Hudsonia ericoides and Hudsonia tomentosa: Anatomy of mycorrhizas of two members in the Cistaceae from Eastern Canada

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Abstract: Most species in the family Cistaceae are found in the Mediterranean basin. Several hosts are of special interest, owing to their associations with truffle species, while many are important as pioneer plants in disturbed areas and in soil stabilization. For these reasons, understanding their root systems and their associated fungal symbionts is important. Most studies of the structure of mycorrhizas in this family involve two genera, Cistus and Helianthemum. The present study examines structural features of mycorrhizas in two North American species, Hudsonia ericoides L. and Hudsonia tomentosa Nutt. Root systems of both species are highly branched with most fine roots colonized by mycorrhizal fungi. Based on morphological features, several mycorrhizal fungi were identified; structural details also provided evidence of more than one fungal symbiont for each host species. All mycorrhizas had a multi-layered fungal mantle and Hartig net hyphae confined to radially elongated epidermal cells; no intracellular hyphae were observed. Although the Hartig net was confined to the epidermis, the outer row of cortical cell walls lacked suberin, a known barrier to fungal penetration. Mycorrhizas in H. ericoides and H. tomentosa differed from those of Cistus and Helianthemum species that have a Hartig net that extends into the root cortex, as well as frequently present intracellular hyphae.

Key words: Cistaceae, Hudsonia, mycorrhiza, anatomy, Hartig net, mantle.

Résumé : La plupart des espèces de la famille des Cistaceae se retrouvent dans le bassin de la Méditerranée. Plusieurs hôtes présentent un intérêt particulier, compte tenu de leurs associations avec des espèces de truffes, alors que d'autres constituent des plantes pionnières importantes dans les endroits perturbés et pour la stabilisation des sols. Pour ces raisons, il importe de comprendre la biologie de leurs systèmes racinaires et de leurs champignons symbiotiques associés. La plupart des études publiées sur la structure des mycorhizes de cette famille portent sur les deux genres Cistus et Helianthemum. On examine ici les caractéristiques structurales des mycorhizes de deux espèces Nord-américaines, l’Hudsonia ericoides L. et l’Hudsonia tomentosa Nutt. Chez les deux espèces, l’on observe des systèmes racinaires fortement ramifiés, la plupart des racines fines étant colonisées par des champignons mycorhiziens. Sur la base des caractéristiques morphologiques, les auteurs ont pu identifier plusieurs espèces de champignons mycorhiziens; les détails des structures fournissent également des preuves de l’existence de plus d’un symbiote fongique chez chaque espèce hôte. Toutes les mycorhizes montrent un manteau fongique à plusieurs couches et des hyphes du réseau de Hartig confinées aux cellules épidermiques radialement allongées; on observe aucune pénétration intracellulaire. Bien que le réseau de Hartig soit confiné à l’épiderme, les parois cellulaires de la couche externe de cellules corticales ne possèdent pas de subérine, une barrière reconnue pour la pénétration fongique. Les mycorhizes chez les H. ericoides et H. tomentosa diffèrent de celles des espèces de Cistus et d’Helianthemum, lesquelles possèdent un réseau de Hartig s’étendant dans le cortex racinaire et montrent la présence d’hyphes intracellulaires souvent présentes.

Mots-clés : Cistaceae, Hudsonia, mycorhize, anatomie, réseau de Hartig, manteau.

[Traduit par la Rédaction]
Figs. 1–8. Figs. 1–5. *Hudsonia ericoides*. Fig. 1. Plant (arrow) growing in sand along an abandoned railroad west of Kingston, Nova Scotia. Fig. 2. Close-up of plant in similar sandy habitat. Trowel scale is in millimetres. Fig. 3. Portion of the root system showing diverse size of mycorrhizal roots and several morphotypes (arrows). Fig. 4. White rhizomorphic mycorrhiza showing beaded appearance due to growth increments (arrowheads) along the length of the root, and large attached rhizomorphs (arrow). Scale as in Fig. 5. Fig. 5. Brown *Tomentella*-like morphotype showing swollen root tips (arrows) and abundant extraradical hyphae. Figs. 6–8. *Hudsonia tomentosa*. Fig. 6. Excavated seedling showing the highly branched root system. Scale is in millimetres. Fig. 7. Portion of the same root system showing two morphotypes: *Cenococcum* mycorrhizas (arrowhead) and white rhizomorphic mycorrhizas (double arrowhead) with large rhizomorphs (arrows). Fig. 8. Mycorrhizal root showing recolonization by *Cenococcum* (at the apex) and the white rhizomorphic mycorrhiza (basal). Large *Ceno- coccum* extraradical hyphae (arrowheads) are present.

