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# *Tryblidiopsis magnesii sp. nov.* from *Picea glauca* in Eastern Canada

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**Abstract:** *Tryblidiopsis pinastri* (*Leotiomycetes*, *Rhytismatales*) was described from *Picea abies* in Europe and was also thought to occur on North American *Picea*. However, previously published sequences of *Picea* foliar endophytes from Eastern Canada suggested the presence of at least two cryptic *Tryblidiopsis* species, distinct from *T. pinastri* and other known species. Our subsequent sampling of *Tryblidiopsis* ascomata from dead attached *Picea glauca* branches resulted in the collection of a putatively undescribed species previously isolated as a *P. glauca* endophyte. Morphological evidence combined with phylogenetic analyses based on nuclear ribosomal internal transcribed spacer (ITS) and large subunit ribosomal (LSU) DNA sequences support the distinctiveness of this species, described here as *T. magnesii*.

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## INTRODUCTION

The type species of *Tryblidiopsis* (*Rhytismatales*, *Rhytismataceae*), *T. pinastri*, is a common associate of *Picea abies* in Europe. *Tryblidiopsis pinastri* is an endophyte of twigs and branches, likely non-pathogenic or only weakly so, and may contribute to the self-pruning of suppressed branches in the lower crown (Smerlis 1973, Kowalski & Kehr 1992, Livsey & Minter 1994, but see Takahashi & Saho 1973). Relatively little is known about the colonization process by *T. pinastri,* although it is commonly isolated from both the outer bark (rhytidome and periderm) and inner bark (phelloderm, phloem, and cambium) of *P. abies* (Kowalski & Kehr 1992, Barklund & Kowalski 1996). The presence of *Tryblidiopsis pinastri* becomes apparent following substrate death, when it produces black, *ca*. 1–2 mm diam, shortly stipitate ascomata erumpent through the bark. The 0–1-septate, clavate ascospores are, like most *Rhytismataceae* species, enveloped in a mucoid sheath, which facilitates surface adhesion and possibly conservation of water. Ascospores are typically released between spring and late summer, depending upon weather (Livsey & Minter 1994, Minter 1996). In moist conditions, the covering stroma opens by longitudinal or radial tears, revealing the hymenium and imparting the ascoma with a stellate or coronal appearance when viewed from above. The covering stroma alternately opens and retracts in moist and dry conditions, respectively.

*Tryblidiopsis pinastri* was thought to have a circumpolar boreal distribution with *Picea* species (Livsey & Minter 1994). However, the delineation of a North American subspecies, *T. pinastri* subsp. *americana*, by Magnes (1997) and recent description of two *Tryblidiopsis* species from *Picea* in China (Wang *et al.* 2014) demonstrate that the traditional morphotaxonomic concept of *T. pinastri* probably encompasses several species and suggests that the natural range of *T. pinastri s. str.* is restricted to Europe. In an ongoing survey of conifer endophytes from Eastern Canada, at least two cryptic *Tryblidiopsis* species were thought to occur based on nuclear ribosomal internal transcribed spacer (ITS) region sequences (Sumarah *et al.* 2008, McMullin *et al.* 2019). Our subsequent field surveys focused on the collection of *Tryblidiopsis* ascomata in Eastern Canada in an effort to acquire samples necessary for morphological identification and characterization. In this study, we describe a new *Tryblidiopsis* species producing ascomata and conidiomata on dead, attached lower branches of *Picea glauca* (white spruce) in Eastern Canada and provide morphological and phylogenetic evidence to support its distinction from *T. pinastri* and related species. We also report the occurrence of *Tryblidiopsis* species as foliar endophytes of *Picea* trees.

## MATERIALS AND METHODS

### Collecting, culturing, and morphological observations

Specimens of ascomata and conidiomata were collected from dead *Picea glauca* branches still attached to living trees in Ontario, Canada. Specimens were cultured by suspending ascomata from the top of Petri dish plate covers using drops of water and allowing ascospores to eject onto the surfaces of Petri dishes containing 2 % malt extract agar (MEA; 20 g Bacto malt extract, Difco Laboratories, Sparks, MD; 15 g agar, EMD Chemicals Inc., NJ; 1 L distilled water) or water agar (WA; 1.5 % water agar with 1 mL trace metal solution; Visagie *et al.* 2014). Attempts to culture from conidia involved streaking conidia on MEA, cornmeal agar (CMA; Acumedia Manufacturers Inc., Lansing, MI), WA, oatmeal agar (OA; Visagie *et al.* 2014), and spruce needle potato agar (SNPA; Su *et al.* 2012). Cultures were maintained on MEA at 16 °C under a 12 h fluorescent light cycle. Representative strains were deposited in the Canadian Collection of Fungal Cultures (DAOMC; Agriculture & Agri-Food Canada, Ottawa, Ontario, Canada).

