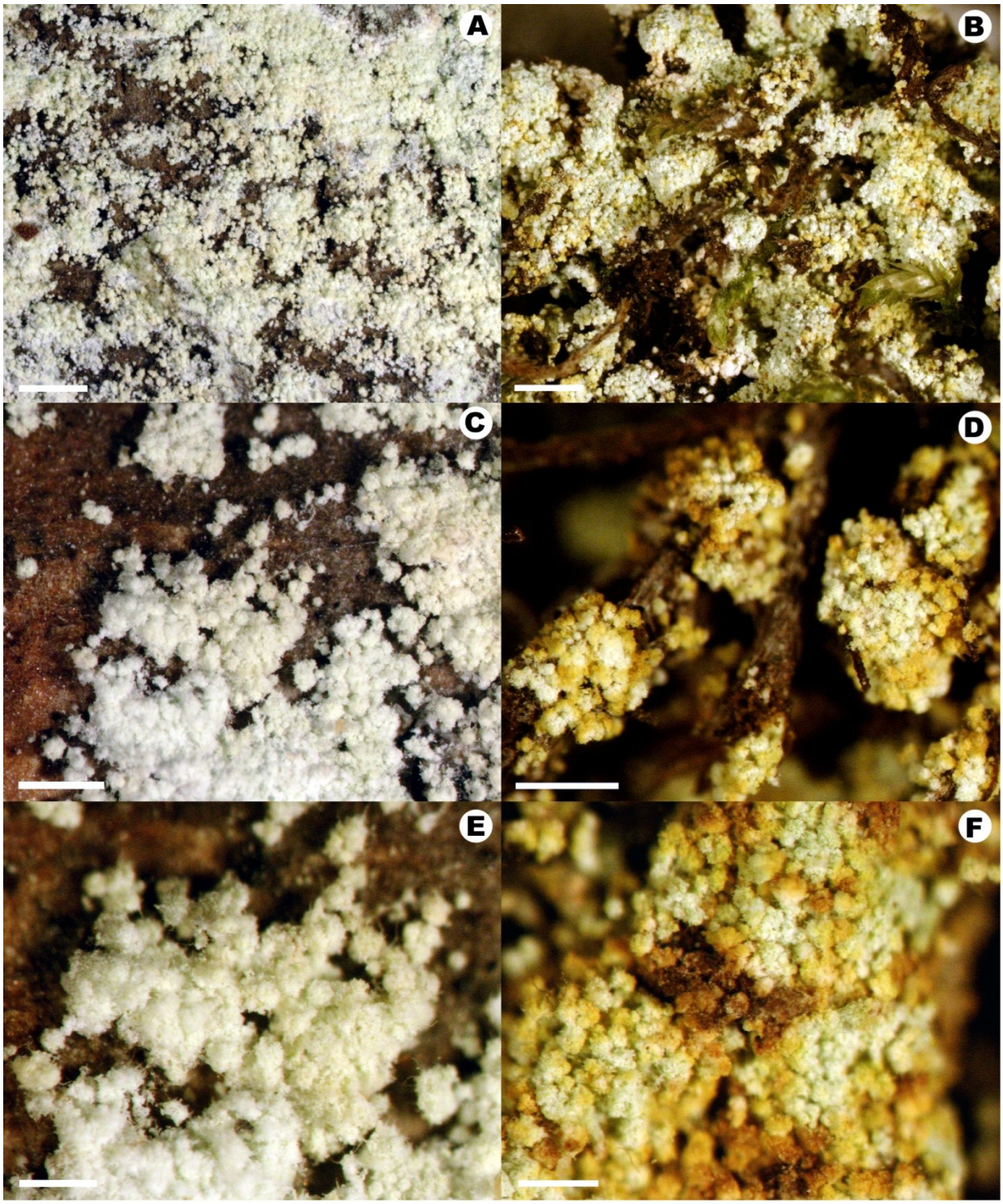

Figure 66, comparison of North American populations of *Lepraria diffusa* (left, from *Lendemer 22344*) and *L. diffusa* "var. *chrysodetoides*" (right, from *McCune 30488*). A–B, gross morphology of the thallus (scales = 1.0 mm). C–D, thallus surface (scales = 0.5 mm). E–F, detail of the granules (scales = 0.25 mm).

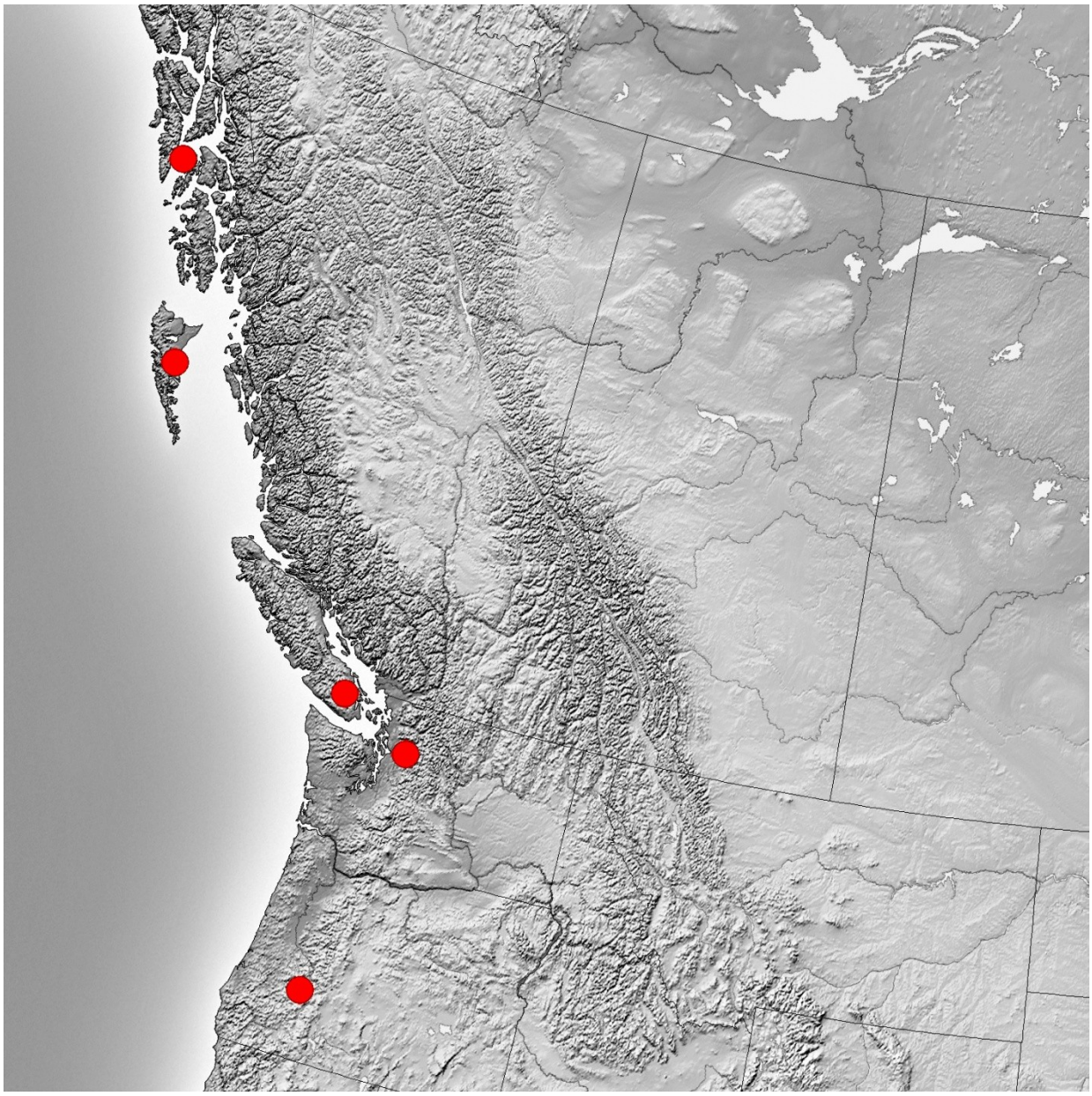

Figure 67, geographic distribution of *Lepraria diffusa* based on the specimens examined for this study.

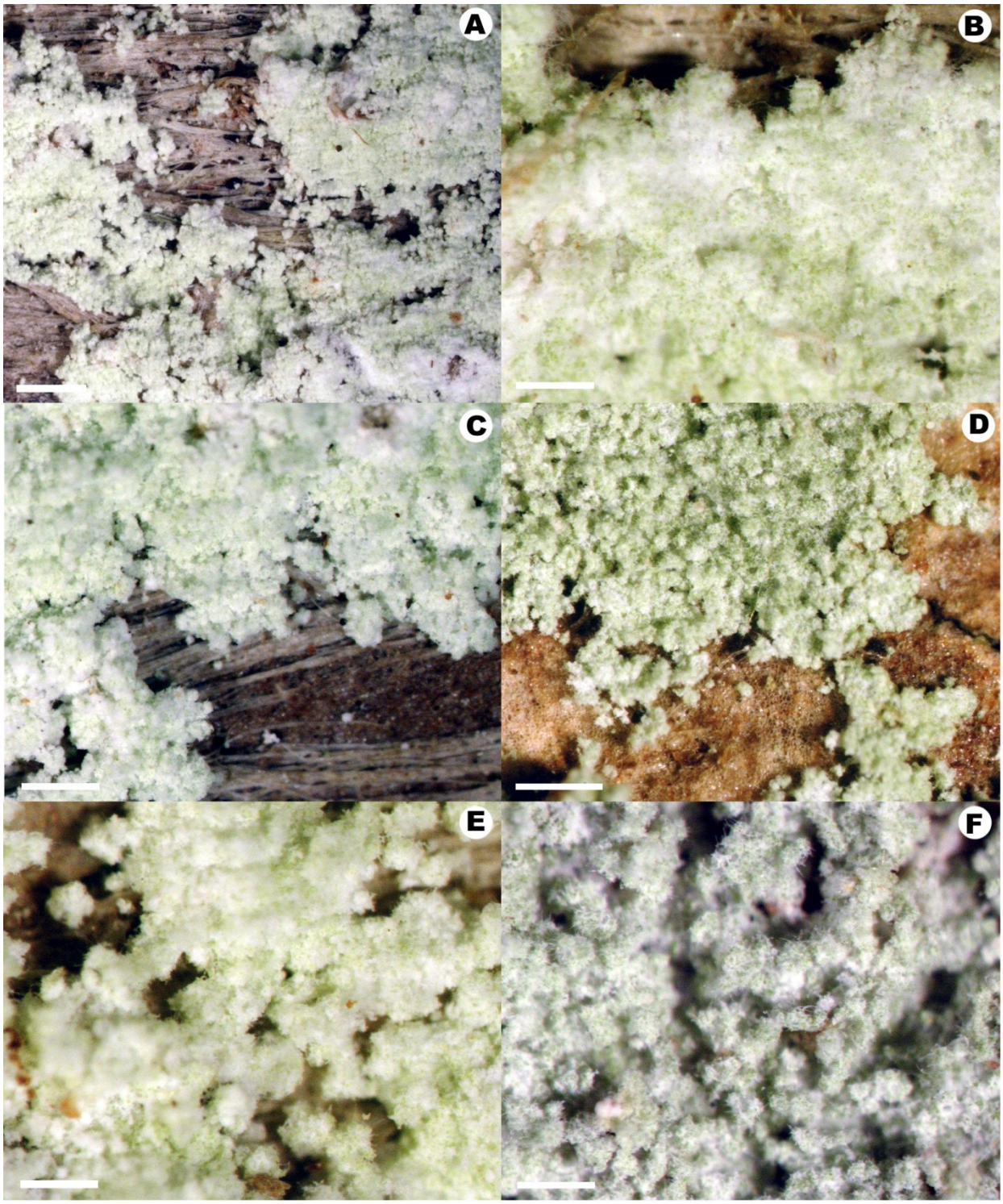

Figure 68, *Lepraria eburnea* (A–C and E from *Lendemer 22734*; D and F from *Lendemer 23046*). A, gross morphology of the thallus (scale = 1.0 mm). B, detail of the thallus margin (scale = 0.25 mm). C–D, aspect of the thallus margin (scales = 0.5 mm). E–F, detail of the granules (scales = 0.25 mm).

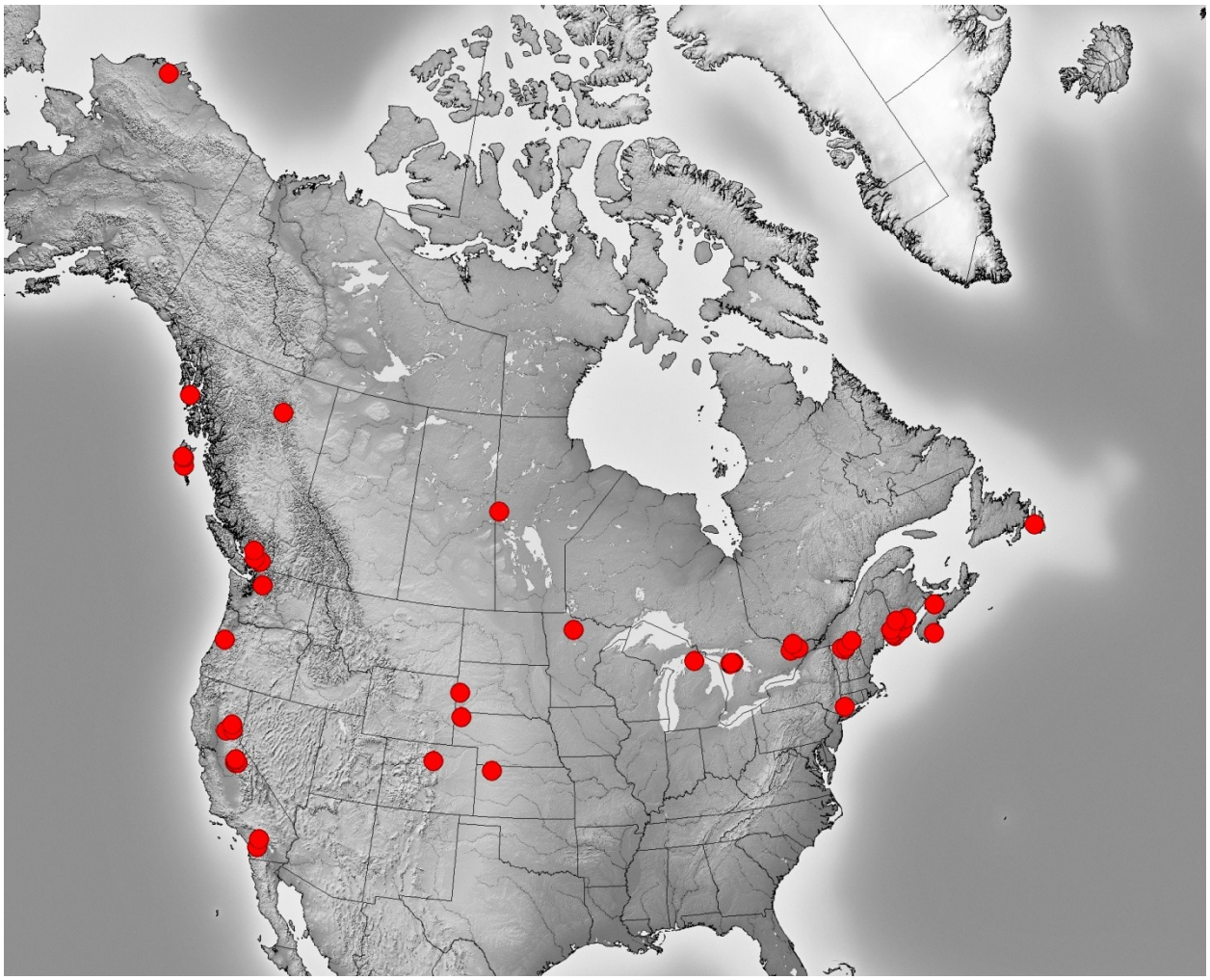

Figure 69, geographic distribution of *Lepraria eburnea* based on the specimens examined for this study.

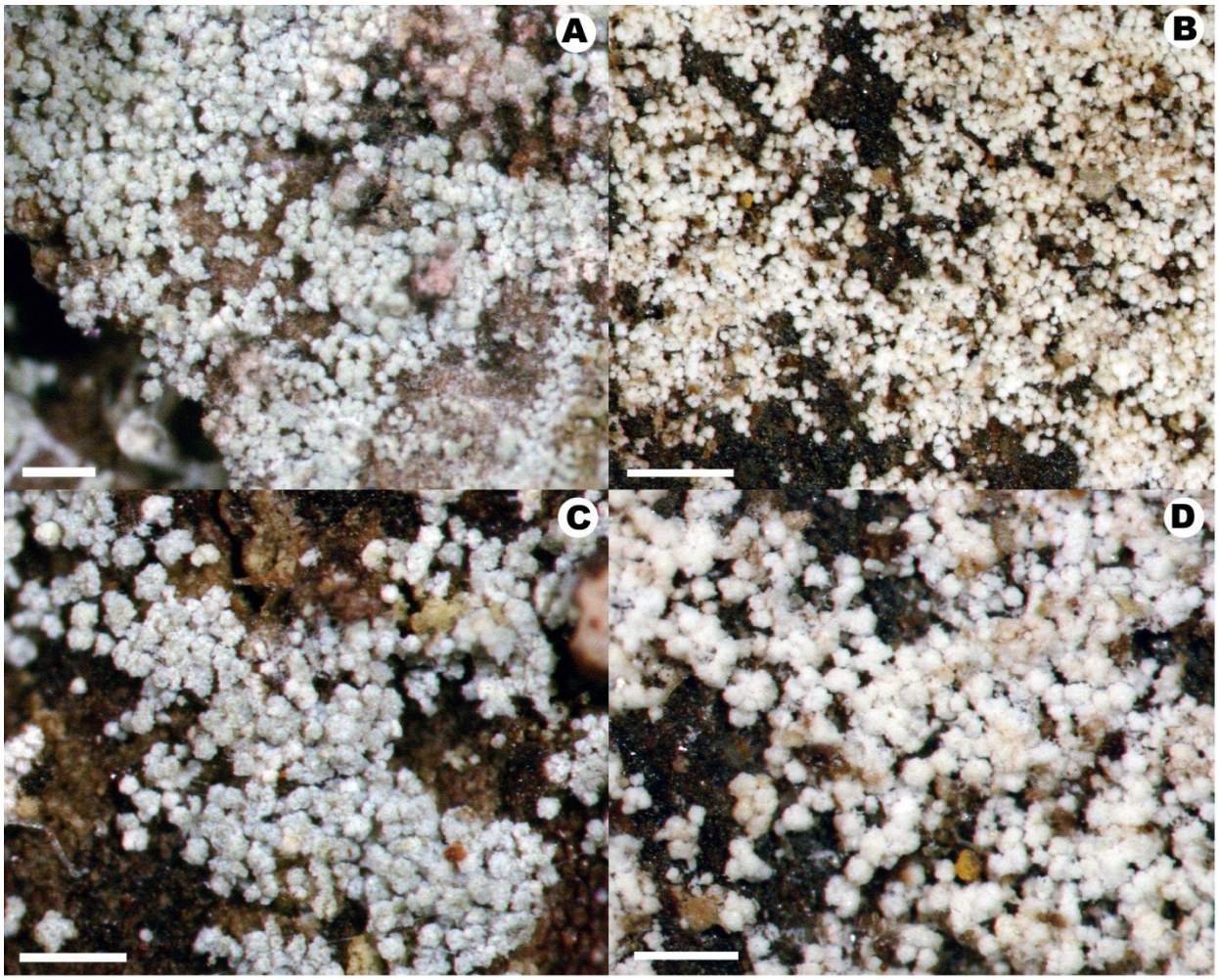

Figure 70, comparison of the holotypes of *Lepraria elobata* (*Tønsberg 17404*, left) and *L. salazinica* (*Tønsberg 30941*, right). A–B, gross morphology of the thallus (scales = 0.25 mm and 0.5 mm respectively). C–D, detail of the granules (scales = 0.25 mm).

Figure 71, North American populations of *Lepraria elobata* (*Lendemer 28116* is left; *Lendemer 28349A* is right). A–B, gross morphology of the thallus (scales = 1.0 mm). C–D, detail of thallus morphology (scales = 0.5 mm). E–F, detail of the granules (scales = 0.25 mm).

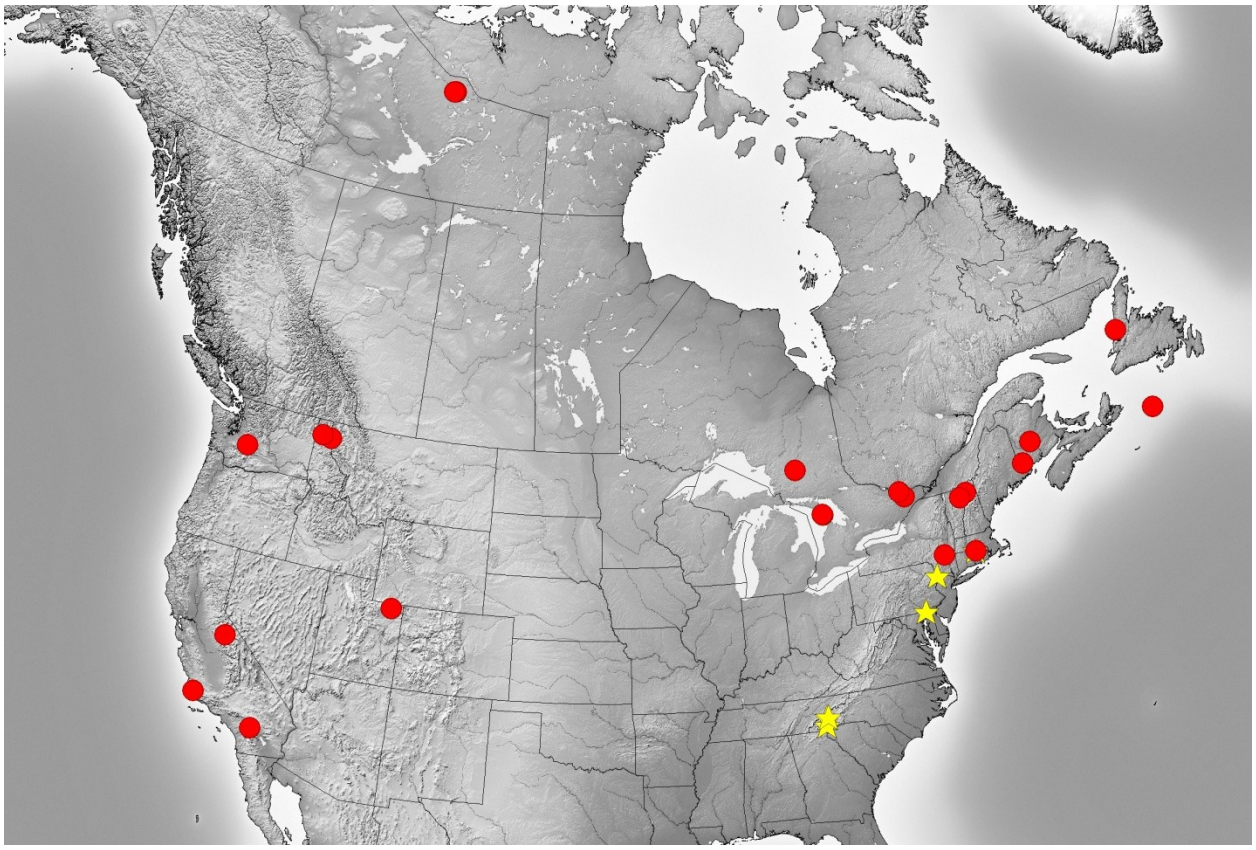

Figure 72, geographic distribution of *Lepraria elobata* based on the specimens examined for this study (red dots = typical chemotype, yellow stars = salazinic acid chemotype).