Introduction

The family Cistaceae (the rock rose family) is composed of eight genera and approximately 180 species (Guzmán and Vargas 2009) centered in temperate Europe and the Mediterranean basin where they are often part of important early successional stages in disturbed habitats (Guzmán and Vargas 2005). The genera, based on plastid rbcL and trnL–trnF sequences (Guzmán and Vargas 2009), include *Cistus*, *Halimium*, *Helianthemum*, *Hudsonia*, *Lechea*, *Tuberaria*, *Fumana*, and *Crocanthemum*. Several genera occur in North America. Members of this family have been known to form associations with mycorrhizal fungi (see Malloch and Thorn 1985), and recently, Comandini et al. (2006) published an extensive list of fungal species associated with the genus *Cistus*. Several species of *Hebeloma* are highly specific to *Cistus* species (Eberhardt et al. 2009). With respect to mycorrhizal associations of Cistaceae species in North America, Malloch and Thorn (1985) examined three *Helianthemum* species and two species each of *Hudsonia* and *Lechea* (misspelled Lechia in their paper). Based on sporocarps found in the vicinity, the authors listed the probable fungal symbionts of these hosts as well as other fungi associated with *Cistus* species.

In spite of the large number of species in the Cistaceae, relatively few have been examined for their mycorrhizal status, and fewer still for the structural details between roots and associated symbiotic fungi. The majority of structural studies have involved *Cistus* species and various fungal symbionts: *C. incanus* L. – *Tuber melanosporum* Vitt. (Fusconi 1983; Giovannetti and Fontana 1982; Wenkart et al. 2001); *Cistus ladanifer* L. – *Laccaria laccata* (Scop.: Fr.) Berk. & Br. (Torres et al. 1995); *C. ladanifer* L. – *Boletus edulis* Bull. ex Fr. (Águeda et al. 2006, 2008); and, C. cf. *ladanifer* L. – *Boletus rhodoxanthus* Kallenb. (Hahn 2001). All are characterized by having a mantle and a Hartig net that involves both epidermal and cortical cell layers, features that are shared with conifer ectomycorrhizas. Similar results were obtained for four other *Cistus* species in combination with various *Tuber* species (Giovannetti and Fontana 1982), and for a *Cistus* sp. associated with both *Lactarius tesquorum* Maletcon (Nuytinck et al. 2004) and with *Lactarius cistophilus* Bon & Trimbach (Comandini and Rinaldi 2008). However, mycorrhizas formed with in-vitro transformed root clones of *C. incanus* and fungal isolates of *Terfezia boudieri* Chatin either developed ectomycorrhizal features similar to other *Cistus* species or had intracellular penetration by hyphae, depending on the clone and culture conditions (Zarotsky et al. 2006).

Cleared roots of *Helianthemum chamaecistus* Mill. – *Ceno- coccum graniforme* (Sow.) Ferd mycorrhizas showed cortical Hartig net formation as well as intracellular hyphae, although no mention was made concerning mantle development (Kianmehr 1978). Dexheimer et al. (1985) described ultrastructural features of Hartig net hyphae, as well as intracellular hyphae of *Helianthemum salicifolium* (L.) Mill. colonized by two *Terfezia* species. Fortas and Chevalier (1992) examined the effect of culture conditions on the colonization of *Helianthemum guttatum* (L.) Mill. by three truffle species; all species formed ectomycorrhizas with a cortical Hartig net but without a mantle in high phosphorus conditions, whereas they developed ectendomycorrhizas (cortical Hartig net and intracellular hyphae) in phosphorus-deficient cultures. Gutiérrez et al. (2003) showed that, under greenhouse conditions, *Helianthemum almeriense* Pau – *T. claveryi* mycorrhizas developed a cortical Hartig net with limited mantle, whereas in-vitro conditions produced a cortical Hartig net and a thick, multi-layered mantle. The same host colonized by *Picoa lefebvrei* (Pat.) Maire under greenhouse conditions had limited mantle, cortical Hartig net, and intracellular hyphae. Similarly, cortical Hartig net and intracellular fungal hyphae formed in in-vitro *Helianthemum ovatum* (Viv.) Dun. – *Terfezia terfezioides* (Matt.) Trappe mycorrhizas (Kovács et al. 2003); however, the authors cautioned that the root–fungus interaction in vitro might not reflect the true mycorrhizal association. Malloch and Thorn’s (1985) examination of whole mounts of field-collected *Lechea intermedia* roots showed an epidermal and cortical Hartig net, but there was no report of intracellular hyphae. The above reports demonstrate how highly variable mycorrhizal structure is in Cistaceae, perhaps not surprising in a family that is also taxonomically so diverse. No information is available for species in the genera *Crocanthemum* and *Tu- beraria*.

