Mollisiaceae: An overlooked lineage of diverse endophytes

J.B. Tanney1, and K.A. Seifert2,3

1Pacific Forestry Centre, Canadian Forest Service, Natural Resources Canada, 506 Burnside Road, Victoria, British Columbia, V8Z 1M5, Canada; 2Ottawa Research and Development Centre, Biodiversity (Mycology and Microbiology), Agriculture and Agri-Food Canada, 960 Carling Avenue, Ottawa, Ontario, K1A 0C6, Canada; 3Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario, K1S 5B6, Canada

*Correspondence: J.B. Tanney, joey.tanney2@canada.ca

Abstract: Mollisia is a taxonomically neglected discomycete genus (Helotiales, Leotiomycetes) of commonly encountered saprotrophs on decaying plant tissues throughout temperate regions. The combination of indistinct morphological characters, more than 700 names in the literature, and lack of reference DNA sequences presents a major challenge when working with Mollisia. Unidentified endophytes, including strains that produced antifungal or antinsect secondary metabolites, were isolated from conifer needles in New Brunswick and placed with uncertainty in Phialocephala and Mollisia, necessitating a more comprehensive treatment of these genera. In this study, morphology and multigene phylogenetic analyses were used to explore the taxonomy of Mollisiaceae, including Mollisia, Phialocephala, and related genera, using new field collections, herbarium specimens, and accessioned cultures and sequences. The phylogeny of Mollisiaceae was reconstructed and compared using the rnc internal transcribed spacer rDNA (ITS) barcode and partial sequences of the 28S rnc rDNA (LSU) gene, largest subunit of RNA polymerase II (RPB1), DNA topoisomerase I (TOP1), and the hypothetical protein Lipin/Ned1/Smp2 (LNS2). The results show that endophytism is common throughout the Mollisiaceae lineage in a diverse range of hosts but is infrequently attributed to Mollisia because of a paucity of reference sequences. Generic boundaries within Mollisiaceae are poorly resolved and based on phylogenetic evidence the family included species placed in Acepahala, Acidomeliina, Barrenia, Bispora, Cheirospora, Cystoderion, Fuscosclera, Hysteroneaevia, Loramyces, Mollisia, Neopyrenopeziza, Oblectodiscus, Ombrophila, Patellariopsis, Phialocephala, Pulvinata, Tapesia (=Mollisia), and Trimmatostroma. Taxonomic novelties included the description of five novel Mollisia species and five novel Phialocephala species and the synonymy of Fuscosclera with Phialocephala, Acidomeliina with Mollisia, and Loramycesceteae with Mollisiaceae.

Key words: discomycete, molecular taxonomy, new taxa, Mollisia, Phialocephala, species identification.

Taxonomic novelties: New combinations: Mollisiaceae, Phialocephala heterosperma (Münzenb. & Bubner) J.B. Tanney & K.A. Seifert, Phialocephala lignicoles (Hern.-Restr., J. Mené & Gené) J.B. Tanney & K.A. Seifert, Mollisia panicicola (E. Walsh & N. Zhang) J.B. Tanney & K.A. Seifert. New species: Mollisia diesbachiann Tanney & Seifert, M. monnilioides Tanney & Seifert, M. novobrunswickensis Tanney & Seifert, M. prismatica Tanney & Seifert, M. rava Tanney & Seifert, Phialocephala amethystea Tanney & Seifert, P. biguturala Tanney & Seifert, P. collarifera Tanney & Seifert, P. helenae Tanney & Seifert, P. vermiculata Tanney & Seifert.

Available online 13 March 2020; https://doi.org/10.1016/j.simyco.2020.02.005.

INTRODUCTION

Mollisia (Mollisiaceae, Helotiales) is a large, cosmopolitan genus comprising species that are common saprotrophs, usually observed forming greyish to bluish, discoid apothecia on decaying plant tissues, especially wood and graminoid culms and leaves. Apothecia are typically 1–3 mm in diameter, sessile, and characterized by an outer layer (ectal excipulum) composed of pigmented, rounded cells (textura globulosa), a hyaline textura intricata inner layer (medullary excipulum), cylindrical paraphyses that when alive contain refractive vascular bodies, and ascospores that are usually 0–1-septate, elliptic-fusoid to fusiform, hyaline, and borne in 8- to 32-spored amyloid asci arising from croziers.

Mycologists collecting and studying Mollisia invariably face a major obstacle: our current understanding of asexual and sexual morphological characters does not permit rapid identification of most Mollisia species in the field or even confident identification following detailed microscopic study. Despite these difficulties, or perhaps because of them, hundreds of species have been named since the inception of the genus in 1871. More than 700 Mollisia names exist and the status of many of these species is mostly unknown. These numbers do not include possibly congeneric species currently placed in other genera, such as Belonidium, Belonopsis, Haglindia, Hysteroneaevia, Hysteronezietella, Nimbomollisia, Niptera, Scutomollisia, and Tapesia (=Mollisia; Hawksworth & David 1989), which were distinguished from Mollisia based on morphological characters such as the presence of long, cylindrical, septate marginal hairs (Haglindia), a well-developed melanized subiculum (Tapesia), septate ascospores with calcium oxalate crystals embedded in the medullary excipulum (Belonopsis), apothecia developing under a shield of radiating hyphae (Scutomollisia), 1-septate ascospores with gelatinous sheaths (Niptera), or multisepitate spores (Trichobolarium) (Nannfeldt 1976, Nauta & Spooner 2000a, b). The name Mollisia has also been applied to phylogenetically distant but sometimes morphologically similar taxa, including Cistella (Hyaloscyphaceae), Leptotrichia (Drepanopezizaceae), Mniaecia (Mniaeciacae), Orbilia (Orbiliaceae, Orbiliales), and especially Pyrenepeziza (Pleutnerulaceae) species, which can be quite similar to Mollisiaceae species but lack refractive vascular bodies in paraphyses.

Although discomycete taxonomists historically did not emphasize cultural studies, axenic cultures of Mollisia are readily made from fresh field collections by allowing ascospores to discharge onto standard media such as 2 % malt extract agar or potato dextrose agar. Asexual morphs may develop in vitro, notably phialocephala-like conidiophores as well as reports of
other distinctive asexual morphs (Le Gal & Mangenot 1956, Hennebert & Bellemere 1979, Tanney et al. 2016a). Altogether, cultural and phylogenetic studies link various morphologically diverse asexual morphs to Mollisia and related genera, including Anavirga dendromorpha (Descals & Sutton 1976, Hamad & Webster 1988), Anguillospora crassa (Webster 1961), Casaresia sphagnicola (Webster & Descals 1975, Webster et al. 1993), Cheirospora botryospora (Crous et al. 2015), Helicodendron giganteum (Fisher & Webster 1983), Paradigmymobrytum oblongum (=Phialocephala oblonga) (Tanney et al. 2016a), and Variocladium giganteum (Baschien et al. 2013), and other morphs referable to Anguillospora, Diplococcium, Filosporella, Septonema, Trimmatostroma, and microsclerotial structures (Webster & Descals 1979, Digby & Goos 1987, Shenoy et al. 2010, Tanney et al. 2016a; see Table 1). Prolonged incubation is often required to induce the development of asexual morphs, while the development of mature apothecia in vitro is rarely documented (Gremmen 1955, Le Gal & Mangenot 1958, Tanney et al. 2016a).

The entangled relationship between Mollisia and Phialocephala (Mollisiaceae, Helotiales), a genus comprising important root and leaf endophytes and known mostly by asexual morphs, suggests that the ecology of Mollisia species is more complex than previously assumed (Day et al. 2012, Tanney et al. 2016a). Phialocephala species are saprotrophs, plant mutualists, potential plant health promoters, producers of industrially significant secondary metabolites, and infrequent causal agents of plant diseases (Frasz et al. 2014, Arneaud & Porter 2015, Wong et al. 2015). The most well-studied Phialocephala species belong to the Ph. fortinii sensu lato (s.l.)–Acephala applanata species complex (PAC), which contains at least 22 cryptic species, eight of which have been formally described (Stroheker et al. 2018, Landolt et al. 2020). The genus Acephala consists of two endophyte species associated with roots of Picea and Pinus. Although Acephala was delineated from Phialocephala by phenotypic characteristics, namely absent sporulation and colony morphology, and molecular (ITS) characteristics, it is likely congeneric with Phialocephala (Grünig & Sieber 2005, Tanney et al. 2016a).

