Lophodermium resinosum sp. nov. from red pine (*Pinus resinosa*) in Eastern Canada

Journal:	<i>Botany</i>
Manuscript ID	cjb-2017-0012.R1
Manuscript Type:	Article
Date Submitted by the Author:	09-Mar-2017
Complete List of Authors:	Tanney, Joey; Ottawa Research and Development Centre, Agriculture and Agri-Food Canada, Biodiversity (Mycology & Botany) Seifert, Keith; Ottawa Research and Development Centre, Agriculture and Agri-Food Canada, Biodiversity (Mycology & Botany)
Please Select from this Special Issues list if applicable:	N/A
Keyword:	cryptic species, Leotiomycetes, molecular systematics, Rhytismataceae, Rhytismatales

SCHOLARONE™
Manuscripts

***Lophodermium resinosum* sp. nov. from red pine (*Pinus resinosa*) in Eastern Canada**

Joey B. Tanney^a

Keith A. Seifert^b

Biodiversity (Mycology & Botany), Ottawa Research and Development Centre, Agriculture and Agri-Food
Canada, Ottawa, Ontario, K1A 0C6 Canada

^aJoey.Tanney@agr.gc.ca

^bKeith.Seifert@agr.gc.ca

Corresponding author:

J.B. Tanney

Biodiversity (Mycology & Botany), Ottawa Research and Development Centre, Agriculture and Agri-Food
Canada, Ottawa, Ontario, K1A 0C6 Canada.

Telephone: (613)-759-1820

Joey.Tanney@agr.gc.ca

Abstract

A morphologically distinct *Lophodermium* species was collected from fallen secondary needles of *Pinus resinosa* over two consecutive years in Eastern Ontario; subsequent herbarium studies confirmed its presence in Nova Scotia, Quebec, and Maine. Symptomatic needles frequently exhibited red bands and completely subepidermal ascomata and conidiomata. Ascospore isolates from specimens were used to reconstruct phylogenies inferred from internal transcribed spacer rDNA and partial actin gene sequences. Both phylogenetic analyses delineated the specimens from other sequenced *Lophodermium* species. Phylogenetic evidence combined with morphological characters of ascomata and conidiomata supported the distinctiveness of this species, described here as *Lophodermium resinosum* sp. nov.

Keywords: cryptic species, Leotiomyces, molecular systematics, Rhytismataceae, Rhytismatales

Introduction

Lophodermium species (Rhytismataceae, Rhytismatales) are common foliar endophytes, saprotrophs, and pathogens of conifer trees. Molecular studies indicate the polyphyly of *Lophodermium*, thus morphological characters considered diagnostic for *Lophodermium*, notably elliptical ascomata opening by a single longitudinal slit, filiform ascospores surrounded by a gelatinous sheath, and leptostroma-like asexual states, are not synapomorphic (Lantz et al. 2011; Gao et al. 2013). Recent work also reveals the presence of species complexes and cryptic species in *Lophodermium* growing on *Pinus* foliage, including examples of novel species delimited among specimens formerly grouped together and misidentified as *L. pinastri* (Sokolski et al. 2004; Hou et al. 2009; Reignoux et al. 2009; Gao et al. 2013; Reignoux et al. 2014; Oono et al. 2015). Given (1) the recent description of novel cryptic *Lophodermium* species, (2) the unaccounted diversity of Rhytismataceae and possibility of narrow host preferences, and (3) the historical propensity of mycologists to identify North American specimens with names originally described from European type material, the delimitation and identity of *Lophodermium* species associated with conifer hosts endemic to North America should be re-examined. The identification of novel bioactive secondary metabolites from strains of *Lophodermium* and other Rhytismataceae species in eastern Canada gives impetus and relevance to this research (Sumarah et al. 2008; Sumarah et al. 2011; Sumarah et al. 2015).

In this study, specimens of a *Lophodermium* species were collected from fallen secondary needles of *Pinus resinosa* in late spring over two successive years in Eastern Canada. Reports of *Lophodermium* from *Pinus resinosa* are scarce, but include *L. australe*, *L. nitens*, *L. pinastri*, and *L. seditiosum* (Dearness 1926; Farr and Rossman 2016); detailed morphological study indicated the specimens we collected were distinct from these species and warranted further examination. Phylogenetic analyses using the internal transcribed spacer rDNA (ITS) marker and partial actin (*ACT*) gene sequences combined with morphological observations of the fresh collections and *Lophodermium*

herbarium specimens provide evidence supporting the distinctiveness and consequent recognition of a novel *Lophodermium* species, described here as *L. resinosum*.

Materials and methods

Sampling and isolation

Field specimens were collected from fallen *Pinus resinosa* secondary needles in a mature *P. resinosa* plantation, a part of the Larose Forest established in 1928 in Clarence-Rockland, Ontario, Canada. Ascospore isolations were made on 2% malt extract agar (MEA; 20 g Bacto malt extract, Difco Laboratories, Sparks, Maryland; 15 g agar, EMD Chemicals Inc., New Jersey; 1 L distilled water) or corn meal agar (CMA; Acumedia Manufacturers Inc., Lansing, MI). Monoascospore and polyascospore cultures were made by suspending mature ascomata from the lids of 6 cm Petri dishes using drops of sterile deionized water and allowing ascospores to eject downward onto the agar surface. Individual ascospores were transferred to 6 cm Petri dishes containing MEA using an electrolytically sharpened tungsten needle (Brady 1965). Ascospores were verified for germination and incubated at 16 °C under 12:12 h light conditions. Dried specimens were accessioned in, and supplementary preserved specimens were obtained from, the Canadian National Mycological Herbarium (Ottawa, Canada; DAOM). Living cultures were deposited in the Canadian Collection of Fungal Cultures (Ottawa, Canada; DAOMC).

Morphological observations

Sections of apothecia and conidiomata were cut by hand using a safety razor blade or with a freezing microtome and mounted in either water, 5% KOH, Lugol's solution with or without 5% KOH pretreatment to test amyloid reactions (Baral 1987), or 85% lactic acid. Colony colours were described using the alphanumeric codes of Kornerup and Wanscher (1978). Morphological descriptions and measurements were made with living material. Microscopic measurements were taken from material mounted in deionized water and are presented as ranges calculated from the mean $\pm$ standard deviation of each measured value with outliers in brackets. Observations were made using an Olympus BX50F4

light microscope (Olympus, Tokyo, Japan) and an Olympus SZX12 stereomicroscope and images were captured with an InfinityX-32 camera (Lumenera Corp., Ottawa, Canada) using Infinity Analyze v6.5.2 (Lumenera Corp.) software. Photographic plates were assembled using Adobe Photoshop v5.5 (Adobe Systems, San Jose, California). Cardinal temperatures were assessed for two strains (DAOMC 251482 and DAOMC 251485) by incubating three-point inoculated 9 cm Petri dishes containing MEA at 5 °C intervals from 5–40 °C. Each treatment was conducted in triplicate and colony diameters were measured two and four wk after inoculation. Plates showing no growth were then incubated at 20 °C to determine viability.