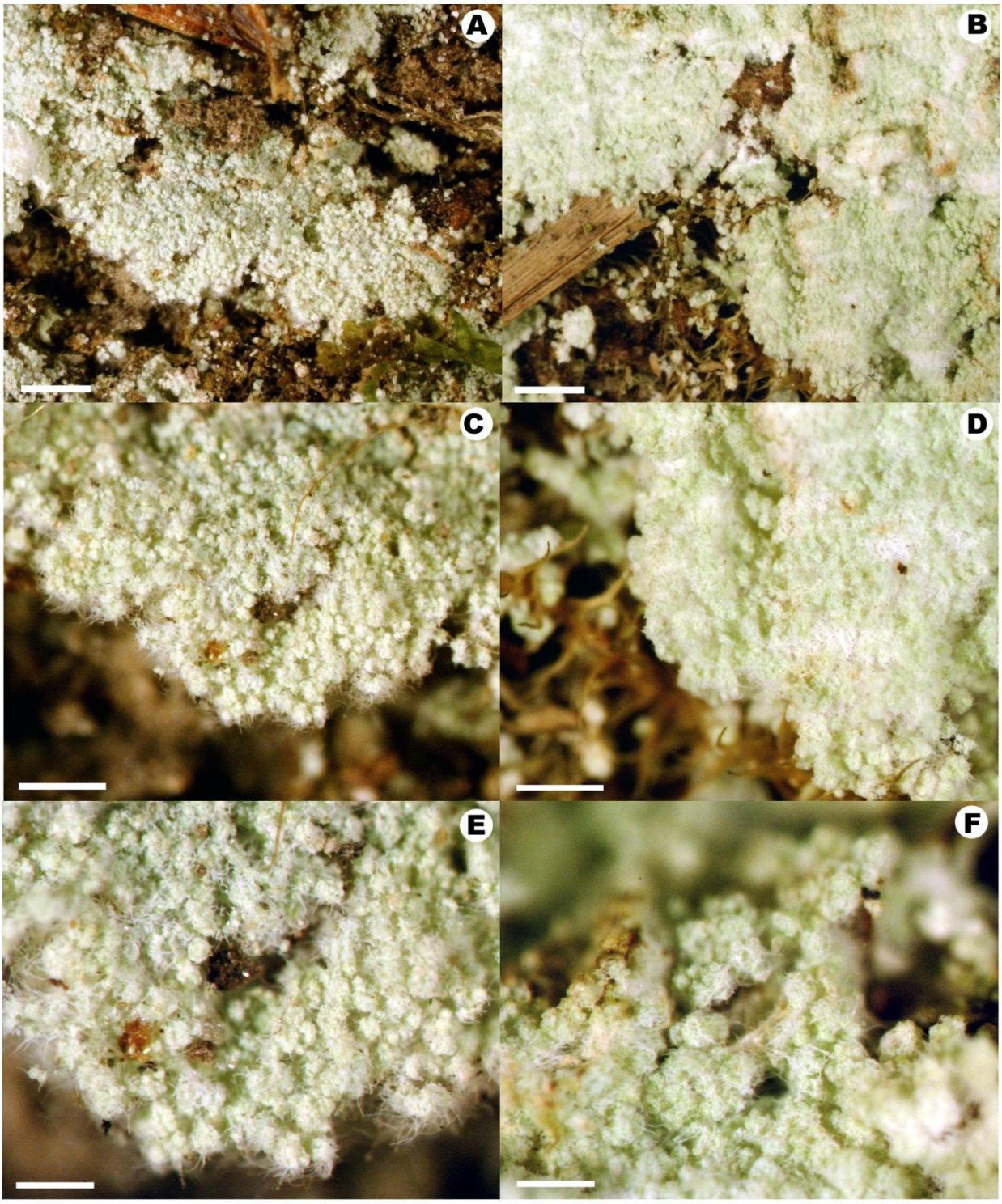

Figure 73, *Lepraria finkii* (*Lendemer 29677* is left; *Lendemer 27521* is right). A–B, gross morphology of the thallus (scales = 1.0 mm). C–D, detail of the thallus margins (scales = 0.5 mm). E–F, detail of the granules (scales = 0.25 mm).

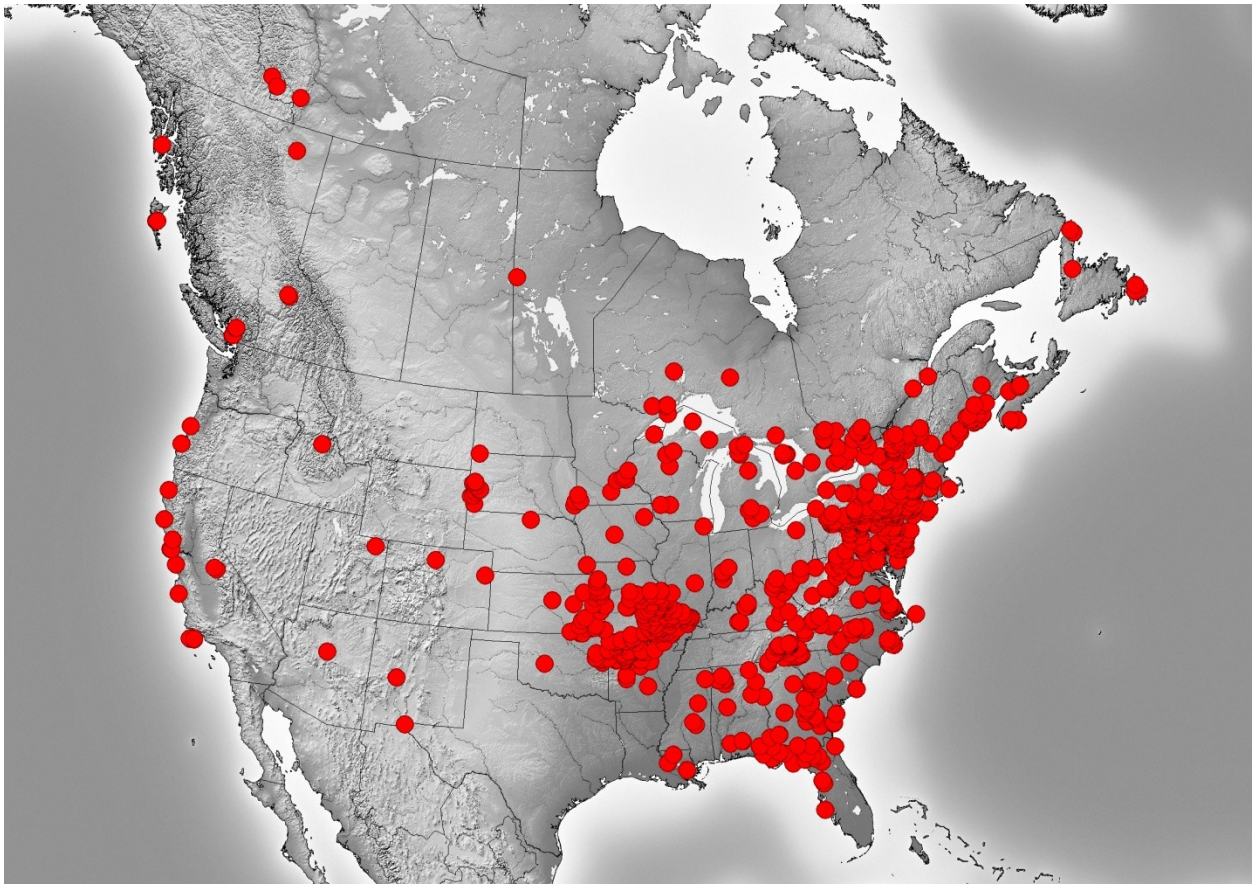

Figure 74, geographic distribution of *Lepraria finkii* based on the specimens examined for this study.

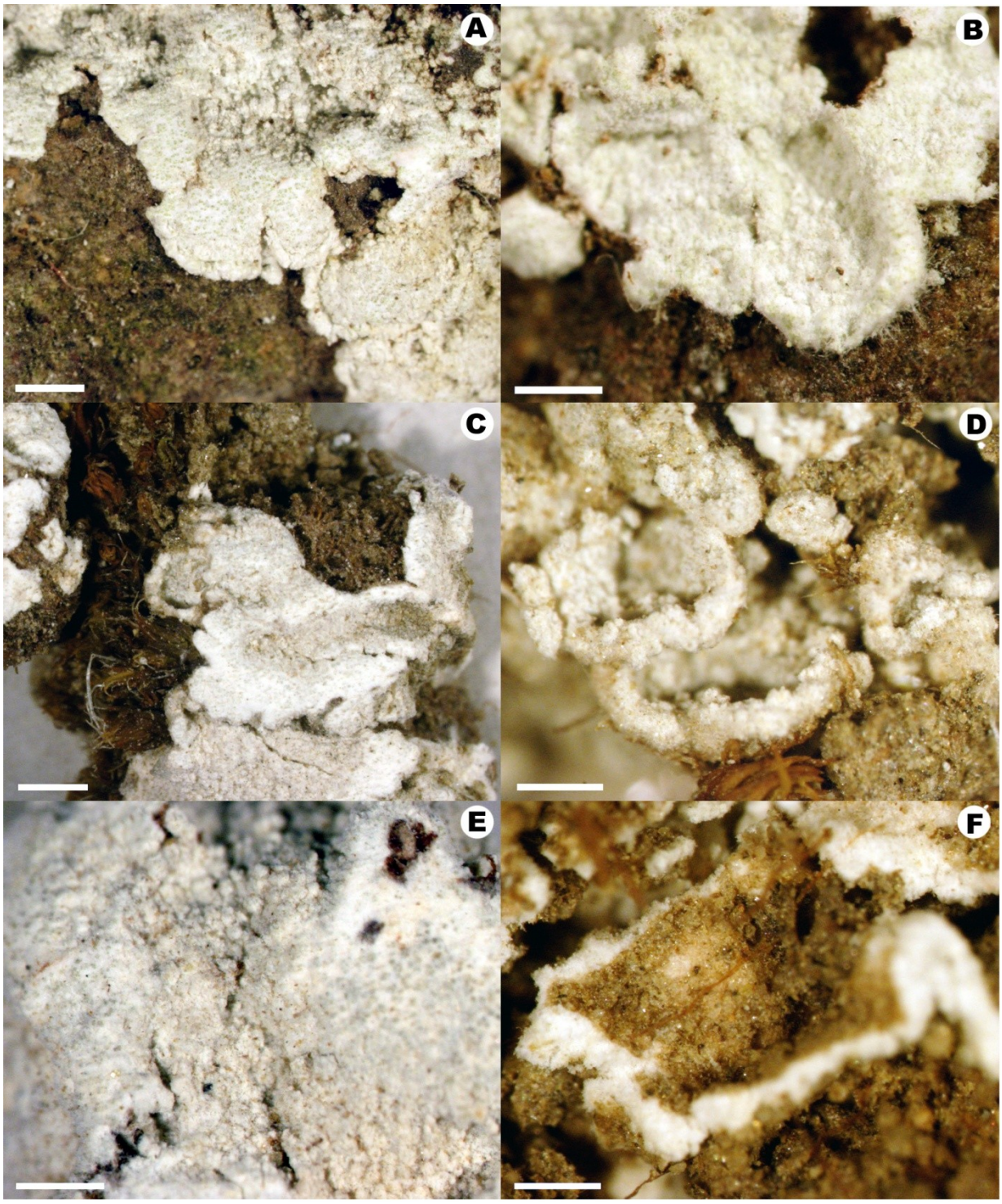

Figure 75, comparison of isotype of *Lepraria santosii* in GZU (A and B) with the lectotype of *L. lobificans* in H-NYL (C–F). A, gross morphology of the thallus in *L. santosii* (scale = 1.0 mm). B, detail of the thallus margin illustrating the crisped edge in *L. santosii* (scale = 0.5 mm). C, gross morphology of the thallus in *L. lobificans* (scale = 1.0 mm). D, detail of the thallus margin illustrating the crisped edge in *L. lobificans* (scale = 0.5 mm). E, thallus surface in *L. lobificans* (scale = 0.5 mm). F, lower surface in *L. lobificans* (scale = 0.5 mm).

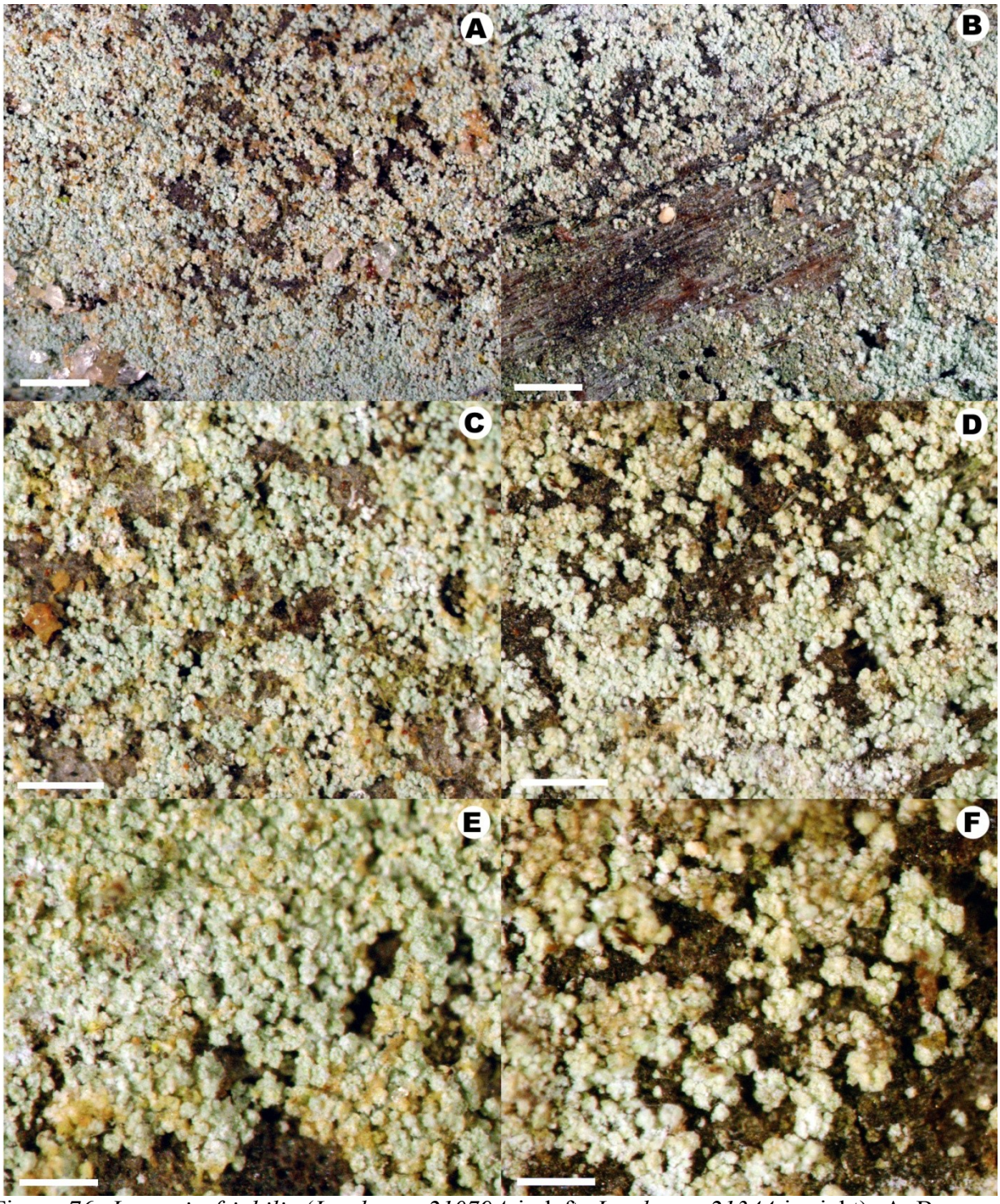

Figure 76, *Lepraria friabilis* (*Lendemer 21079A* is left; *Lendemer 21344* is right). A–B, gross morphology of the thallus (scales = 1.0 mm). C–D, detail of the thallus (scales = 0.5 mm). E–F, detail of the granules (scales = 0.25 mm).

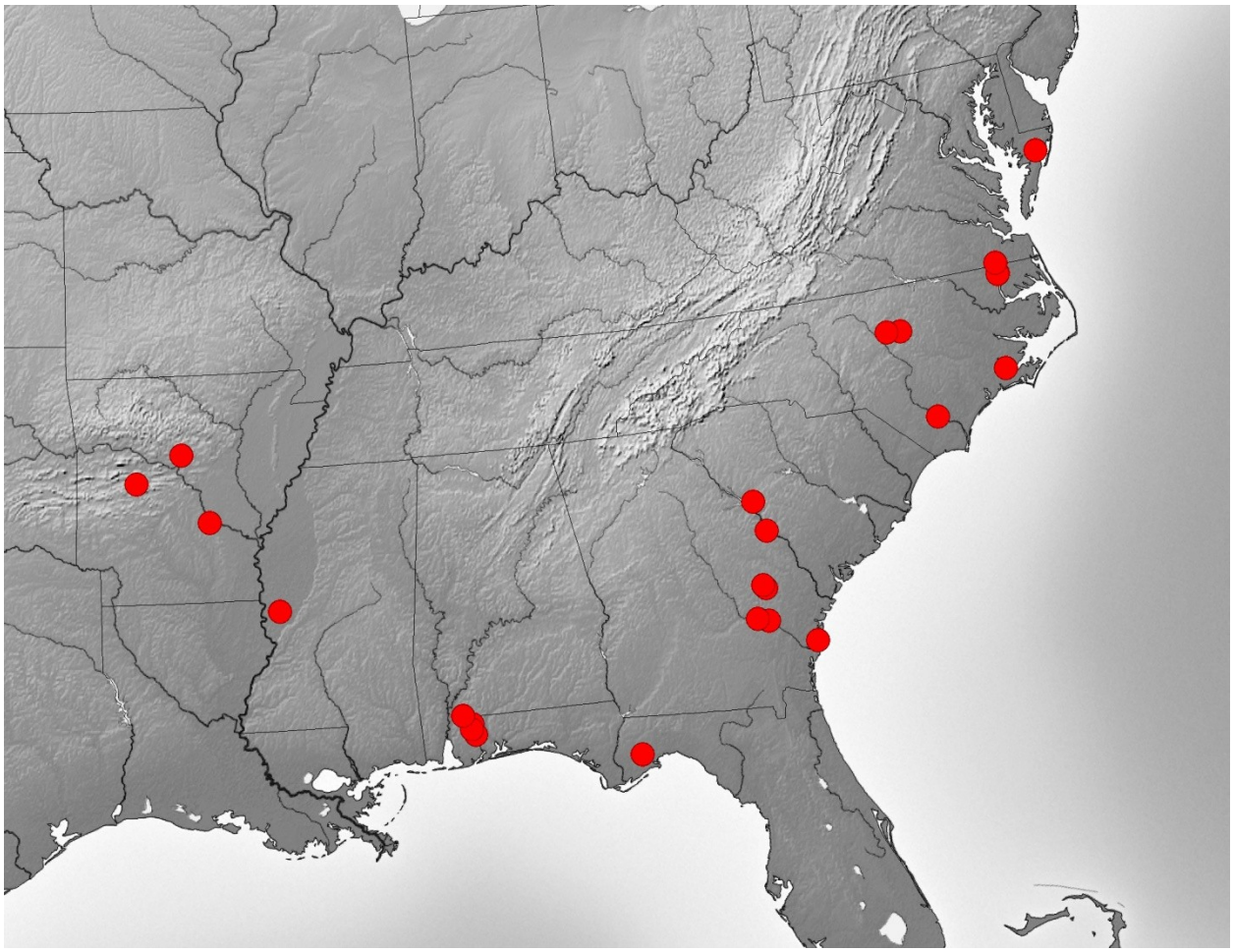

Figure 77, geographic distribution of *Lepraria friabilis* based on the specimens examined for this study.

Figure 78, *Lepraria harrisiana* (all from *Lendemer 55730*). A, gross morphology of the thallus (scale = 1.0 mm). B–D, detail of the thallus, and thallus surface (scales = 0.5 mm). E–F, detail of the thallus edge, illustrating rhizohyphae and ascending margins (scales = 0.25 mm).