### Table 1. Examples of diverse asexual morphs attributed to Mollisiaceae.

| Taxa                          | Morphological description                                                                 | References                  |
|-------------------------------|-------------------------------------------------------------------------------------------|-----------------------------|
| Acephala macroscelerotium     | Sclerotia with thick-walled highly melanized outer cells and less thick-walled hyaline inner cells | Münzenberger et al. (2009) |
| Anavirga dendromorpha         | Brown stauroconidia with 2–4 septate arms                                                 | Descals & Sutton (1976), Hamad & Webster (1988) |
| Anguillospora crassa          | Hyaline sclecoconidia                                                                    | Webster (1961)              |
| Bispora betulina              | Monoblastic, brown (1–)2(–3)-septate didymospores in unbranched and branched acrpetal chains with phialocephala-like synaexual morphs | Wang (1989)                 |
| Casaresia sphagnorum          | Monoblastic stauroconidia with 5–6 recurved arms                                          | Webster et al. (1993)       |
| Cheiriospora botryospora      | Bulbils composed of brown globose cells surrounded in a gelatinous sheath arising from sporodochia or acervuli | Crous et al. (2015)          |
| Cystodendron dryophilimum     | More or less phialocephala-like conidiophores and phialides forming sporodochia; also see M. discolor var. longispora and M. benesuada | Le Gal & Mangenot (1956), Aebi (1972) |
| Diplococcium spicatum         | Brown, 1-septate, cylindrical conidia in chains from branched conidiophores                | Seifert et al. (2011)       |
| Fuscoscella lignicola         | Multisepitate, dark brown to black, irregular propagules from unbranched septate conidiophores (cf. P. catenospora) | Hernández-Restrepo et al. (2017) |
| Helicodendron giganteum       | Hyaline to brown three-dimensional helicoid conidia                                        | Fisher & Webster (1983)     |
| Loramyces juncicola           | Anguillospora-like hyaline sclecoconidia                                                   | Digby & Goos (1987)         |
| Mollisia ligni                | Black, synnema-like, arising from black stroma with sterile inflated vesicles interspersed among cystodendron-like conidiophores (‘forme Pyrodiella’ sensu Le Gal & Mangenot 1956) | Tulasne & Tulasne (1865), Le Gal & Mangenot (1956) |
| Ph. fortinii sensu lato (s.l.)–Acephala applanata complex | Microsclerotia lacking differentiation of rind and medulla | Yu et al. (2001)            |
| Phialocephala cladophialophoroides | Monilioid hypha interpreted by Crous et al. (2017) as a cladophialophora-like asexual state | Crous et al. (2017)          |
| P. catenospora                | Diplococcium-like chains of didymo- and phragmoconidia                                      | Tanney et al. (2016a)       |
| P. compacta                   | Sclerotized conidiophores                                                                  | Kowalski & Kehr (1995)      |
| P. dimorphospora              | Brown penicillate conidiophores bearing deep collarettes yielding dimorphic conidia in slimy chains | Harrington & McNew (2003)   |
| P. hiberna                   | Phialocephala-like conidiophores and phialides forming sporodochia (up to 2.5 × 0.5 cm)   | Bills (2004)                |
| P. nodosa                    | Darkly pigmented sclerotia comprised of moniliform cells arising from helicoid initials    | Tanney et al. (2016a)       |
| P. oblonga                    | Didymospores in short acropetal chains arising from synnemata                              | Tanney et al. (2016a)       |
| Trimmatostroma salcis         | Brown moniliform arthroconidia                                                             | Crous et al. (2007)         |
| Variocladium giganteum       | Hyaline tetraradiate conidia with 2+ septate arms                                          | Baschien et al. (2013)      |
et al. 2016a). The PAC comprises so-called dark septate root endophytes, which are ubiquitous in the Northern Hemisphere and form complex communities in the roots of conifers and ericaceous plants. Members of the PAC are apparently restricted to roots and corresponding apothecia have never been observed in nature. Other Phialocephala species are reported as foliar and branch endophytes that form apothecia on decomposing tissues. For example, P. scopiformis is a well-studied, common foliar and branch endophyte of Picea in N. America and Europe that produces apothecia on decomposing Picea wood, while P. piceae is an endophyte of Picea and Pinus strobus that produces apothecia on decomposing hardwood such as Acer saccharum (Tanney et al. 2016a).

While generally considered to be “just” saprotrhops, some studies also report Mollisia as endophytes of leaves and twigs in diverse host plants (Sieber 1989, Barklund & Kowalski 1996, Shamoun & Sieber 2000, Kowalski & Andruch 2012, Anderson Stewart et al. 2019, Lee et al. 2019). However, Phialocephala and Mollisia are polyphyletic and the delineation of these two genera remains unclear; consequently, endophytes identified by ITS sequences are often arbitrarily designated as Phialocephala, Mollisia, or Acephala. While the type species of Phialocephala, P. dimorphospora, is designated by an ex-type strain and is phylogenetically well-defined, the precise identification of the type species of Mollisia, M. cinerea, is unclear and the holotype is lost, although efforts to epitypify M. cinerea are underway (A. Gminder, pers. comm.). Understanding the relationship between Phialocephala and Mollisia is crucial for defining generic boundaries within Mollisiaceae.

Additionally, close phylogenetic relationships between Phialocephala, Mollisia, and other related genera comprising species that inhabit aquatic habitats, including Loramyces (Loramyctaceae, Helotiales) and Vibrissea (Vibrissectaceae, Helotiales) (Wang et al. 2006b), along with reports of conidial adapted for aquatic dispersal (Webster 1961, Webster et al. 1993), suggest unexpected ecological and morphological diversity within the lineage. As the list of possible Mollisiaceae genera grows, so does the list of potential taxonomic and nomenclature issues. Even the consensus remains unclear on which family to place Mollisiaceae and related mollisioid discomycetes is notoriously difficult (Greenleaf & Korf 1980, Nauta 2010). The major obstacles hindering the progress of taxonomic and phylogenetic studies of Mollisia include: (1) an absence of authenticated reference sequences; (2) a dearth of ex-type cultures; (3) difficulties identifying or sequencing exsiccatae because of the absence of vital characters, poor condition, or loss; (4) difficulty identifying field and herbarium specimens based on indistinct morphological characters; and (5) the absence of a usable taxonomic treatment with identification keys. These obstacles have effectively deterred any concerted effort to confront Mollisia, and the shortage of traditional taxonomists and a growing dependence on working with previously identified specimens or sequences, sometimes of questionably accuracy, compounds the problem.

More than 120 years ago, Crossland (1896) stated that a thorough revision of Mollisia was out of the question and described the difficulty in defining the type species M. cinerea, which still remains unresolved. In the aptly titled paper, “Mollisia in Macaronesia: an exercise in frustration”, Greenleaf and Korf (1980) pointed out, “how little, not how much, we know about Mollisia today”—a sentiment that unfortunately still holds true 40 years later. Taxonomic research on Mollisia and related taxa has effectively stagnated, with a few exceptions (e.g. Gminder 2006, 2012, Day et al. 2012, Hosoya et al. 2015, Tanney et al. 2016a).

New descriptions of Mollisia species have dwindled in the last 100 years (Fig. 1) and while a search for the keyword, “Mollisia” in Google Scholar shows a rise in its use over the last 20 years (Fig. 2), most references to Mollisia in such publications are cursory, such as biodiversity checklists, references to accessioned GenBank sequences included in unrelated phylogenetic studies, or mentioning unidentified Mollisia endophytes. A query of the keyword, “Phialocephala” shows its use surpassing that of “Mollisia”, most likely from PAC and other endophyte research. We predict a resurgence of interest in Mollisia, and Mollisiaceae in general, because of emerging evidence showing their ubiquity and significance as endophytes. Thus, the taxonomic neglect of Mollisiaceae must end sooner than later to facilitate growing research interest in non-clavicipitaceous endophytes and to prevent the accumulation of new taxonomic discrepancies.

In this study, the phylogeny of Mollisiaceae is explored using DNA sequences from multiple loci and data derived from new field collections, cultures and herbarium specimens. The objectives of this study are to: (1) assist users by providing reference data and more comprehensive phylogenies; (2) test LNS2, RPB1, and TOP1 as phylogenetic markers and secondary barcodes; (3) provide rationale for the various approaches to addressing some of the major taxonomic and nomenclatural issues of Mollisiaceae; (4) evaluate the significance of endophytism throughout the lineage; and (5) generate interest for this important but neglected family. Given the overall lack of recent research into the taxonomy and ecology of Mollisiaceae, we provide some extended discussion on the taxonomy, biology, and ecology of select clades, genera, and species. One of the original goals of this study was to generate phylogenetic data and make taxonomic changes accordingly, promoting taxonomic stability and practicality in this lineage. It soon became evident that such actions would be premature and likely initiate a turbulent taxonomic phase marked by ephemeral name changes and more confusion for taxonomists and users alike. In this respect, this
study may serve as a prodromus for the impending and necessary revision of Mollisiaeaceae. Sampling is presently too inadequate to enable wide sweeping and stable taxonomic changes. While previous workers depending solely on morphological characters could not make enough progress because of confounding characters, DNA sequence-based methods now facilitate rapid species delineation, identification, and phylogenetic reconstruction. Molecular phylogenetic methods combined with detailed phenotypic and ecological studies will provide robust and cohesive taxonomic concepts for this notoriously
difficult family. The future of *Mollisia* taxonomy is finally encouraging.

**MATERIALS AND METHODS**

**Sampling and isolation of fungi**

Field collections of mostly lignonicolous apothecia were made in New Brunswick, Ontario, and Quebec, Canada (Table 2). Herbarium specimens and cultures were kept in the personal collection of J.B. Tanney and representative materials of interest were accessioned into the Canadian National Mycological Herbarium (DAOM) and Canadian Collection of Fungal Cultures (CCFC/DAOMC). Other cultures of *Mollisia* and related genera were obtained from the CCFC and the Westerdijk Fungal Biodiversity Institute (CBS) culture collection.

Cultures derived from ascospores were made by suspending mature ascocarps from the lid of a 6 cm Petri dish using petroleum jelly or drops of water for up to 24 h and allowing ascospores to eject downward or upward onto the agar surface. 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). Monospore cultures were obtained from individual ascospores whenever feasible and transferred to 6 cm Petri dishes containing MEA. Endophyte cultures were isolated following the methods of Tanney et al. (2016a). All cultures were incubated in the dark at 16 °C.

**Morphological studies**

Vertical sections of apothecia were cut by hand and mounted in either water, 85 % lactic acid, Melzer’s reagent, cresyl blue, 5 % KOH, or Lugol’s solution with or without 5 % KOH pretreatment to test amyloid reactions (Baral 1987b). Culture tissues were cut from agar using a scalpel or dissected using pins and mounted in either water, 85 % lactic acid, 5 % KOH, or lactofuchsin. Morphological observations of specimens were made on living material whenever possible. The length of the space occupied by ascospores in living asci is defined as *pars sporifera*. In some *Mollisiaceae* species, the refractive vacuolar bodies in the paraphyses show a yellow reaction of varying intensity when placed in KOH. To assess this character, we introduced apothecia to a raphyses show a yellow reaction of varying intensity when placed in KOH. To assess this character, we introduced apothecia to a

**Phylogenetic studies**

Total genomic DNA was extracted from 4–12-wk-old cultures using the Ultraclean Microbial DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA) or NucleoSpin Plant II Kit (Macherey-Nagel, Düren, Germany) following the manufacturers’ protocols. DNA extractions from herbarium specimens were made using the NucleoMag Trace kit (Macherey-Nagel, Düren, Germany) with an initial tissue grinding stage in liquid nitrogen using an Ayxygen polypropylene pestle (PES-15-B-SI, Union City, CA, USA).