DNA extraction and analyses

Total genomic DNA was extracted from 12-wk-old cultures using the Ultraclean Microbial DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, California) following the manufacturer's protocol. The primers ITS4 and ITS5 (White et al. 1990) were used to amplify and sequence the ITS region and ACT-512F and ACT-783R were used to amplify and sequence part of the actin (*ACT*) gene (Carbone and Kohn 1999). Translation elongation factor-1 α (*TEF1 α*) gene was partially amplified and sequenced using EF1-983 and EFgr (Rehner 2001) and partial LSU region was amplified using the primer pairs LR0R and LR8 and sequenced using LR0R, LR3R, LR5, and LR7 (Vilgalys and Hester 1990). DNA was amplified using the methods described in Nguyen et al. (2013). All loci were amplified using the following PCR profile: 95 °C for 3 min, then 35 cycles at 95 °C for 1 min, 56 °C for 45 s, and 72 °C for 1.5 min, followed by a final extension at 72 °C for 10 min.

For a herbarium specimen of *Meloderma dezmazerii* (DAOM 89807), DNA was extracted from fragments of symptomatic needles using the NucleoMag Trace kit (Macherey-Nagel, Düren, Germany) with an initial tissue grinding stage in liquid nitrogen using an Axygen polypropylene pestle (PES-15-B-SI, Union City, California), with the same PCR protocol as above including the addition of 0.5 μ L of 20mg/ml bovine serum albumin (BSA; Thermo Fisher Scientific, Waltham, MA) to the PCR reaction. All PCR

products were verified by agarose gel electrophoresis and sequenced with Big Dye Terminator (Applied Biosystems, Foster City, California).

Sequence contigs were assembled, trimmed, and manually checked using Geneious v8.1.5 (Biomatters, Auckland, New Zealand) and each individual gene dataset aligned by the MAFFT v7.017 algorithm within Geneious (Katoh et al. 2002). The resulting alignments were trimmed and manually checked within Geneious.

The ITS dataset included 64 sequences and 494 positions and the *ACT* dataset consisted of 42 sequences and 297 positions. *TEF1 α* was excluded from phylogenetic analysis because of a lack of representative Rhytismatales sequences available. Bayesian analyses were performed for each individual gene dataset using MrBayes v3.2 (Ronquist et al. 2012) and the most suitable sequence evolution models selected for each gene based on the optimal Akaike information criterion scores in MrModeltest v2.2.6 (Nylander 2004): GTR+I+G for ITS and GTR+G for *ACT*. *Lophodermium piceae* (FR837918) was selected as outgroup for the ITS analysis because previous studies place it at the base of Rhytismatales (Ortiz-García et al. 2003; Lantz et al. 2011). *Hymenoscyphus epiphyllus* (Helotiaceae, Helotiales; FJ005192) was outgroup for the *ACT* analysis based on previous analyses (Koukol et al. 2015; Li et al. 2016). For each alignment, three independent Metropolis-coupled Markov chain Monte Carlo (MCMCMC) runs were simultaneously performed with four chains (three heated and one cold) with sampling 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 kept and combined into one 50% majority-rule consensus tree. Convergence was assessed from the three independent runs using Tracer v1.6 (Rambaut et al. 2014). Consensus trees were imported into FigTree v1.4.2 (Rambaut 2014) and exported as SVG vector graphics for assembly in Adobe Illustrator v10 (Adobe Systems). All novel sequences used in this study were accessioned in GenBank (Table 1) and taxonomic novelties and associated metadata were deposited in MycoBank (www.MycoBank.org).

Results

The ITS and *ACT* phylogenies show branches with low–moderate support overall and many polytomous clades, which is expected given the sampling gaps in sequences available for *Lophodermium* and related genera (Figs. 1–2). The lack of available *ACT* sequences for *Lophodermium* and other Rhytismataceae species did not allow for a combined analysis, therefore asymmetrical datasets were used. The new species *Lophodermium resinosum* formed a well-supported distinct clade in both the ITS and *ACT* phylogenetic analyses. The ITS phylogeny placed *L. resinosum* sister to *L. molitoris* (posterior probability (PP) = 0.96) while the *ACT* phylogeny, lacking available *L. molitoris* sequences, placed *L. resinosum* sister to *Lophodermium kumaunicum* (PP = 0.99).

A GenBank BLAST of *Lophodermium resinosum* ITS sequences revealed three closely related matches with unidentified *Picea glauca* endophytes: DQ068338, DQ068340, (identities = 464/465 (99%), gaps = 0/465) and DQ068339 (identities = 463/465 (99%), gaps = 0/465). The next closest related sequences in GenBank included unidentified endophytes isolated from *Pinus taeda* (KM519348: identities = 462/478 (97%), gaps = 3/478 (0%) and *Pinus monticola* (HQ535910: 471/492 (96%), 4/492(0%); HQ535923, HQ535897: 470/492 (96%), 2/492(0%).

A study of *Lophodermium pinastri* and *L. seditiosum* DAOM herbarium specimens from *Pinus resinosa* confirmed the presence of *Lophodermium resinosum* from additional localities, listed below. *Lophodermium resinosum* was represented in the DAOM collection by 11 specimens identified as *L. pinastri* and one specimen identified as *L. seditiosum* (DAOM 198724).

Taxonomy

Lophodermium resinosum J.B. Tanney & Seifert, **sp. nov.**

Fig. 3

MycoBank MB819741.

Etymology: Named to correspond with the host epithet, *Pinus resinosa*.

Diagnosis: Morphologically similar to *Lophodermium seditiosum*, but with smaller ascomata, covering stroma that extends as far as the basal wall, inostiolate conidiomata, and an ITS sequence distinct from *L. seditiosum* ex-type (ATCC 28345). ITS (KY485127), *ACT* (KY485130), and LSU (KY485133) sequences are distinct from all available *Lophodermium* and Rhytismataceae sequences.

Typus: **Canada**, Ontario, Clarence-Rockland, Larose Forest, ex fallen secondary needles of *Pinus resinosa*, coll. May 31, 2015, J.B. Tanney & B. Tanney (holotype DAOM 745753; culture ex-type DAOMC 251482).

ITS barcode: KY485127 (alternative markers: *ACT* = KY485130; *TEF1 α* = KY702582; LSU = KY485133).