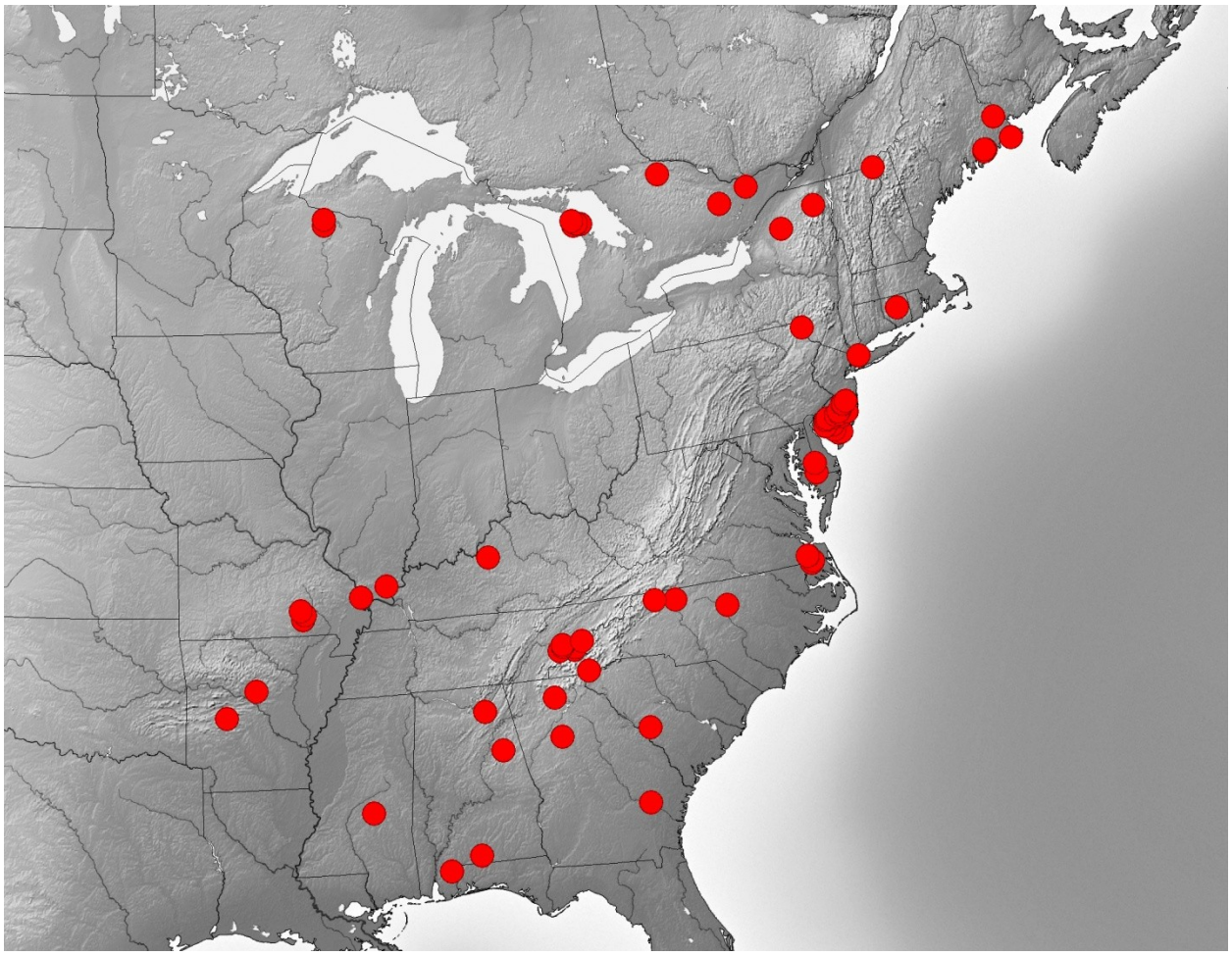

Figure 79, geographic distribution of *Lepraria harrisiana* based on the specimens examined for this study.

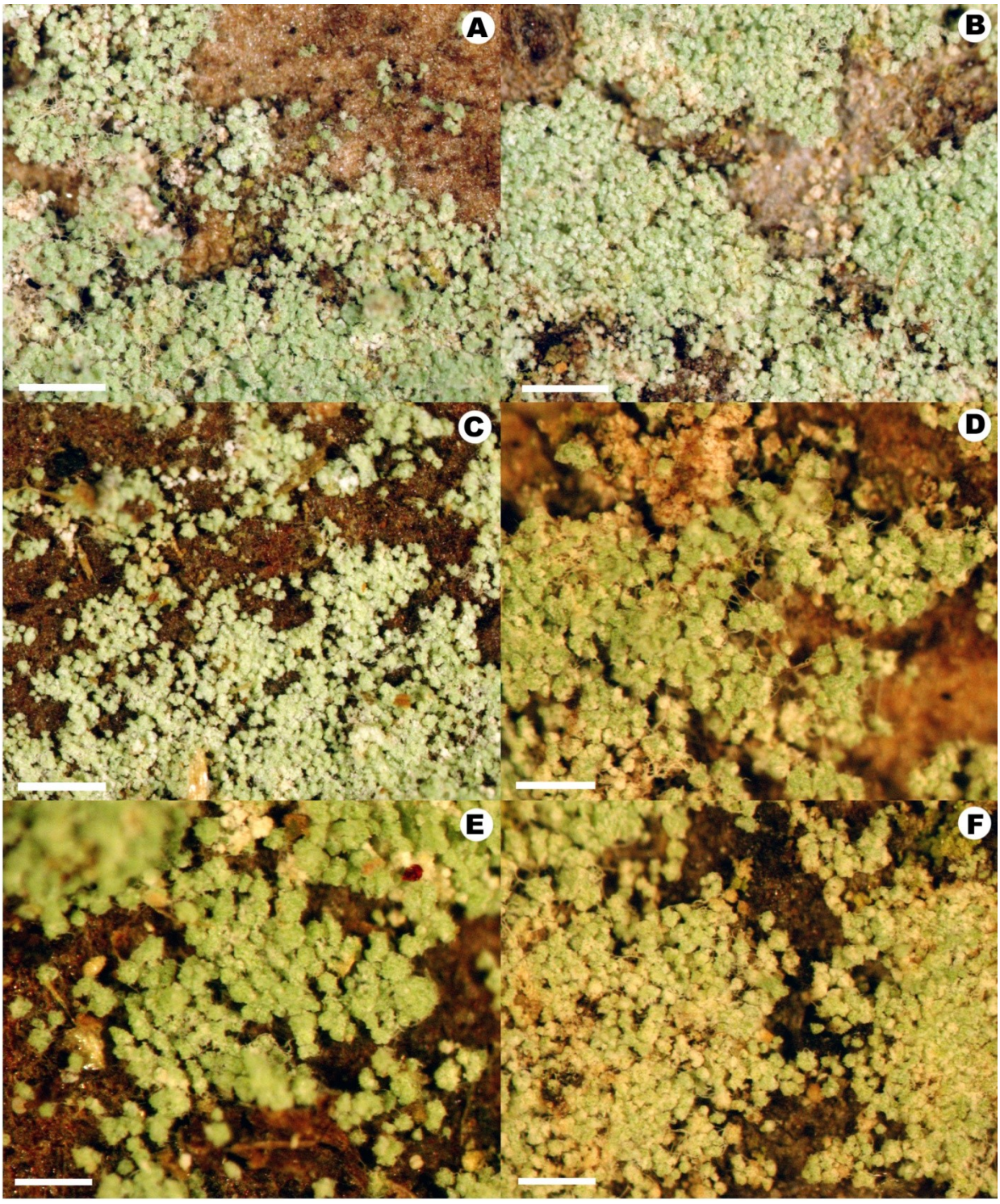

Figure 80, *Lepraria hodkinsoniana* (A, B, D and F are from *Lendemer 24535*; C and E are from *Lendemer 28250*). A–C, gross morphology of the thallus (scales = 0.5 mm). D–F. Detail of the granules (scales = 0.25 mm).

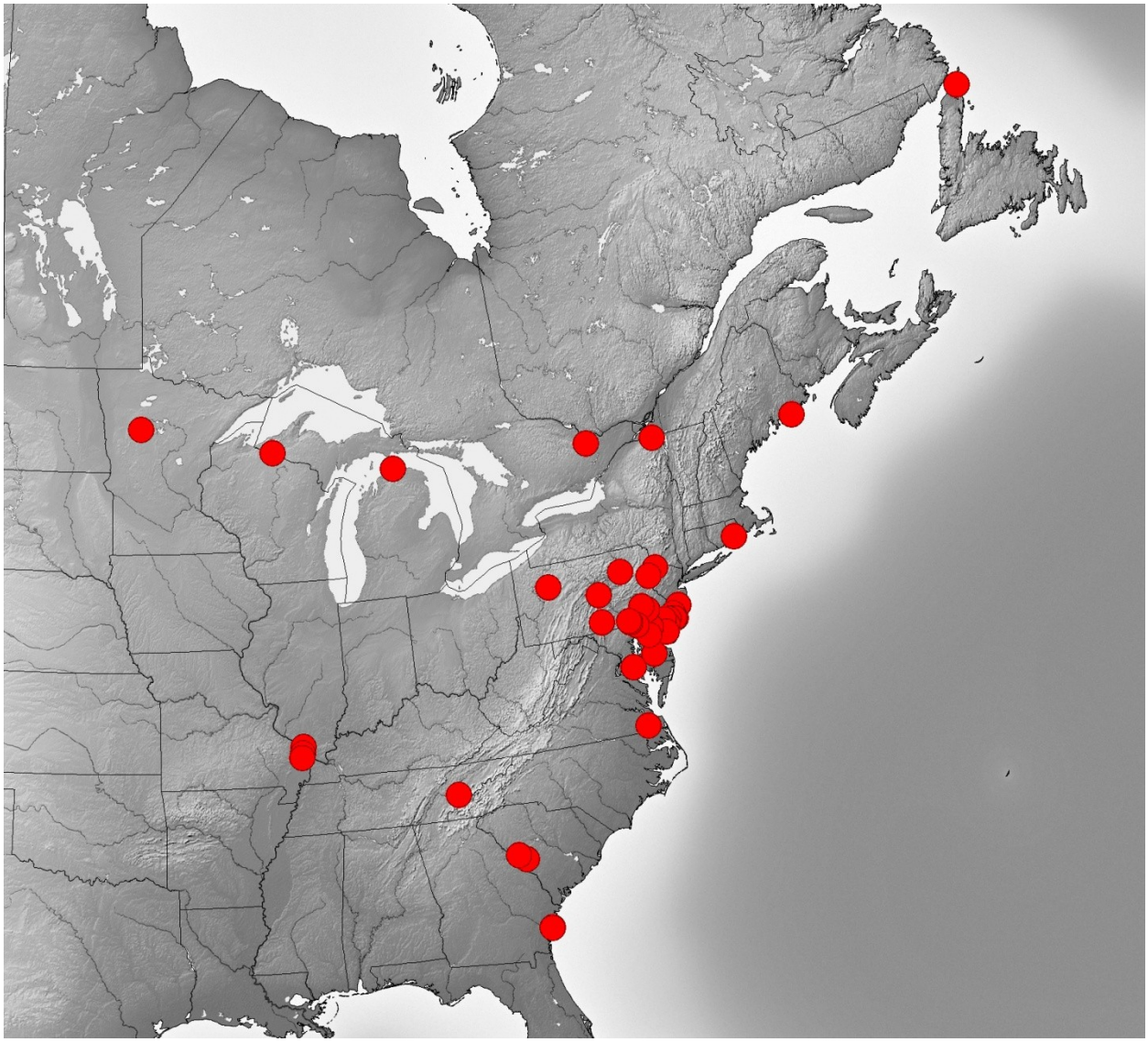

Figure 81, geographic distribution of *Lepraria hodkinsoniana* based on the specimens examined for this study.

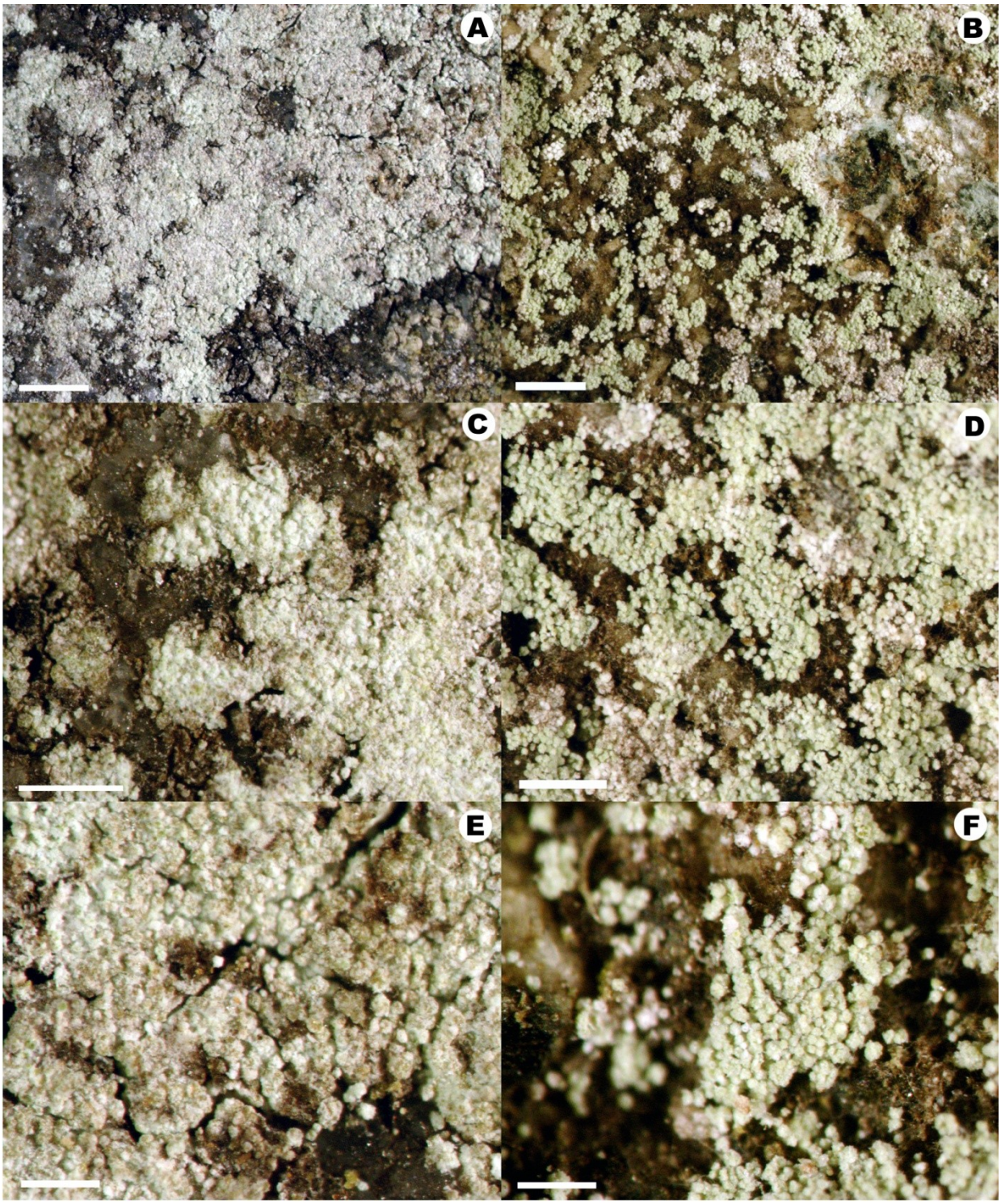

Figure 82, *Lepraria humida* (*Lendemer 12651* is left; *Lendemer 22865* is right). A–B, gross morphology of the thallus, note the tendency to form dispersed aggregations (scales = 1.0 mm). C–D, detail of the thallus (scales = 0.5 mm). E–F, detail of the granules (scales = 0.25 mm).

Figure 83, geographic distribution of *Lepraria humida* based on the specimens examined for this study.

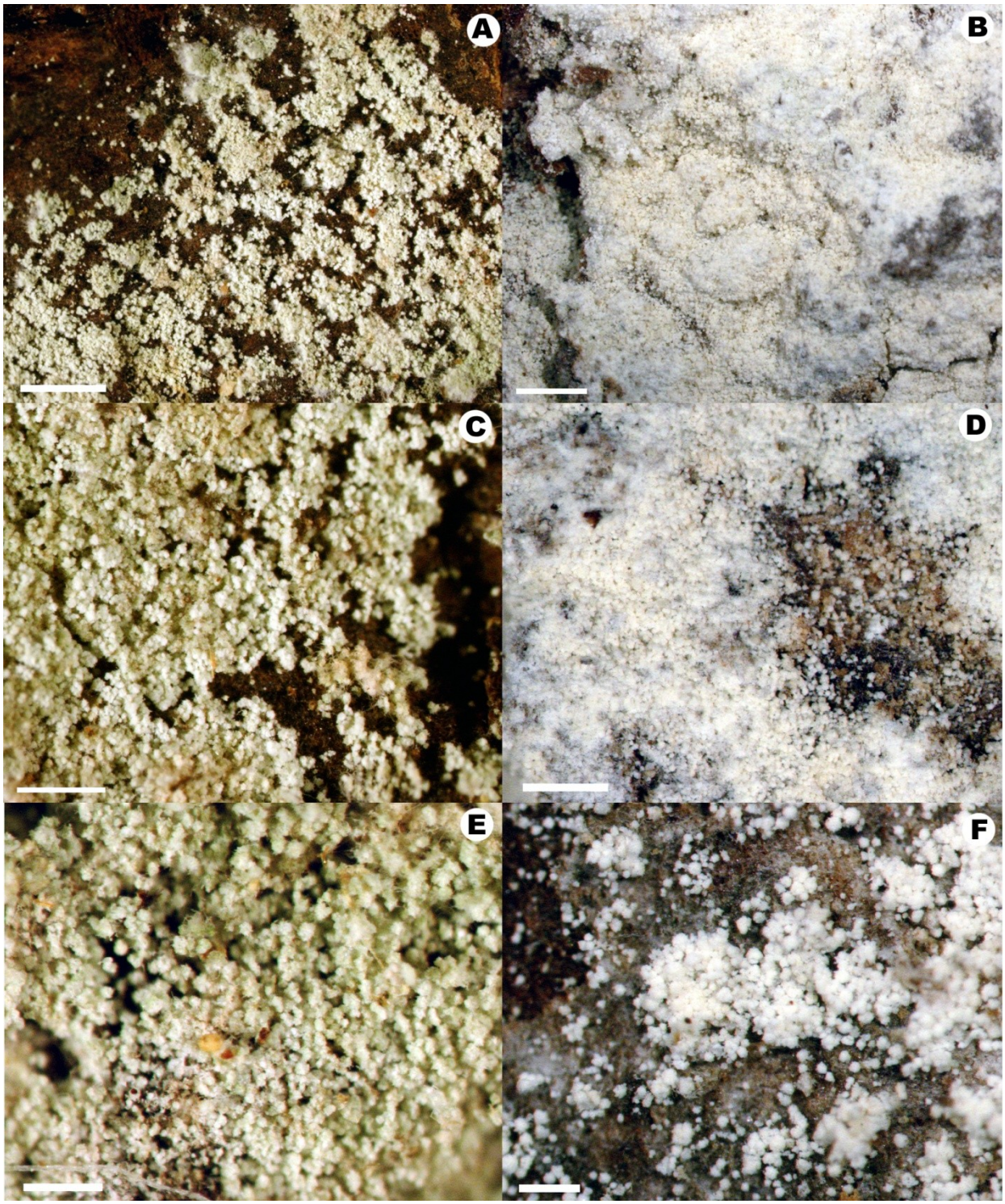

Figure 84, comparison of holotype of *Lepraria jackii* (*Tønsberg 6176*, right) to North American population (*LaGreca 1980*, left). A–B, gross morphology of the thallus (scales = 0.5 mm and 1.0 respectively). C–D, detail of the thallus (scales = 0.5 mm). E–F, detail of the granules (scales = 0.25 mm).

Figure 85, geographic distribution of *Lepraria jackii* based on the specimens examined for this study.

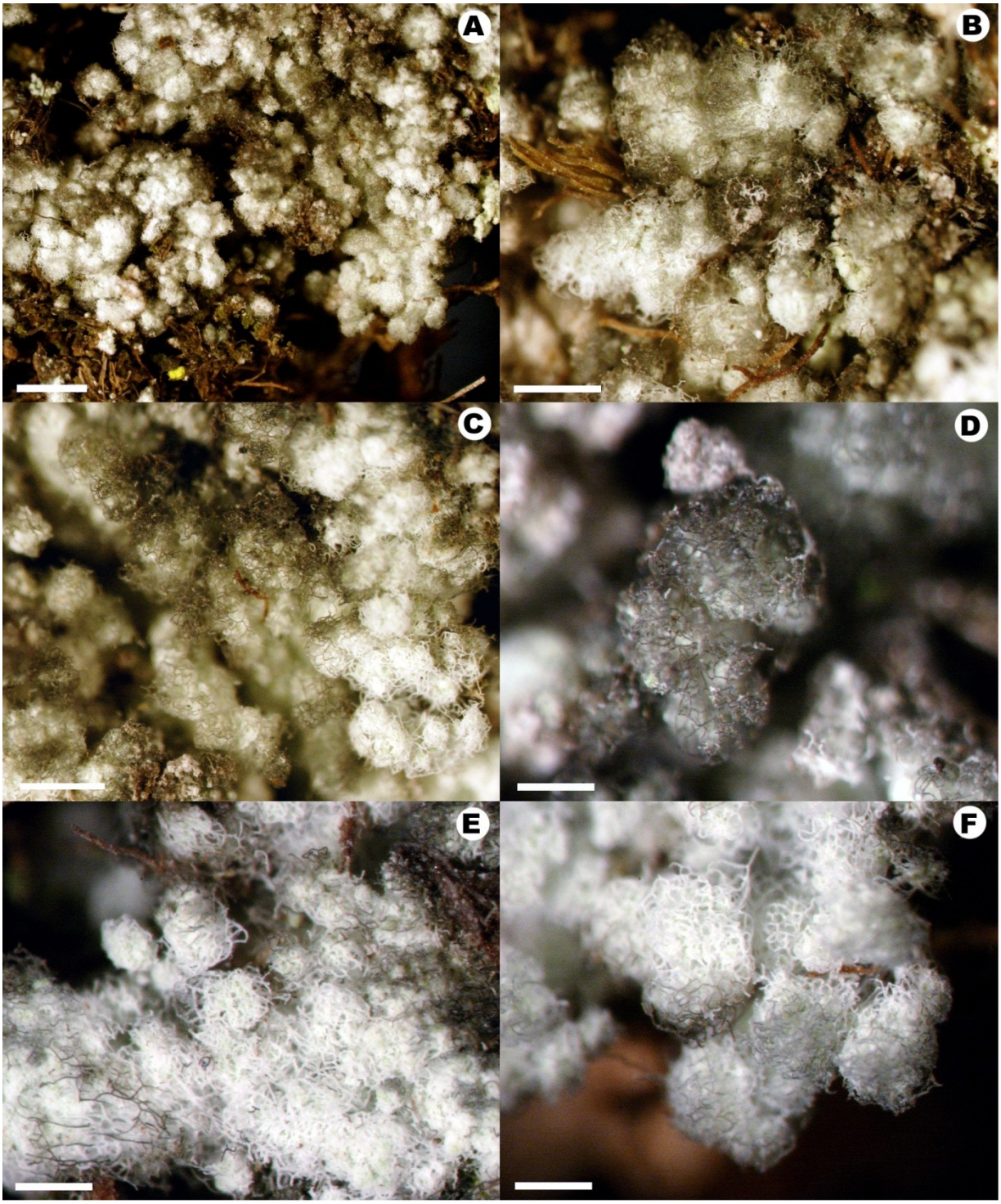

Figure 86, *Lepraria lanata* (all from *Lendemer 23306*). A, gross morphology of the thallus (scale = 1.0 mm). B–C, detail of the thallus (scales = 0.5 mm). D–F, detail of the granules, note the tendency for some hyphae to become darkly pigmented (scales = 0.25 mm).