The primer pairs ITS1 and ITS4 (White et al. 1990) or ITS4A and ITS5 (Larena et al. 1999) were used to amplify and sequence the ITS region, which is the primary barcode for fungi (Schoch et al. 2012). Partial 28S nuc rDNA (LSU) gene, selected because of its discriminatory power for taxonomic assignment at family and higher taxonomic levels (Vu et al. 2019), was amplified and sequenced following the methods of Tanney et al. (2015). The largest subunit of RNA polymerase II (RPB1), selected because of its ability to distinguish species and provide good phylogenetic resolution in *Mollisiaceae* and other Holotiales families (Walsh et al. 2015, Tanney et al. 2016a, Pärtel et al. 2017, Johnston et al. 2019), was amplified and sequenced using RPB1-Af and RPB1-6Rlasc (Stiller & Hall 1997, Hofstetter et al. 2007). Three protein-coding genes identified by Stielow et al. (2015) as promising supplementary DNA barcodes were tested: Lipin/Ned1/Smp2 (LNS2) was amplified using the primers LNS2_468-F and LNS2_468-R, DNA topoisomerase I (TOP1) was amplified using the primers TOP1_501-F and TOP1_501-R. DNA was amplified using a PCR master mix consisting of 0.5 μL 2 μM dNTPs, 0.04 μL 20 μM forward primer, 0.04 μL 20 μM reverse primer, 1 μL 10× Titanium Taq buffer (Clontech, Mountain View, CA, USA), 0.1 μL 50× Titanium Taq enzyme (Clontech), 1 μL of DNA template, and 7.32 μL sterile Milli-Q water (Millipore, Bedford, MA, USA) per reaction (Allain-Boulé et al. 2004). For herbarium specimens, 0.5 μL of 20 mg/mL bovine serum albumin (BSA; Thermo Fisher Scientific, Whitman, MA, USA) was added to each reaction. 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. TOP1 and LNS2 were initially amplified using the Touchdown PCR (68–58 °C) described in Stielow et al. (2015). PCR troubleshooting included adjusting annealing temperature, dilution of DNA, addition of BSA, and using different Taq polymerase (Ex Taq HS DNA Polymerase, Takara Bio Inc., Otsu, Japan). PCR products were verified by agarose gel electrophoresis and sequenced with BigDye Terminator (Applied Biosystems, Foster City, CA, USA).

Sequence contigs were assembled and trimmed using Geneious Prime 2019.0.4 (Biomatters Ltd., Auckland, New Zealand). Individual gene sequences were aligned using MAFFT v. 7 (Katoh & Standley 2013) and the resulting alignments trimmed and manually checked using Geneious Prime.
| Name | Substrate | Location | Sequence source or specimen | GenBank accession no. | Reference |
|------|-----------|----------|-----------------------------|-----------------------|-----------|
| **Name** | **Substrate** | **Location** | **Sequence source or specimen** | **ITS** | **LSU** | **RPB1** | **TOP1** | **LNS2** | **Reference** |
| Acephala applanata | Endophyte of Picea abies root | Switzerland | CBS:109321T | NR_119482 | MT026532 | MT018410 | MT039071 | MT009650 | Grünig et al. (2002), this study |
| A. macroscleroticum | Ectomycorrhizal root tip of Pinus sylvestris | Germany | CBS:123555T | NR_121349 | MT026487 | MT018414 | MT039026 | MT009505 | Münzenberger et al. (2009), this study |
| Acephala sp. | Root of Pinus banksiana | Canada | D_3_1 | EUA34830 | — | — | — | — | Grünig et al. (2009) |
| | Root of Picea abies | Finland | 2811 | KC480052 | — | — | — | — | Terhonen et al. (2014) |
| | Unspecified | Unspecified | AL6m1 | KJ188688 | — | — | — | — | Luo et al. (2014) |
| | Sugarcane cultivar SP80-1842 | Brazil | ASR-174 | GU973728 | — | — | — | — | Romao & Araujo (direct submission) |
| | Sugarcane cultivar IMI | Brazil | ASR-197 | GU973749 | — | — | — | — | Romao & Araujo (direct submission) |
| Dichanthelium acuminatum | USA | CM14_RG32 | KU597356 | — | — | — | — | Luo et al. (direct submission) |
| Dichanthelium acuminatum | USA | CM14_RG90_1A | KU597353 | — | — | — | — | Luo et al. (direct submission) |
| Unspecified | Unspecified | CM7m4 | KJ188687 | — | — | — | — | Luo et al. (2014) |
| Peat | Germany | JU-A-2/DSM:27592 | HG530746 | — | — | — | — | Singh et al. (2014) |
| Root of Pseudorchis albida | Czech Republic | PA 150/9 MV-2011 | JN655568 | — | — | — | — | Kohout et al. (2013) |
| Root of Pseudorchis albida | Czech Republic | PA 205/7 MV 2011 | JN655564 | — | — | — | — | Kohout et al. (2013) |
| Root of Pseudorchis albida | Czech Republic | PB 001/4 MV-2011 | JN655562 | — | — | — | — | Kohout et al. (2013) |
| Root of Pseudorchis albida | Czech Republic | PB 075/4 MV-2011 | JN655563 | — | — | — | — | Kohout et al. (2013) |
| Root of Leucorchis albida | Czech Republic | PFO_041/7 CRG-2011 | HQ713749 | — | — | — | — | Grüning & Sieber (direct submission) |
| Cymbidium insigne | China | W2-5 | HQ889709 | — | — | — | — | Huang et al. (direct submission) |
| Pinus rigida | USA | WSF14_P14 | KU597350 | — | — | — | — | Luo et al. (direct submission) |
| Panicum virgatum | USA | WSF14_SW51 | KU597351 | — | — | — | — | Luo et al. (direct submission) |
| Unspecified | China | Y-007 | MN59787 | — | — | — | — | Yuan (direct submission) |
| Name                        | Substrate                        | Location       | Sequence source or specimen       | GenBank accession no. | Reference                      |
|-----------------------------|----------------------------------|----------------|-----------------------------------|-----------------------|--------------------------------|
| **Acidomelania panicicola** | Roots of Panicum virgatum        | Switzerland    | CdV_6_D.4.4b                      | EU434832              | Grünig et al. (2009)           |
| **Apostemidium vibrisseoides** | Sticks in water                | Canada: Ontario | DAOM:120405                       | MT026442              | This study                     |
| **Ascomycete sp.**          | Unspecified                      | Norway         | HK-S236                           | AM084465              | Kauserud et al. (2005)         |
| **Ascomycete**              | Sticks in water                 | Canada: Quebec | DAOM:46436                        | MT026443              | This study                     |
| **Barrenia panicia**        | Roots of Digitaria sp.           | USA            | A15M2                             | —                     | Walsh et al. (2015)            |
| **B. taeda**                | Roots of Panus rigida           | USA            | CM11M2                             | —                     | Walsh et al. (2015)            |
| **Belonium excelsior**      | Roots of Pinus rigida           | USA            | CM14P64                            | —                     | Walsh et al. (2015)            |
| **Bispora betulina**        | Roots of Pinus rigida           | USA            | RUTPP:WSF14P22T                    | —                     | Walsh et al. (2015)            |
| **Cadophora hiberna**       | Robinia pseudoacacia wood       | USA: New Jersey | GB5129                            | AF530461              | Bills (2004)                   |
| **Calluna vulgaris root**   | Calluna vulgaris                | Germany        | agrKH075                          | FM172774              | Pietrowski (direct submission) |

(continued on next page)
| Name                          | Substrate                                  | Location          | Sequence source or specimen | GenBank accession no. | Reference                        |
|-------------------------------|--------------------------------------------|-------------------|----------------------------|-----------------------|----------------------------------|
| *Calluna vulgaris*            | Germany                                    | agrKH079          | FM172778                   | —                     | Pietrowski (direct submission)   |
| *Calluna vulgaris*            | Germany                                    | agrKH104          | FM172803                   | —                     | Pietrowski (direct submission)   |
| *cf. Niptera* sp.             | Bark and wood of rotten branch in drying stream | Canada: New Brunswick | MT026433                   | —                     | This study                       |
| *Cheirospora botryospora*     | Branches of *Fagus sylvatica*              | Germany           | CPC 24603                  | KR611870              | Crous et al. (2015)               |
|                              | Branches of *Fagus sylvatica*              | Germany           | CPC 24605                  | KR611871              | Crous et al. (2015)               |
|                              | Branches of *Fagus sylvatica*              | Germany           | CPC 24611                  | KR611873              | Crous et al. (2015)               |
| *Chlorenceocelia versiformis* | Rotten hardwood log                        | Canada: Quebec    | DAOMC:251598               | MH457140              | McMullin et al. (2019), this study |
| *Chlorosplenium chlora*       | Fallen branch USA: Massachusetts           | FH:BHI-F736       | MG553993                   | —                     | Haelewaters et al. (2018)         |
|                              | Wood                                       | FH:BHI-F737       | MG553994                   | —                     | Haelewaters et al. (2018)         |
| *Cystodendron dryophilum*    | *Juniperus communis* needle                 | Switzerland       | CBS:295.81                 | MT026425              | This study                       |
| *Cystodendron sp.*           | *Abies alba* Switzerland: Alptal           | TS_, 90, 233      | EU434835                   | —                     | Grüning et al. (2009)             |
|                              | *Castanea sativa* Switzerland: Bellinzona  | UAMH 10850        | EU434834                   | —                     | Grüning et al. (2009)             |
| *Durella connivens*          | Broken hardwood stick USA: Massachusetts   | FH:BHI-F627       | MF161306                   | —                     | Haelewaters et al. (2018)         |
|                              | *Branch of Populus tremula*                | Luxembourg        | KY462810                   | —                     | Hermant (direct submission)      |
|                              | *Branch of Salix* Luxembourg               | Luxembourg        | KY462811                   | —                     | Hermant (direct submission)      |
| *Epacris microphylla* root associated fungus | *Epacris microphylla* Australia | 27 | AY268211 | — | — | — | Williams et al. (direct submission) |
|                              | *Epacris pulchella* Australia              | EP19              | AY627823                   | —                     | Bougoure & Cairney (2005)         |
| *Fungal endophyte*           | *Picea mariana* Canada                     | 3395              | DQ979586                   | —                     | Higgins et al. (2007)             |
|                              | *Picea mariana* Canada                     | 4608              | DQ979674                   | —                     | Higgins et al. (2007)             |
|                              | *Avenella flexuosa* Norway                 | 36-54t            | GUS81235                   | —                     | Jensen et al. (2011)              |
| Name                     | Substrate                          | Location          | Sequence source or specimen | GenBank accession no. | Reference                                |
|--------------------------|------------------------------------|-------------------|-----------------------------|-----------------------|------------------------------------------|
| *Avenella flexuosa*      | Norway                             | 51-54t            | GU581250                    | —                     | Jensen et al. (2011)                     |
| *Avenella flexuosa*      | Norway                             | 52-54t            | GU581251                    | —                     | Jensen et al. (2011)                     |
| *Avenella flexuosa*      | Norway                             | 57-4rct           | GU581256                    | —                     | Jensen et al. (2011)                     |
| *Calluna vulgaris*       | Germany                            | AP509             | FM200687                    | —                     | Pietrowski (direct submission)           |
| *Elymus mollis*          | USA                                | C339J             | KT203037                    | —                     | David (direct submission)                |
| Leaf and root of         | USA                                | SV1702            | MK036902                    | —                     | Kimbrough et al. (2019)                  |
| Taxodium distichum       |                                    |                   |                             |                       |                                          |
| Fungal sp.               |                                    |                   |                             |                       |                                          |
| Root tips of *Pinus*     | Finland                            | 2.2.4C            | KM068396                    | —                     | Sarjala et al. (direct submission)       |
| *sylvestris*             |                                    |                   |                             |                       |                                          |
| Root tips of *Pinus*     | Finland                            | 2.2.4D            | KM068397                    | —                     | Sarjala et al. (direct submission)       |
| *sylvestris*             |                                    |                   |                             |                       |                                          |
| Root tips of *Pinus*     | Finland                            | 3.12.4B           | KM068428                    | —                     | Sarjala et al. (direct submission)       |
| *sylvestris*             |                                    |                   |                             |                       |                                          |
| Root tips of *Pinus*     | Finland                            | 3.16.3A           | KM068431                    | —                     | Sarjala et al. (direct submission)       |
| *sylvestris*             |                                    |                   |                             |                       |                                          |
| Root tips of *Pinus*     | Finland                            | 3.44.4D           | KM068438                    | —                     | Sarjala et al. (direct submission)       |
| *sylvestris*             |                                    |                   |                             |                       |                                          |
| Root tips of *Pinus*     | Finland                            | 3.46.4A           | KM068442                    | —                     | Sarjala et al. (direct submission)       |
| *sylvestris*             |                                    |                   |                             |                       |                                          |
| Withered plant material  | Norway                             | A4-3              | AM231338                    | —                     | Mysterud et al. (2007)                   |
| Wood                     | Antarctica                         | AB45              | FJ235978                    | —                     | Arszen & Blanchette (2009)               |
| *Myotis septentrionalis*| USA                                | APA-2013 clone    | KF212280                    | —                     | Johnson et al. (2013)                   |
| wing                     | LJ75Mg10w                          |                   |                             |                       |                                          |
| *Myotis septentrionalis*| USA                                | APA-2013 clone    | KF212281                    | —                     | Johnson et al. (2013)                   |
| wing                     | LJ81Mg10w                          |                   |                             |                       |                                          |
| *Thuja koraiensis*       | South Korea: Gyeonggi              | JE-2              | LC163521                    | —                     | Eo et al. (2016)                         |
| *Carex bigelowii*        | Finland                            | K7TJ2 AR-2014     | KF527816                    | —                     | Ruotsalainen et al. (direct submission)  |
| *Phragmites australis*   | USA                                | OTU26             | KT923245                    | —                     | Clay et al. (2016)                      |
| var. *australis*         |                                    |                   |                             |                       |                                          |
| *Phragmites australis*   | USA                                | OTU5              | KT923252                    | —                     | Clay et al. (2016)                      |
| var. *australis*         |                                    |                   |                             |                       |                                          |