Colony description based on DAOMC 251482 at 20 °C after 14 d in dark: 12–15 mm diam; planar with sparse to moderate aerial hyphae; margin diffuse and hyaline; surface hyaline to pale orange to greyish orange (5A3–5B4); reverse orange grey to greyish orange (5B2–5B3). Exudates and soluble pigments absent. Conidiomata sometimes forming within 4 wk, occurring singly, scattered, on agar surface or submerged, morphologically different from conidiomata on host, up to 1 mm diam, dark brown to black, globose, with conidia dimensions same as those from host. Mycelium consisting of hyaline to pale brown, smooth, septate, branched, hyphae 1.5–4(–6) μ m diam.

Ascomata mostly on adaxial and less frequently abaxial surfaces of needles, subepidermal, (500–)600–950(–1100) $\times$ 300–500(–600) μ m, scattered, occasionally coalescing, elliptical, shiny, black, margin paler, opening by a longitudinal fissure with grey to black lips. Lip cells ovoid to cylindrical-clavate, hyaline to subhyaline, up to 17 μ m long, arranged more or less radially, often invested in mucus, not always evident. Covering stroma dark brown, 18–25 μ m thick towards edges, 20–55 μ m thick towards lips, covered by host epidermis, extending to basal stroma, consisting of *textura angularis* to *textura epidermoidea*, cells 3–8 μ m diam, thick-walled (ca. 1 μ m thick). Basal stroma dark brown, concave,

occurring within host epidermal cells, poorly developed, consisting of *textura angularis* to *textura epidermoidea*, cells 2.5–4 µm diam, thin- to thick-walled. Subhymenium 14–28 µm thick, hyaline, consisting of *textura angularis* and *textura intricata*. Paraphyses septate, filiform, unbranched, hyaline, smooth-walled, covered in 1–2 µm thick gelatinous sheath that frequently appears discontinuous and granular with maturity, 4–5.5 µm thick at base, tapering to 2.5 µm, apices rounded to irregularly subclavate, as long as mature asci, connected at base by hyphal bridges. Asci (120–)125–150(–165) × 11–14(–16) µm, cylindrical to clavate-cylindrical, apex rounded to subpapillate, more or less sessile, inamyloid, 8-spored, thin-walled, maturing sequentially. Ascospores (83.5–)104.5–125.5(–133.5) × 2–2.5(–3) µm (length: $n = 54$, $\bar{x} = 115$ µm, $SD = 10.5$ µm, $SE = 1.43$ µm, $95\% CI = 2.79$; width: $n = 54$ µm, $\bar{x} = 2.5$ µm, $SD = 0.2$ µm, $SE = 0.03$ µm, $95\% CI = 0.05$), filiform, ends rounded, tapered towards base, hyaline, contents sometimes appearing granular, smooth-walled, covered by a well-developed, more or less uniform, 1.5–2(–2.5) µm thick gelatinous sheath that appears denser and bulbous at both ends, sheath (3–)4–5(–5.5) µm thick at apex and (2–)2.5–4(–4.5) µm thick at base, arranged parallel or sometimes helically coiled.

Conidiomata mostly on adaxial and less frequently abaxial surfaces of needles, subepidermal, co-occurring with ascomata, scattered to crowded or coalescing, appearing as flat to raised, irregularly oval to elliptical, blister-like swellings on tissue, dull to shiny, concolorous or paler than surrounding host tissue, becoming darker with age, margin often brown or dark brown, 300–500 (–800) × (100–)150–300 µm, opening by lateral fissures. Subconidiogenous layer 7–14 µm thick, hyaline to subhyaline, consisting of *textura globulosa*, thin-walled cells. Conidiogenous cells (9.5–)10–16(–19) × 2–3 µm, cylindrical to broadly ampulliform, tapering towards apex, sometimes swollen at base or above midcentre, hyaline, indeterminate, discrete, extending sympodially. Conidia (3.5–)4.5–6(–8) × 1–1.5 µm (length: $n = 100$, $\bar{x} = 5.25$ µm, $SD = 0.1$ µm, $SE = 0.08$ µm, $95\% CI = 0.16$; width: $n = 100$, $\bar{x} = 1.45$ µm, $SD = 0.12$ µm, $SE = 0.01$

µm, 95% CI = 0.02), oblong, ends rounded and sometimes broadly tapered at base, straight, hyaline, smooth-walled, exuded in slimy pale to buff mass from irregular rupture in covering host epidermis.

Cardinal temperatures: Range 5–30 °C, optimum 25 °C, minimum 5 °C, maximum slightly >30 °C.

Habitat: On fallen secondary needles of *Pinus resinosa*.

Distribution: Canada: Ontario, Quebec, Nova Scotia; USA: Maine.

Notes: Infected needles rarely exhibiting black zone lines, instead frequently having red bands 100–400 µm wide, and ascomata and conidiomata usually mature May–June. *Lophodermium resinosum* is distinguished from the morphologically similar *L. seditiosum* by its smaller ascomata (vs. 800–1600 µm sensu Minter 1981), covering stroma extending as far as the basal wall, and inostiolate conidiomata. Other species that form completely subepidermal ascomata differ from *L. resinosum* by their lack of lips and splitting along a line of stomata (*L. baculiferum*; Minter 1980), conidia >15 µm long (*L. ravenelii*; Minter 1981), or smaller ascospores, e.g.: *L. pini-pumilae* (87–91 × 2 µm; Minter 1981) and *L. orientale* (55–85 × 2 µm; Minter 1981). *Lophodermium resinosum* differs morphologically from *L. staleyi* by its larger ascospores (vs. 70–115 × 9–12 µm; Minter 1981) and smaller conidia (vs. 8–12 × 1 µm; Minter 1981). Ascomata of *L. resinosum* are readily distinguished from those of *L. australe*, *L. conigenum*, *L. pinastri*, and *L. pini-excelsae* by being totally subepidermal. *Lophodermium resinosum* is morphologically distinct from *L. molitoris*, the closest relative in the ITS phylogeny, most notably by its subepidermal vs. subcuticular ascomata (Minter 1980). It differs morphologically from *L. kumaunicum*, the closest relative in the ACT phylogeny, by its longer ascospores (vs. 60–90 × 1–1.5 µm; Minter and Sharma 1982) and subepidermal vs. subcuticular or partly subepidermal ascomata (Fig. 3).