Sections of specimens were made using a freezing microtome or by hand using a safety razor blade and then mounted in either deionized water, 5 % KOH, or 85 % lactic acid for microscopy. Observations were made using an Olympus BX50F4 light microscope (Olympus, Tokyo, Japan) and an Olympus SZX12 stereomicroscope. Morphological descriptions and measurements were made with living material and colors were described using the alphanumeric codes of Kornerup and Wanscher (1978). Images were captured with an InfinityX-32 camera (Lumenera Corp., Ottawa, Canada) with Infinity Analyze (Lumenera Corp.) software. The photographic plate was assembled using Adobe Photoshop CC 2017 (Adobe Systems, San Jose, CA). Select specimens were deposited in the Canadian National Mycological Herbarium (DAOM; Agriculture & Agri-Food Canada, Ottawa, Ontario, Canada) and Pacific Forestry Centre Forest Pathology Herbarium (DAVFP; Victoria, British Columbia, Canada).

### DNA sequencing and phylogenetic analyses

Total genomic DNA was extracted from 8–12-wk-old cultures using the Ultraclean Microbial DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA) following the manufacturer's protocol. Nuclear ribosomal internal transcribed spacer (ITS) region, partial nuclear large subunit of the ribosomal DNA (LSU), and partial actin (*ACT*) gene sequences were amplified and sequenced according to the methods of Tanney & Seifert (2017). Sequence contigs were assembled and trimmed using Geneious Prime 2019.0.4 (http://www.geneious.com).

GenBank sequences were used to populate both an ITS and a concatenated ITS-LSU dataset, which were aligned using the MAFFT v. 7.017 algorithm within Geneious Prime (Katoh *et al*. 2002). The purpose of the ITS dataset was to explore the phylogeny of *Tryblidiopsis* and related but unidentified endophyte sequences, while the concatenated ITS-LSU dataset was used to construct a phylogeny of identified *Tryblidiopsis* species with available ITS and LSU sequences. *Coccomyces strobi* (*Rhytismataceae*) was used as the outgroup for phylogenetic analysis of both datasets based on the results of Lantz *et al.* (2011) and McMullin *et al.* (2019). The best nucleotide substitution models for each dataset (SYM+G for ITS, GTR+G for ITS-LSU) were determined with jModelTest v. 2.1.10 (Darriba *et al.* 2012). For each alignment, MrBayes v. 3.2 was used to perform Bayesian inference (BI) phylogenetic reconstruction (Ronquist *et al.* 2012) with three independent Metropolis-coupled Markov chain Monte Carlo (MCMCMC) samplings performed with 11 heated chains and one cold chain. Sampling occurred every 500 generations until the standard deviation of split frequencies reached a value < 0.01. The first 25 % of trees were discarded as burn-in and the remaining trees were retained and combined into one 50 % majority rule consensus tree. The consensus tree was visualized in FigTree v. 1.4.2 (available at http://tree.bio.ed.ac.uk/software/figtree/) and exported in SVG format and edited in Adobe Illustrator v. 10 (Adobe Systems, San Jose, CA, USA). While *ACT* appears to be a sufficient barcode marker for *Rhytismatales* species (Tanney & Seifert 2017), we did not use this gene for phylogenetic reconstruction because of the paucity of available reference sequences. Sequences generated in this study were accessioned in GenBank (accession numbers in Table 1).

## RESULTS

*Tryblidiopsis* specimens collected from white spruce branches consisted of ascomata and sometimes co-occurring conidiomata. Polyspore strains were easily obtained from ascospores ejected on MEA but strains were not obtained from conidia, which did not germinate on any of the tested media. Initial study showed that ascomata were morphologically distinct from *T. pinastri* by the conspicuous pigmentation of the inner layer of the covering stroma (Fig. 1). ITS sequences of cultures derived from ascomata collections made in Dunsford and Thunder Bay, Ontario were identical and, based on an NCBI BLAST query, distinct from available sequences of known *Tryblidiopsis* species.