The two species examined in the present study, *Hudsonia ericoides* L. and *Hudsonia tomentosa* Nutt., are native to North America. *Hudsonia ericoides* (pine barren golden-heather; false heather) is an evergreen subshrub that grows in nutrient poor, sandy soil in isolated areas restricted to northeastern Canada and USA. It is specifically adapted to drought, soil erosion, and extreme soil conditions. Malloch and Thorn (1985) published images of root tips with *Cenococcum*-like mantles, from a collection made in Nova Scotia. The second host, *H. tomentosa* (sand heather; woolly beach heather) is found in sandy pine woods and clearings from Nova Scotia to Alberta, Canada, and in eastern USA. It is often found growing on sand dunes (Smeric et al. 1997). Whole mounts of roots previously collected in New Brunswick and Ontario showed a Hartig net that appeared to be restricted to the epidermis (Malloch and Thorn 1985). Allen and Allen (1992) noted that *H. tomentosa* (described
by them as an ericaceous shrub) had arboutoid-like mycorrhizas; however, this observation was not documented by images.

The objective of the present study was to use a variety of microscopical methods to more fully describe the anatomical features of the mycorrhizal associations of *H. ericoides* and *H. tomentosa* from field-collected specimens. Of interest was to compare the results with what is known for these species and for species in the other genera in this large family.

### Materials and methods

#### Plant material

Samples of *H. ericoides* were collected on 22 May 2009, west of Kingston, Nova Scotia (approximately 44.988°N, 64.949°W), in sandy habitats along an abandoned railway bed. Ectomycorrhizal hosts in the vicinity included *P. strobus, P. resinosa* L., as well as *P. banksiana* Lamb., *P. tomentosa* Aiton, *P. stroblos* L., as well as *Populus tremuloides* (Michx.) A & D Löve., *Populus grandidentata* Michx., and *Betula populifolia* Marsh. Collections of *H. tomentosa* were made on 9 October 2009, from a sandy beach site in Petawawa, Ontario (approximately 46.0°N, 77.3°W); mature *P. strobus, P. resinosa*, and smaller *Populus balsamifera* L., *P. tremuloides*, and *Betula papyrifera* Marsh. trees were in the vicinity. Entire plants with attached roots and soil were excavated and transported to the University of Guelph where they were stored briefly at 4 °C; subsequently, roots were washed in preparation for examination with a stereo binocular microscope for morphotype characterization and for fixation for further microscopy.

#### Morphological assessment

Standard light microscopy techniques were used to determine the different *Hudsonia* morphotypes (Agerer 1987–2008; Ingleby et al. 1990; Goodman et al. 1996). Mycorrhizas were characterized according to colour, texture, lustre, dimensions, tip shape, branching pattern, and presence or absence of rhizomorphs; root squash mounts were used to describe mantle features, emanating hyphae, rhizomorphs, and other distinguishing features. Preliminary identifications to fungal families or genera were designated when possible; otherwise, a descriptive name was assigned.

#### Resin embedding and light microscopy

Root samples from several plants for each host species were fixed in 2.5% glutaraldehyde in 0.10 mol/L 4-2-hydroxyethyl-1-piperazine ethane sulfonic acid (HEPES) buffer at pH 6.8, dehydrated in an ascending series of ethanol, and embedded in LR White resin (London Resin Company Ltd., Reading, Berkshire, England) using conventional protocols (Ruzin 1999). Thick sections (1–1.5 μm) were cut on a Porter-Blum Ultra-Microtome MT-1 (DuPont Co., Newton, Connecticut, USA) using glass knives, stained for light microscopy using aqueous 0.05% toluidine blue O (Sigma-Aldrich, St. Louis, Missouri, USA) in 1% sodium borate, and viewed on a Leitz Orthoplan (Leica, Mississauga, Ontario, Canada) microscope. Images were captured using a Nikon Coolpix 4500 digital camera (Nikon, Canada, Mississauga, Ont.). At least five samples of roots from both sites were examined for each mycorrhiza type.