Other specimens examined (all ex *Pinus resinosa* needles): **CANADA:** Nova Scotia: Pictou Co., Blue Mountain, 31 Aug. 1933, *I.H. Crowell* (DAOM 24828); Ontario: Prescott and Russell Co., Clarence-Rockland, Larose Forest, 8 Jun. 2015, *J.B. Tanney & B. Tanney* (DAOM 745754, DAOMC 251483, DAOMC 251844, DAOMC 251485, DAOMC 251486, DAOMC 251514, DAOMC 251515); Carleton Co., Buckham

Bay, 17 Sep. 1960, *G.D. Darker* (DAOM 74359); Lake Temagami, Gull Lake Portage, 26 Jun. 1929, *G.E. Thompson* (DAOM 86496); Lake Temagami, Spawning Bay Portage, 26 Jun. 1932, *R.F. Cain*, (DAOM 86494); Lake Temagami, Bear Island, 19 Jun. 1932, *R.F. Cain*, (DAOM 86498); Lake Temagami, Bear Island, 3 Jul. 1929, *G.E. Thompson* (DAOM 86497); Petawawa, Orange Road, 4 Jul. 1940, *C.G. Riley* (DAOM 24042); Petawawa, Orange Road Plantation, 28 Jun. 1940, *A.J. Skolko* (DAOM 6103); South Plantagenet Township, 9 Jun. 1988, *A. Keizer* (DAOM 198724); Quebec: Rimouski Co., Bic sur Mer, 13 Sep 1961, *G.D. Darker* (DAOM 84530); Île Perrot, 20 May 1939, *I.H. Crowell* (DAOM 218306); **U.S.A.:** Maine: Eustis, needles of cut trees, 29 Jul 1935, *J.R. Hansbrough* (DAOM 48675).

Discussion

Lophodermium resinosum forms abundant ascomata and conidiomata on fallen secondary needles of *Pinus resinosa* across northeastern North America. The ecology of *L. resinosum* is currently unknown, for example whether it exhibits a prolonged endophytic infection preceding a saprotrophic phase initiated by foliage senescence and abscission, or if it is an opportunistic pathogen.

Lophodermium resinosum is only known from fallen secondary needles; the presence of *L. resinosum* on attached needles from cut trees (DAOM 48675) suggests the fungus exists endophytically and sporulates following needle death.

The distinctiveness and novelty of *L. resinosum* was overlooked by previous workers examining *Lophodermium* on *Pinus resinosa*, probably because of the broad application of the name *L. pinastri* to specimens bearing superficially-similar ascomata that actually represent distinct species (Sokolski et al. 2004). For example, our ITS phylogeny (Fig. 1) shows the names *L. pinastri* and *L. conigenum* are applied to sequences forming phylogenetically distinct clades, probably representing species that are undescribed or named-but-unsequenced, such as *L. staleyi* (Reignoux et al. 2014). In general, the historical misapplication of names to Rhytismatales specimens resulted in erroneous species concepts

that are too broad and potentially conceal diagnostic or distinctive biological and ecological traits. North American Rhytismatales biodiversity is undersampled and underestimated, especially given the frequent application of European species names to North American specimens based on superficial morphological similarities. Several studies reveal the presence of novel cryptic or overlooked species misidentified as European species in North America, such as *Rhytisma americanum* (Hudler et al. 1998), *Lophodermium macci* (Sokolski et al. 2004), and *L. "piceae"* (Stefani and Bérubé 2006a), while Chinese species of *Lophodermium* and *Tryblidiopsis* have also been distinguished from their European look-alikes (Gao et al. 2013; Wang et al. 2014). While Oono et al. (2014) suggested that all *Pinus* species can potentially host most *Lophodermium* species, this is unlikely given the polyphyly of *Lophodermium* and apparent narrow host preferences observed in other Rhytismataceae species, for example *Lirula macrospora*, *Lophodermium piceae* s.s., and *Tryblidiopsis pinastri* restricted to *Picea abies* (Lantz et al. 2011; Tanney unpub data), *Isthmiella faullii* restricted to *Abies balsamea* (Darker 1932), *Lophophacidium dooksii* restricted to *Pinus strobus* (Corlett and Shoemaker 1984; Laflamme et al. 2015), and *Rhytisma salicinum* and *Cryptomyces maximus* restricted to *Salix* (Lantz et al. 2011). However, closely-related ITS sequences of *Lophodermium* species derived from disjunct locales and more distantly related *Pinus* hosts indicates that some species are not restricted to specific *Pinus* species or subsections (Ortiz-García et al. 2003; Johnston et al. 2003; Oono et al. 2014). Further sampling of *Lophodermium* and other Rhytismatales from endemic North American hosts is therefore encouraged to elucidate biodiversity, host ranges, and provide evidence of host-tracking coevolution and speciation.

Previous observations of *Lophodermium* on *Pinus resinosa* name *Lophodermium pinastri* and *L. seditiosum* as causal agents of needle cast causing mortality or severe damage to *Pinus resinosa* and *P. sylvestris* in North American nurseries and plantations during the 1960–1980s (Nicholls and Skilling 1970; Ostry and Nicholls 1989). Skilling and Nicholls (1975) described what they interpreted as two *Lophodermium pinastri* biotypes isolated from symptomatic *Pinus resinosa* and *P. sylvestris* trees in

Christmas tree plantations: one type was pathogenic, isolated primarily from 1- and 2-year-old needles and produced brown mycelia in culture, while the other type was isolated from older needles and produced white mycelia in culture. Other workers have noted differences in pathogenicity, cultural and apothecial morphology, and ecology of *Lophodermium pinastri* “types” on *Pinus sylvestris*, which in consideration of recent molecular work, provides compelling evidence for the existence of undescribed species (Darker 1932; Millar and Watson 1971; Reignoux et al. 2014; Koukol et al. 2015). Wilson et al. (1994) identified 27 distinct genotypes of *Lophodermium pinastri* isolated as *Pinus resinosa* endophytes in Eastern Canada, but again their identities cannot be confirmed without morphological evidence or available sequences. Connecting precise taxonomic names to such records would allow valuable field observations (e.g.: pathogenicity reports) to be reinterpreted.

The epitypification of species using material from original host species and type locales is crucial to provide benchmarks for species and generic concepts within Rhytismatales, an order consisting of numerous large polyphyletic families that must be resolved (Lantz et al. 2011). New collections and epitypification efforts should focus on taxonomically important species, such as *Lophodermium arundinaceum*, *L. piceae*, and *L. pinastri*, and unsequenced type species of genera such as *Isthmiella abietis* and *Lirula nervisequa*. *Lophodermium* is polyphyletic and the type species, *L. arundinaceum*, occupies a clade consisting primarily of Poaceae associates (Lantz et al. 2011). This is an unfortunate lectotype species, because in the pursuit of monophyletic taxa, *Lophodermium* is likely to be eventually restricted to this clade of largely graminicolous species, resulting in the taxonomic upheaval of more frequently reported species such as important conifer associates. Additional sampling and phylogenetic study is required, especially for type species and genera unrepresented by sequence data, before making drastic taxonomic and nomenclatural changes to these genera.