The resulting ITS dataset consisted of 18 taxa and 469 characters and the ITS-LSU dataset consisted of 7 taxa and 1 074 characters. The ITS and ITS-LSU phylogenies revealed that the *Tryblidiopsis* sp. formed a strongly supported (posterior probability (PP) = 1.0) clade distinct from known *Tryblidiopsis* species (Figs 2, 3). This *Tryblidiopsis* sp. is therefore described as a new species, *T. magnesii*, below based on morphological and phylogenetic evidence. The ITS phylogeny contained sequences of unidentified *Picea* endophytes, including a *Picea glauca* endophyte from New Brunswick now identified as *T. magnesii* [KX901893, CBS 120380; identities = 441/443 (99%), gaps = 0/443 (0%)] (Fig. 2). A well-supported (PP = 0.99) clade sister to *T. magnesii* and *Tryblidiopsis* sp. (KC312675, HOU 662) consisted of foliar endophytes isolated from *Picea glauca* in Alaska, *P. mariana* in Quebec, and *P. rubens* in New Brunswick. The concatenated ITS-LSU phylogeny placed *T. sichuanensis* as the basal species and *T. magnesii* sister to a clade comprised by *T. pinastri*, *T. sinensis*, and *Tryblidiopsis* sp.

### Taxonomy

***Tryblidiopsis magnesii*** J.B. Tanney & K.A. Seifert, ***sp. nov.*** MycoBank MB829496. Fig 1.

*Etymology*: Named for the Austrian mycologist Dr. Martin Magnes, who distinguished North American *T. pinastri* subsp. *americana* from European *T. pinastri* specimens based on ascoma morphology.

*Diagnosis*: *Tryblidiopsis magnesii* differs from *T. pinastri* by the pigmented inner layer of the covering stroma, from *T. sichuanensis* by larger ascospores (18–24 × 4–6 µm in *T. sichuanensis*) and unilocular conidiomata, and from *T. sinensis* by the absence of hyaline apical appendages and larger, more obtuse or clavate ascospores (18–30 × 2.5–4.5 µm in *T. sinensis*).

*Description*: *Conidiomata* co-occurring with ascomata on substratum, superficially similar in appearance to immature ascomata, 150–700 µm diam, globose to peg-shaped, smooth or wrinkled in appearance, semi-immersed, erumpent from bark, opening by irregular tear without ostiole, covering layer 22–40 µm thick, unilocular, locule 120–500 µm diam. *Conidiophores* hyaline, septate, simply branched, lining entire locular cavity. *Conidiogenous cells* holoblastic, sympodial, 10–15(–20) × (1.5–) 2–3(–4) µm, hyaline, thin-walled, smooth, ampulliform, often swollen above midpoint before tapering to a long cylindrical collulum. *Conidia* hyaline, filiform, falcate, or sigmoid, (18–)20–26(–29) × (1–)1.5–2 µm (length: n = 20, $\bar{x}$ = 23 µm, SD = 2.9 µm, SE = 0.6 µm, 95 % CI = 1.27; width: n = 20, $\bar{x}$ = 1.6 µm, SD = 0.4