(continued on next page)
| Name                     | Substrate                       | Location      | Sequence source or specimen | GenBank accession no. | Reference                  |
|--------------------------|---------------------------------|---------------|-----------------------------|-----------------------|----------------------------|
| P. australis var. australis | USA                             | OTU54         | KT23222                     |                       | Clay et al. (2016)         |
| Tetrastigma hemiselyanum  | China                           | TH11          | KY607743                    |                       | Song et al. (2017)         |
| Fuscosclera lignicola    | Dead wood                       | CBS:142287T   | NR, 164252                  |                       | Hemández-Restrepo et al. (2017) |
| Grass root mycorrhizal sp. | Netherlands                     | PPO-1         | AYS99235                    |                       | Baar et al. (direct submission) |
| Helgardia anguioides     | Unspecified                     | CBS:496.80    | MH861290                    |                       | Vu et al. (2019)           |
| Helotiales sp.           | Bottom sediment of bog          | Russia        | 65 OA-2013                  |                       |                           |
|                         | Bottom sediment of bog          | Russia        | 73 OA-2013                  |                       |                           |
| Black sclerotium         | USA: Florida                    | BA4b011       | A8986446                    |                       | Obase et al. (direct submission) |
| Hair roots of Phylloclode aleutica | Japan                  | EF804         | LC130995                    |                       | Shimono & Hirose (direct submission) |
| Green herbarized         | Populus euphratica              | China         | POPeuph60                   |                       | Unterseher et al. (2012)   |
| Lonicera caerulea        | Japan                           | Tok4-6        | LC180195                    |                       | Tamai et al. (direct submission) |
| Hysteronaevia scirpina   | Scirpus acutus                  | Canada: Ontario | DAOM: 147320               | MT026444             | This study                 |
| Leotiomycetes sp.        | Flavoparmelia caperata          | USA           | ARIZ: NC1044                | JQ761691             | U’Ren et al. (2012)        |
|                         | Tsuga canadensis                | USA           | ARIZ: NC1274                | KX908506             | U’Ren & Arnold (2016)      |
|                         | Picea abies wood                | Latvia        | SR95                        | MK911701             | Burnevica & Bruna (direct submission) |
|                         | Betula pendula                  | Latvia        | Z45                         | MK907715             | Burnevica & Bruna (direct submission) |
| L. macrosporus           | Submerged dead internode of Equisetum limosum | UK          | CBS:235.53T                | MH857170             | Vu et al. (2019), this study |
|                         | Eleocharis palustris            | UK            | CBS:235.53T                | MH857170             | Vu et al. (2019), this study |
|                         | Submerged dead internode of Equisetum limosum | UK          | CBS:235.53T                | MH857170             | Vu et al. (2019), this study |
| Name                | Substrate                          | Location                | Sequence source or specimen | GenBank accession no. | Reference                  |
|---------------------|------------------------------------|-------------------------|----------------------------|-----------------------|----------------------------|
|                     |                                    |                         |                            | ITS                   | LSU | RPB1 | TOP1 | LNS2   |                          |
| Mollisia benesuada  | Fallen branch                      | Switzerland             | DAOM:56135                 | MT026445              |     |      |      |        | This study                |
| M. caesia           | Unspecified                        | Netherlands             | CBS:220.56                 | MT026389              | MT026503 | MT018366 | MT039042 | MT009521 | This study                |
|                     | Symphoricarpos occidentalis        | Canada: Manitoba        | DAOM:86792                 | MT026446              |     |      |      |        | This study                |
| M. cf. cinerea      | Decaying wood                      | Canada: Ontario         | DAOMC:251569               | MT026401              | MT026515 | MT018353 | MT039054 | MT009533 | This study                |
|                     | Decaying log                       | Canada: Ontario         | DAOMC:251576               | MT026402              | MT026516 | MT018354 | MT039055 | MT009534 | This study                |
|                     | Rotten branch of Picea glauca      | Canada: Alberta         | DAOMC:251594               | MT026447              |     |      |      |        | This study                |
|                     | Rotten wood of Betula alleghaniensis | Canada: New Brunswick   | DAOMC:252029               | MT026403              | MT026517 | MT018352 | MT039056 | MT009535 | This study                |
| M. cf. diesbacha    | Decaying branch in wet culvert     | Canada: Quebec          | JBT-36-1                   | MT026448              |     |      |      |        | This study                |
| M. cf. fusca        | Decaying log of Betula papyrifera  | Canada: New Brunswick   | DAOMC:251565               | MT026434              |     |      | MT025204 |         | This study                |
| M. cf. melaluca     | Endophyte of Abies balsamea needle | Canada: New Brunswick   | DAOMC:250733               | MT026408              | MT026524 | MT018365 | MT039063 | MT009542 | This study                |
| M. cf. nigrescens   | Endophyte of Picea rubensneedle    | Canada: New Brunswick   | DAOMC:250738               | MT026414              | MT026535 | MT018415 | MT039074 | MT009553 | This study                |
| M. cf. undulatocephalodesulata | Decaying Betula papyrifera branch on ground | Canada: New Brunswick | DAOMC:250746               | MT026449              |     |      |      |        | This study                |
|                     | Decaying decoricated hardwood branch | Canada: New Brunswick   | NB563                      | MT026450              |     |      |      |        | This study                |
| M. cinerea          | Fallen log                         | USA                     | AFTOL 76                   | DQ491498              |     |      |      |        | Schoch (direct submission) |
|                     | Fallen log                         | USA: Oregon             | CBS:122029                 | MT026426              | MT026558 | MT018426 | MT039097 | MT009576 | This study                |
|                     | Erica umbellata                    | Morocco                 | ER47M                      | KU986797              |     |      |      |        | Hamim et al. (2017)       |
|                     | Leaves of Embothrium coccineum     | Chile                   | FE21                       | KU743962              |     |      |      |        | Gonzalez Teuber (direct submission) |
|                     | Luma apiculata                     | Argentina               | UFMGCB 3900                | JQ346201              |     |      |      |        | Vaz et al. (2014)         |
| M. cinereola        | Calluna vulgaris                   | Morocco                 | ER366                      | KU986768              |     |      |      |        | Hamim et al. (2017)       |
| M. cinereolivascens | Fallen stem of Betula sp.          | France                  | CBS:553.63                 | MT026371              | MT026477 | MT018350 | MT039016 | MT009495 | This study                |
| M. dextrinospora    | Decaying wood                      | Spain: Macaronesia      | CBS:401.78T                | NR_119489             | MT026542 | MT018437 | MT039081 | MT009560 | Crous et al. (2003), this study |