Unfortunately, asymmetrical datasets of available ITS and *ACT* sequences resulted in discrepancies in our phylogeny. For example the ITS tree places *L. resinosum* sister to *L. molitoris* (PP =

0.96) and the *ACT* phylogeny places *L. resinosum* sister to *L. kumaunicum* (PP = 0.99). To reduce such difficulties caused by missing data, cultures of unrepresented and novel species should be generated whenever possible and deposited in publicly available collections and sequences of the ITS barcode, LSU, and protein-coding phylogenetic markers such as *ACT* and *TEF1 α* made available in NCBI GenBank. In consideration of the taxonomic issues surrounding Rhytismatales, we discourage the description of novel Rhytismatales species without representative sequences of at least the ITS barcode.

Rhytismatales species may be readily distinguished using the ITS barcode and other secondary barcodes. *Lophodermium resinosum* ITS sequences showed 99% similarity to three sequences in GenBank (DQ068338, DQ068340, and DQ068339) originating from endophyte cultures from surface sterilized *Picea glauca* needles sampled from separate trees within a plot in eastern Canada (Stefani and Bérubé 2006b). *Lophodermium resinosum* has not been recovered in other *Picea* endophyte studies and Stefani and Bérubé (2006b) described the sampling site as a *Picea glauca* plantation surrounded by a mixture of mature *Pinus resinosa* and *P. strobus*. It is unexpected that *Lophodermium resinosum* would occur on both *Pinus resinosa* and *Picea glauca* and plausible that *L. resinosum* ascospores adhering to *Picea glauca* needles survived the surface sterilization process, resulting in its detection as an apparent *P. glauca* endophyte. However, the ability of *Lophodermium resinosum* to endophytically infect *Picea glauca*, while doubtful, cannot be ruled out.

In general, Rhytismatales sequence data are scant, especially for non-rDNA loci; therefore amassing available *ACT* and other gene sequences (e.g.: *TEF1 α*) by the relatively small community focussing on Rhytismatales is imperative to provide data for phylogeny-based taxonomic treatments. Several studies adopted *ACT* as a secondary phylogenetic marker for *Lophodermium* (e.g.: Oono et al. 2014; Reignoux et al. 2014; Koukol et al. 2015; Li et al. 2016). Species resolution and phylogenetic reconstruction using *ACT* appears satisfactory; for example, Reignoux et al. (2014) presented three *Lophodermium pinastri* clades that were congruent in both ITS and *ACT* phylogenies, although tree

topologies differed (e.g.: *L. pinastri* clades were polytomous in *ACT* phylogeny). *ACT* is readily amplified in *Lophodermium* species and sequences are typically <300 bp, making it a potentially useful secondary barcode despite its short length. It is also appealing for sequencing Rhytismatales herbarium specimens, where DNA is often fragmented or degraded, especially if it might be possible to develop Rhytismatales-specific *ACT* primers. Stielow et al. (2015) reported an 84% amplification success for *ACT* in a diverse sampling of Ascomycota and Basidiomycota samples, but different primer pairs were tested and no universal performing set was found.

At the time of writing, Rhytismatales species are poorly represented by *TEF1 α* sequences in GenBank; therefore species identification and phylogenetic analysis using *TEF1 α* is currently infeasible. Stielow et al. (2015) identified *TEF1 α* as the most promising universal secondary barcode candidate and, given its wide usage as a phylogenetic marker, *TEF1 α* should be considered in future Rhytismatales phylogenetic studies.

Pinus resinosa or red pine is an economically and ecologically important tree species in northeast North America and represents the only extant subsection *Pinus* species native to the continent. Despite its occurrence across a wide geographic range, phenotypic and genetic variation in *Pinus resinosa* is very low, probably resulting from a population bottleneck during the last glaciation event (Fowler and Morris 1977; Boys et al. 2005; Walter and Epperson 2005). Ascomycota records from *Pinus resinosa* are relatively sparse compared to other commercial North American conifers (Farr and Rossman 2016); the unique evolutionary history of *P. resinosa* should encourage sampling from this host. For example, did its expansion from smaller refugia populations also result in a biodiversity bottleneck for symbionts, or are many *Pinus resinosa* symbionts shared with other co-occurring conifer hosts?

The detection of a novel *Lophodermium* species on an endemic North American host with a poorly characterized microbiota is expected given the recent descriptions of novel *Lophodermium* and

other Rhytismataceae species worldwide (e.g.: Wang et al. 2014; Koukol et al. 2015; Masumoto et al. 2015; Zhang et al. 2015). Furthering our understanding of the biodiversity and taxonomy of Rhytismatales species will reveal insight into the coevolution of these fungi and their hosts, provide reference sequences facilitating the identification of sequences from endophyte and other ecological surveys, and potentially lead to the identification of novel bioactive secondary metabolites. The detection and identification of the novel species *Lophodermium resinosum* from *Pinus resinosa* should therefore stimulate further taxonomic work on fungi associated with native plant hosts in Canada.

Acknowledgements

This study was supported by the Natural Sciences and Engineering Research Council of Canada PGSD2-459312-2014 to J.B. Tanney and the NSERC CRDPJ 421782-11 to JD Miller. We are grateful to the Microbiology Molecular Technologies Laboratory (MMTL) group of ORDC-AAFC, Ottawa, for processing DNA sequencing and Quinn Eggertson for advice on sequencing herbarium specimens.

References

- Baral, H.O. 1987. Lugol's solution/IKI versus Melzer's reagent: hemiamyloidity, a universal feature of the ascus wall. *Mycotaxon*, **29**: 399–450.
- Boys, J., Cherry, M., and Dayanandan, S. 2005. Microsatellite analysis reveals genetically distinct populations of red pine (*Pinus resinosa*, Pinaceae). *Am. J. Bot.*, **92**(5): 833–841.
- Brady, J. 1965. A simple technique for making very fine, durable dissecting needles by sharpening tungsten wire electrolytically. *Bulletin of the World Health Organization*, **32**(1): 143.
- Carbone, I., and Kohn, L.M. 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia*, **91**(3): 553–556.
- Corlett, M., and Shoemaker, R.A. 1984. *Lophophacidium dooksii* n. sp., a phacidiaecous fungus on needles of white pine. *Can. J. Bot.*, **62**(9): 1836–1840.
- Darker, G.D. 1932. The Hypodermataceae of Conifers. *Contrib. Arnold Arboretum*, **1**:1–131.
- Dearness, J. 1926. New and Noteworthy Fungi: IV. *Mycologia*, **18**(5): 236–255.
- Farr, D., and Rossman, A. 2016. Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA [online]. Available from <http://nt.ars-grin.gov/fungalatabases/> [accessed 2 December 2016].