#### Fluorescence microscopy

Free-hand sections of roots of both species that had been stored in 50% ethanol were stained for 1 h with 0.1% (w/v) berberine hemi-sulphate (Sigma, C.I. No. 75160) in dH₂O, rinsed several times with dH₂O, and mounted in 50% glycerol. Sections were viewed under UV and blue light (350–460 excitation wavelength) on a Leitz SM-LUX epifluorescence microscope (Leica, Mississauga, Ont.). Images were captured using the same camera system described above.

#### Scanning electron microscopy

Samples fixed in 2.5% glutaraldehyde were dehydrated through an ascending series of ethanol to 100%, critical-point dried, and mounted on aluminum stubs with two-sided sticky tape. After coating with gold–palladium, they were examined with a Hitachi S-570 (Nissei-Sangyo, Tokyo, Japan) scanning electron microscope. Images were taken with an attached digital camera.

#### Transmission electron microscopy

Thin sections (~0.1 μm) of LR-White-embedded material were sectioned with glass knives on a Reichert ultramicrotome (Model OM-U3; Reichert-Jung (Leica), Wetzlar, Germany), collected on formvar-coated copper grids, stained for 10 min in 2% ethanolic uranyl acetate, and counter-stained for 5 min with 1.0% lead citrate. Sections were examined with a Philips CM10 transmission electron microscope (Philips Electron Optics, Eindhoven, Germany) at 80 kV and images were collected with a digital camera.

### Results

#### Morphology of root systems

Both *H. ericoides* and *H. tomentosa* occurred as small patches in open areas of sandy soil or in semi-open areas.
near the tree(shrub)–sand zones (Figs. 1 and 2). Branched shoots consisted of scale-like leaves (Fig. 2). The two species shared similarities in growth form, rooting patterns, and fungal colonization. Both hosts had a mostly fibrous root system from which grew different order laterals. Most roots were extremely small (mycorrhizal tips were up to 200 μm in diameter with approximately 0.5–2.0 mm long) and almost all root tips that were examined were mycorrhizal (Fig. 3); root systems for each host species were colonized by several different symbiotic fungi.

Hudsonia ericoides morphotypes included a white rhizomorphic mycorrhiza that had a compact mantle of felt to net synencyhyma, emanating hyphae (EH) 2–4 μm in diameter with clamp connections, and numerous rhizomorphs (Fig. 4) with large vesicle-like hyphae. This host also produced a brown Tomentella-like morphotype with a thick net to angular synencyhyma mantle, EH 4–5 μm in diameter with clamp connections, and no obvious rhizomorphs (Fig. 5). Minor components of H. ericoides mycorrhizas were formed by dark septate ascomycetes; these consisted of MRA (Mycelium radicis atroviens Melin)-like fungi (Ingleby et al. 1990) and Cenococcum geophilum Fr. with isodiametric mantle cells forming a regular synencyhyma (see Ingleby et al. 1990) and abundant, large EH. Hudsonia tomentosa roots (Fig. 6) were dominated by two morphotypes: a white rhizomorphic mycorrhiza similar to that seen for H. ericoides; and Cenococcum geophilum mycorrhizas (Fig. 7). White rhizomorphic mycorrhizas had few emanating hyphae; these were often short and had a determinant length with enlarged (swollen) hyphal tips, or took the form of enlarged surface mantle cells. No other associated fungi were identified for H. tomentosa. However, many roots showed frequent successional colonization patterns with numerous tips being re-colonized by the other fungal morphotype (i.e., either Cenococcum was at the tip and the white morphotype basal (Fig. 8), or the white mycorrhiza was at the tip and Cenococcum basal). The position of the two fungi was variable in re-colonized roots.