**Table 1.** Sequences generated and used in the phylogenetic analyses in this study.

| Species | Voucher No. | GenBank Accession No. | | | Collection data | Reference |
|---|---|---|---|---|---|---|
| | | ITS | LSU | Act | | |
| *Tryblidiopsis magnesii* | DAOMC 252096$^{T}$ | MK748209 | MK748168 | MK733765 | Dead attached *Picea glauca* branch, Ontario, Canada | This study |
| *Tryblidiopsis magnesii* | DAOMC 252041 | MK748207 | — | MK733763 | Dead attached *Picea glauca* branch, Ontario, Canada | This study |
| *Tryblidiopsis magnesii* | DAOMC 252042 | MK748208 | — | MK733764 | Dead attached *Picea glauca* branch, Ontario, Canada | This study |
| *Tryblidiopsis magnesii* | CBS 120380 | KX901893 | — | — | *Picea glauca* endophyte, New Brunswick, Canada | McMullin *et al.* (2019) |
| *Tryblidiopsis* sp. | HOU 662 | KC312675 | KC312682 | — | Dead *Picea jezoensis* branch, Jilin, China | Wang *et al.* (2014) |
| *Tryblidiopsis pinastri* | HOU 198 | KC312678 | KC312680 | — | Dead *Picea abies* branch, Germany | Wang *et al.* (2014) |
| *Tryblidiopsis pinastri* | 1974-20/2 | JF793679 | — | — | Dead *Picea abies* branch, Nord-Trøndelag, Norway | Solheim *et al.* (2013) |
| *Tryblidiopsis pinastri* | CBS 445.71 | JF793678 | — | — | *Picea abies*, Slovakia | Solheim *et al.* (2013) |
| *Tryblidiopsis pinastri* | 1974-21/8 | JF793680 | — | — | *Picea abies*, Nord-Trøndelag, Norway | Solheim *et al.* (2013) |
| *Tryblidiopsis sinensis* | HOU 814$^{T}$ | KC312674 | KC312681 | — | Dead *Picea asperata* branch, Hebei, China | Wang *et al.* (2014) |
| *Tryblidiopsis sichuanensis* | HOU 300 | KC312677 | KC312679 | — | Dead *Picea retroflexa* twigs, Sichuan, China | Wang *et al.* (2014) |
| *Tryblidiopsis sichuanensis* | HOU 306$^{T}$ | KC312676 | KC312683 | — | Dead *Picea brachytyla* subsp. *complanata* twigs, Sichuan, China | Wang *et al.* (2014) |
| *Tryblidiopsis* sp. | 06-265B | MK748206 | MK748167 | — | *Picea rubens* endophyte, New Brunswick, Canada | This study |
| *Tryblidiopsis* sp. | 4724A | DQ979687 | — | — | *Picea mariana* endophyte, Quebec, Canada | Higgins *et al.* (2007) |
| *Tryblidiopsis* sp. | 4503 | DQ979641 | — | — | *Picea mariana* endophyte, Quebec, Canada | Higgins *et al.* (2007) |
| *Tryblidiopsis* sp. | 4473 | DQ979636 | — | — | *Picea mariana* endophyte, Quebec, Canada | Higgins *et al.* (2007) |
| *Tryblidiopsis* sp. | ARIZ:AK1644 | KX909081 | — | — | *Picea glauca* endophyte, Alaska, U.S.A. | U'Ren & Arnold (2016) |
| *Coccomyces strobi* | DAOMC 251589 | MH457130 | MH457157 | — | Self-pruned *Pinus strobus* branches, Ontario, Canada | McMullin *et al.* (2019) |

$^{T}$ denotes type specimens or ex-type strains.

µm, SE = 0.1 µm, 95 % CI = 0.16), germination not observed on CMA, MEA, OA, or WA.

*Ascomata* on dead, often brittle, twigs and branches still attached to living stem of host, usually found in lower crown where self-pruning occurs. External appearance: outline circular to slightly undulate when viewed from above, pseudostipitate, black, matte to slightly glossy, texture leathery to pebbly, erumpent from bark, occurring singly to gregariously; young ascomata spherical to peg-shaped, becoming urceolate to discoid with maturity; 1.25–2 mm diam when mature and fresh or rehydrated, up to 1.3 mm tall, pedicel up to 350 µm tall and more evident in young ascomata. *Covering stroma* often opening first by slit then 3–6(–9) irregular radial fissures in humid conditions with outline appearing stellate and revealing disc-shaped, pale orange to orange grey (5A3–5B2) hymenium, subsequently retracting and covering the hymenium in dry conditions and reopening when humid conditions arise, (40–)46–75(–90) µm thick, +/- consistent thickness enveloping ascoma, sometimes thinner towards base, composed of three distinct layers: (1) outer layer carbonaceous, *textura angularis*, 13.5–23 µm wide, composed of (1–)2–3(–4) rows of dark brown (6F6) to black, melanized, thick-walled (1–2 µm), globose to angular cells, (4.5–)6.5–11(–12) × (4.5–)6–9(–9.5) µm; (2) middle layer *textura angularis*, 12–26 µm wide, composed of (4–)5–6(–7) rows of hyaline, thin- to thick-walled (1 µm), globose to angular cells, (4.5–)5.5–9(–10.5) × (4–)4.5–7.5(–9) µm, embedded in gel; (3) inner layer *textura angularis*, 9–34 µm wide, composed of 2–4 rows of brown (5F8) thin- to thick-walled (1 µm), globose to angular cells, (4.5–)5–8(–8.5) × (4–)5–6.5(–7.5) µm. *Basal stroma* well developed, 500–700 µm deep, comprised of hyaline branching hyphae embedded in gelatinous substrate, crystalline material often present, especially towards base. *Subhymenium* 18–30 µm thick, hyaline, *textura intricata*. *Paraphyses* exceeding length of asci, 1–2 µm wide, thin-walled, hyaline, filiform, unbranched, septate, apices rounded to clavate or occasionally ossiform, 3–4.5(–5) µm wide, frequently linked near the base by hyphal bridges. *Asci* arising from croziers, maturing sequentially, (120–)125–150(–165) × (12–)13–15.5(–17) µm, cylindrical-clavate, apex obtuse to rounded, thin-walled, inamyloid, eight-spored. *Ascospores* biseriate, sometime uniseriate towards base, (22–)25–30(–31) × (4.5–)5–6(–6.5) µm (length: n = 30, x̄ = 27.5 µm, SD = 2.4 µm, SE = 0.4 µm, 95 % CI = 0.86; width: n = 30, x̄ = 5.4 µm, SD = 0.4 µm, SE = 0.1 µm, 95 % CI = 0.15), ellipsoidal-fusiform to fusiform-clavate, apical