Fowler, D., and Morris, R. 1977. Genetic diversity in red pine: evidence for low genic heterozygosity.

Can. J. Forest. Res., **7**(2): 343–347.

Gao, X.-M., Lin, Y.-R., Huang, H.-Y., and Hou, C.-L. 2013. A new species of *Lophodermium* associated with the needle cast of Cathay silver fir. Mycol. Prog., **12**(1): 141–149.

Hou, C.-L., Li, L., and Piepenbring, M. 2009. *Lophodermium pini-mugonis* sp. nov. on needles of *Pinus mugo* from the Alps based on morphological and molecular data. Mycol. Prog., **8**(1): 29–33.

Hudler, G.W., Jensen-Tracy, S., and Banik, M.T. 1998. *Rhytisma americanum* sp. nov.: a previously undescribed species of *Rhytisma* on maples (*Acer* spp.). Mycotaxon, **68**: 405–416.

Johnston, P., Park, D., Dick, M., Ortiz-García, S., and Gernandt, D. 2003. Identifying pine-inhabiting *Lophodermium* species using PCR-RFLP. New Zealand J. Forestry Sci., **33**(1): 10–24.

Katoh, K., Misawa, K., Kuma, K.i., and Miyata, T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res., **30**(14): 3059–3066.

Kornerup, A., and Wanscher, J.H. 1978. Methuen handbook of colour. 3rd ed. Eyre Methuen, London, United Kingdom.

Koukol, O., Pusz, W., and Minter, D. 2015. A new species of *Lophodermium* on needles of mountain pine (*Pinus mugo*) from the Giant Mountains in Poland. Mycol. Prog., **14**(5): 1–13.

Laflamme, G., Broders, K., Côté, C., Munck, I., Iriarte, G., and Innes, L. 2015. Priority of *Lophophacidium* over *Canavirgella*: taxonomic status of *Lophophacidium dooksii* and *Canavirgella banfieldii*, causal agents of a white pine needle disease. *Mycologia*, **107**(4): 745-753.

Lantz, H., Johnston, P., Park, D., and Minter, D.W. 2011. Molecular phylogeny reveals a core clade of Rhytismatales. *Mycologia*, **103**(1): 57–74.

Li, Z.-J., Taylor, J., and Hou, C.-L. 2016. An unusual *Lophodermium* species on needles of *Pinus taiwanensis* from China. *Mycol. Prog.*, **15**(12): 1229–1237.

Masumoto, S., Tojo, M., Uchida, M., and Imura, S. 2015. Morphological and molecular characterization of *Rhytisma filamentosum* sp. nov. from Nagano Prefecture, Japan. *Mycol. Prog.*, **14**(6): 1–6.

Millar, C., and Watson, A.R. 1971. Two biotypes of *Lophodermium pinastri* in Scotland. *Eur. J. For. Pathol.*, **1**(2): 87–93.

Minter, D.W. 1980. *Leptostroma* on pine needles. *Botany*, **58**(8): 906–917.

Minter, D.W. 1981. *Lophodermium* on pines. *Mycol. Pap.*, **147**: 1–54.

Minter, D.W. and Sharma, M.P. 1982. Three species of *Lophodermium* from the Himalayas. *Mycologia*, **74**(5):702–711.

Nguyen, H.D., Nickerson, N.L., and Seifert, K.A. 2013. *Basidioascus* and *Geminibasidium*: a new lineage of heat-resistant and xerotolerant basidiomycetes. *Mycologia*, **105**(5): 1231–1250.

Nicholls, T.H., and Skilling, D.D. 1970. *Lophodermium pinastri* outbreak in lake states forest nurseries. Plant Dis. Rep., **54**(9): 731–733.

Nylander, J. 2004. MrModeltest 2.2. Program distributed by the author., Evolutionary Biology Centre, Uppsala University, Uppsala.

Oono, R., Lefèvre, E., Simha, A., and Lutzoni, F. 2015. A comparison of the community diversity of foliar fungal endophytes between seedling and adult loblolly pines (*Pinus taeda*). Fungal Biol., **119**(10): 917–928.

Oono, R., Lutzoni, F., Arnold, A.E., Kaye, L., U'Ren, J.M., May, G., and Carbone, I. 2014. Genetic variation in horizontally transmitted fungal endophytes of pine needles reveals population structure in cryptic species. Am. J. Bot., **101**(8): 1362–1374.

Ortiz-García, S., Gernandt, D.S., Stone, J.K., Johnston, P.R., Chapela, I.H., Salas-Lizana, R., and Alvarez-Buylla, E.R. 2003. Phylogenetics of *Lophodermium* from pine. Mycologia, **95**(5): 846–859.

Ostry, M.E., and Nicholls, T.H. 1989. Effect of *Lophodermium seditiosum* on growth of pine nursery seedlings in Wisconsin. Plant Dis., **73**: 798–800.

Rambaut, A. 2014. FigTree v1. 4.2. Edinburgh: University of Edinburgh.

Rambaut, A., Suchard, M., Xie, D., and Drummond, A. 2014. Tracer ver. 1.6. Available from <http://beast.bio.ed.ac.uk/Tracer>.

Rehner SA, 2001. EF1-alpha primers. USDA, ARS, PSI Insect Biocontrol Laboratory. Available from <http://www.aftol.org/pdfs/EF1primer.pdf>

Reignoux, S.N., Aitken, E.A.E., Green, S., and Ennos, R.A. 2009. Two new species of *Lophodermium* colonise Scots Pines needles in Scotland. Proceedings of the conference IUFRO working party 7.02.02, ISSN: 1302-7082, 15–23.

Reignoux, S.N., Green, S., and Ennos, R.A. 2014. Molecular identification and relative abundance of cryptic *Lophodermium* species in natural populations of Scots pine, *Pinus sylvestris* L. Fungal Biol., **118**(9): 835–845.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., and Huelsenbeck, J.P. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol., **61**(3): 539–542. doi: 10.1093/sysbio/sys029.

Skilling, D., and Nicholls, T. 1975. The development of *Lophodermium pinastri* in conifer nurseries and plantations in North America. Eur. J. For. Pathol., **5**(4): 193–197.

Sokolski, S., Piché, Y., and Bérubé, J.A. 2004. *Lophodermium macci* sp. nov., a new species on senesced foliage of five-needle pines. Mycologia, **96**(6): 1261–1267.