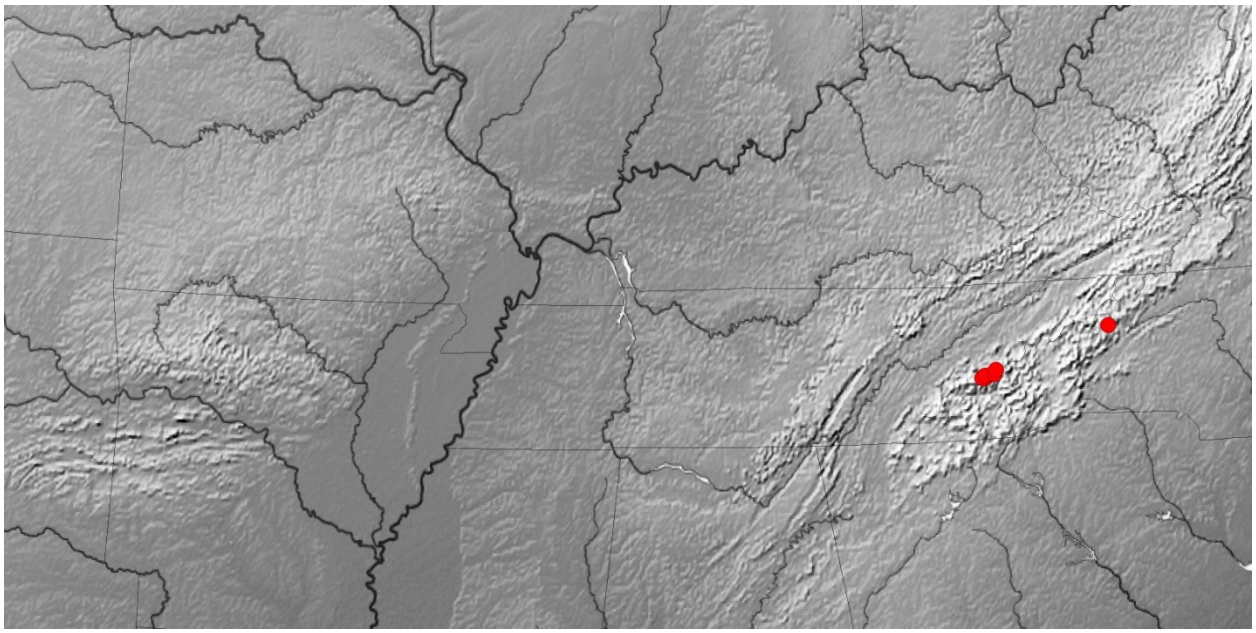

Figure 87, geographic distribution of *Lepraria lanata* based on the specimens examined for this study.

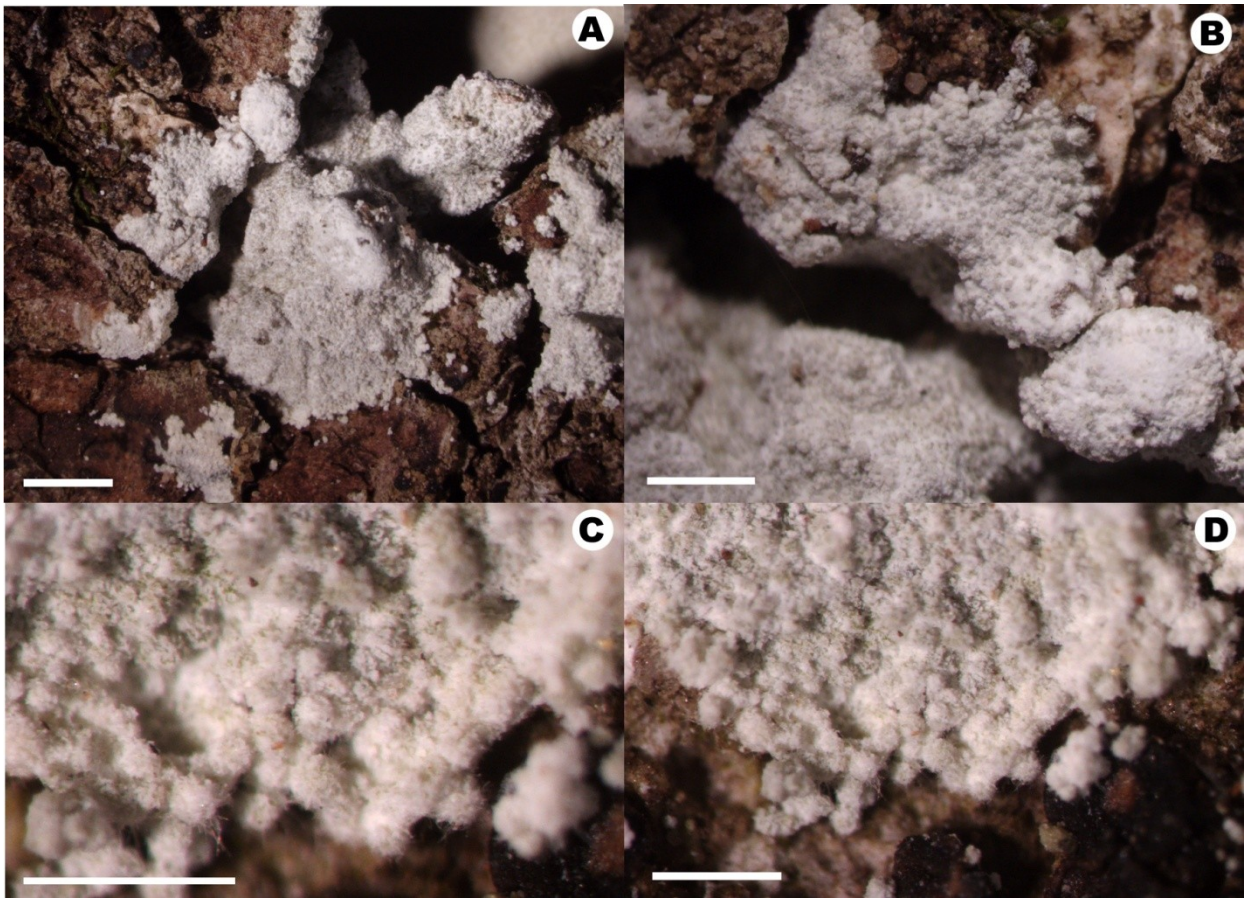

Figure 88. *Lepraria lecanorica* (all from the holotype). A–B, macromorphology of the thallus (scales = 2.0 mm and 1.0 mm respectively). C, detail of the granules (scale = 0.5 mm). D, detail of the thallus margin (scale = 0.5 mm).

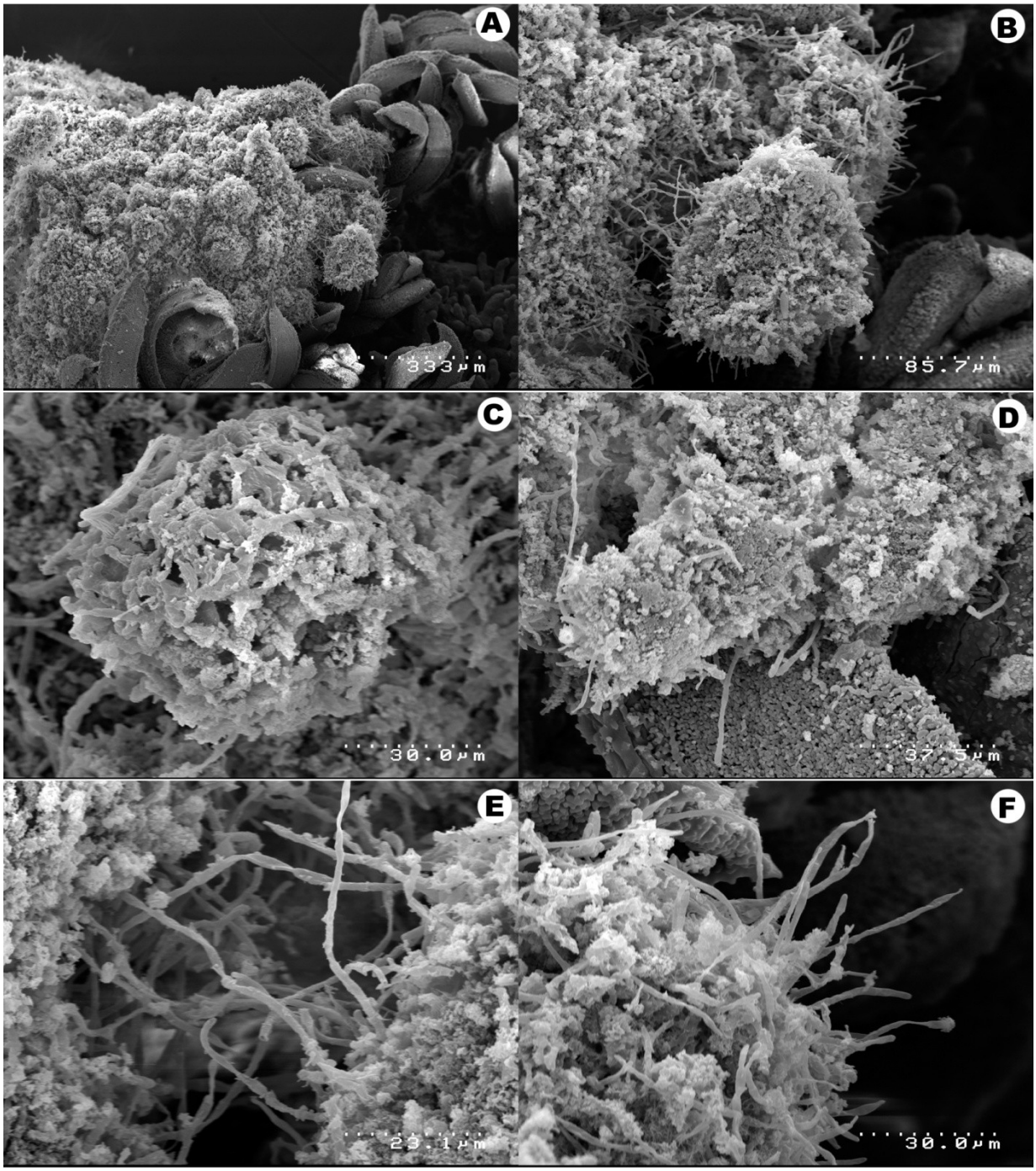

Figure 89, SEMs of *Lepraria lecanorica* (all from the holotype, scales as indicated). A, macromorphology of the thallus. B, thallus edge with prothalline hyphae extending outward from the granules. C, young ecorticate granule. D, mature granules with pseudocortex. E–F, detail of prothalline hyphae extending outward from the granules.

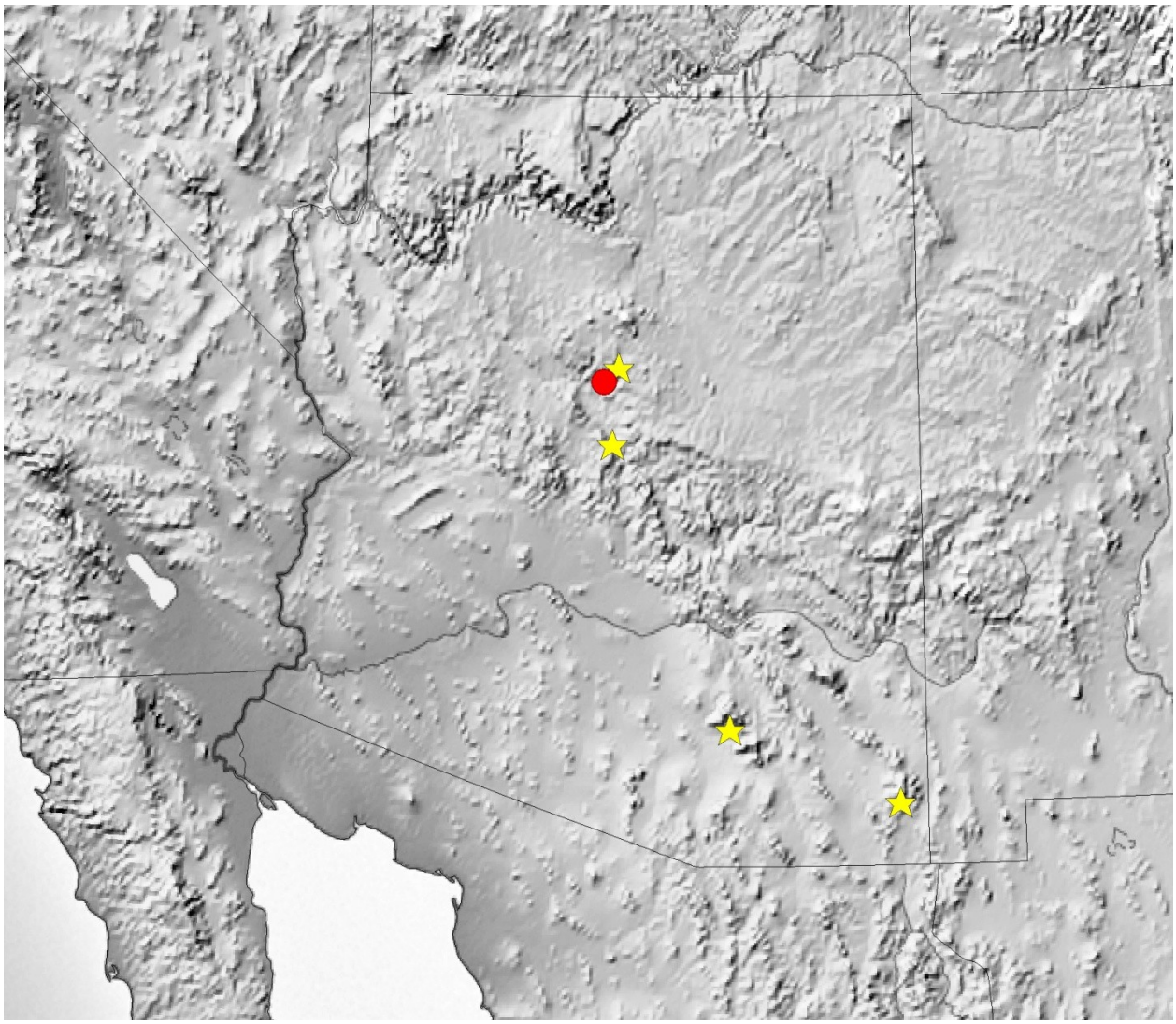

Figure 90, geographic distribution of *Lepraria lecanorica* based on the specimens examined for this study (red dot = specimens examined for this study; yellow stars = vouchers from CNALH and protologue note seen for this study).

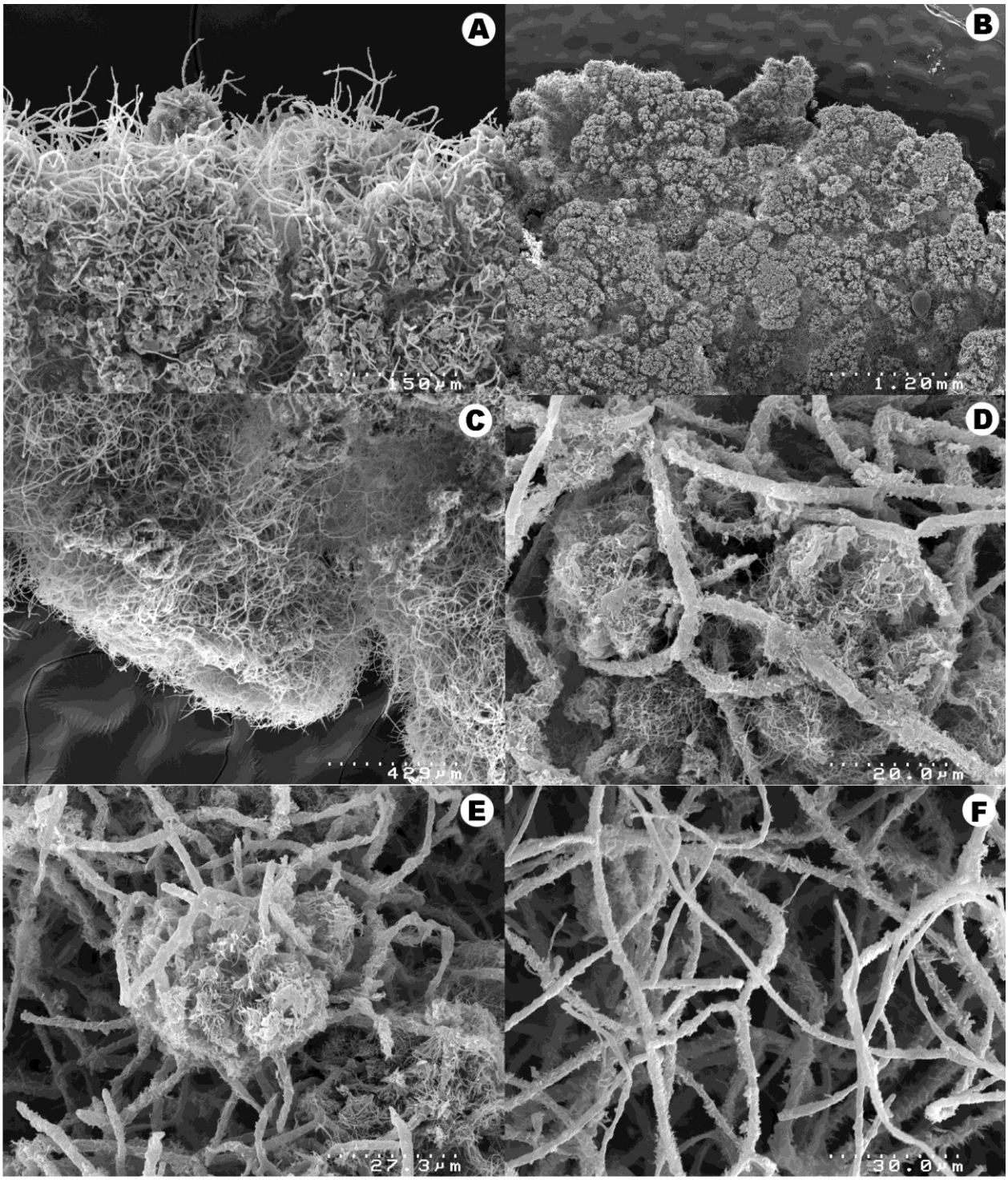

Figure 91, SEMs of *Lepraria leprolomopsis* (all from *Harris 28456* unless otherwise indicated). A, detail of thallus edge. B, gross morphology of thallus. C, detail of thallus edge from below (*Guccion 1663*). D, granules encased in network of thalline hyphae. E, young granule developing on thallus surface. F, detail of hyphae forming the hypothallus (*Guccion 1663*).

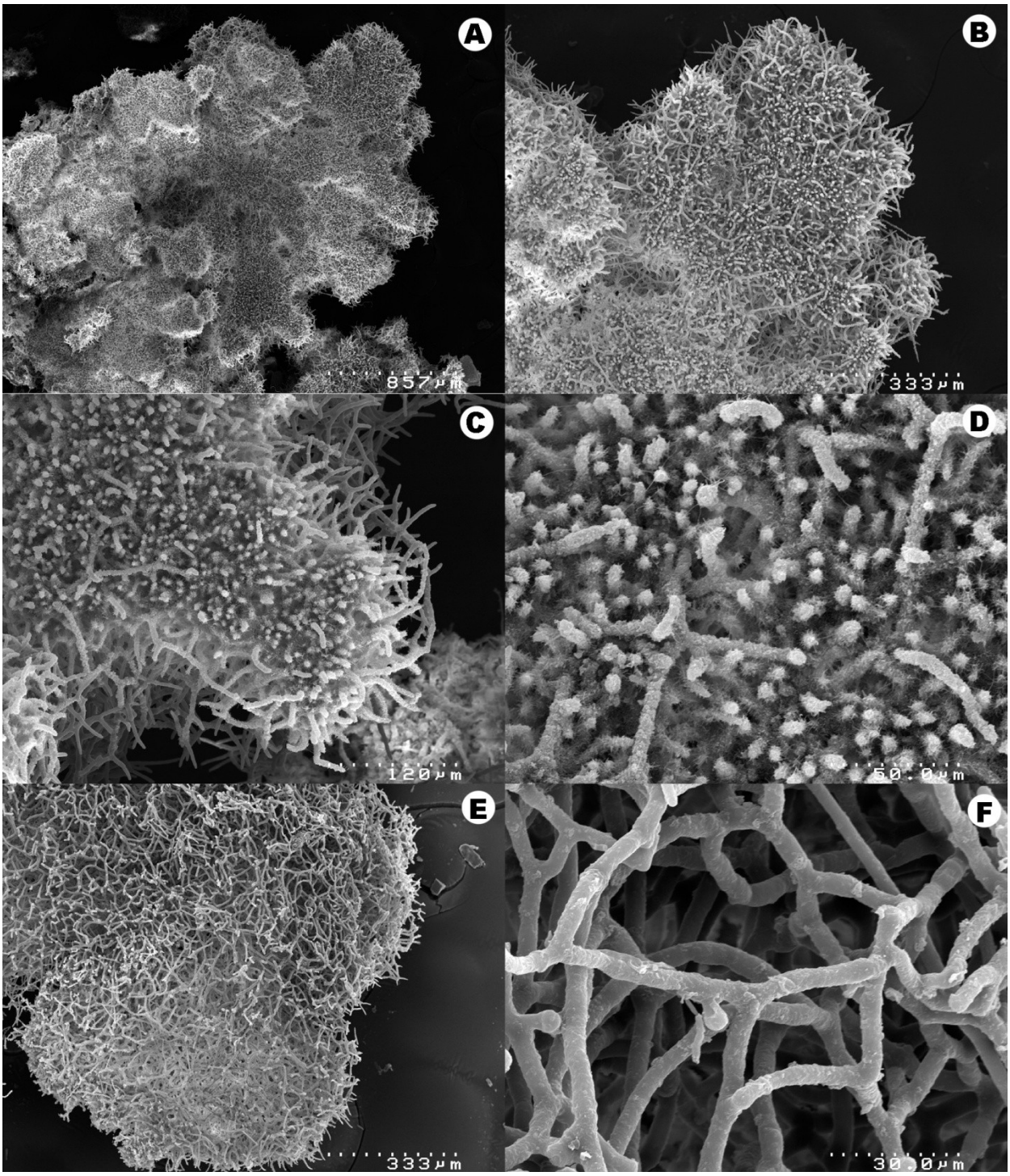

Figure 92, SEMs of *Crocynia pyxinoides* (all from *Lendemer 9067*, NY). A, gross morphology of thallus. B, detail of "lobe". C, detail of thallus margin illustrating both the upper surface and hypothallus extending outward from the underside of the "lobe" tips. D, detail of upper surface. E, thallus margin from below. F, detail of hyphae forming the hypothallus.