Scanning electron microscopy

Roots of H. ericoides – Cenococcum mycorrhizas, when viewed by SEM, often had enlarged root apices with large prominent EH (Fig. 9); other morphotypes formed compact mantles (Fig. 10) with branched mantle hyphae (Fig. 11) and few to abundant EH with clamp connections (Fig. 12). White mycorrhizas that associated with both host species (as seen in Fig. 7) had abundant, large, branching rhizomorphs (Fig. 13). Figure 14 shows a H. tomentosa – Cenococcum mycorrhiza with numerous, large EH. Some H. tomentosa mycorrhizas had a compact mantle structure with fewer EH (Fig. 15), or showed successional re-colonization by two fungal species (Fig. 16).

Light microscopy

Sectioned roots from both plant hosts showed that colonized root tips had a simple anatomy: an epidermis of radially elongated cells, only 2–3 rows of cortical cells, and a vascular cylinder consisting of a few xylem tracheary elements and phloem. Longitudinal sections of LR-White-embedded mycorrhizal root tips of H. ericoides (Figs. 17–20) showed that mycorrhizas had a compact, multi-layered mantle (Figs. 17 and 18) and a Hartig net confined to the radially elongated epidermal cells; intracellular hyphae were not present (Figs. 17 and 18). Other H. ericoides morphotypes also had compact, fungal mantles of varying thickness that extended the length of the root and covered the root apex (Fig. 19). The Hartig net was again limited to the radially elongated epidermal cells and intracellular hyphae were not present (Figs. 19 and 20). Longitudinal sections of H. tomentosa mycorrhizas (the section illustrated is a Cenococcum mycorrhiza) (Figs. 21 and 22) showed similar structural features to those of H. ericoides; the mantle was multi-layered with the outer mantle hyphae showing the melanized walls of Cenococcum and dense inter-hyphal deposits (Fig. 22). Hartig net hyphae were associated only with epidermal cells (as in H. ericoides) and intracellular hyphae were not present (Fig. 22).

Fluorescence microscopy

Transverse sections of both H. ericoides and H. tomentosa mycorrhizal roots, when stained with berberine hemisulphate, showed that lignified walls of tracheary elements and suberized walls of endodermal cells floresced when viewed with UV–blue light (Figs. 23 and 24). Walls of the outer layer of cortical cells (hypodermis) and epidermal cells did not fluoresce although these cells and mantle hyphae showed some background fluorescence (Fig. 23).
Transmission electron microscopy

Transmission electron microscopy confirmed that neither the walls of the hypodermal cells (Fig. 25) nor the epidermal cells (not shown) contained suberin lamellae.

Discussion

Root systems of both *H. ericoides* and *H. tomentosa* consisted of mostly fibrous roots with numerous laterals, some of a diameter similar to that of “hair roots” of Ericaceae species (Peterson et al. 2004). Owing to their minute size, microscopic examination of the fine lateral roots for entire root systems was essential to determine that most root tips for both species were heavily colonized. Despite this, only four distinct fungal morphotypes were characterized for the two *Hudsonia* hosts. Based on observations of other systems (Smith and Read 2008), the number of fungal symbionts involved in colonization of both species would possibly increase with the use of molecular methods. However, this was not the intent of this study; rather, emphasis was on determining the structure of the root–fungus associations, to compare with results of other genera in the Cistaceae.

The presence of several morphotypes, some with an abundance of EH, may contribute to the success of these hosts in nutrient-poor soils by increasing the absorbing surface of the plant. Also, the well-developed mantles of most morphotypes and the prevalence of rhizomorphs on some may enable these species to exist in drought prone habitats (Smreciu et al. 1997). Morte et al. (2000) showed experimentally that *Helianthemum almeriense* was able to withstand imposed drought conditions when colonized by the desert truffle, *T. claveryi*. Elsewhere, and in the two habitats sampled, *Hudsonia* spp. and their mycorrhizal fungal associates may play a significant role in soil structure, stabilization and restoration in sandy soils based on the prevalence of mycorrhizal root tips and the abundance of EH (Perry et al. 1990).

