Discovery of Epiphytic Lichens in Connecticut Suggests Novel Introduction and Reintroduction via Horticultural Practices

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Discovery of epiphytic lichens in Connecticut suggests novel introduction and reintroduction via horticultural practices

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ABSTRACT. The discovery of Teloschistes chrysophthalmus in Connecticut more than one hundred years since its last known occurrence is argued to result from human introduction. The species only occurred on the horticultural tree, Gleditsia triacanthos var. inermis, planted on the University of Connecticut campus. Gleditsia triacanthos is not indigenous to northeastern North America, but is widespread in the central United States. Other epiphytic macrolichens also recorded on this phorophyte include Punctelia bolliana and Parmotrema austrosinense, both widespread in the central United States, and new to Connecticut and New England, respectively. This is likely the first reported case of combined introductions of lichenized fungi in North America through the import of ornamental trees.

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Humans do not immediately come to mind as direct dispersers of lichens. However, they frequently transport substrates with lichens growing on them, e.g., firewood, soils and horticultural plants (Aptroot 2011; Huiskes et al. 2014; Osyczka 2010), and similarly, directly collect and transport lichens as crafting materials. Despite this frequent human-mediated transport, inadvertent human introductions of lichens to new areas are rarely explicitly documented. One example is *Lecanora conizaeoides* Nyl. ex Cromb., a crustose and pollution tolerant lichen native to Europe that was likely introduced to North America through trade of imported timber carrying soredia (LaGreca & Stutzman 2006). In contrast to the many mycorrhizal fungi and plant diseases dispersed via the horticultural trade (Healy et al. 2016; Shear & Stevens 1926), only two lichenized fungi are thought to have had expanded ranges caused by distribution of ornamental trees in North America: *Physcia millegrana* Degel., considered introduced to California via elm trees sold from a New Jersey nursery (Thomson 1963), and *Xanthoria parietina* (L.) Th.Fr., which is extending its predominantly coastal distribution in the Pacific Northwest inland through the distribution of ornamental trees (Fraser et al. 2016). Similar range expansions driven by the horticultural (trees) or landscaping (rocks) trade were also reported from Europe (Aptroot 2010, 2011; Aptroot & Toetenel 2011; Lepista & Aptroot 2016; Nygaard & Tønsberg 2015), New Zealand (Galloway 1998), Hawaii (Moncada et al. 2014), and circumstantially in Mexico (Gregorio-Cipriano et al. 2016).
While collecting on the campus of the University of Connecticut, the first author observed *Teloschistes chrysophthalmus* (L.) Th.Fr. (Fig. 1) occurring on four ornamental thornless honeylocusts (*Gleditsia triacanthos* L. var. *inermis*), a tree native to the central United States and widely distributed for horticultural purposes (Thompson et al. 1999). Twenty-one thalli of *T. chrysophthalmus* occurred on these trees, and this species was not observed on any of the nearby trees. Other epiphytes associated with these four honeylocusts appeared also to not be known from Connecticut, though their geographic range overlapped with that of *Gleditsia triacanthos*. This prompted the hypothesis that the import of these trees from nurseries resulted in the introduction of these lichens. We report on the identity of these species and discuss the circumstantial evidence supporting this hypothesis.

**Materials and Methods**

The lichens were collected on four trees occurring in a walkway on the campus of the University of Connecticut (Tolland County, Storrs) near the Thomas J. Dodd Center (405 Babbidge Road Storrs, UCONN arboretum tree numbers 6385–8, 41.806278°N, 72.250691°W, 1 July 2020; Fig. 3F). The trees are part of the University of Connecticut Arboretum, and were planted at least 15 years ago (all are ~9.5 cm diameter at breast height). Records of their purchases could not be located. All lichen vouchers collected by H. Frye or H. Frye & B. Goffinet are deposited in the Herbarium of the University of Connecticut (CONN).