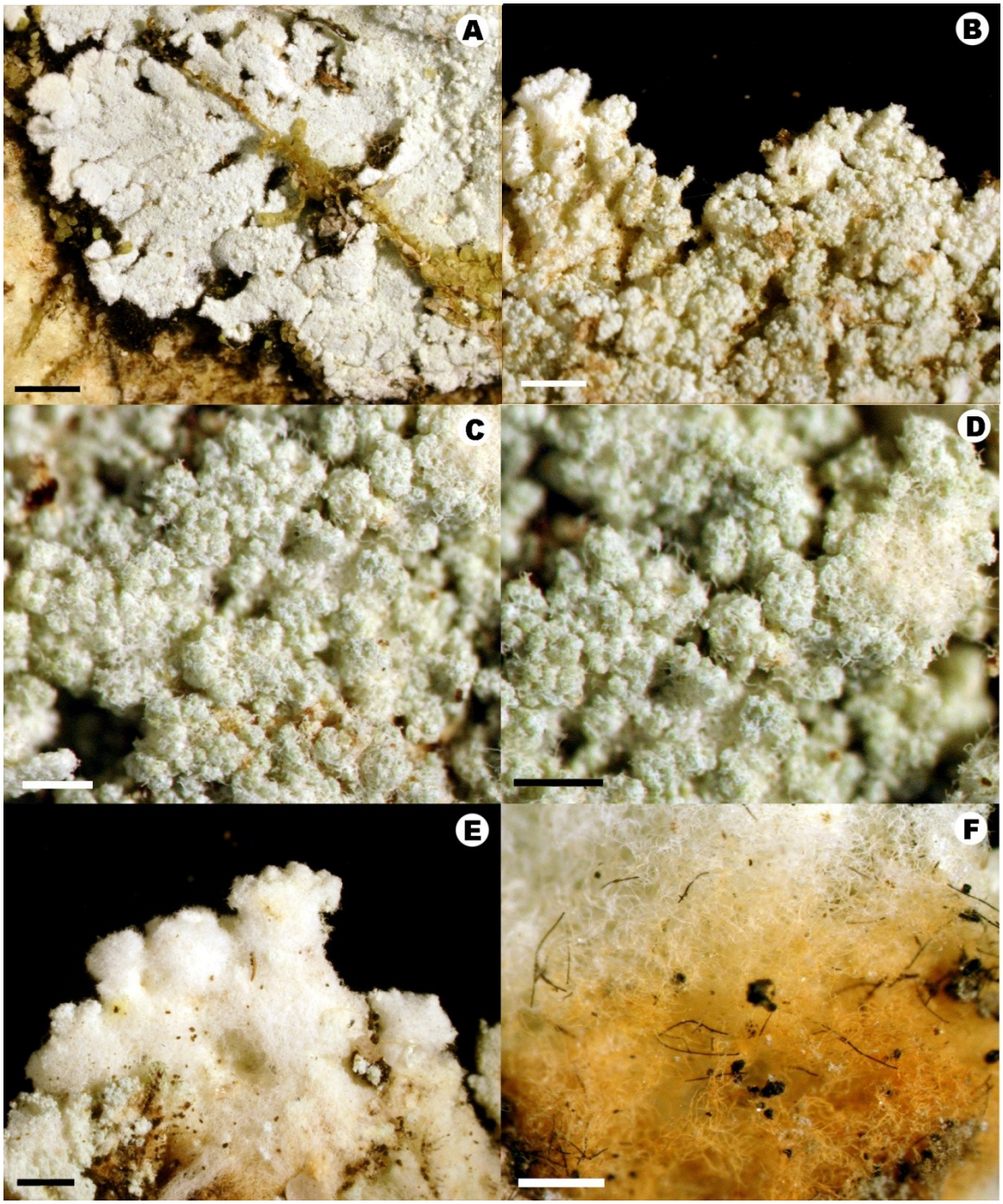

Figure 93, color illustrations of *Crocynia pyxinoides* (A) and *Lepraria leprolomopsis* (B–F). A, gross morphology of *C. pyxinoides*, note well developed black hypothallus surrounding the "lobes" (*Lendemer 9067*, scale = 2 mm). B, gross morphology of thallus of *L. leprolomopsis* (*Harris 28456* , scale = 2 mm). C–D, detail of supper surface and aggregations of granules (*Harris 28456*, scales = 0.25 mm). E–F, underside of thallus illustrating unpigmented margins (E) and pigmented portions near center of thallus (F) (both from *Guccion 1663*, scales = 1.0 and 0.5 mm respectively).

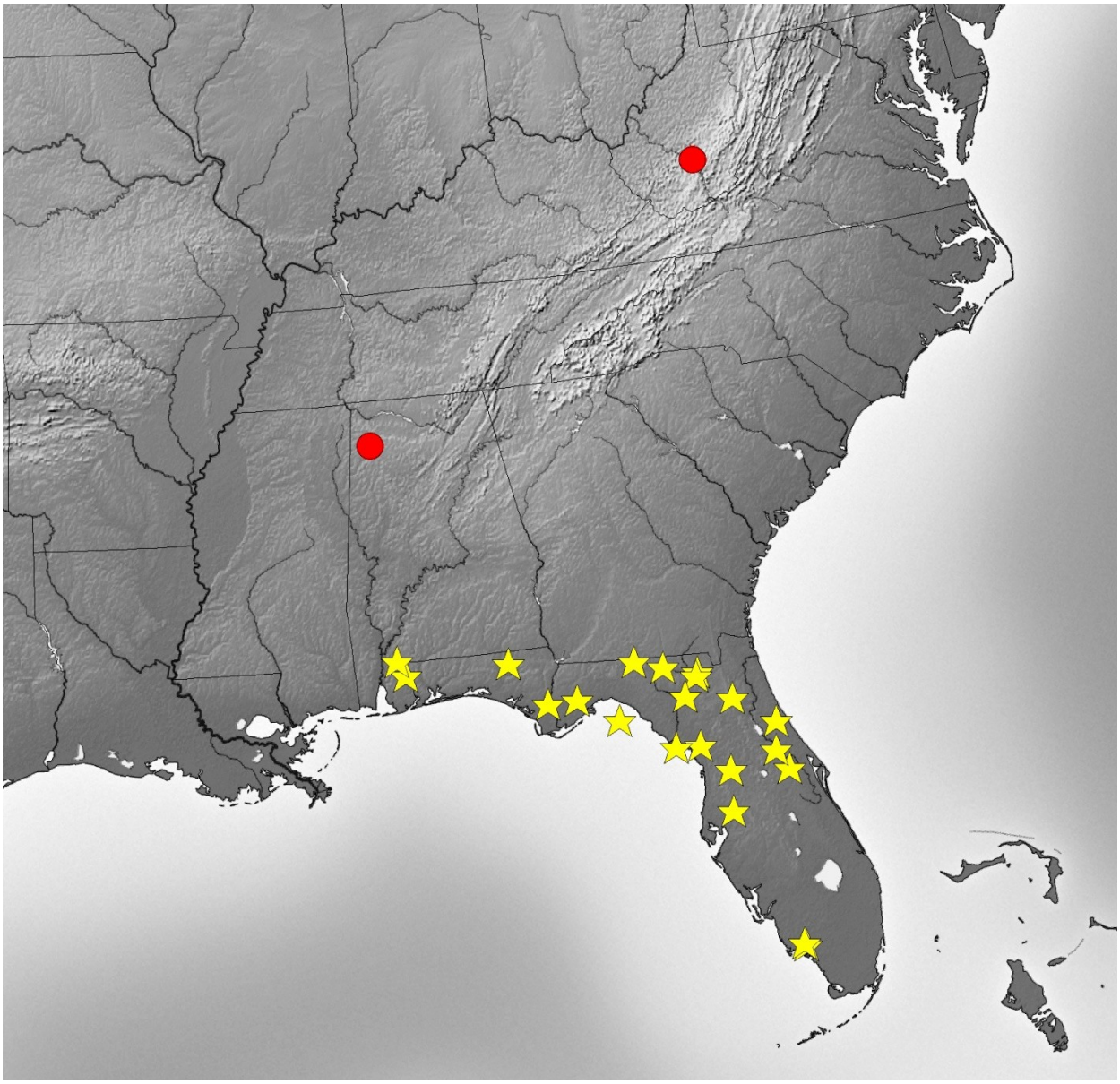

Figure 94, geographic distribution of *Lepraria leprolomopsis* (red dots) based on the specimens examined for this study compared to the distribution of *Crocynia pyxinoides* (yellow stars) based on herbarium specimens at NY.

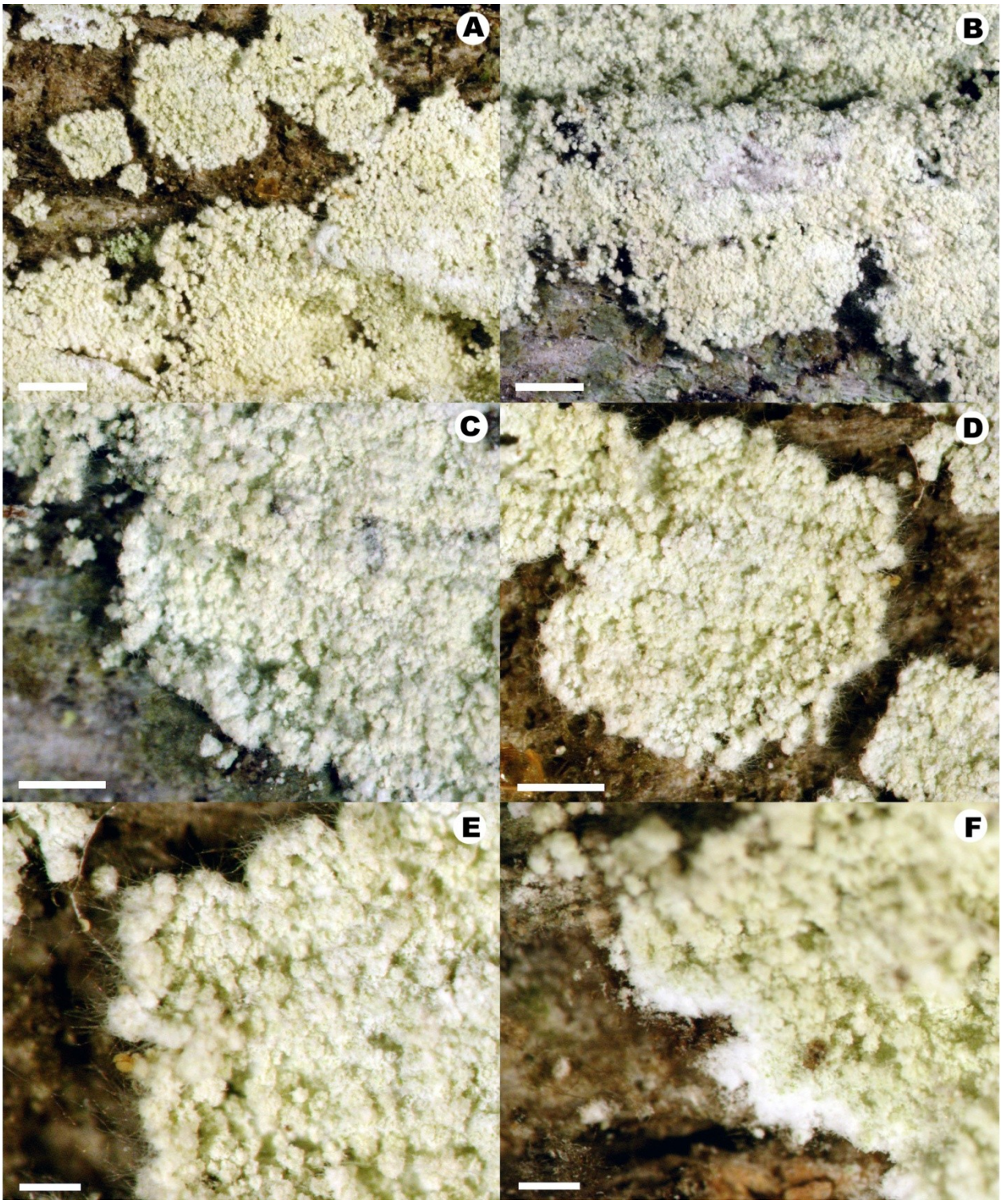

Figure 95, *Lepraria membranacea* (all from *Lewis 450*). A–B, macromorphology of the thallus (scales = 1.0 mm). C–D, detail of the thallus (scales = 0.5 mm). C, detail of the granules and thallus edge (scale = 0.25 mm). F, section of the thallus illustrating thick white hypothallus (scale = 0.25 mm).

Figure 96, geographic distribution of *Lepraria membranacea* based on the specimens examined for this study.

Figure 97, *Lepraria neglecta* s.l., comparison of a saxicolous population (A–B, *Lendemer 24995*), a corticolous population (C–D, *Lendemer 23717*), and a population with an *alpina*-type thallus (E–F, *Buck 55113*). A, C and E, macromorphology of the thallus, note the tendency to form radiating rosettes (scales = 1.0 mm). B, D and F, detail of the granules (scales = 0.25 mm).

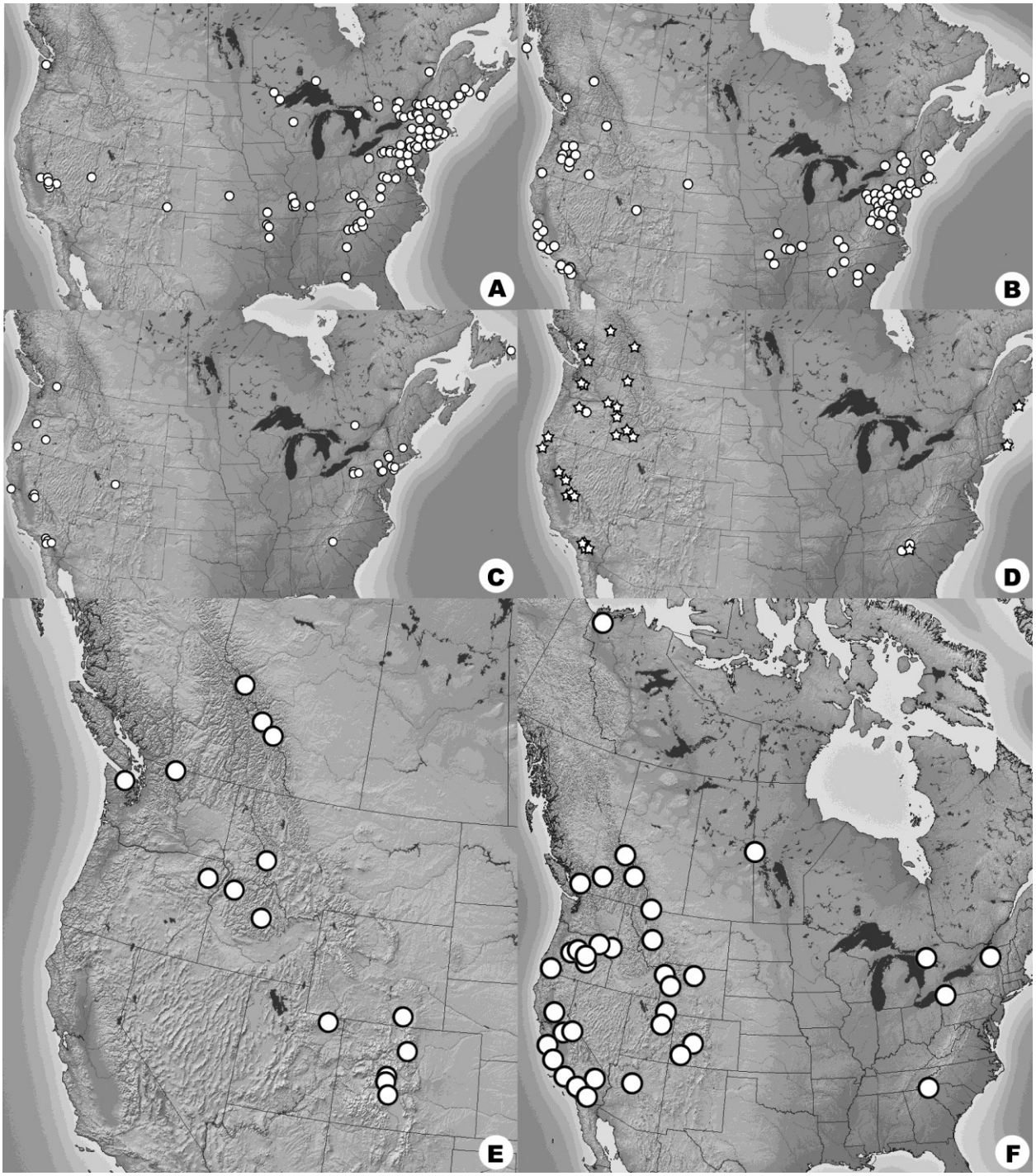

Figure 98, geographic distributions of the chemotypes of *Lepraria neglecta* s.l. in North America based on specimens examined for this study. A, alectorialic acid chemotype (*L. neglecta* s.str.). B, fumarprotocetraric acid chemotype (*L. caesioalba*). C, psoromic acid chemotype (unnamed). D, stictic acid (stars, *L. svalbardensis*) and norstictic acid (circles, unnamed) chemotypes. E, porphyrilic acid chemotype (*L. alpina*). F, fatty acid chemotypes (populations with roccellic/angardianic acid were unnamed, those with rangiformic/jackinic acid were referred to *L. borealis*).

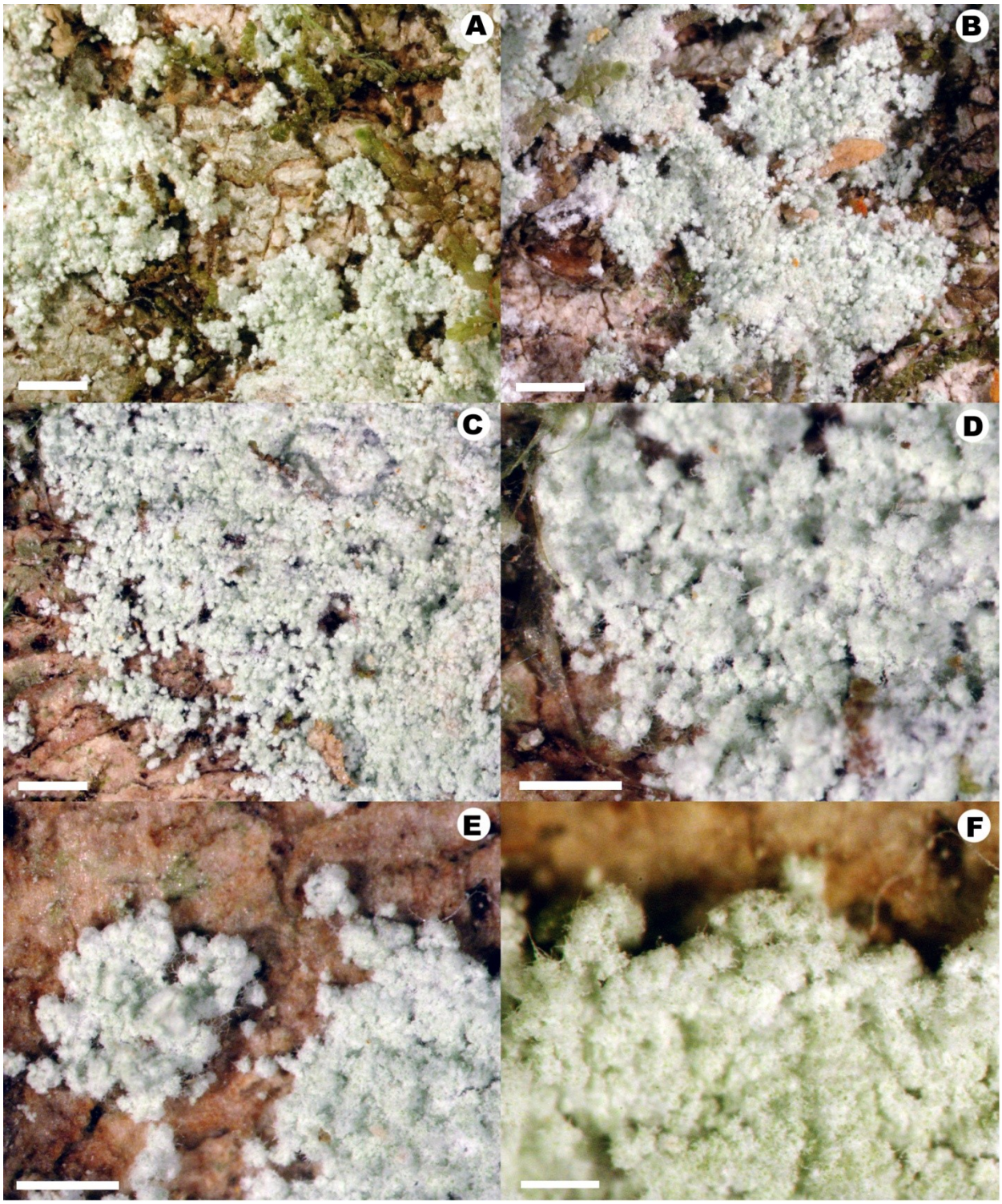

Figure 99, *Lepraria nivalis* (all from *Lendemer 22375*). A–C, macromorphology of the thallus (scales = 1.0 mm). D–E, detail of the thallus (scales = 0.5 mm). F, detail of the granules and thallus margin (scale = 0.25 mm).