end often more obtuse to clavate with basal end acute, hyaline, 0–1-septate, 2-septate ascospores rarely observed, septum median or supramedian, covered with (1.5–)2.5–3.5(–4.5) µm thick gelatinous sheath.

*Culture characteristics*: Colonies 6–11 mm diam after 14 d in the dark at 20 °C on MEA, flat, sparse aerial hyphae, margin entire, hyaline, surface and reverse white to pale yellow or light orange (4A3–5A5), exudates and soluble pigments absent; older colonies show high variability in colouration and exudates, with some colonies turning dark brown and exuding brown pigments into surrounding agar. Mycelium consisting of hyaline, smooth, septate, branched, hyphae 1.5–3.5 µm diam. Cardinal temperatures range 5–30 °C, optimum 20 °C, minimum slightly < 5 °C, maximum slightly > 30 °C.

*Ecology and distribution*: On dead *Picea glauca* branches, often the suppressed branches of lower canopy, and as a foliar endophyte of *P. glauca*. Common in Eastern Canada (Ontario, Quebec, New Brunswick).

*Typus*: **Canada**, Ontario, Kawartha Lakes, Dunsford, 44.4834 -78.6524, alt. 254 m, on dead attached *Picea glauca* branch, 24 Aug. 2014, *J.B. Tanney* NB-630 (**holotype** DAVFP 29737; ex-type strain DAOMC 252096).

*Additional materials examined*: **Canada**, Ontario, Kawartha Lakes, Dunsford, 44.4834 -78.6524, alt. 254 m, on dead attached *Picea glauca* branch, 24 May 2015, *J.B. Tanney* NB-647; Ontario, Kawartha Lakes, Dunsford, 44.4834 -78.6524, alt. 254 m, on dead attached *Picea glauca* branch, 17 Aug. 2016, *J.B. Tanney* NB-790; Ontario, Thunder Bay, Centennial Park, on dead attached *Picea glauca* branch, 29 Jul. 2015, *J.B. Tanney* NB-678/DAOM 867427/DAOMC 252041; Ontario, Thunder Bay, Centennial Park, on dead attached *Picea glauca* branch, 29 Jul. 2015, *J.B. Tanney* NB-679/DAOM 250760/DAOMC 252042; Ontario, Thunder Bay, Lakehead University, on dead attached *Picea glauca* branch, 29 Jul. 2015, *J.B. Tanney* NB-680; New Brunswick, Sussex, isolated as *Picea glauca* foliar endophyte, 28 Nov. 2003, *M. Sumarah* CBS 120380.

*Notes*: *Tryblidiopsis magnesii* occurs in eastern Canada, *T. pinastri* in Europe, and *T. sichuanensis* and *T. sinensis* in China. The primary morphological difference between *T. magnesii* and other *Tryblidiopsis* species is the melanized inner wall of the covering stroma. Magnes (1997) differentiated N. American specimens of *T. pinastri* from their European counterparts based on their larger ascomata and the melanized interior stromatal covering and subsequently erected *T. pinastri* subsp. *americana*. ITS sequences of *Tryblidiopsis* strains isolated as foliar endophytes from *Picea rubens* and *P. mariana* in New Brunswick, Canada suggest the presence of additional cryptic *Tryblidiopsis* species (McMullin *et al.* 2017). Therefore, the concept of *T. pinastri* subsp. *americana* probably encompasses a species complex given evidence suggesting additional cryptic species and the broad geographic and host range attributed to *T. pinastri* subsp. *americana* (Magnes 1997). *Tryblidiopsis magnesii* is probably distinct from *T. pinastri* subsp. *americana sensu stricto*, given that the ascospores reported for *T. pinastri* subsp. *americana* are narrower, 3–4(–5.5) µm, and the type specimen is from *Picea engelmannii* in Oregon, USA; however this distinction requires confirmation. Morphological differences combined with rDNA phylogenetic analysis support the recognition of *T. magnesii*, distinct from *T. pinastri* and the Asian species *T. sichuanensis* and *T. sinensis*.