**Lichen identification.** Lichen thalli were examined for morphological characters under a Leica MZ7.5 stereoscopic microscope. Standard chemical spot tests were used to identify lichen secondary compounds, and thin-layer chromatography using solvent C was performed to confirm the identity of each of these compounds (Orange et al. 2001). The morphological and chemical
characters scored are presented in Supplementary Table S1A. The lichen thalli were identified using a variety of regional taxonomic keys (e.g., Brodo et al. 2001; Harris & Ladd 2005; Hinds & Hinds 2007). We also measured thallus size for lichens that could be distinguished by morphology alone. This excluded some species within Parmotrema because morphological species concepts may not be upheld by phylogenetic analyses of DNA data.

**DNA extraction, amplification, and sequencing.** To confirm the chemical and morphological identification of the specimens, exemplars from each morphological group were selected for DNA extraction. DNA was extracted using the NuceloSpin® Plant II kit. Following the successful extraction of DNA, PCR was conducted to amplify fungal ITS using primers ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990). Purified PCR products were sent for dual direction sequencing at Eurofins Genomic, and sequences were assembled and manually edited in Geneious Prime 2020.2.2 (Auckland, New Zealand).

**RESULTS & DISCUSSION**

Morphological and chemical analysis resulted in the identification of one Teloschistes species and five prominent parmelioid taxa (within Parmotrema and Punctelia) and the identity of a subsection of these was largely corroborated with our molecular data. Fungal DNA sequences were deposited in GenBank under the accession numbers MW311305—MW311317 and are listed with their corresponding collections in Supplementary Table S1A.

**Teloschistes chrysophthalmus** (L.) Th. Fr. (Fig. 1)

The golden-eye lichen is most distinctive by its bright color and fruticose growth form. The species occurs on all four Gleditsia trees, and individual thalli reach 0.7 cm in height and
roughly 1.5 cm at the largest diameter (See Supplementary Table 1B for thalli measurements). All individuals are apotheciate. This species was exclusively found on these trees and not observed on other nearby, native or ornamental, trees.

While *Teloschistes chrysophthalmus* commonly appears on a variety of substrates and was historically distributed widely across North America (Brodo 2001; Howe 1915), its range severely declined with extant populations considered endangered/vulnerable in Canada (Lewis & Brinker 2016). In New England (Fig. 2), the last record of *T. chrysophthalmus* dates from a 1938 observation in Nantucket (Hinds & Hinds 2007) and in Connecticut, the species has not been recorded since 1911 (FH-00480839), despite subsequent surveys in Connecticut (Evans & Meyrowitz 1926; Metzler 1980) and New England (Brodo 1968). Habitat loss and air pollution may be major factors underlying lichen decline in the northeastern United States (Hinds & Hinds 2007), while the hurricane of 1938 might, earlier, have caused enough habitat loss to extirpate *T. chrysophthalmus* from Long Island and coastal New England (Brodo 1968). Recent reports of this species from Ohio (Riley 2011) and New York (pers. comm. R. C. Harris 2019) suggest an eastward expansion of *T. chrysophthalmus* to areas that it once inhabited. This expansion, perhaps mediated in part by the horticultural trade, suggests that ameliorating atmospheric conditions are allowing a return into a once native range. Indeed, a recent collection of the species in Oswego Co., New York (*P. DeSanto s.n. (NY)*) was also made in a residential lawn on an exotic tree, *Cercis canadensis* L. (eastern redbud), a species predominantly found in the central and southeastern United States, and widely planted elsewhere.