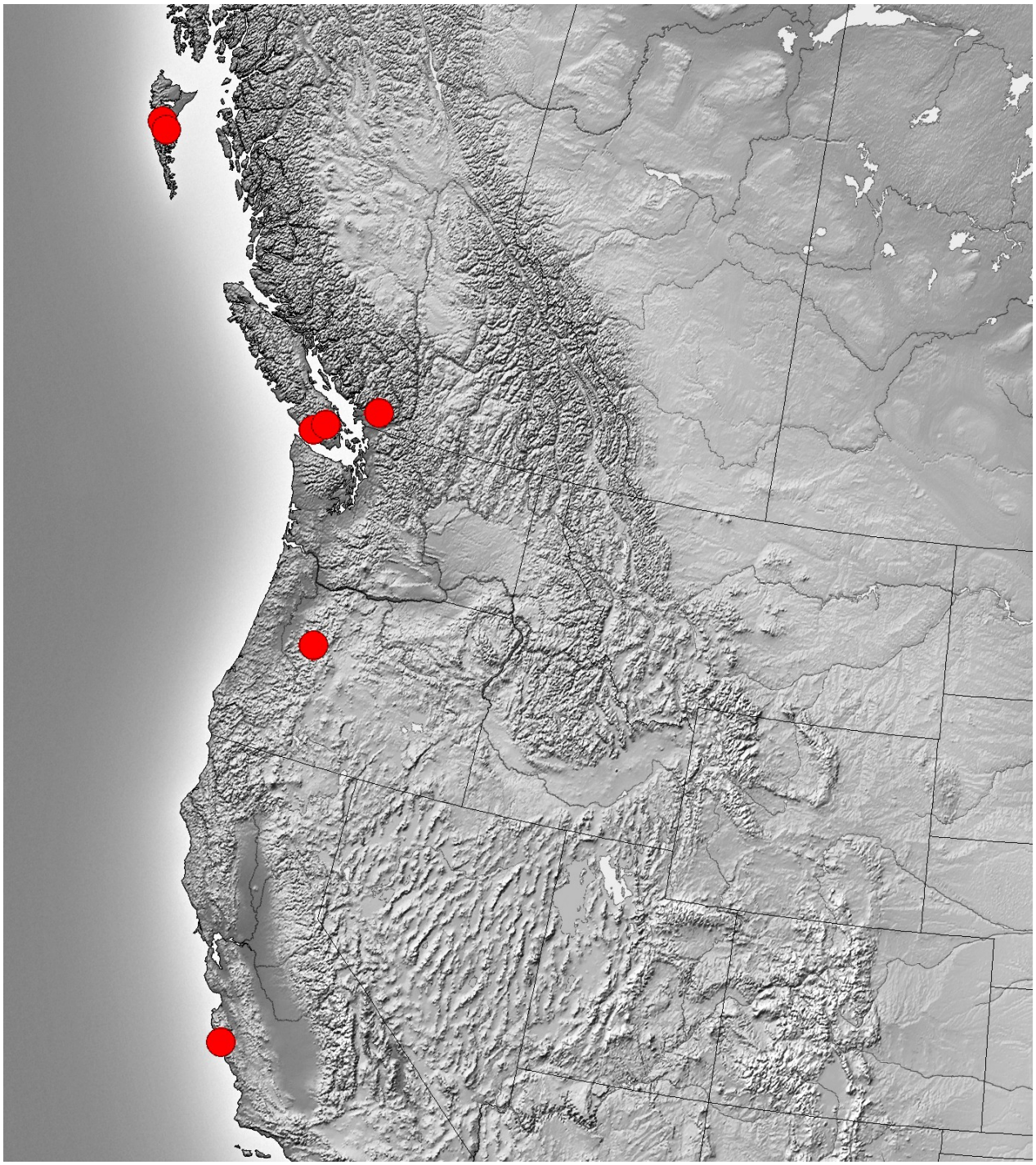

Figure 100, geographic distribution of *Lepraria nivalis* based on the specimens examined for this study.

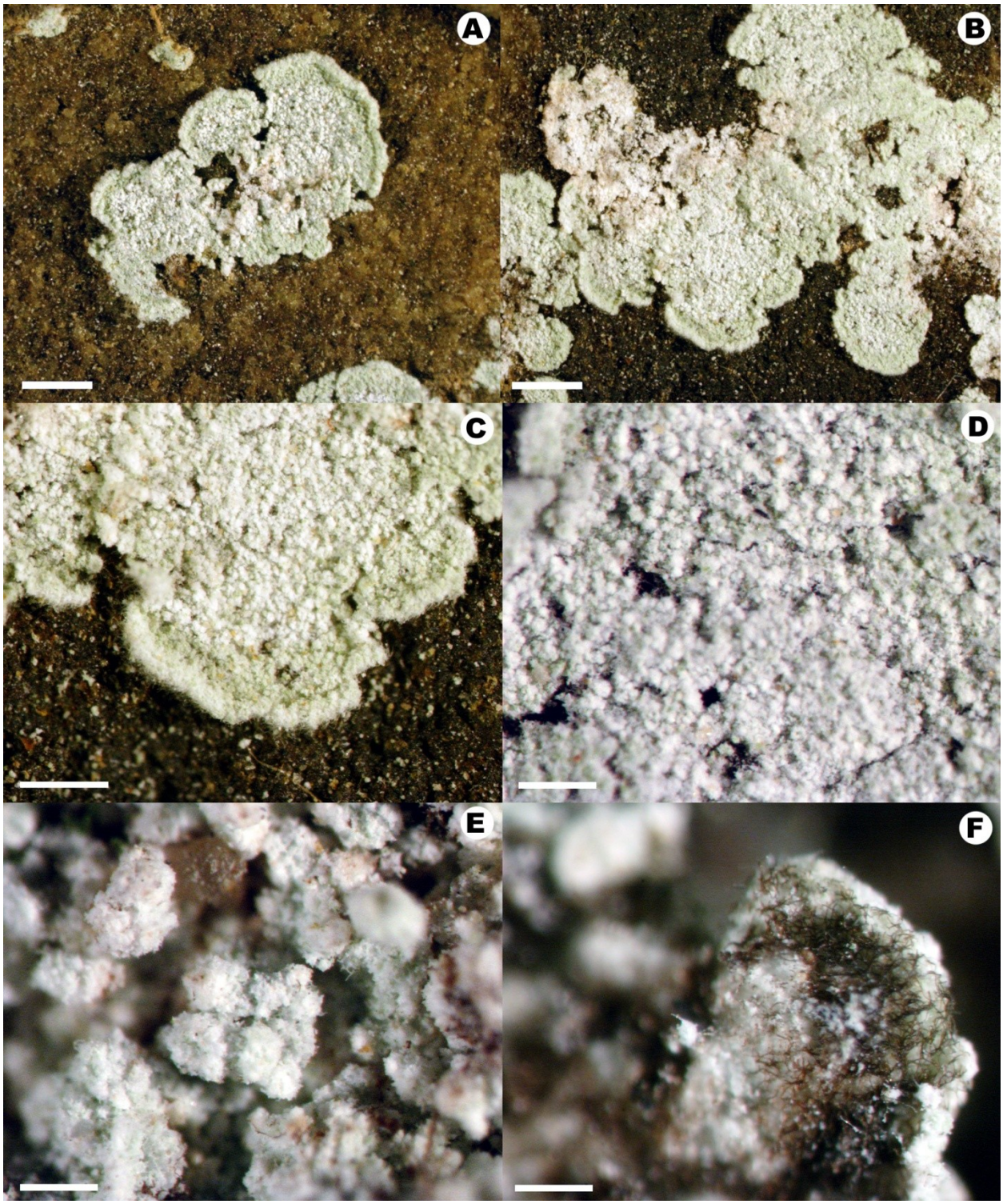

Figure 101, *Lepraria normandinoides* (all from *Hollinger 2229* unless otherwise indicated). A–B, gross morphology of the thallus (scales = 1.0 mm). C, detail of the thallus margin (scale = 0.5 mm). D, detail of the thallus surface (scale = 0.25 mm). E, aggregation of granules becoming separated from the hypothallus (*Lendemer 25913*, scale = 0.25 mm). F, dark pigmented hyphae extending from the lower surface of the thallus (*Lendemer 26222*, scale = 0.25 mm).

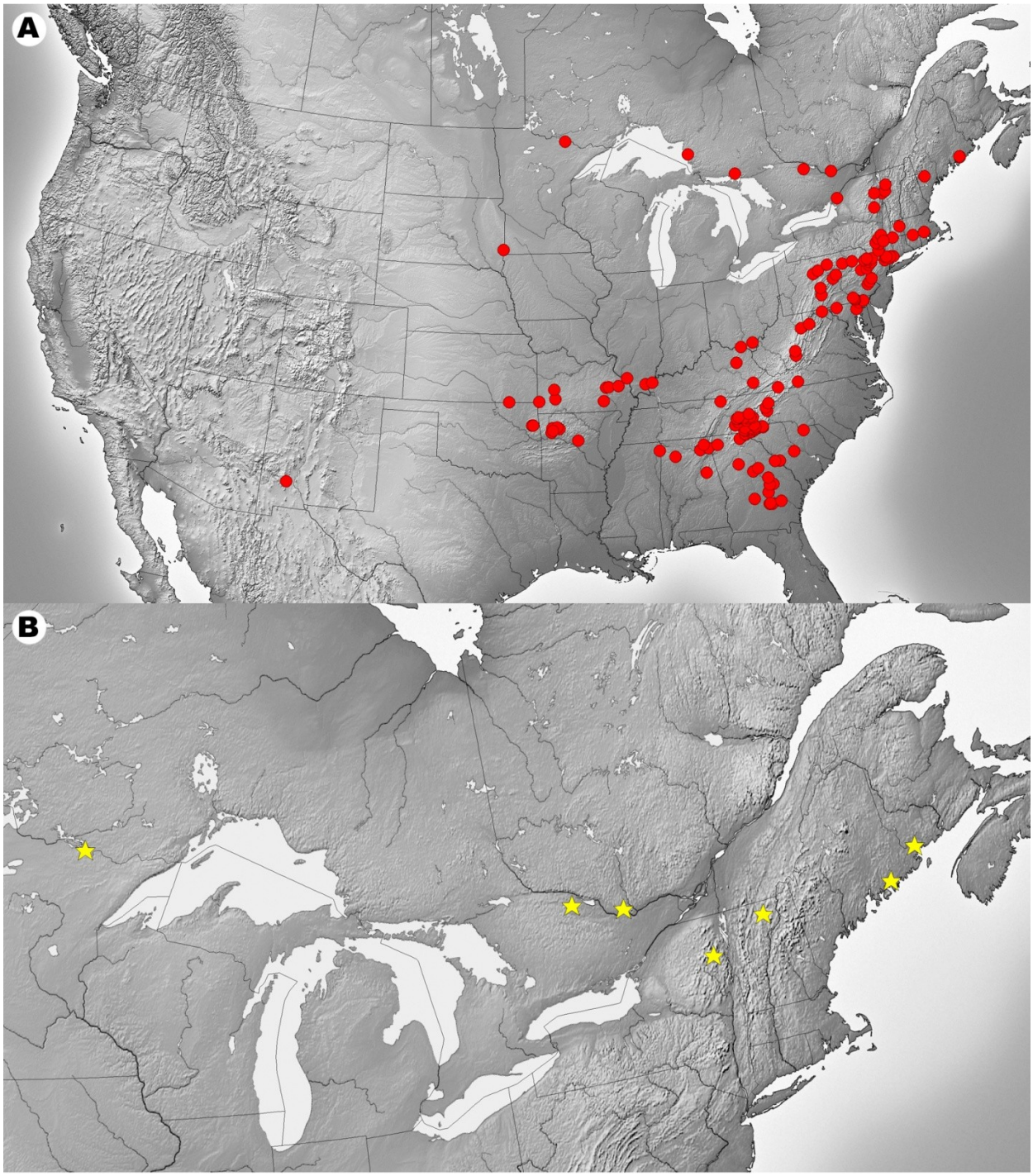

Figure 102, geographic distribution of *Lepraria normandinoides* based on the specimens examined for this study. A, protocetraric acid chemotype. B, acid deficient chemotype.

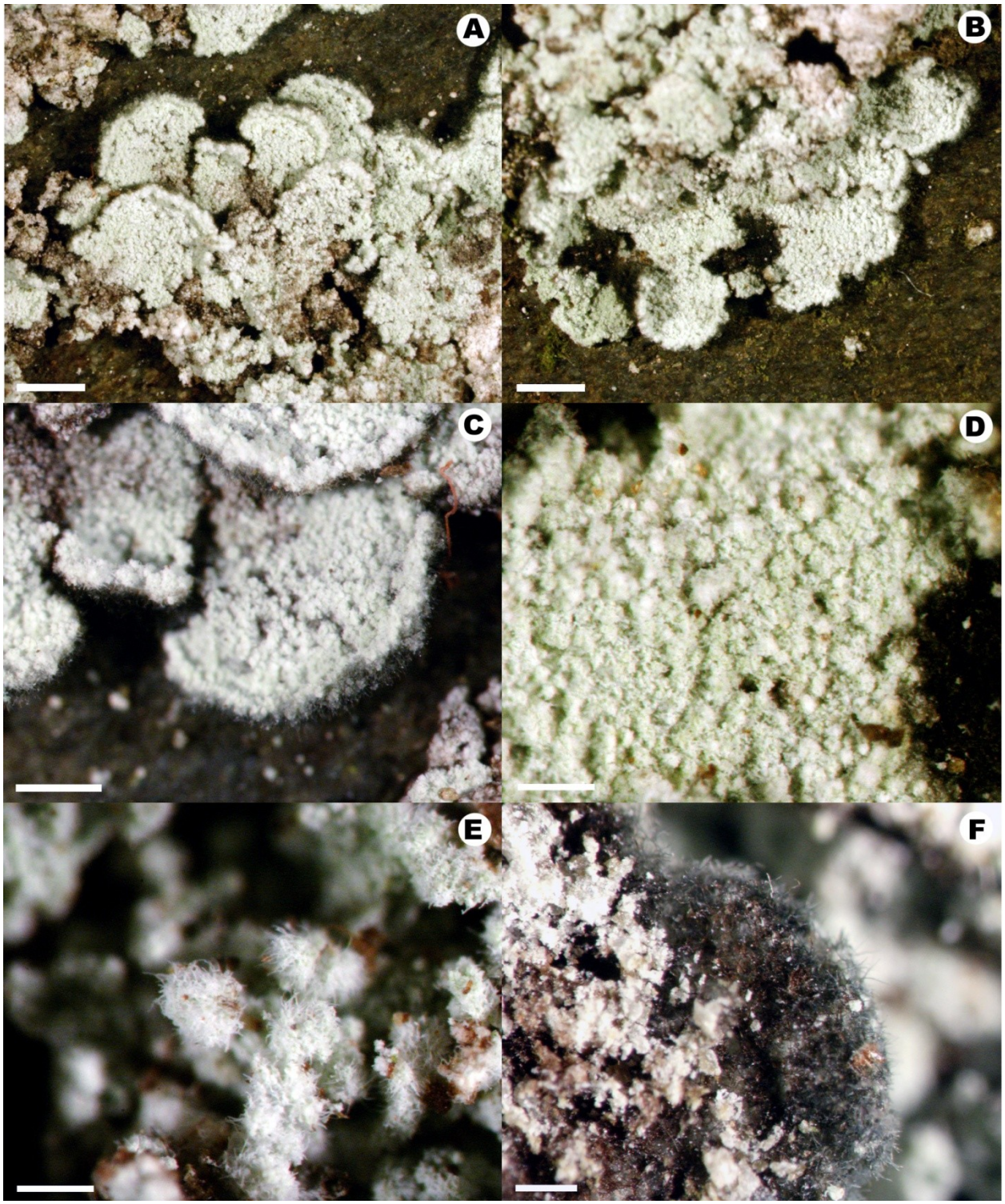

Figure 103, *Lepraria oxybapha* (all from *Lendemer 29526*). A–B, gross morphology of the thallus (scales = 1.0 mm). C, detail of the thallus margin (scale = 0.5 mm). D, detail of the thallus surface (scale = 0.25 mm). E, aggregation of granules becoming separated from the hypothallus (scale = 0.25 mm). F, dark pigmented rhizohyphae extending from the lower surface of the thallus (scale = 0.25 mm).

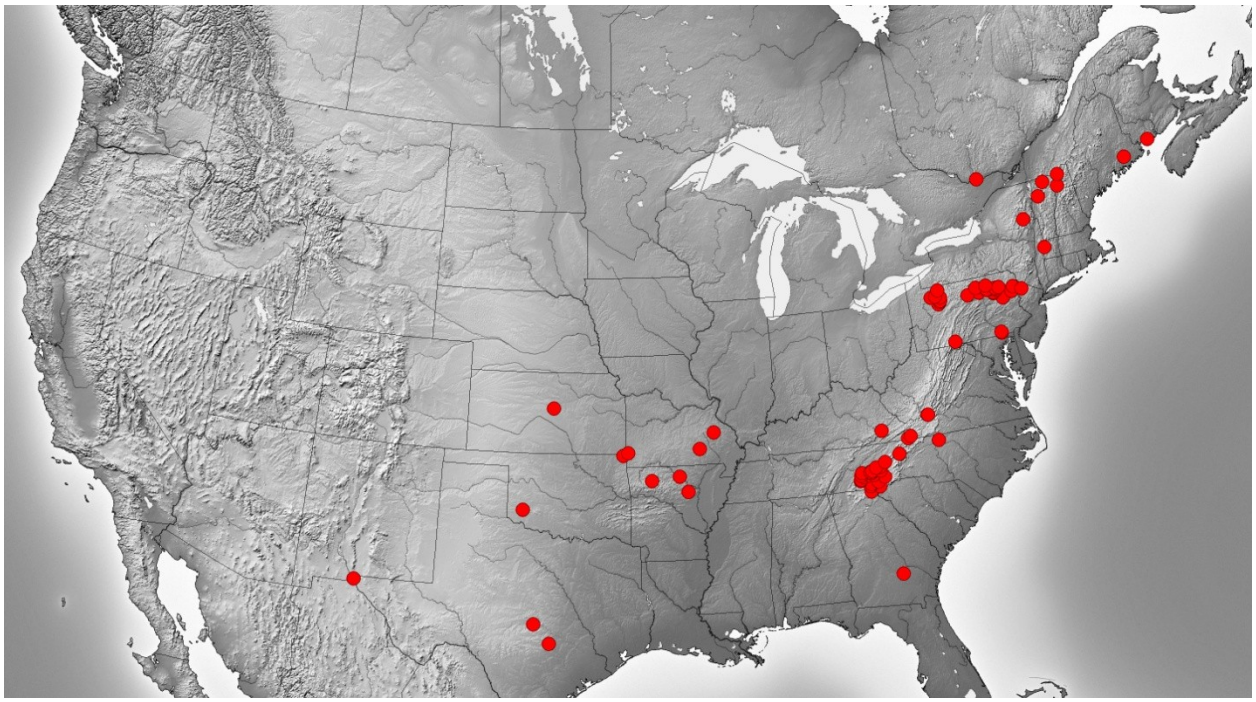

Figure 104, geographic distribution of *Lepraria oxybapha* based on the specimens examined for this study.

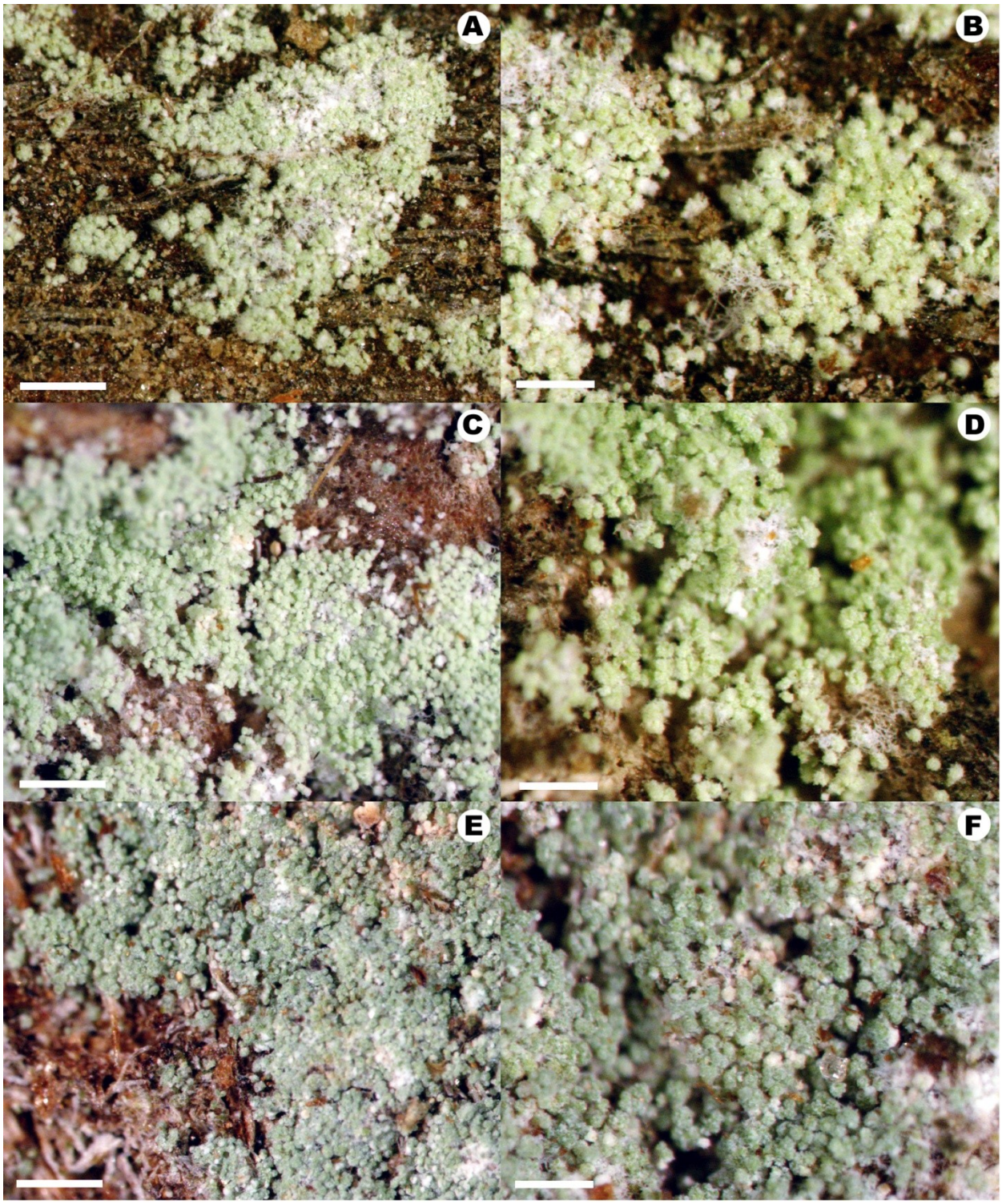

Figure 105, *Lepraria pacifica* (A–B from *Buck 53923*, C–D from *Lendemer 19532*, E–F from *Lendemer 19562*). A,C and E, gross morphology of the thallus (scales = 1.0 mm). B, D and F, detail of the granules (scales = 0.25 mm).