### Key to *Tryblidiopsis* species

1. Inner wall of covering stroma melanized ..... *T. magnesii*
1. Inner wall of covering stroma hyaline ..... 2

2. Conidiomata multilocular, ascomata single or in clusters of 2–3 ..... *T. sichuanensis*
2. Conidiomata unilocular, ascomata single or in clusters of 2–6 ..... 3

3. Ascospores clavate, bottom usually more acute than top, 1-septate, 25–32 × 4–6 µm ..... *T. pinastri*
3. Ascospores fusiform, slightly acute ends, 0–1-septate, 18–30 × 2.5–4.5 µm ..... *T. sinensis*

## DISCUSSION

The identification of a novel *Tryblidiopsis* species in N. America is not unexpected given Magnes' (1997) morphological distinction of N. American *T. pinastri* specimens and subsequent introduction of *T. pinastri* subspp. *americana* and *pinastri*. *Tryblidiopsis* diversity is probably still underestimated in both N. America and Asia, where host *Picea* species diversity is greater than in Europe. For example, Wang *et al.* (2014) described two novel *Tryblidiopsis* species from *Picea* in China and also recognized a third novel species (*Tryblidiopsis* sp. HOU 662) based on an rDNA phylogeny, but declined to formally describe it because it was represented by only one collection of immature ascomata. Based on the ITS phylogeny presented here, *T. magnesii* and this undescribed Asian *Tryblidiopsis* sp. (HOU 662) are sister to *T. pinastri* with strong support (PP = 1; Fig. 2). The ITS phylogeny also reveals an unidentified cryptic *Tryblidiopsis* species known only from strains isolated as foliar

**Fig. 1.** *Tryblidiopsis magnesii* (DAVFP 29737, holotype). **A–D.** Ascomata erumpent from bark of dead attached *Picea glauca* branch. **E.** Longitudinal section of ascoma with black arrow denoting melanised inner wall of the covering stroma. **F.** Close-up of covering stroma with white arrow denoting melanised inner wall. **G.** Carbonaceous outer layer of covering stroma. **H.** Asci with ascospores showing gelatinous sheath. **I.** Asci and paraphyses. **J.** Ascospores. **K.** Longitudinal section of conidioma. **L.** Conidiogenous cells and conidia. **M.** Conidiomata erumpent from bark of dead attached branch. **N.** Conidiogenous cells and conidia. **O–P.** Conidia. Scale bars: E = 1 000 µm; F, K = 100 µm; G–J, L, N–P = 10 µm.