(continued on next page)
| Name                  | Substrate                  | Location                  | Sequence source or specimen                        | GenBank accession no. | Reference                          |
|-----------------------|----------------------------|---------------------------|---------------------------------------------------|-----------------------|------------------------------------|
| M. diesbachiana       | Decaying Betula alleghaniensis wood | Canada: New Brunswick      | DAOMC:250732                                       | MT026405 MT026521 MT018377 MT039060 MT009539         | This study                        |
| M. discolor           | Unspecified                | France                    | CBS:289.59                                        | MT026390 MT026504 MT018367 MT039043 MT009522         | This study                        |
| M. endocrystallina    | Fallen decorticated Picea abies tree trunk | Croatia                  | CNF 2/1005ST                                      | MK088059              | Matocec et al. (direct submission) |
| M. epiypsyha          | Typha latifolia            | Canada: Ontario           | DAOM:15077                                        | MT026451              | This study                         |
| M. fallens            | Unspecified                | Netherlands               | CBS:221.56                                        | MT026391 MT026505 MT018368 MT039044 MT009523         | This study                        |
| M. fusca              | Decorticated log           | USA: Massachusetts        | BHI-F660a                                         | MF161318              | Haaselwaters et al. (2018)          |
| M. fuscoparaphysata   | Unspecified                | Czech Republic            | J1106_36                                          | MH492942              | Vasutova (direct submission)       |
| M. heterosperma       | Unspecified                | France                    | CBS:292.59                                        | KP768364 MT026481 MT018382 MT039020 MT009499         | Tanney et al. (2016a), this study |
| M. hydrophila         | Phragmites australis       | France                    | CBS:556.63                                        | MT026436              | This study                         |
| M. ligni var. ligni   | Unspecified                | France                    | CBS:290.59                                        | MT026404 MT026520 MT018378 MT039059 MT009538         | This study                        |
| M. ligni var. olivascens | Unspecified                | France                    | CBS:291.59                                        | MT026437              | This study                         |
| M. lividofusca        | Lonicera coerulea          | Switzerland               | CBS:231.71                                        | MT026438              | This study                         |
| M. melaleuca          | Endophyte of Picea abies needle | Germany                 | CBS:589.84                                        | MH861785 MT026519 MT018364 MT039058 MT009537         | Vu et al. (2019)                  |
| M. minutella          | Picea abies                | Sweden                    | JAB5                                              | D0008242              | Allmer et al. (2006)               |
|                       | Ledum palustre             | China                     | X12                                               | KJ817294              | Yang & Yan (direct submission)     |
| M. moniloides         | Endophyte of Picea rubens needle | Canada: New Brunswick     | DAOMC:250734T                                     | MT026427 MT026559 MT018427 MT039098 MT009577         | This study                        |
|                       | Endophyte of Picea rubens needle | Canada: New Brunswick     | DAOMC:250735                                      | MT026428 MT026560 MT018428 MT039099 MT009578         | This study                        |
| M. nigrescens         | Decaying wood              | France                    | CBS:556.63                                        | MT026415 MT026536 MT018416 MT039075 MT009554         | This study                        |
|                       | Unknown hardwood           | Canada: Ontario           | DAOMC:250739                                      | MT026416 MT026537 MT018417 MT039076 MT009555         | This study                        |
| M. novobrunsvicensis  | Endophyte of Abies balsamea needle | Canada: New Brunswick     | DAOMC:250736                                      | MT026439              | This study                         |
| Name | Substrate | Location | Sequence source or specimen | GenBank accession no. | Reference |
|------|-----------|----------|-----------------------------|-----------------------|-----------|
|      | Rotten wood of *Betula alleghaniensis* | Canada: New Brunswick | DAOMC:251495 | MT026453 | This study |
|      | Decaying log | Canada: New Brunswick | DAOMC:251538 | MT026383 | This study |
|      | Decaying log of *Betula alleghaniensis* | Canada: New Brunswick | DAOMC:251631 | MT026452 | This study |
|      | Old decaying mossy log of *Betula papyrifera* | Canada: New Brunswick | DAOMC:252263T | MT026382 | This study |
|      | Decaying *Betula alleghaniensis* branch on ground | Canada: New Brunswick | NB579 | MT026384 | This study |
| *M. olivascens* | Unspecified | France | CBS:293.59 | MT026440 | This study |
|      | Decaying stick of *Acer saccharum* | Canada: Quebec | DAOMC:250740 | MT026394 | This study |
|      | Rotten hardwood | Canada: Ontario | DAOMC:251496 | MT026432 | This study |
|      | Rotten wood of *Acer saccharum* | Canada: Quebec | DAOMC:251599T | MT026395 | This study |
| *M. prismatica* | Decaying stick of *Acer saccharum* | Canada: New Brunswick | DAOMC:250742 | MT026417 | This study |
|      | Decaying hardwood branch submerged in stream | Canada: New Brunswick | DAOMC:250743 | MT026393 | This study |
|      | Endophyte of *Picea rubens* needle | Canada: New Brunswick | DAOMC:250744 | MT026385 | This study |
|      | Decaying hardwood stick on ground | Canada: Quebec | DAOMC:250745 | MT026418 | This study |
|      | Endophyte of *Picea rubens* needle | Canada: New Brunswick | DAOMC:250747 | MT026418 | This study |
|      | Decaying wood | Canada: Ontario | DAOMC:251578 | MT026418 | This study |

(continued on next page)
| Name | Substrate | Location | Sequence source or specimen | GenBank accession no. | Reference |
|------|-----------|----------|-----------------------------|----------------------|-----------|
|      |           |          |                             | ITS                  | LSU       | RPB1     | TOP1    | LNS2    |           |
|      |           |          |                             | This study           | Johnston & Park (direct submission) |
| Endophyte of Picea rubens needle | Canada: New Brunswick | DAOMC:251642 | MT026378 MT026488 MT018356 MT039027 MT009506 | This study |
| Dead stem of Betula papyrifera | Canada: New Brunswick | DAOMC:252005 | MT026420 MT026543 MT018423 MT039082 MT009561 | This study |
| Endophyte of Picea mariana needle | Canada: New Brunswick | NB2502I | MT026386 MT026497 MT018363 MT039036 MT009515 | This study |
| Endophyte of Picea rubens needle | Canada: New Brunswick | NB5342C | MT026375 MT026484 MT018355 MT039023 MT009502 | This study |
| Decaying wood | Canada: Ontario | NB655 | — MT025209 — — | This study |
| Dead leaf of Carex appressa | New Zealand | PDD:108711 | MG195529 — — | Johnston & Park (direct submission) |
| Wood in running water | New Zealand | PDD:108713, D1302 | MG195536 — — — | Johnston & Park (direct submission) |
| Dead wood of Nothofagus sp. | New Zealand | PDD:108714 | MG195537 — — — — | Johnston & Park (direct submission) |
| Lophozonia moorei | Australia | PDD:108715 | MG195538 — — — — | Johnston & Park (direct submission) |
| Dead wood of Nothofagus sp. | New Zealand | PDD:57544 | MG195531 — — — — | Johnston & Park (direct submission) |
| Dead leaf of Carex sp. | New Zealand | PDD:61852 | MG195535 — — — — | Johnston & Park (direct submission) |
| Dead wood | New Zealand | PRJ D1876 | MG195463 — — — — | Johnston & Park (direct submission) |
| Dead wood | New Zealand | PRJ D372 | MG195486 — — — — | Johnston & Park (direct submission) |
| Dead wood of Nothofagus sp. | New Zealand | PRJ D638 | MG195465 — — — — | Johnston & Park (direct submission) |
| Dead wood of Nothofagus sp. | New Zealand | PRJ D655 | MG195461 — — — — | Johnston & Park (direct submission) |
| Dead wood of Nothofagus sp. | New Zealand | PRJ D703 | MG195467 — — — — | Johnston & Park (direct submission) |
| Unspecified | New Zealand | PRJ D728 | MG195462 — — — — | Johnston & Park (direct submission) |
| Decorticated wood | New Zealand | PRJ D2011 | MG195530 — — — — | Johnston & Park (direct submission) |
| Decaying wood | New Zealand | TTT1406 | MG195466 — — — — | Johnston & Park (direct submission) |
| Name | Substrate | Location | Sequence source or specimen | GenBank accession no. | Reference |
|------|-----------|----------|-----------------------------|----------------------|-----------|
|      |           |          | ITS | LSU | RPB1 | TOP1 | LNS2 |
| **M. subcornea** | Dead wood of *Ulex europaeus* | New Zealand | TTT2238 | MG195464 | — | — | — | Johnston & Park (direct submission) |
| **M. undulatodepressula** | Half submerged twig | France | CBS:559.63 | MT026400 | MT026514 | MT018351 | MT039053 | MT009532 | This study |
| **M. ventosa** | Branch of angiosperm tree | Wood | CBS:322.77 | MT026392 | MT026506 | MT018369 | MT039045 | MT009524 | This study |
| **Neomollisia gelatinosa** | Unspecified | Thailand | KUS-F52181 | NR_163788 | — | — | — | Han et al. (2014) |
| **Neopyrenopeziza nigripigmentata** | Unspecified | Thailand | MFLU 18-0701T | MT026400 | MT026514 | MT018351 | MT039053 | MT009532 | This study |
| **Niptera discolor** | On dead sticks in wet bog | Canada: Ontario | DAOM:86811 | MT026456 | — | — | — | This study |
| **N. pulla** | Unspecified | UK | CBS:271.53 | M1857193 | — | — | — | Vu et al. (2019) |
| **N. ramincola** | On dead sticks on moist ground in woods | Canada: Ontario | DAOM:86812 | MT026457 | — | — | — | This study |
| **Nipterella parksi** | Alnus rubra slash | Canada: British Columbia | DAOM:56610 | MT026458 | — | — | — | This study |
| **Obtectodiscus aquaticus** | Carex rostrata | Switzerland | CBS:553.79 | MH872998 | MT026501 | MT018373 | MT039040 | MT009519 | Vu et al. (2019), this study |
| **Ombrophila hemiamyloidea** | Decorticated branch in stream | Canada: New Brunswick | DAOMC:251536 | MT026429 | MT026561 | MT018374 | MT039100 | MT009579 | This study |
| **Patellaropsis atrovinosa** | Branch of *Cornus sanguinea* | Luxembourg | G.M. 2014-06-15-1 | KY462814 | — | — | — | Hermant (direct submission) |
| **P. dennisii** | Wood of *Eucalyptus sp.* | France | G.M.2017-09-04.3 | MK120898 | — | — | — | Marson (direct submission) |
| **Phialocephala ametystea** | Fallen branch of *Acer saccharum* | Canada: New Brunswick | DAOMC:251552T | MT026387 | MT026499 | MT018412 | MT039038 | MT009517 | This study |