*Parmotrema austrosinense* (Zahlbr.) Hale (Fig. 3A)

Eighteen thalli were observed on the four *Gleditsia* trees, and individual thalli reached 10.0 cm in diameter (Supplementary Table S1C). *Parmotrema austrosinense* has never been
documented in New England, but primarily occurs in the lower central and southeastern United States (CNALH 2021). Within the past decade, two specimens were collected in New Jersey (
\textit{D.P. Watters 1759 (NY); Lendemer 51290 (NY)}) perhaps indicating a recent range expansion, though the mechanism is unclear. Moreover, a lichen survey of Virginia (Hodkinson & Case 2008) noted the presence of \textit{P. austrosinense} outside of its range on another cultivated tree (\textit{Buxus sempervirens} L.) suggesting that the species may have been introduced to new areas via human-mediated activity. The samples from Connecticut produced lecanoric acid and developed marginal soredia. DNA sequences of the three specimens share >98\% similarity with other sequences from GenBank identified as \textit{P. austrosinense}, confirming our morphological and chemical identifications.

\textit{Parmotrema perforatum} s.str. (Jacq.) A.Massal. & \textit{P. hypotropum} s.l. (Nyl.) Hale (Fig. 3B)

Among the collections, ten \textit{Parmotrema} lichen thalli contained norstictic acid and identified as either \textit{P. perforatum} or \textit{P. hypotropum} based on the absence or presence of soredia, respectively. However, ITS sequences for a subset of our specimens suggest that all specimens belong to \textit{P. perforatum} s.str. (\textit{fide} Widhelm et al. 2016), an observation congruent with the recent hypothesis that the mode of reproduction does not distinguish two monophyletic taxonomic entities (Lendemer et al. 2015; Widhelm et al. 2016). Historical floristic inventories did not have the luxury of using DNA to identify material, necessitating the exclusive use of morphological and chemical species concepts. The morphological/chemical species concept has likely been applied to the majority of the material in New England and global herbaria and thus may have resulted in misidentification of members of the \textit{P. perforatum} group in historical collections. Neither \textit{P. perforatum} nor \textit{P. hypotropum} are regarded as rare in New England;
however, reevaluating historical collections in the light of the work by Widhelm et al. (2016) may yield a different conclusion.

*Parmotrema reticulatum* (Taylor) M. Choisy (Fig. 3C)

Two specimens were identified as *Parmotrema reticulatum* based on chemical and morphological traits. Molecular data corresponding to these collections confirm these identifications and place these specimens within *P. reticulatum* s.l., a paraphyletic species complex (Del-Prado et al. 2011, 2016; Divikar et al. 2005). *Parmotrema reticulatum* is known from multiple collections in New England, and from other collections across much of the United States (CNALH 2021).

*Punctelia bolliana* (Müll. Arg.) Krog (Fig. 3D)

The four collected epiphytic thalli match the morphological and chemical concept of *Punctelia bolliana* (Harris & Ladd 2005). The largest thallus reached 5.5 cm in diameter (Supplementary Table S1D). Given the lack of reference sequences for this taxon, we could not corroborate our identification on the basis of DNA sequences. Our sequences (i.e., MW311315 & MW311316) are nearly identical to the GenBank entry GU994579 labeled “*Punctelia* sp. Cole 11219” (see Crespo et al. 2010), a collection for which further details are scant (i.e., locality is simply referred to as “U.S.A.”) and that could not be located in any public herbarium database. While we are confident in our morphological and chemical identification, it is possible that the “sp.” designation represents a novel cryptic species potentially suggested by Crespo et al. (2010). *Punctelia bolliana* is known from four collections in New England (one in Vermont and three in New Hampshire), but occurs in New York and is increasingly frequent toward the central United States (CNALH 2021).

*Punctelia rudecta* s.l. (Ach.) Krog (Fig. 3E)
An additional Punctelia collection (CONN 227227B) is unique in that its morphology matches that of *P. hypoleucites*, as it is abundantly pycnidiate and lacking soredia and isidia. ITS sequence data align this specimen to the *Punctelia rudecta* s.l. clade (fide Alors et al. 2016). Other specimens from GenBank identified as *P. hypoleucites* (e.g., HQ650685 & MK213353) form a monophyletic group within *P. rudecta* s.l., however, *P. hypoleucites* may be polyphyletic (Alors et al. 2016). *Punctelia rudecta* is common across New England and much of the eastern and central United States (CNALH 2021), but the morphology of this specimen is unlike others reported from the region.