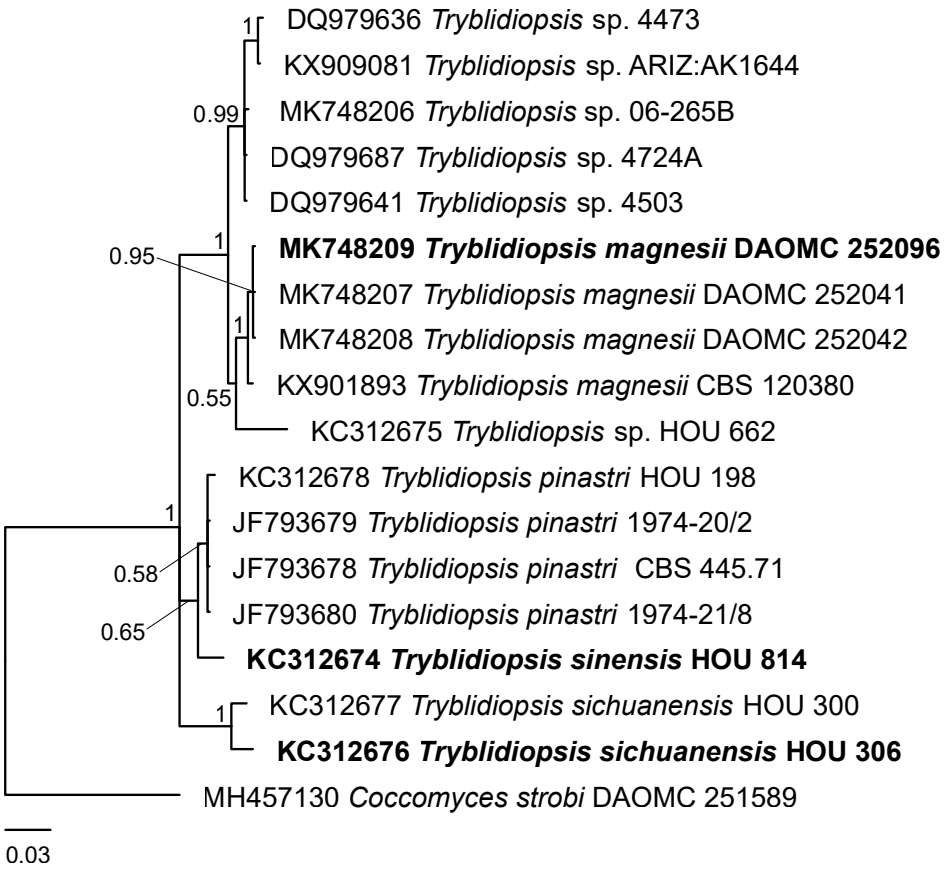


**Fig. 2.** Bayesian 50 % majority consensus tree from the ITS analysis showing relationships of *Tryblidiopsis* species with unidentified foliar endophytes. The tree was rooted to *Coccomyces strobi* and the scale bar represents number of substitutions per site. Posterior probability values are shown at branch nodes and ex-type sequences are in bold. GenBank accession numbers precede species names followed by strain or collection ID.

endophytes of *Picea glauca* in Alaska (U'Ren & Arnold 2016), *P. mariana* in Quebec (Higgins *et al.* 2007), and *P. rubens* in New Brunswick (McMullin *et al.* 2017) (Fig. 2). In an ongoing survey of foliar endophytes of the Acadian Forest Region, we also isolated several corresponding *Tryblidiopsis* sp. strains as endophytes of *Picea mariana* and *P. rubens* needles in New Brunswick; however, we defer the description of this species pending the eventual collection of its ascomata (J.B. Tanney, unpubl. info.). The diversity of *Tryblidiopsis* in N. America is clearly more complex than previously realized and future work should involve additional field collections from eastern and western N. America.

*Tryblidiopsis magnesii* is another example of a cryptic *Rhytismataceae* species endemic on N. American hosts and long misidentified as its European counterpart. Overall, European *Rhytismataceae* species ostensibly reported from endemic N. American hosts potentially represent undescribed species and warrant reexamination. For example, Tanney & Seifert (2017) described *Lophodermium resinosum*, a cryptic species from *Pinus resinosum* needles, which was also present in herbarium collections under the name *L. pinastri*. Similarly, *L. macci* is a N. American species previously misidentified as *L. pinastri* in collections from *Pinus strobus* (Sokolski *et al.* 2004). *Rhytisma americanum*, the causal agent of tarspot on red maple (*Acer rubrum*) and silver maple (*A. saccharinum*) in N. America, was generally assumed to be *R. acerinum*, a species found *on A. platanoides* trees native to Europe and planted abroad (Hudler *et al.* 1998). Salas-Lizana & Oono (2018) recently described *L. fissuratum*, a species occurring sympatrically with the morphologically similar *L. nitens*, from *Pinus lambertiana* and *P. monticola* in the Pacific Northwest. Furthermore, the ongoing descriptions of novel *Rhytismataceae* species, cryptic and otherwise, from Asia also indicate a greater global species diversity than previously recognized (*e.g.*: Kaneko 2003, Fan *et al.* 2012, Masumoto *et al.* 2015, Zhang *et al.* 2015, Li *et al.* 2016). *Rhytismataceae* host preferences should be further investigated, especially for putative host-jumping in introduced trees. For example, does *T. magnesii* occur on ornamental *Picea abies* trees planted in eastern N. America and do *T. magnesii* and *T. pinastri* occur sympatrically among *Picea glauca* trees planted in Europe?