(continued on next page)
| Name                | Substrate                                      | Location               | Sequence source or specimen | GenBank accession nos. | Reference                  |
|---------------------|-----------------------------------------------|------------------------|-----------------------------|------------------------|----------------------------|
|                     |                                               |                        |                             | ITS        | LSU          | RPB1       | TOP1      | LNS2      |                           |
|                     |                                               |                        |                             | MT026388   | MT026500     | MT018413   | MT039039  | MT009518  | This study                |
| Endophyte of Picea rubens needle | Canada: New Brunswick | NB3824F                |                             |                        |                           |
|                       |                                               |                        |                             | MT026388   | MT026500     | MT018413   | MT039039  | MT009518  | This study                |
| P. aylmerensis       | Decaying hardwood on ground                   | Canada: Quebec         | DAO:MC:250106T              | NR,136124  | MT026489     | MT018394   | MT039028  | MT009507  | Tanney et al. (2016a), this study |
|                       | Decaying hardwood on ground                   | Canada: Quebec         | DAO:MC:250107              | MT026379   | MT026490     | MT018395   | MT039029  | MT009508  | Tanney et al. (2016a), this study |
|                       | Decaying log                                  | Canada: Quebec         | DAO:MC:251592              | MT026462   | —            | —          | —         | —         | This study                |
|                       | Decaying Betula papyrifera branch on ground   | Canada: Quebec         | NB684                      | MT026463   | —            | —          | —         | —         | This study                |
| P. bamuru            | Pinus sylvestris var. mongolica roots         | China                  | A024                       | MN006137   | —            | —          | —         | —         | Xun & Song (direct submission) |
|                       | Pinus sylvestris var. mongolica roots         | China                  | A083                       | MN006138   | —            | —          | —         | —         | Xun & Song (direct submission) |
|                       | Root of Cynodon dactylon                      | Australia              | DAR 82498                  | KJ877191   | —            | —          | —         | —         | Wong et al. (2015)         |
|                       | Root of Cynodon dactylon                      | Australia              | DAR 82499                  | KJ877192   | —            | —          | —         | —         | Wong et al. (2015)         |
|                       | Root of Pennisetum clandestinum               | Australia              | DAR 82500                  | KJ877193   | —            | —          | —         | —         | Wong et al. (2015)         |
|                       | Root of Pennisetum clandestinum               | Australia              | DAR 82501                  | KJ877194   | —            | —          | —         | —         | Wong et al. (2015)         |
|                       | Unspecified                                   | China                  | EF-395                     | MG066498   | —            | —          | —         | —         | Wang et al. (direct submission) |
| Dead leaf of Baumea sp. | New Zealand                                  | PDD:56863              | MG195534                   | —          | —            | —          | —         | —         | Johnston & Park (direct submission) |
| Dead leaf of Baumea sp. | New Zealand                                  | PDD:56864              | MG195533                   | —          | —            | —          | —         | —         | Johnston & Park (direct submission) |
| Unspecified           | South Africa                                  | RB275.1                | MH035706                   | —          | —            | —          | —         | —         | Jacobs (direct submission) |
| Unspecified           | South Africa                                  | RB275.2                | MH035707                   | —          | —            | —          | —         | —         | Jacobs (direct submission) |
| Unspecified           | South Africa                                  | RB275.3                | MH035708                   | —          | —            | —          | —         | —         | Jacobs (direct submission) |
| Unspecified           | South Africa                                  | RB305                  | MH035709                   | —          | —            | —          | —         | —         | Jacobs (direct submission) |
| Name       | Substrate                                      | Location             | Sequence source or specimen | GenBank accession no. | Reference                        |
|------------|------------------------------------------------|----------------------|-----------------------------|-----------------------|-----------------------------------|
| Dead leaf of *Baumea* sp. | New Zealand | TTT2287              | MG195532                    | —                     | Johnston & Park (direct submission) |
| Root of *Cynodon dactylon* | Australia   | DAR 82497T           | KJ877190                    | —                     | Wong et al. (2015)               |
| *P. biguttulata* | Under bark of fallen *Pinus strobus* log | Canada: Ontario      | DAOMC:250754T              | MT026373             | This study                       |
| *P. botulispora* | Unspecified                                      | Unspecified          | DAOM:75261T               | NR_155609            | McKemy et al. (direct submission) |
| *P. catenospora* | Decaying *Betula papyrifera* branch on ground | Canada: New Brunswick | DAOMC:250108T             | NR_136122            | Tanney et al. (2016a), this study |
| *P. cf. nodosa* | Old canker on *Betula* (?) sp. | Canada: Quebec       | JBT-47                     | MT026465             | This study                       |
| *P. cladophialophoroides* | Human toenail                                 | Chile                | HM87T                      | KY798313             | Crous et al. (2016)              |
| *P. collarifera* | Decaying *Betula papyrifera* log               | Canada: Quebec       | DAOMC:250755T             | KP768359             | Tanney et al. (2016a), this study |
| *P. compacta* | Living bark of *Alnus glutinosa*                | Germany              | CBS:507.94T                | MH862480             | Vu et al. (2019), this study      |
| *P. dimorphospora* | Parchment                                     | Portugal             | a209                       | KT896775             | de Carvalho et al. (2016)        |
| Pear tree  | Greece                                         | AXL1SP1              | KX881592                   | —                    | Markakis et al. (2017)           |
| *Fagus sylvatica* leaves | Italy                                         | CBS:112411           | MT026413                   | MT026534             | This study                       |
| Decaying hardwood on ground | Canada: Ontario | DAOMC:250111            | KP768360                   | MT026478             | Tanney et al. (2016a), this study |
| Pulp mill slime | Canada: New Brunswick | DAOMC:87232T             | KP972464                   | MT026479             | Tanney et al. (2016a), this study |
| Creosote-treated crosstie | Korea                                | KUC5023              | GQ241290                   | —                    | Kim et al. (2010)                |