Stefani, F., and Bérubé, J. 2006a. Biodiversity of foliar fungal endophytes in white spruce (*Picea glauca*) from southern Québec. *Botany*, **84**(5): 777–790.

Stefani, F., and Bérubé, J. 2006b. Evaluation of foliar fungal endophyte incidence in field-grown transgenic Bt white spruce trees. *Botany*, **84**(10): 1573–1580.

Stielow, J., Lévesque, C., Seifert, K., Meyer, W., Irinyi, L., Smits, D., Renfurm, R., Verkley, G., Groenewald, M., and Chaduli, D. 2015. One fungus, which genes? Development and assessment of universal primers for potential secondary fungal DNA barcodes. *Persoonia*, **35**(1): 242–263.

Sumarah, M.W., Kesting, J.R., Sørensen, D., and Miller, J.D. 2011. Antifungal metabolites from fungal endophytes of *Pinus strobus*. *Phytochemistry*, **72**(14): 1833–1837.

Sumarah, M.W., Puniani, E., Blackwell, B.A., and Miller, J.D. 2008. Characterization of polyketide metabolites from foliar endophytes of *Picea glauca*. *J. Nat. Prod.*, **71**(8): 1393–1398.

Sumarah, M.W., Walker, A.K., Seifert, K.A., Todorov, A., and Miller, J.D. 2015. Screening of fungal endophytes isolated from eastern white pine needles. *In* The Formation, Structure and Activity of Phytochemicals. *Edited by* R. Jetter. Springer International Publishing. pp. 195–206.

Vilgalys, R., and Hester, M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *J. Bacteriol.*, **172**(8): 4238–4246.

Walter, R., and Epperson, B.K. 2005. Geographic pattern of genetic diversity in *Pinus resinosa*: contact zone between descendants of glacial refugia. *Am. J. Bot.*, **92**(1): 92–100.

Wang, S., Cannon, P., Li, Z.-J., and Hou, C.-L. 2014. Multigene phylogenetic analysis detects cryptic species of *Tryblidiopsis* in China. *Mycologia*, **106**(1): 95–104.

White, T., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* *PCR Protocols: A Guide to Methods and Applications*. Edited by M. Innis and D. Gelfand and J. Sninsky and T. White. Academic Press, Inc., New York. pp. 315–322.

Wilson, R., Wheatcroft, R., Miller, J.D., and Whitney, N.J. 1994. Genetic diversity among natural populations of endophytic *Lophodermium pinastri* from *Pinus resinosa*. *Mycol. Res.*, **98**(7): 740–744.

Zhang, T.-T., Tong, X., Lin, Y.-R., and Hou, C.-L. 2015. A new species and a new combination of *Terriera* based on morphological and molecular data. *Mycol. Prog.*, **14**(8): 1–6.

Table 1. Isolates used in phylogenetic analyses in this study; new sequences generated in this study are in bold.

Species	Strain/specimen identifier	GenBank accession no.		
		ITS	ACT	LSU
<i>Elytroderma deformans</i>	CBS 181.68	AF203469	—	—
<i>Hymenoscyphus epiphyllus</i>	HB7054	—	FJ005192	—
<i>Hypoderma junipericola</i>	R104a	GU138754	—	—
<i>Leptostroma decipiens</i>	ja2n4t5	AY100654	—	—
<i>Leptostroma decipiens</i>	ma4n7m1	AY100653	—	—
<i>Lophodermium australe</i>	2V2	—	KM106324	—
<i>Lophodermium australe</i>	CMW 34720	KF636499	—	—
<i>Lophodermium australe</i>	DP_SL1_1c	—	KM106346	—
<i>Lophodermium baculiferum</i>	loba2 Oregon	AY100658	—	—
<i>Lophodermium baculiferum</i>	mon2zem Nuevo Leon	AY100656	—	—
<i>Lophodermium baculiferum</i>	monbacp Morelos	AY100657	—	—
<i>Lophodermium cathayae</i>	HOU827	—	KX443648	—
<i>Lophodermium cathayae</i>	HOU837	HQ992810		
<i>Lophodermium cathayae</i>	Hou837M	HQ992811		
<i>Lophodermium conigenum</i>	Ab1	HM060648	HM060669	—
<i>Lophodermium conigenum</i>	Ab2	—	HM060670	—
<i>Lophodermium conigenum</i>	Ab3	HM060650	HM060671	—
<i>Lophodermium conigenum</i>	Ab4	—	HM060672	—
<i>Lophodermium conigenum</i>	CMW 39040	KF636501	—	—
<i>Lophodermium conigenum</i>	HOU1227D	KX443659	KX443646	—
<i>Lophodermium conigenum</i>	R114	FJ861974	—	—
<i>Lophodermium conigenum</i>	R117	FJ861976	—	—
<i>Lophodermium corconticum</i>	CCF 4780	HG939565	—	—
<i>Lophodermium corconticum</i>	CCF 4781	—	HG965439	—
<i>Lophodermium corconticum</i>	CCF 4868 ^T	LM654179	LM654182	—
<i>Lophodermium corconticum</i>	L178	—	HG965447	—
<i>Lophodermium corconticum</i>	L204	—	HG965440	—
<i>Lophodermium indianum</i>	CMW 39036	KF636505	—	—
<i>Lophodermium indianum</i>	CMW 39038	KF636507	—	—
<i>Lophodermium indianum</i>	CMW 39125	KF636510	—	—
<i>Lophodermium juniperinum</i>	HOU1210	KX443661	—	—
<i>Lophodermium juniperinum</i>	ICMP 17306	KF033120	—	—
<i>Lophodermium kumaunicum</i>	HOU1112B	—	KX443651	—
<i>Lophodermium kumaunicum</i>	HOU514A	KX443660	KX443649	—
<i>Lophodermium kumaunicum</i>	HOU812A	—	KX443650	—
<i>Lophodermium macchi</i>	S-171	AF540560	—	—
<i>Lophodermium macchi</i>	S-172	AF540561	—	—