*Tryblidiopsis magnesii* was previously isolated as an unidentified foliar endophyte of *Picea glauca* (CBS 120380; Sumarah *et al.* 2008, McMullin *et al.* 2017). To our knowledge, this is the first identified record of *Tryblidiopsis* occurring as a foliar endophyte; however, based on GenBank accessions, previous studies detected unidentified *Tryblidiopsis* sequences from *Picea* endophytes (Table 1; Higgins *et al.* 2007, U'Ren & Arnold 2016). Tanney *et al*. (2018) suggested that while *Tryblidiopsis* is known to sporulate only from dead branches and twigs, it is conceivable that foliar infections or colonization represent an important life history phase, for example as a pathway of infection allowing the fungus to gain entry into the host plant from the needle (e.g. stomata) and spread to the inner bark. This proposed pathway is known in the pathogen *Elytroderma deformans* (*Rhytismataceae*), causal agent of a needle cast and broom disease of *Pinus* species in western N. America, which is capable of colonizing branches and stems

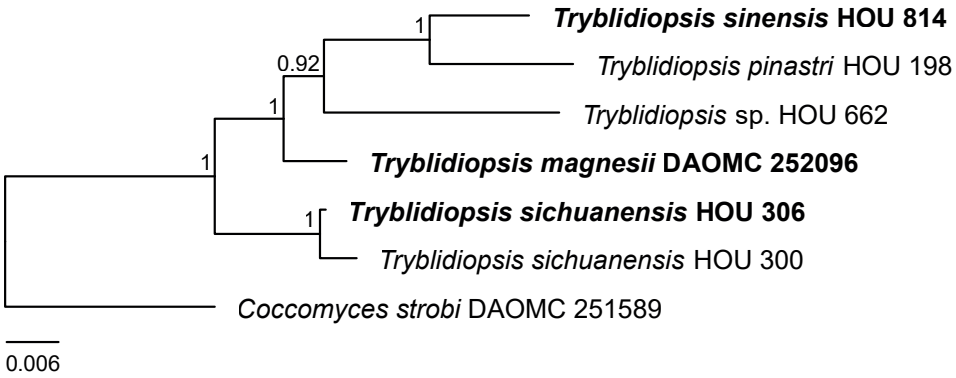


**Fig. 3.** Bayesian 50 % majority consensus tree from the ITS-LSU analysis showing relationships between *Tryblidiopsis* species. The tree was rooted to *Coccomyces strobi* and the scale bar represents number of substitutions per site. Posterior probability values are shown at branch nodes and ex-type sequences are in bold. Strain or collection accession numbers follow the binomial names.

by means of initial foliar infections (Waters 1962). Additionally, Darker (1932) noted claims of mycelium invading young stems and branches from needles by the *Rhytismataceae* species *Meloderma desmazieri* (Fron 1911) and *Lophodermium pinastri* (Tubeuf 1901). Foliar colonization might also permit the persistence and dispersal of *Tryblidiopsis* in the absence of the primary host substrate (*sensu* Carroll 1999). *Coccomyces strobi* and *Therrya* species, which occur on *Pinus* and appear to have a similar biology and ecology as *Tryblidiopsis* (*i.e.*: associated with dead attached lower branches), have also been isolated as foliar endophytes from their *Pinus* hosts (Botella & Diez 2011, Solheim *et al.* 2013, McMullin *et al.* 2019). The overall life histories and role of needle, cambium, and bark colonization should be further investigated for *Tryblidiopsis* and other branch-inhabiting *Rhytismataceae* species.

This study is part of an ongoing research program concerning endophyte diversity of conifers in the Acadian forest, which has resulted in: (1) the identification of novel and biologically active compounds; (2) the description of novel species and insight into the biology and ecology of conifer endophytes; and, (3) large-scale field testing of select endophytes strains to assess their role in mitigating damage caused by the important forest pest *Choristoneura fumiferana* (spruce budworm) (Tanney 2018). *Rhytismataceae* strains are of particular interest because of their secondary metabolite diversity and evidence of undescribed cryptic species (Sumarah *et al.* 2011, McMullin *et al.* 2015, Sumarah *et al.* 2015, McMullin *et al.* 2017). For example, *Tryblidiopsis magnesii* (CBS 120380) culture filtrate extracts incorporated into the diet of spruce budworm larvae significantly reduced budworm weight and head capsule size and disc diffusion tests showed antifungal effects of the extracts against *Saccharomyces cerevisiae* (Sumarah *et al.* 2008). Overall, these findings should provide stimulus to further progress in elucidating the biodiversity and life histories of *Rhytismataceae* species in N. America.

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