The structural features observed for both *H. ericoides* and *H. tomentosa* mycorrhizas (a multi-layered mantle, Hartig net restricted to radially elongated epidermal cells, and lack of intracellular hyphae) are shared by the majority of woody mycorrhizas (a multi-layered mantle, Hartig net restricted to radially elongated epidermal cells, and lack of intracellular hyphae) are shared by the majority of woody mycorrhizas (Massicotte et al. 1998). However, in contrast to some ectomycorrhizas (Massicotte et al. 1990; Peterson et al. 2004; Smith and Read 2008), as well as by *Polygonum viviparum* L. (Polygonaceae) and *Kobresia bellardii* (All.) Degl. (Cyperaceae), two herbaceous angiosperms (Massicotte et al. 1998). However, in contrast to some ectomycorrhizas that have a Hartig net confined to the epidermis, which but which also have a suberized hypodermis believed to restrict hyphal penetration into deeper cortical layers (e.g., Massicotte et al. 1986), the two *Hudsonia* species lacked suberized walls in the outer cortical cell layer (hypodermis). The lack of suberization suggests that other properties of hypodermal cell walls may exist that impede fungal penetration; however, absence of suberin in outer cortical cell walls might also allow, under different environmental conditions or with different fungal associates, the possibility of intracellular colonization. This has not been explored. Reports that *H. tomentosa* has arbutoid mycorrhizas (Allen and Allen 1992) were not supported by our findings. Although a mantle and Hartig net developed, intracellular hyphae were not present.

Results confirmed true ECM formation for *H. ericoides* and *H. tomentosa*. Jakucs et al. (1999) also reported that the species *Fumana procumbens* (Dun.) Gr. Godr. is an ECM host (with mantle and Hartig net restricted to the epidermis) for the fungus *Helobela annuophillum* Bohus. These mycorrhizas differ structurally from some of the other members in the Cistaceae. For example, mycorrhizas of all *Cistus* species that associate with a range of fungal symbionts have a mantle and Hartig net involving both epidermal and cortical cells (e.g., Agueda et al. 2006; Fusconi 1983; Giovannetti and Fontana 1982; Nuytinck et al. 2004; Torres et al. 1995; Wenkart et al. 2001). These features are typical of conifer ectomycorrhizas (in contrast to the woody angiosperm ectomycorrhizas) (Peterson et al. 2004). Structurally, if sectioned material is not median longitudinal (i.e., is submedian or tangential), neighbouring epidermal cells may be cut obliquely or at an angle that suggests two cell rows, and this could falsely lead to the conclusion that the Hartig net is involving cortical cells. In addition, it has been reported that some mycorrhizas formed with in-vitro transformed root clones of *C. inanus* colonized by *T. boudieri* had intracellular penetration by hyphae as well as epidermal and cortical Hartig net formation, depending on culture conditions (Zaręsky et al. 2006). This outcome may reflect more the artificial situation under which the roots were colonized.

The mycorrhizal condition reported for species of *Helianthemum* is highly variable although most host–fungus symbioses show both epidermal and cortical Hartig net formation as well as the existence of intracellular hyphae (Dexheimer et al. 1985; Kianmehr 1978; Kovács et al. 2003), features characteristic of ectendomycorrhizas (Peterson et al. 2004; Yu et al. 2001). Kovács and Jakucs (2001) reported the Hartig net in *H. ovatum* (Viv.) was restricted to the outer row of cortical cells and absent from the epidermis. Structural differences may partly depend on growth and soil conditions, as well as the fungal symbiont involved (Gutiérrez et al. 2003). The two *Hudsonia* species examined in our study both came from sandy, mineral soil habitats and, despite seasonal and geographical sampling differences, showed remarkably similar ECM associations and structure. A larger study might further explore the limits of location and sampling times for *Hudsonia* populations, and correlate mycorrhizal status with these and other variables such as soil properties. Fortas and Chevalier (1992) reported that phosphorus concentration was important in determining the type of mycorrhiza formed between three truffle species and *H. guttatum* under in-vitro culture conditions; whether this might be important in natural systems is not known. Although a few studies have explored plant–fungus signaling in the Cistaceae, these have only involved *Cistus* species and mechanisms underlying these interactions are still being investigated (see Coughlan and Piché 2005).

For many species in the Cistaceae, the mycorrhizal status remains undetermined and, as a result, our ability to assess the range of mycorrhizal categories present in this diverse family is constrained. Evidence that some species can form arbuscular mycorrhizal associations (Harley and Smith 1983) was not seen in the present study but is of interest for further investigation. The host–fungus species involved in each association, host age, and (or) the habitat (including growth conditions) are most likely all important contributing factors.
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