<i>Lophodermium molitoris</i>	CV1_3a	KM106803	—	—
<i>Lophodermium molitoris</i>	ICMP 14900	AY247752	—	—
<i>Lophodermium nitens</i>	102C27_CP	—	DQ342126	—
<i>Lophodermium nitens</i>	19_CP	—	DQ342122	—
<i>Lophodermium nitens</i>	41a3	—	DQ342124	—
<i>Lophodermium nitens</i>	DAOMC 250027	KR494247	—	—
<i>Lophodermium nitens</i>	NB-283-2D	KY485136	—	—
<i>Lophodermium petrakii</i>	R60	FJ861984	—	—
<i>Lophodermium piceae</i>	ZK53/08	FR837918	—	—
<i>Lophodermium pinastri</i>	6	EU696766	—	—
<i>Lophodermium pinastri</i>	Aa1	HM060657	HM060673	—
<i>Lophodermium pinastri</i>	Aa12	—	HM060681	—
<i>Lophodermium pinastri</i>	Aa13	HM060659	HM060677	—
<i>Lophodermium pinastri</i>	Aa2	—	HM060682	—
<i>Lophodermium pinastri</i>	Aa23	—	HM060678	—
<i>Lophodermium pinastri</i>	Aa28	—	HM060674	—
<i>Lophodermium pinastri</i>	Aa29	—	HM060675	—
<i>Lophodermium pinastri</i>	Aa4	—	HM060679	—
<i>Lophodermium pinastri</i>	Aa6	HM060661	HM060680	—
<i>Lophodermium pinastri</i>	Aa7	—	HM060683	—
<i>Lophodermium pinastri</i>	Aa8	HM060655	HM060676	—
<i>Lophodermium pinastri</i>	Aa9	—	HM060684	—
<i>Lophodermium pinastri</i>	HOU1227C	KX443658	KX443645	—
<i>Lophodermium pinastri</i>	L230	AY100650	—	—
<i>Lophodermium pinastri</i>	L23pinLo	AY775698	—	—
<i>Lophodermium pinastri</i>	L24pinLo	AY775699	—	—
<i>Lophodermium pinastri</i>	loppon	AY100649	—	—
<i>Lophodermium pinastri</i>	LUBC	AF462434	—	—
<i>Lophodermium pinastri</i>	R109	FJ861985	—	—
<i>Lophodermium pinastri</i>	R110	FJ861986	—	—
<i>Lophodermium pinastri</i>	Yinggao	AY422490	—	—
<i>Lophodermium pini-bungeanae</i>	R112	FJ861989	—	—
<i>Lophodermium pini-excelsae</i>	R108	FJ861988	—	—
<i>Lophodermium pini-excelsae</i>	R85	FJ861987	—	—
<i>Lophodermium pini-taiwanensis</i>	HOU1073AM		KX443640	—
<i>Lophodermium pini-taiwanensis</i>	HOU1073A ^T	KX443654	KX443641	—
<i>Lophodermium pini-taiwanensis</i>	HOU1075B	—	KX443642	—
<i>Lophodermium pini-taiwanensis</i>	HOU1222B	—	KX443643	—
<i>Lophodermium pini-taiwanensis</i>	HOU1227B	—	KX443644	—
<i>Lophodermium resinosum</i>	DAOMC 251482^T	KY485127	KY485130	KY485133
<i>Lophodermium resinosum</i>	DAOMC 251484	KY485128	KY485131	KY485134
<i>Lophodermium resinosum</i>	DAOMC 251485	KY485129	KY485132	KY485135

<i>Lophodermium seditiosum</i>	Ac1	HM060652	HM060667	—
<i>Lophodermium seditiosum</i>	Ac2	—	HM060668	—
<i>Lophodermium seditiosum</i>	Ac4	HM060654	—	—
<i>Lophodermium seditiosum</i> ^T	ATCC 28345	AF462435		
<i>Lophodermium seditiosum</i>	L225	AF203468		
<i>Meloderma desmazieresi</i>	ATCC MYA-2460	AF426056		
<i>Meloderma desmazierii</i>	DAOM 89807	KY485137		

ATCC = American Type Culture Collection, Manassas, Virginia, U.S.A.; CBS = CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CMW = Tree Pathology Co-operative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; DAOM = Canadian National Mycological Herbarium, Agriculture and Agri-Food Canada, Ottawa, Canada; DAOMC = Canadian Collection of Fungal Cultures, Agriculture and Agri-Food Canada, Ottawa, Canada; ICMP = International Collection of Microorganisms from Plants, Landcare Research, Auckland, New Zealand (<http://www.landcareresearch.co.nz/resources/collections/icmp>).

^T = Holotype or ex-type culture

Draft

Figure captions

Fig. 1. Bayesian 50% majority rule consensus tree based on ITS dataset containing representative *Lophodermium* species and related genera. GenBank accession numbers precede the species name followed by culture collection accession numbers, with ex-type strains in bold. Branch Bayesian posterior probability values below 1.0 are presented at the nodes. The tree was rooted to *Lophodermium piceae* and the scale bar indicates the number of substitutions per site.

Fig. 2. Bayesian 50% majority rule consensus tree based on *ACT* dataset containing representative *Lophodermium* species and related genera. GenBank accession numbers precede the species name followed by culture collection accession numbers, with ex-type strains in bold. Branch Bayesian posterior probability values below 1.0 are presented at the nodes. The tree was rooted to *Hymenoscyphus epiphyllus* and the scale bar indicates the number of substitutions per site.

Fig. 3. *Lophodermium resinosum* morphology. (A–G) Symptomatic *Pinus resinosa* needles showing ascomata and conidiomata (arrows denoting conidiomata). (H) Vertical section showing subepidermal position of mature ascoma. (I) Basal and covering stroma of ascoma. (J, M) Covering stroma of ascoma underneath host epidermis. (K) Basal stroma, subhymenium, and hymenium of ascoma. (L) Close-up of ascoma lip cells embedded in mucous. (N) Asci and paraphyses mounted in KOH. (O) Subepidermal conidiomata. (P) Conidiomata and conidia with covering host epidermis torn free. (Q) Gelatinous sheathes of ascospores evident within asci mounted in water. (R) Discharged ascospores mounted in water. (S) Conidia. (T) Conidiogenous cells. Scale bars: H, O–P = 100 µm; I–N, Q–T = 10 µm.

Lophodermium resinosum morphology. (A–G) Symptomatic *Pinus resinosa* needles showing ascomata and conidiomata (arrows denoting conidiomata). (H) Vertical section showing subepidermal position of mature ascoma. (I) Basal and covering stroma of ascoma. (J, M) Covering stroma of ascoma underneath host epidermis. (K) Basal stroma, subhymenium, and hymenium of ascoma. (L) Close-up of ascoma lip cells embedded in mucous. (N) Asci and paraphyses mounted in KOH. (O) Subepidermal conidiomata. (P) Conidiomata and conidia with covering host epidermis torn free. (Q) Gelatinous sheathes of ascospores evident within asci mounted in water. (R) Discharged ascospores mounted in water. (S) Conidia. (T) Conidiogenous cells. Scale bars: H, O–P = 100 μ m; I–N, Q–T = 10 μ m.

Fig. 3

180x261mm (300 x 300 DPI)