(continued on next page)
| Name                   | Substrate                           | Location                        | Sequence source or specimen | GenBank accession no. | Reference                  |
|------------------------|-------------------------------------|---------------------------------|----------------------------|-----------------------|----------------------------|
| **Table 2. (Continued)** |                                     |                                 |                            |                       |                            |
| **Picea abies**        | 7-yr-old stump                      | Sweden                          | aurim1051                  | AY606307              | Menkis et al. (2004)       |
|                        |                                     |                                 |                            |                       |                            |
|                        | 6-yr-old stump                      | Sweden                          | aurim1061                  | AY606305              | Menkis et al. (2004)       |
|                        |                                     |                                 |                            |                       |                            |
|                        | 4-yr-old stump                      | Sweden                          | aurim1067                  | AY606302              | Menkis et al. (2004)       |
|                        |                                     |                                 |                            |                       |                            |
|                        | 5-yr-old stump                      | Sweden                          | aurim1068                  | AY606308              | Menkis et al. (2004)       |
|                        |                                     |                                 |                            |                       |                            |
|                        | 7-yr-old stump                      | Sweden                          | aurim107                   | AY606303              | Menkis et al. (2004)       |
|                        |                                     |                                 |                            |                       |                            |
|                        | 5-yr-old stump                      | Sweden                          | olrim301                   | AY606304              | Menkis et al. (2004)       |
|                        |                                     |                                 |                            |                       |                            |
|                        | 5-yr-old stump                      | Sweden                          | olrim315                   | AY606306              | Menkis et al. (2004)       |
|                        |                                     |                                 |                            |                       |                            |
|                        | 7-yr-old stump                      | Sweden                          | olrim380                   | AY606309              | Menkis et al. (2004)       |
|                        |                                     |                                 |                            |                       |                            |
| **P. europaea**        | Endophyte of Picea abies root       | Switzerland                     | CBS:119271T                | AY347399              | Grünig et al. (2004), this study |
|                        |                                     |                                 |                            |                       |                            |
| **P. fortinii**        | Pinus sylvestris root               | Finland                         | CBS:443.86T                | NR_103577             | Girlanda et al. (2002), this study |
|                        |                                     |                                 |                            |                       |                            |
| **P. glacialis**       | Root of Vaccinium myrtillus         | Switzerland                     | UAMH:10852                 | NR_111320             | Grünig et al. (2009)       |
|                        |                                     |                                 |                            |                       |                            |
| **P. helenae**         | Fallen Acer saccharum branch on ground | Canada: New Brunswick            | DAOMC:250756T             | MT026398              | This study                |
|                        | Decaying Betula alleghaniensis branch along river | Canada: New Brunswick            | DAOMC:251553              | MT026380              | This study                |
|                        | Fallen log                          | Canada: Ontario                 | DAOMC:252040              | MT026466              | This study                |
|                        | Endophyte of Picea rubens needle    | Canada: New Brunswick           | NB36510N                  | MT026381              | This study                |
|                        | Decaying Betula cordifolia branch on ground | Canada: New Brunswick           | NB457B                    | MT026399              | This study                |
| Name       | Substrate                                      | Location        | Sequence source or specimen | GenBank accession no. | Reference                        |
|------------|------------------------------------------------|-----------------|----------------------------|-----------------------|----------------------------------|
| P. helvetica | Endophyte of Picea abies root | Switzerland     | CBS:119273T | MT026409 | MT026525 | MT018403 | MT039064 | MT009543 | This study |
| P. hiberna  | Decorticated wood of Robinia pseudoacacia     | USA; Pennsylvania | CBS:110521T | NR_119465 | MT026538 | MT018418 | MT039077 | MT009556 | Bills (2004), this study |
| P. lagerbergii | Unspecified                     | Unspecified     | CBS:266.33   | NR_119426 | —       | —       | —       | —       | McKerny et al. (direct submission) |
|             | Rotted Picea log                  | USA; Alaska     | CFMR:FP-170134 | KU668951 | —       | —       | —       | —       | Palmer et al. (direct submission) |
|             | Crop field soil                   | South Korea     | KNU14-11     | KP055600 | —       | —       | —       | —       | Babu et al. (direct submission) |
| P. letzii   | Burned Pinus mugo tree            | Lithuania       | VL274         | JF440608 | —       | —       | —       | —       | Lygis et al. (2014) |
| P. mallochii | Endophyte of Picea abies root      | Switzerland     | CBS:119268T  | AY347391 | MT026527 | MT018407 | MT039066 | MT009545 | Grüning et al. (2004), this study |
| P. nodosa   | Decaying Alnus alnobetula subsp. crispa stem on ground | Canada: New Brunswick | DAOMC:250112T | NR_136123 | MT026544 | MT018384 | MT039083 | MT009562 | Tanney et al. (2016a), this study |
|             | Fallen branch of Betula alleghaniensis  | Canada: New Brunswick | DAOMC:250113 | KP768363 | MT026545 | MT018385 | MT039084 | MT009563 | Tanney et al. (2016a), this study |
| P. oblonga  | Endophyte of Pinus strobus needle    | Canada: New Brunswick | NB1052B      | KP768355 | —       | —       | —       | —       | Tanney et al. (2016a) |
|             | Endophyte of Picea mariana needle   | Canada: New Brunswick | NB-249-2D    | KP768353 | —       | —       | —       | —       | Tanney et al. (2016a) |
|             | Decaying decorticated log of Betula papyrifera | Canada: New Brunswick | NB-439 | KP768354 | —       | —       | —       | —       | Tanney et al. (2016a) |
|             | Decaying Betula cordifolia branch on ground | Canada: New Brunswick | NB452        | MT026421 | MT026547 | MT018390 | MT039086 | MT009565 | This study |
|             | Fallen branch of Betula cordifolia | Canada: New Brunswick | NB-452 | KP768358 | —       | —       | —       | —       | Tanney et al. (2016a) |
|             | Downed hardwood log                | New Zealand     | BHI-F752a    | MG553996 | —       | —       | —       | —       | Haelewaters et al. (2018) |
| Name                                                                 | Substrate                                                                 | Location                  | Sequence source or specimen | GenBank accession no. | Reference                              |
|----------------------------------------------------------------------|---------------------------------------------------------------------------|---------------------------|----------------------------|-----------------------|----------------------------------------|
| Decaying mossy Betula alleghaniensis log                              | Canada: New Brunswick                                                     | DAOMC:250117              | KP768373                   | MT026552               | MT018393 | MT039091 | MT009570 | Tanney et al. (2016a), this study |
| Decaying Acer saccharum branch on ground                              | Canada: New Brunswick                                                     | DAOMC:250118              | KP768370                   | MT026553               | MT018392 | MT039092 | MT009571 | Tanney et al. (2016a), this study |
| Rotten wood                                                           | Canada: Ontario                                                          | DAOMC:250119              | MT026422                   | MT026551               | MT018391 | MT039090 | MT009569 | Tanney et al. (2016a), this study |
| Decaying fallen hardwood branch                                       | Canada: Quebec                                                           | DAOMC:251588              | MT026467                   |                       |                       |          |          | This study                         |
| Rotten hardwood log                                                   | Canada: New Brunswick                                                    | DAOMC:251633              | MT026468                   |                       |                       |          |          | This study                         |
| Rotten hardwood log                                                   | Canada: New Brunswick                                                    | DAOMC:251634              | MT026469                   |                       |                       |          |          | This study                         |
| Wood of broken stem of Arbutus menziesii                             | Canada: British Columbia                                                 | JBT-7-52                  | MT026470                   |                       |                       |          |          | This study                         |
| Unknown rotten hardwood                                               | Canada: Ontario                                                          | KAS:3688                  | KP768385                   |                       |                       |          |          | Tanney et al. (2016a)              |
| Decaying log of Betula alleghaniensis                                | Canada: New Brunswick                                                    | NB-376                    | KP768368                   |                       |                       |          |          | Tanney et al. (2016a)              |
| Decaying wood of Betula alleghaniensis                               | Canada: New Brunswick                                                    | NB-548                    | KP768374                   |                       |                       |          |          | Tanney et al. (2016a)              |
| Decaying log of Betula papyrifera                                    | Canada: New Brunswick                                                    | NB-565                    | KP768371                   |                       |                       |          |          | Tanney et al. (2016a)              |
| Decaying log of Acer saccharum                                       | Canada: New Brunswick                                                    | NB-568                    | KP768369                   |                       |                       |          |          | Tanney et al. (2016a)              |
| Decaying log of Betula alleghaniensis                                | Canada: New Brunswick                                                    | NB-597                    | KP768372                   |                       |                       |          |          | Tanney et al. (2016a)              |
| Unknown hardwood branch                                              | Canada: Quebec                                                           | NB653                     | MT026471                   |                       |                       |          |          | This study                         |
| Decaying hardwood log (Fagus grandifolia?)                            | Canada: New Brunswick                                                    | NB696                     | MT026472                   |                       |                       |          |          | This study                         |
| Deocorticated wood                                                    | New Zealand                                                              | PRJ D1117                 | MG195483                   |                       |                       |          |          | Johnston & Park (direct submission) |
| Deocorticated wood                                                    | New Zealand                                                              | PRJ D1391                 | MG195480                   |                       |                       |          |          | Johnston & Park (direct submission) |
| Deocorticated wood                                                    | New Zealand                                                              | PRJ D1657                 | MG195484                   |                       |                       |          |          | Johnston & Park (direct submission) |
| Name                        | Substrate                                | Location          | Sequence source or specimen | GenBank accession no. | Reference                          |
|-----------------------------|------------------------------------------|-------------------|----------------------------|-----------------------|------------------------------------|
| Decorticated wood           | New Zealand                             | PRJ D1957         | MG195474                   |                       | Johnston & Park (direct submission) |
| Rotten wood                 | New Zealand                             | PRJ D2394         | MG195475                   |                       | Johnston & Park (direct submission) |
| Dead wood                   | New Zealand                             | PRJ D593          | MG195478                   |                       | Johnston & Park (direct submission) |
| Dead wood of Nothofagus sp. | New Zealand                             | PRJ D615          | MG195477                   |                       | Johnston & Park (direct submission) |
| Dead wood of Nothofagus sp. | New Zealand                             | PRJ D636          | MG195468                   |                       | Johnston & Park (direct submission) |
| Decorticated wood of Nothofagus sp. | New Zealand                 | PRJ D651          | MG195481                   |                       | Johnston & Park (direct submission) |
| Decorticated wood of Nothofagus sp. | New Zealand                 | PRJ D652          | MG195479                   |                       | Johnston & Park (direct submission) |
| Dead wood                   | New Zealand                             | PRJ D689          | MG195469                   |                       | Johnston & Park (direct submission) |
| Decorticated wood of Nothofagus sp. | New Zealand                 | PRJ D698          | MG195482                   |                       | Johnston & Park (direct submission) |
| Dead wood                   | New Zealand                             | PRJ D726          | MG195470                   |                       | Johnston & Park (direct submission) |
| Dead wood                   | New Zealand                             | PRJ D727          | MG195471                   |                       | Johnston & Park (direct submission) |
| Dead wood                   | New Zealand                             | PRJ D843          | MG195472                   |                       | Johnston & Park (direct submission) |
| Bark on fallen wood         | New Zealand                             | PRJ D870          | MG195473                   |                       | Johnston & Park (direct submission) |
| Dead wood                   | New Zealand                             | PRJ D903          | MG195485                   |                       | Johnston & Park (direct submission) |
| Decaying wood               | New Zealand                             | TTT1501           | MG195476                   |                       | Johnston & Park (direct submission) |
| *P. piceae*                 | Decaying fallen branch of *Acer saccharum* | DAOMC:250101     | MT026396                   |                      | This study                          |
|                            | Decaying fallen branch of *Acer saccharum* | DAOMC:250103     | MT026397                   |                      | This study                          |
|                            | Needle of *Picea abies*                | UAMH:10851T       | NR_111319                  |                      | Grünig et al. (2009)               |