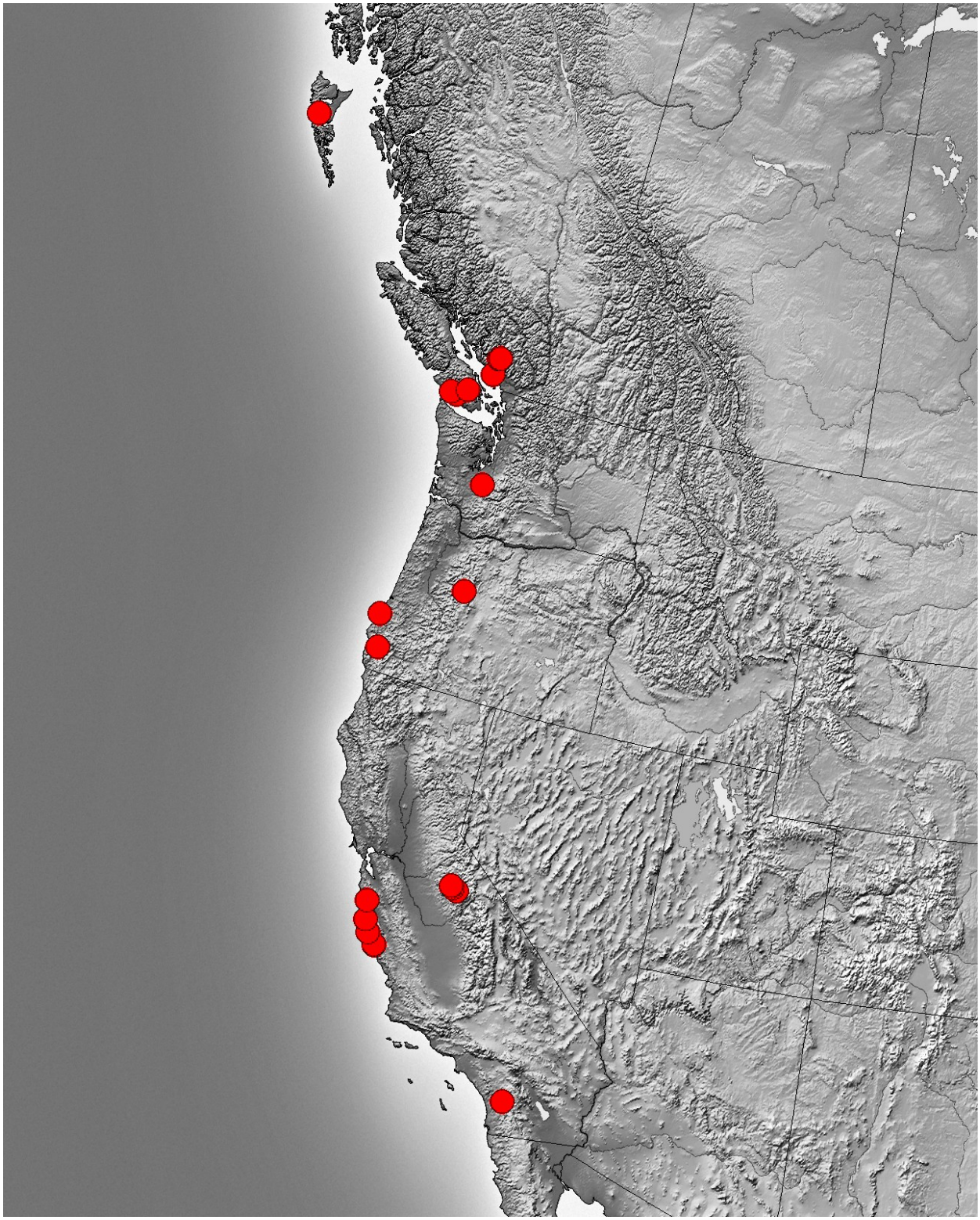

Figure 106, geographic distribution of *Lepraria pacifica* based on the specimens examined for this study.

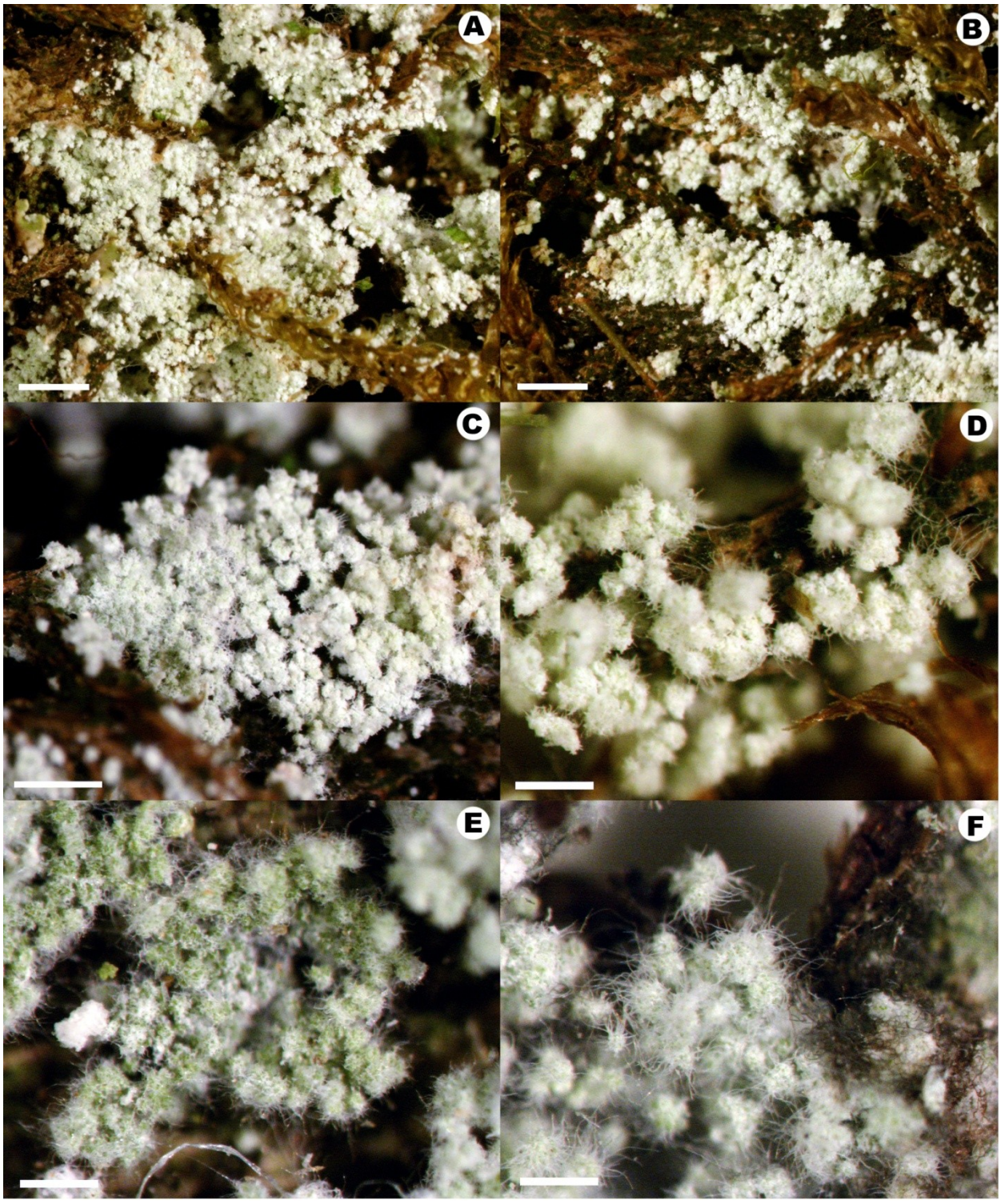

Figure 107, *Lepraria rigidula* (A–D from *Lendemer 22691*, E from *McCune 30967*, F from *Lendemer 14831*). A–B, gross morphology of the thallus (scales = 1.0 mm). C, detail of the thallus (scale = 0.5 mm). D–F, detail of the granules (scales = 0.25 mm).

Figure 108, geographic distribution of *Lepraria rigidula* based on the specimens examined for this study.

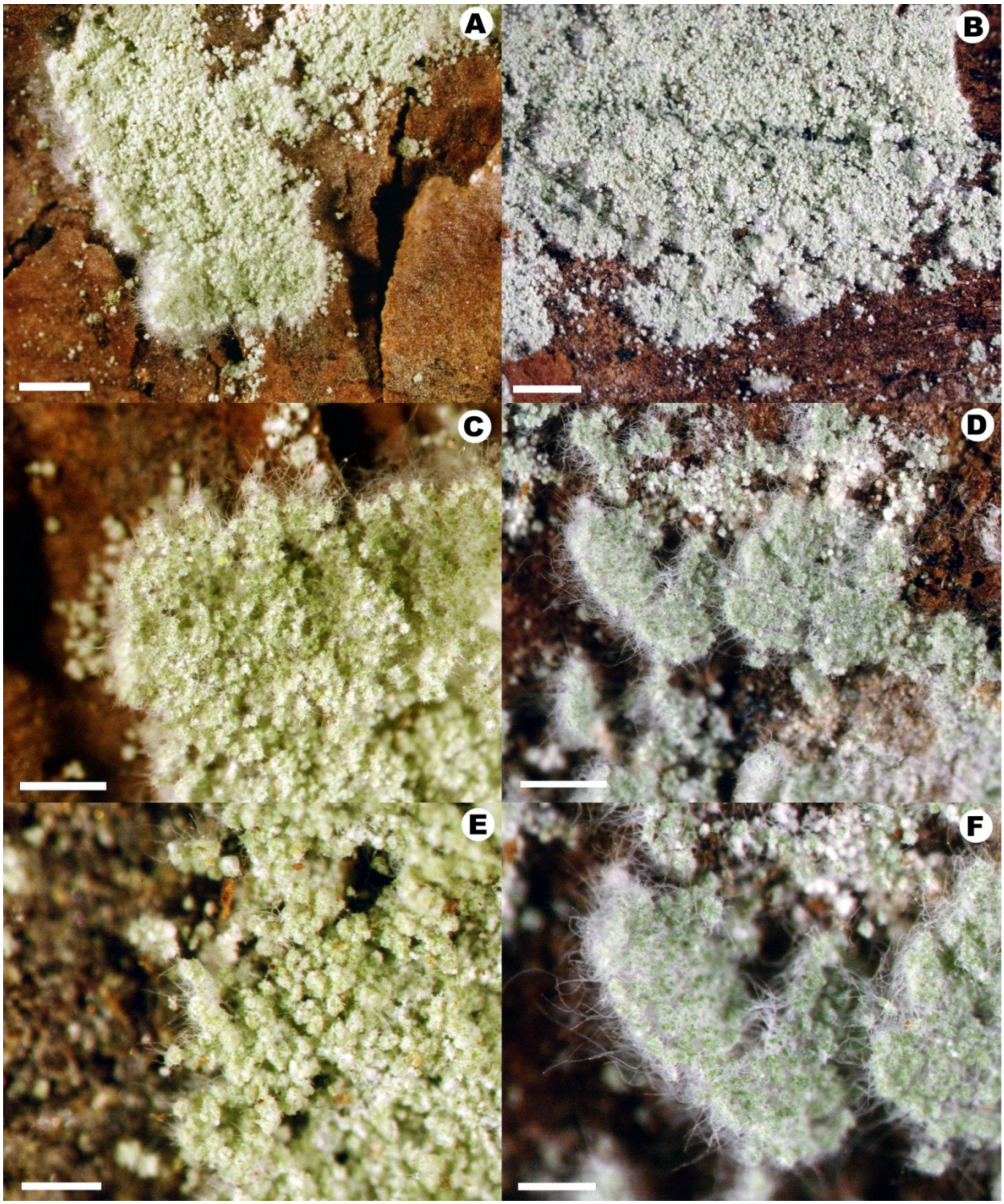

Figure 109, comparison of populations of *Lepraria squamatica* from North America (left, *Buck 54362*) and Australia (right, *Elix 37755*). A–B, gross morphology of the thallus (scales = 1.0 mm). C–D, detail of the thallus (scales = 0.5 mm). E–F, detail of the granules and thallus margins (scales = 0.25 mm).

Figure 110, geographic distribution of *Lepraria squamatica* based on the specimens examined for this study.

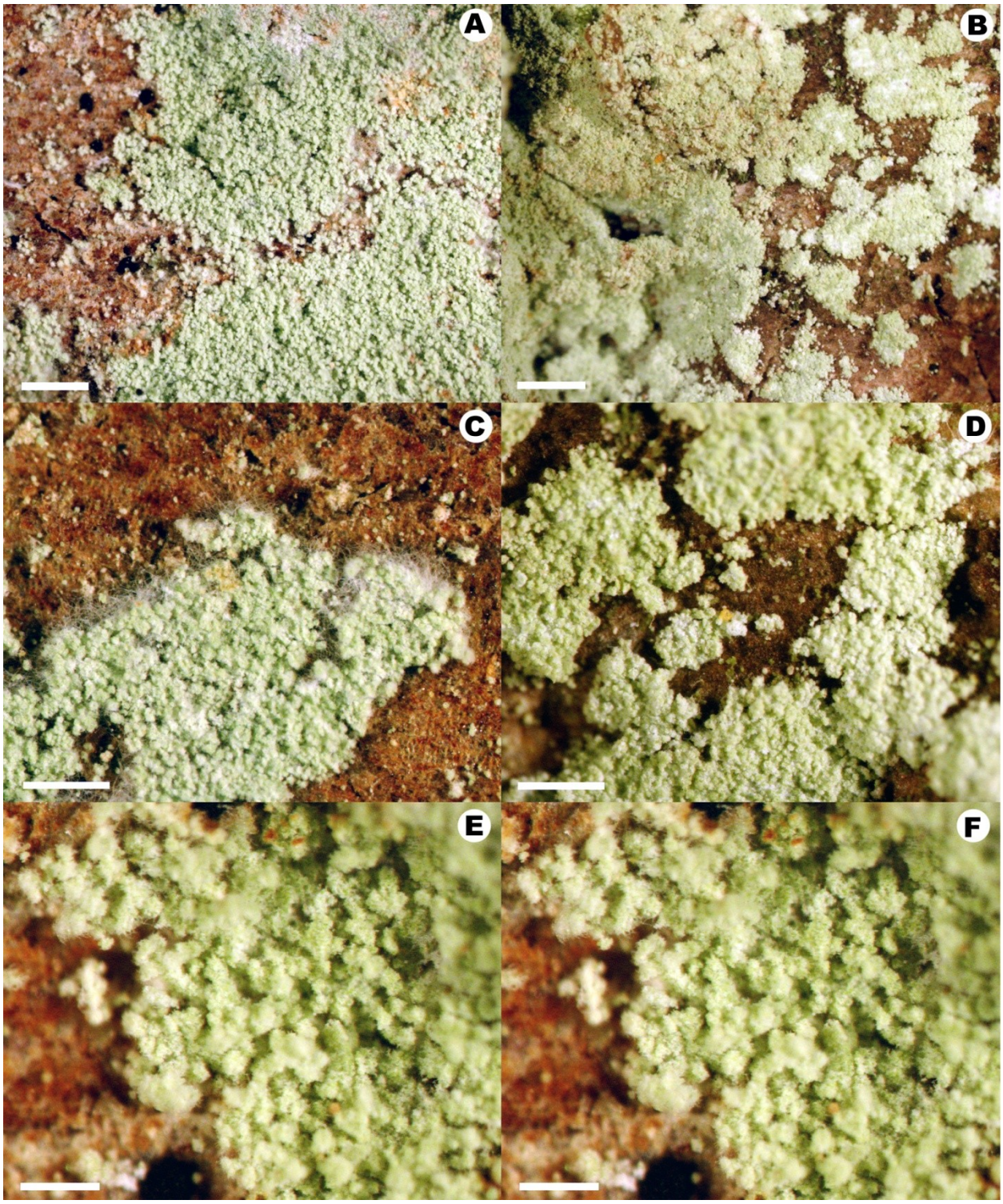

Figure 111, comparison of populations of *Lepraria torii* from western North America (left, *Lendemer 22423*) and eastern North America (right, *Lendemer 27536*). A–B, gross morphology of the thallus (scales = 1.0 mm). C–D, detail of the thallus (scales = 0.5 mm). E–F, detail of the granules and thallus margins (scales = 0.25 mm).

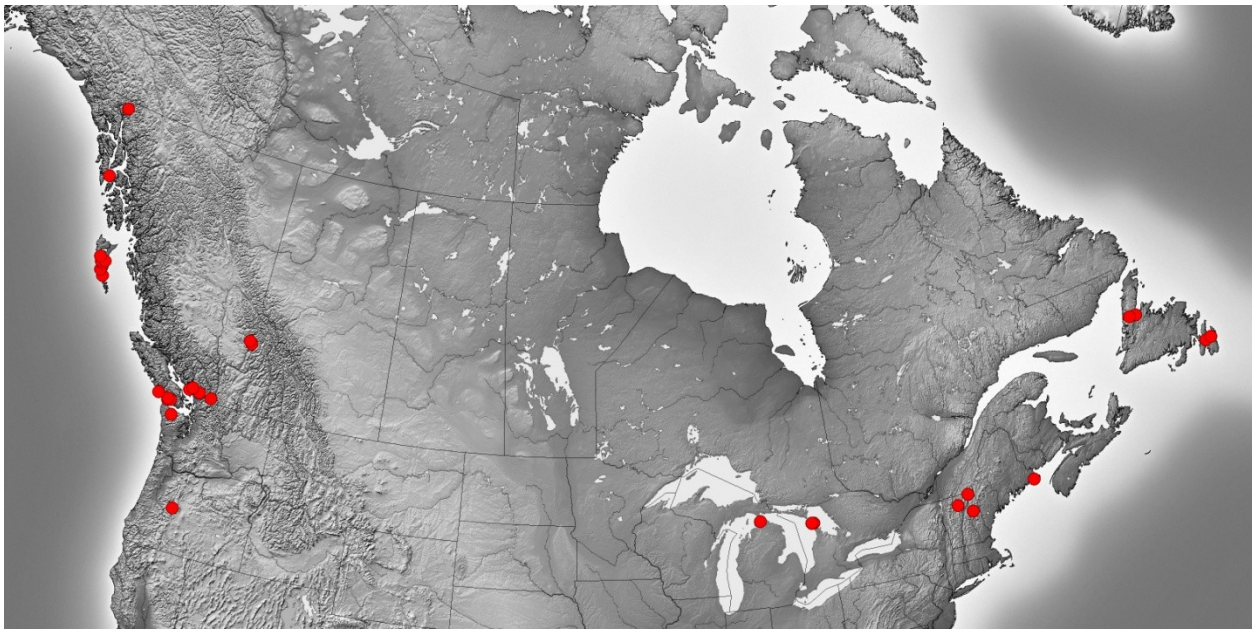

Figure 112, geographic distribution of *Lepraria torii* based on the specimens examined for this study.