**A CASE FOR HUMAN-MEDIATED DISPERSAL**

At least three of the lichenized fungi recorded on ornamental *Gleditsia triacanthos* planted on the campus of the University of Connecticut are either newly reported in New England (*Parmotrema austrosinense*), the state of Connecticut (*Punctelia bolliana*), or they are recorded for the first time in Connecticut in a century (*Teloschistes chrysophthalmus*). Given that the species co-occur on the same four imported trees, are absent on taller neighboring indigenous trees, and have established ranges outside of New England in the central and eastern United States, where they are sympatric with *Gleditsia triacanthos*, their occurrence in Connecticut is likely the result of an introduction linked to the importing of the ornamental trees. We hypothesize that the trees were acquired from a nursery in the central United States and that these plants harbored lichens from their home range prior to their transport to Connecticut. Although we only provide circumstantial evidence, a combined, highly localized co-occurrence of these species outside of their main range is unlikely due to random, converging immigrations.
For *Teloschistes chrysophthalmus* the case can be made that its discovery represents a re-introduction into its original native range (Fig. 2). To our knowledge, this is one of the few documented examples of a lichen being re-introduced to a plausible native range via the horticulture trade (Aptroot 2010). *Parmotrema austrosinense* (Fig. 3A) may represent an introduction of an “exotic” species and evidence from previous surveys indicates its capacity to be transported on cultivated trees as well (Hodkinson & Case 2008). It is, however, possible that *P. austrosinense* was extirpated from New England in the 1800’s during a time of heavy land use (i.e., conversion of forest to pasture) and industrialization. It may not have returned naturally due to the lack of suitable conditions or to natural geographic barriers to its dispersal, such as the Appalachian Mountains. As for *Punctelia bolliana* (Fig. 3D), the species is known from New England from a single record from Vermont and three records from New Hampshire. It becomes increasingly frequent from New York westward to the central United States and Canada, with a scattered distribution in the Southwest and being absent from the remainder of western North America except for rare (unconfirmed) collections in western Canada (Brodo 14962). Given it is largely sympatric with *T. chrysophthalmus* and *Parmotrema austrosinensis*, and that all three species are sympatric with *Gleditsia triacanthos*, it seems indeed as most likely that all four species were imported together to Connecticut. Previous evidence suggests that while imported lichens are likely to survive on their phorophyte after relocalization, their chances of dispersal to surrounding areas are low (Sparrius et al. 2014). It remains to be seen whether or not this small community of introduced species will spread to surrounding phorophytes and either expand or re-establish its range.

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**Literature Cited**


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Supplementary documents online:

Supplementary Tables S1A–D. Chemical and morphological characteristics of lichen.

Table 1A: Chemical and morphological characters for parmelioid lichens.
Table 1B: Counts and measurements of Teloschistes chrysophthalmus.
Table 1C: Counts and measurements of Parmotrema austrosinensis.
Table 1D: Counts and measurements of Punctelia bolliana.
Figure 1. A thallus of *Teloschistes chrysophthalmus* growing on *Gleditsia triacanthos*, an ornamental tree planted on the University of Connecticut campus.
**Figure 2.** Maps comparing historic and recent *Teloschistes chrysophthalmus* occurrences at the county level in New England and surrounding states. The year 1938 marked the last time the species was found in New England until 2019.
Figure 3. Images of lichen species discussed in the text. A. *Parmotrema austrosinense*. B. *Parmotrema perforatum*. C. *Parmotrema reticulatum*. D. *Punctelia bolliana*. E. *Punctelia rudecta* s.l. (*P. hypoleucites* morphology). F. *Gleditsia triacanthos*, the phorophyte on which the lichens were found.