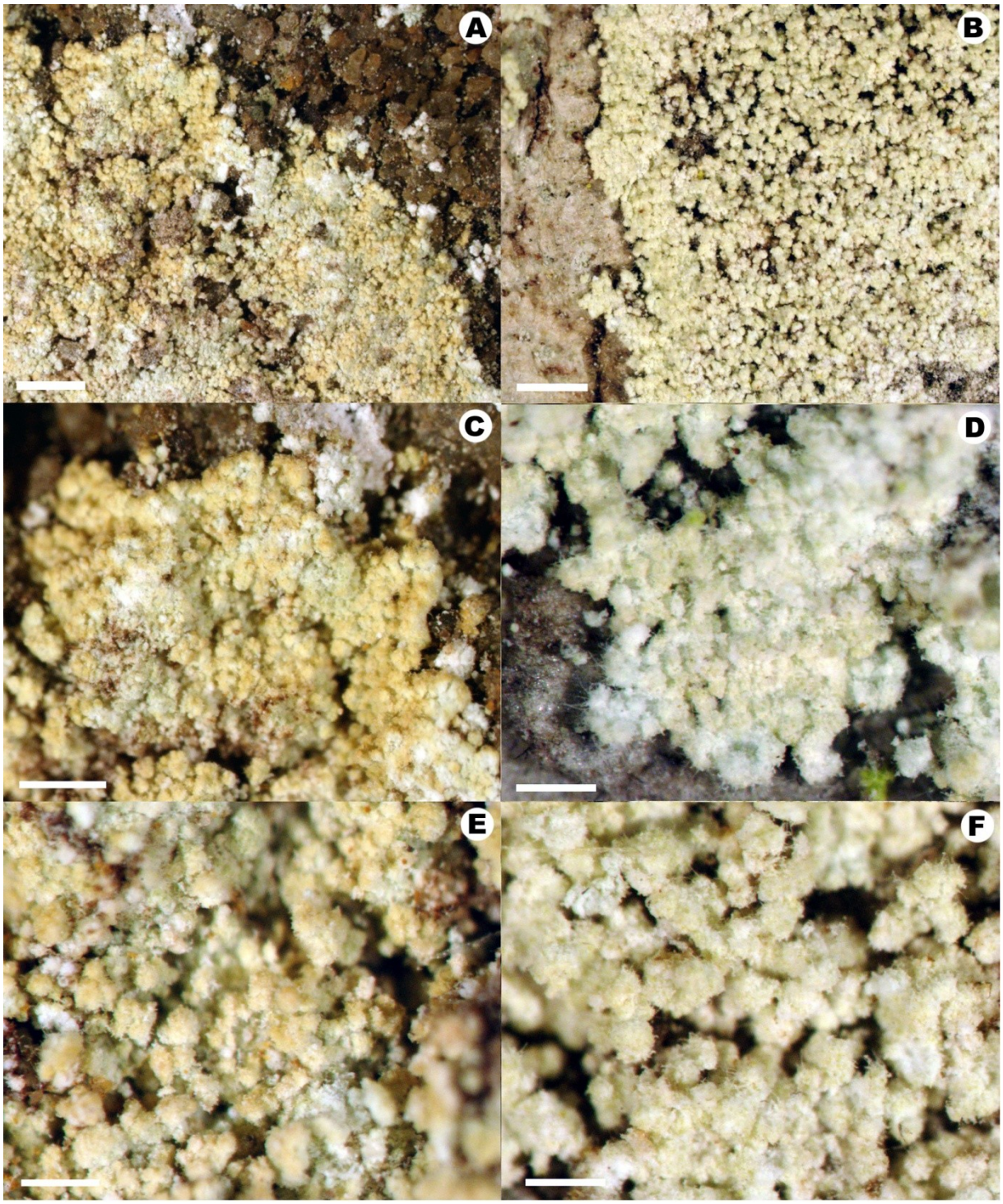

Figure 113, comparison of typical *Lepraria vouauxii* (right, *Lendemer 18816*) and its unnamed "pigmented" variant (left, *Buck 49831*). A–B, gross morphology of the thallus (scales = 1.0 mm). C–D, detail of the thallus margin (scales = 0.5 mm). E–F, detail of the granules (scales = 0.25 mm).

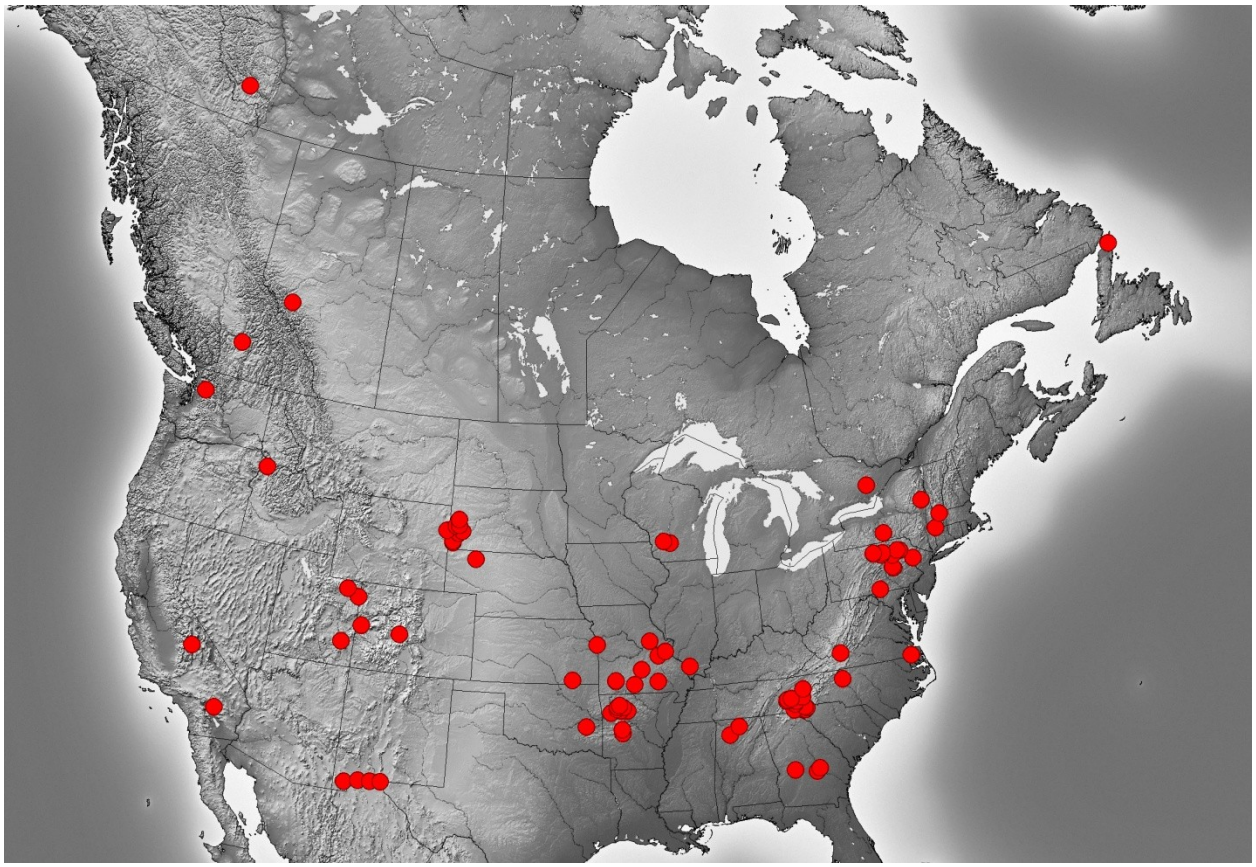

Figure 114, geographic distribution of *Lepraria vouauxii* based on the specimens examined for this study.

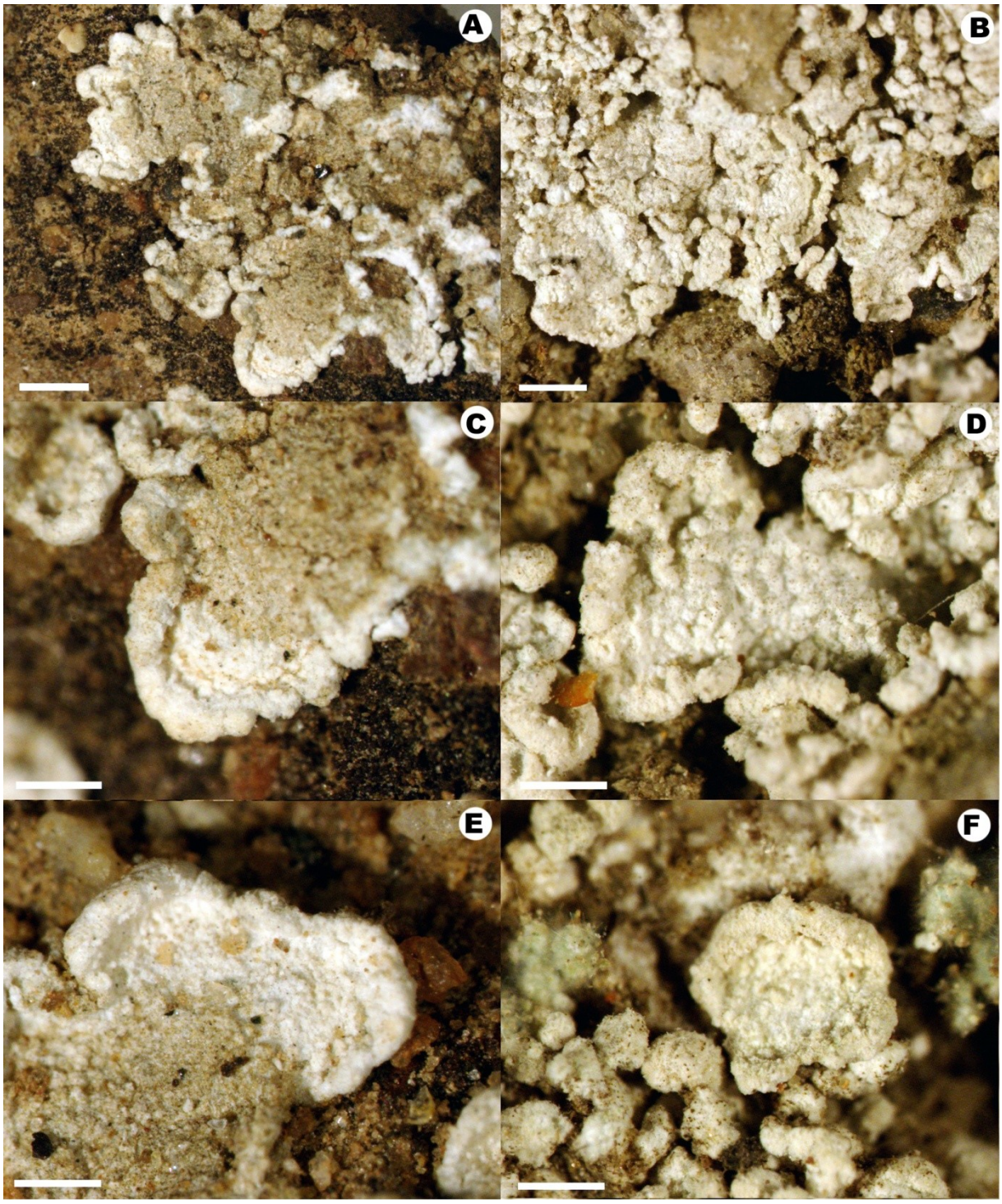

Figure 115. Comparison of the typical chemotype of *Lepraria xerophila* (left, *Bratt 5636*) and the chemotype that produces norascomatic acid (right, *Knudsen 7624*). A–B, gross morphology of the thallus (scales = 1.0 mm). C–E, detail of the thallus margins (scales = 0.5 mm). F, young thallus and large aggregations (scale = 0.5 mm).

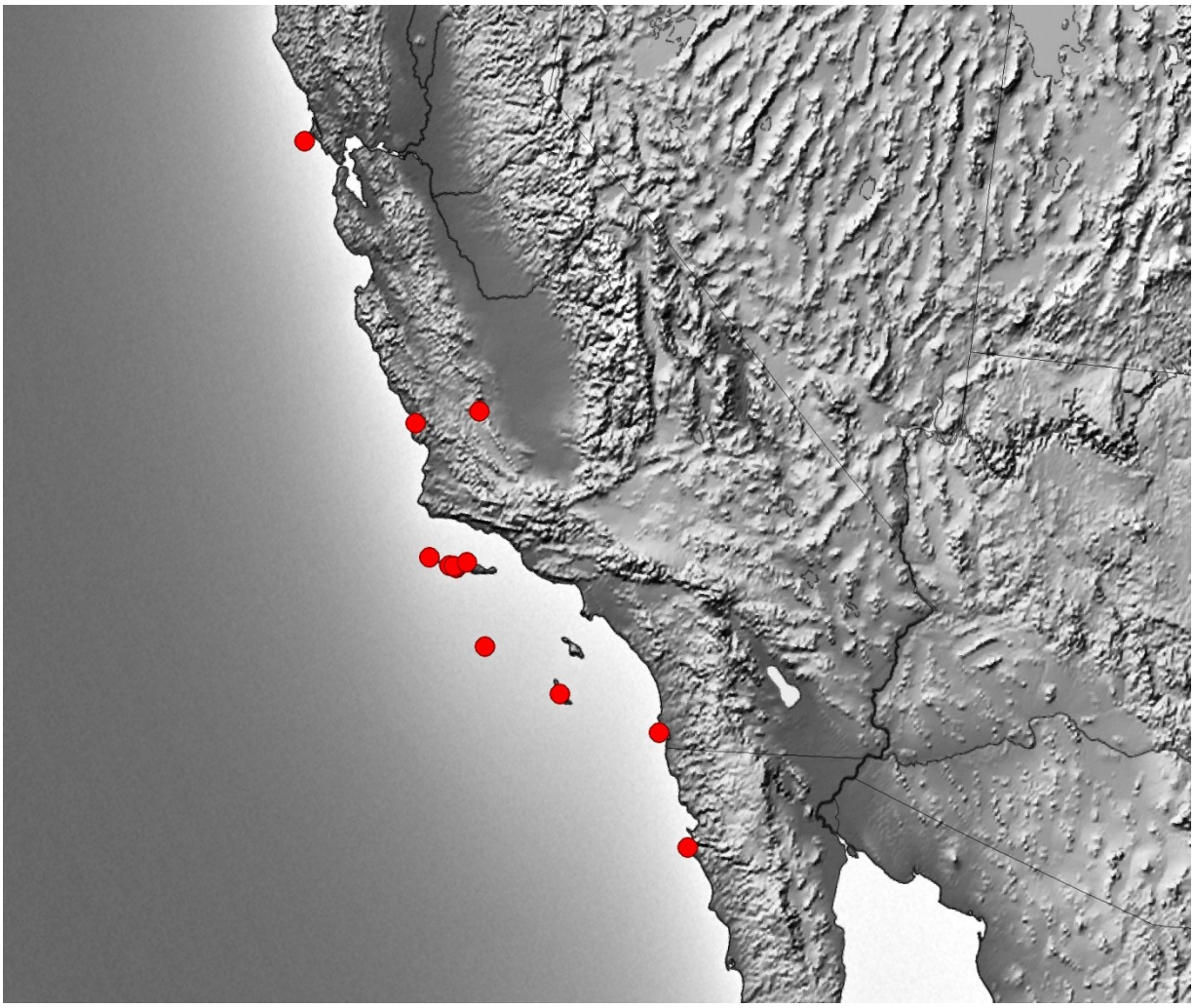

Figure 116, geographic distribution of *Lepraria xerophila* based on the specimens examined for this study.

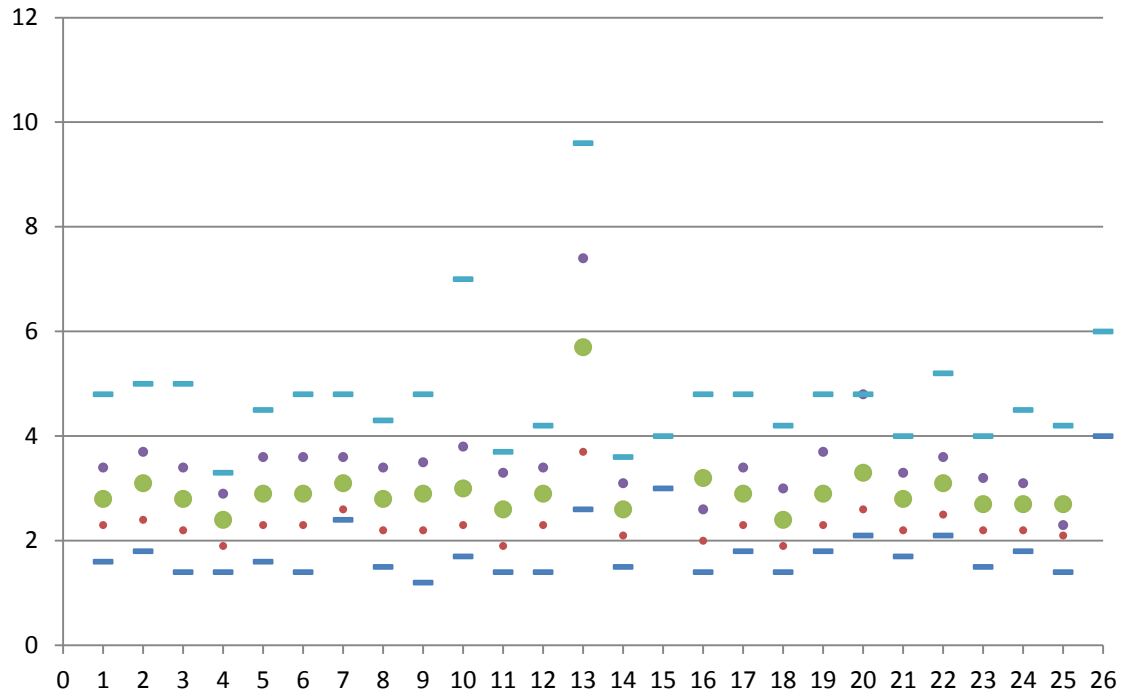

Figure 117, graphical representation of variability in hyphal width between species of *Lepraria* treated here. Numbers on the x-axis correspond to the species accounts herein, the y-axis refers to µm. Numerical ranges are illustrated as the average (large dot) +/- 1 standard deviation (small dots) and bounded by the extreme values observed (bars).

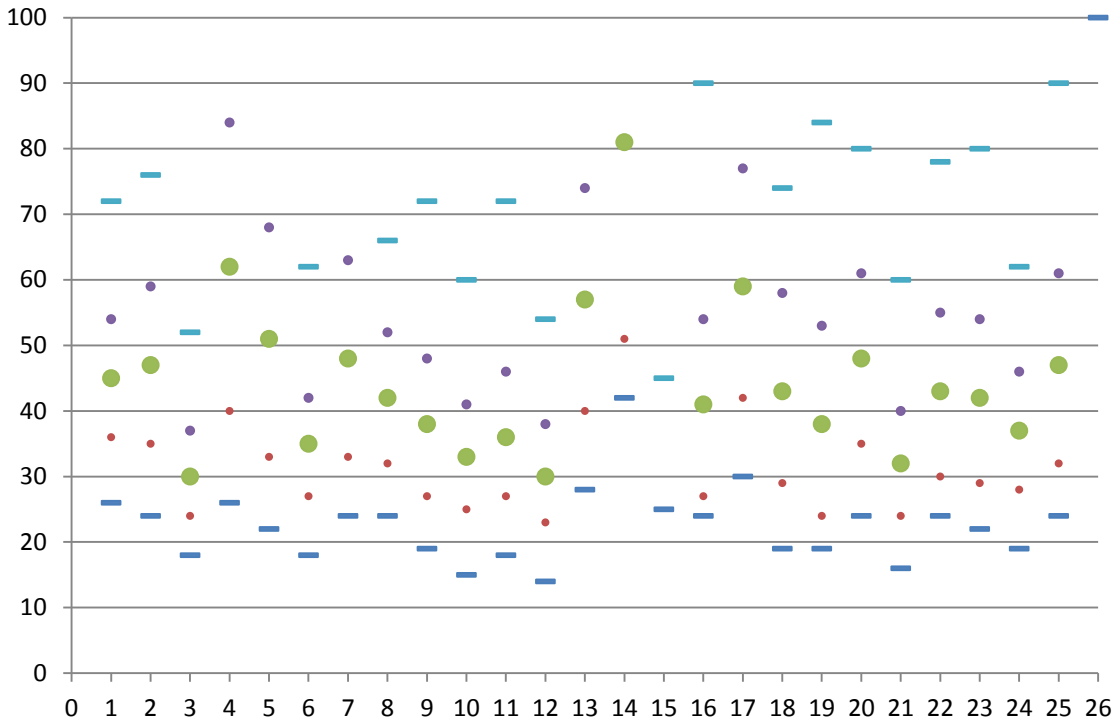

Figure 118, graphical representation of variability in granule size between species of *Lepraria* treated here. Numbers on the x-axis correspond to the species accounts herein, the y-axis refers to µm. Numerical ranges are illustrated as the average (large dot) +/- 1 standard deviation (small dots) and bounded by the extreme values observed (bars).

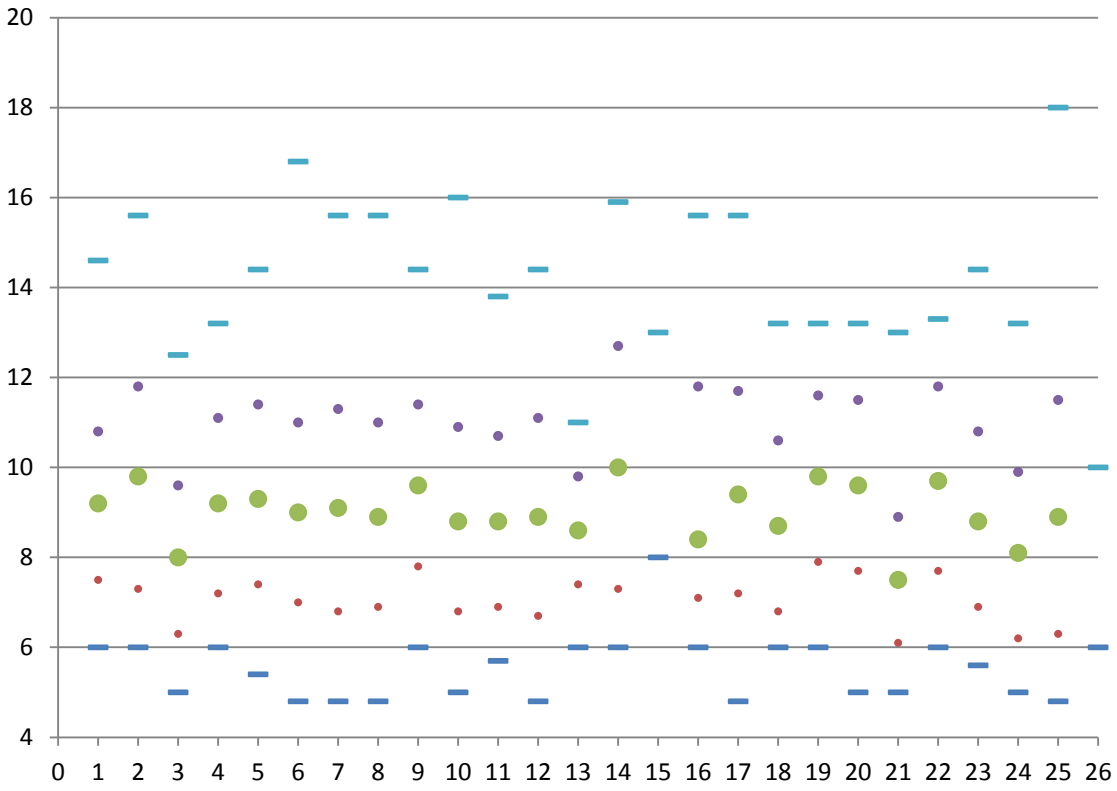

Figure 119, graphical representation of variability in photobiont cell diameter between species of *Lepraria* treated here. Numbers on the x-axis correspond to the species accounts herein, the y-axis refers to µm. Numerical ranges are illustrated as the average (large dot) +/- 1 standard deviation (small dots) and bounded by the extreme values observed (bars).

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