(continued on next page)
| Name                 | Substrate                        | Location          | Sequence source or specimen | GenBank accession no. | Reference                                      |
|----------------------|----------------------------------|-------------------|-----------------------------|-----------------------|------------------------------------------------|
| *P. repens*          | Populus sp.                      | Canada            | MUCL1849T                   | EU434847              | Grünig et al. (2009)                           |
| *P. scopiformis*      | Living bark of *Picea abies*     | Germany           | CBS:468.94T                 | NR_119460 MT026556 MT018432 MT039095 MT009574 | Grünig et al. (2002), this study |
|                      | Endophyte of *Picea rubens*      | Canada: New Brunswick | DAOMC:250122              | MT026423 MT026554 MT018430 MT039093 MT009572 | Tanney et al. (2016a), this study |
|                      | Decaying *Picea rubens* branch on ground | Canada: New Brunswick | DAOMC:250126              | MT026424 MT026555 MT018431 MT039094 MT009573 | Tanney et al. (2016a), this study |
| *Phialocephala* sp. | Roots of *Bletilla striata*      | Korea             | 15P005                      | MG581182              | Lee & Eom (direct submission)                 |
|                      | Unspecified                      | UK: Wales         | AU_BD15/FBOL:1725          | JN995646              | Griffith (direct submission)                  |
|                      | *Pinus sylvestris*               | Sweden            | C73                         | KF156325              | Stenström et al. (2014)                        |
|                      | *Picea abies* stump              | Latvia            | C8                          | FJ803314              | Arhipova et al. (2011)                        |
|                      | *Pinus rigida*                   | USA               | CM14_P25                    | KU597349              | Luo et al. (direct submission)                 |
|                      | Unspecified                      | Unspecified       | CM16s1                      | KJ188684 KT591691     | Luo et al. (2014)                              |
|                      | Wood                             | Antarctica        | Dl276-1                     | KC514879              | Held & Blanchette (2017)                       |
|                      | Wood                             | Antarctica        | Dl84-2                      | KC514878              | Held & Blanchette (2017)                       |
|                      | Root of *Schizachyrium scoparium*| USA               | DS1029                      | MK808065              | Porras-Alfaro (direct submission)              |
|                      | Root of *Schizachyrium scoparium*| USA               | DS1032                      | MK808069              | Porras-Alfaro (direct submission)              |
|                      | Root of *Schizachyrium scoparium*| USA               | DS1262                      | MK808243              | Porras-Alfaro (direct submission)              |
|                      | Root of *Schizachyrium scoparium*| USA               | DS1263                      | MK808244              | Porras-Alfaro (direct submission)              |
|                      | Unspecified                      | Unspecified       | DWS3m2                      | KJ188689              | Luo et al. (2014)                              |
|                      | *Pinus pinea*, forest nursery    | Spain             | HP089                       | KT323171              | Martinez-Álvarez et al. (2016)                 |
|                      | *Pinus pinea*, forest nursery    | Spain             | HP094                       | KT323172              | Martinez-Álvarez et al. (2016)                 |
|                      | Wood                             | Iceland           | ICE2-C3                     | KX100389              | Blanchette et al. (2016)                       |
|                      | Unspecified                      | New Zealand       | ICMP 21725                  | MH682239              | Johnston & Park (direct submission)            |
| Name                           | Substrate                                                                 | Location  | Sequence source or specimen | GenBank accession no. | Reference                                |
|-------------------------------|----------------------------------------------------------------------------|-----------|-----------------------------|-----------------------|------------------------------------------|
| Rock art cave airborne        | organism                                                                  | France    | J042                        | MF788202              | Leplat (direct submission)              |
| Roots of Alnus incana         | subsp. rugosa                                                             | Quebec    | JP10                        | MH029265              | Lalancette (direct submission)          |
| Dactylorhiza majalis          |                                                                             | Austria   | JSE22014                    | KY271861              | Schiebold et al. (2018)                 |
| Unspecified                   |                                                                             | USA       | LF1BA12D2                   | JQ272328              | Baird (direct submission)               |
| Alnus glutinosa wood          |                                                                             | Latvia    | M49                         | JF340261              | Arhipova et al. (2012)                  |
| Dead attached branches of     | Fagus sylvatica                                                           | Germany   | OUT_017                     | HE998708              | Unterseher et al. (2013)                |
| Podophyllum peltatum          | USA                                                                        | PHIL Porter399 | KMO42204                     |                      | Ameaud et al. (direct submission)       |
| Rhizome of Podophyllum peltatum| USA; Delaware                                                             | PPE7      | KMO42204                    |                      | Ameaud & Porter (direct submission)     |
| Wood                          | Russia: Siberia                                                           | Sib5-4-5  | KX100391                    |                      | Blanchette et al. (2016)                |
| Wood of Picea abies           | Latvia                                                                    | SR16      | MK911655                    |                      | Burnevica et al. (direct submission)    |
| Carex aquatilis               | Canada                                                                    | UAMH_10206| EU434851                    |                      | Grünig et al. (2009)                    |
| Inner root of Vochysia        | divergens                                                                 | Brazil    | V1-431                      | KJ439193              | Biz et al. (direct submission)          |
| Unspecified                   | China                                                                     | Y-003     | MN579558                    |                      | Yuan (direct submission)                |
| Unspecified                   | China                                                                     | y-005     | MN208065                    |                      | Lanfang (direct submission)             |
| Unspecified                   | China                                                                     | y-013     | MN563117                    |                      | Yuan (direct submission)                |
| Root from Vaccinium           | vitis-idaea                                                               | China     | Y11                         | KJ817299              | Yang & Yan (direct submission)          |
| P. sphaeroides                | Unspecified                                                               | Canada    | UAMH 10279T                 | NR_121302             | Hambleton (direct submission)           |
| P. subalpina                  | Fine root of Pinus sylvestris                                           | Finland   | CBS:134513                  | MT026411              | This study                              |

(continued on next page)
| Name                  | Substrate                  | Location                  | Sequence source or specimen | GenBank accession no. | Reference                  |
|----------------------|----------------------------|---------------------------|----------------------------|-----------------------|---------------------------|
| **P. turicensis**    | Endophyte of Picea abies root | Switzerland               | CBS:119234T                | JN091488 MT026531 MT018409 MT039070 MT009549 | Dué et al. (2012), this study |
| **P. uotilensis**    | Endophyte of Picea abies root | Switzerland               | CBS:119277T                | MT026410 MT026528 MT018408 MT039067 MT009546 | This study                   |
| **P. urceolata**     | Commercial heparin solution | USA: Missouri             | UAMH:10827T                | NR_111285 — — — — | Wang et al. (2009)         |
| **P. vermiculata**   | Endophyte of Picea glauca needle | Canada: New Brunswick     | DAOMC:229535T              | MT026374 MT026483 MT018396 MT039022 MT009501 | This study                   |
| **Pulvinata tomentosa** | Unspecified                | Thailand                  | MFLU:18-1819               | NR_163775 — — — — | Ekanayaka et al. (2019)    |
| **Pyrenopeziza sp.** | Unspecified                | Canada: Ontario           | DAOMC:251530               | MT026419 MT026541 MT018436 MT039080 MT009559 | This study                   |
| **Pyrenopeziza velebitica** | Lonicera borbasiana       | Croatia                   | CNF:2/10097T               | NR_158942 — — — — | Jadan et al. (direct submission) |
| **Rhoxocercosporidium panacis** | Panax quinquefolius | Canada                    | DAOM:235605T               | NR_119568 — — — — | Reeleder et al. (2006)    |
| **Septonema sp.**    | Decaying wood chip of Fraxinus sp. | Canada: Ontario           | DAOMC:251597               | MT026441 — MT025210 — — — | This study                   |
| **Tapesia cinerea**  | Apothecium on unspecified substrate | Norway                   | ARON3188.H                 | AJ430228 — — — — | Vrålstad et al. (2002)    |
| **T. fusca**         | Decaying twigbark          | Norway                    | ARON 3154                  | AJ430229 — — — — | Vrålstad et al. (2002)    |
| **T. hydrophila**    | Phragmites australis       | Switzerland               | CBS:233.71                 | MT026412 MT026533 MT018420 MT039072 MT009551 | This study                   |
| **T. villosa**       | Alnus ainoetula            | Switzerland               | CBS:228.71                 | MH860087 — MT025203 — — — | This study                   |
| **Trichobelonium obscurum** | Calluna vulgaris         | Sweden                    | DAOM:56173                 | MT026474 — — — — | This study                   |
| **Trimmatostrona betulinum** | Unspecified               | Thailand                  | MFLU:15-2991               | MK584993 — — — — | Ekanayaka et al. (2019)    |
| **T. fusca**         | Decaying twigbark          | Norway                    | ARON 3154                  | AJ430229 — — — — | Vrålstad et al. (2002)    |
| **T. hydrophila**    | Phragmites australis       | Switzerland               | CBS:233.71                 | MT026412 MT026533 MT018420 MT039072 MT009551 | This study                   |
| **T. villosa**       | Alnus ainoetula            | Switzerland               | CBS:228.71                 | MH860087 — MT025203 — — — | This study                   |
| **Trichobelonium obscurum** | Calluna vulgaris         | Sweden                    | DAOM:56173                 | MT026474 — — — — | This study                   |
| **Trimmatostrona betulinum** | Unspecified               | Thailand                  | MFLU:15-2991               | MK584993 — — — — | Ekanayaka et al. (2019)    |
| **T. fusca**         | Decaying twigbark          | Norway                    | ARON 3154                  | AJ430229 — — — — | Vrålstad et al. (2002)    |
| **T. hydrophila**    | Phragmites australis       | Switzerland               | CBS:233.71                 | MT026412 MT026533 MT018420 MT039072 MT009551 | This study                   |
| **T. villosa**       | Alnus ainoetula            | Switzerland               | CBS:228.71                 | MH860087 — MT025203 — — — | This study                   |
| **Trichobelonium obscurum** | Calluna vulgaris         | Sweden                    | DAOM:56173                 | MT026474 — — — — | This study                   |
| **Trimmatostrona betulinum** | Unspecified               | Thailand                  | MFLU:15-2991               | MK584993 — — — — | Ekanayaka et al. (2019)    |
| **T. fusca**         | Decaying twigbark          | Norway                    | ARON 3154                  | AJ430229 — — — — | Vrålstad et al. (2002)    |
| **T. hydrophila**    | Phragmites australis       | Switzerland               | CBS:233.71                 | MT026412 MT026533 MT018420 MT039072 MT009551 | This study                   |
| **T. villosa**       | Alnus ainoetula            | Switzerland               | CBS:228.71                 | MH860087 — MT025203 — — — | This study                   |
| **Trichobelonium obscurum** | Calluna vulgaris         | Sweden                    | DAOM:56173                 | MT026474 — — — — | This study                   |
| Name                        | Substrate                  | Location                  | Sequence source or specimen | GenBank accession no. | Reference                     |
|-----------------------------|----------------------------|---------------------------|-----------------------------|-----------------------|-------------------------------|
| Uncultured Acephala        | Kobresia sp.               | China                     | SGSF217                     | MK192902              | Li & Xu (direct submission)  |
|                            | Root of Pseudorchis        | Czech Republic            | C128_5                      | FJ378718              | Gao & Yang (2010)             |
|                            | albida                     |                           | PA A2 14                    | JN655570              | Kohout et al. (2013)          |
| Uncultured Atheliaceae     | Roots of Kobresia sp.      | China                     | d109c_3_1                   | JQ346839              | Gao & Yang (direct submission)|
| clone                      |                            |                           |                             |                       |                               |
| Uncultured fungus          | Ophiocordyceps sinensis    | China                     | 197T-2                      | HO446078              | Zhang et al. (2010)           |
| Wood stump                  | Finland                    |                           | 3_56                        | KF274391              | Terhonen et al. (2014)        |
| Picea mariana forest soil  | USA: Alaska                |                           | 3312116                     | KF617331              | Taylor et al. (2014)          |
| soil, mineral horizon      |                            |                           |                             |                       |                               |
| Soil                       | Canada                     | 65_NA6_P32_B9             | KC965409                    |                       | Timling et al. (2014)         |
| Peat                       | UK                         | C505                      | AM260869                    |                       | Artz et al. (2007)            |
| Peat                       | UK                         | C510                      | AM260871                    |                       | Artz et al. (2007)            |
| Peat                       | UK                         | C759                      | AM260913                    |                       | Artz et al. (2007)            |
| Peat                       | UK                         | C822                      | AM260920                    |                       | Artz et al. (2007)            |
| Peat                       | UK                         | C92                       | AM260810                    |                       | Artz et al. (2007)            |
| Peat                       | UK                         | C942                      | AM260929                    |                       | Artz et al. (2007)            |
| Paddy field soil           | China                      | ck-105                    | KUS34827                    |                       | Li et al. (2017)              |
| Quercus fabr. ectomycorrhizal root tip | China: Hunan | clone 09-437              | AB769887                    |                       | Huang et al. (2014)           |
| Root of Cypripedium acaule | USA                        | fy_3_3_8                  | JX857262                    |                       | Bunch et al. (direct submission)|
| Picea mariana forest soil | USA: Alaska                | OTU501/3323D1             | KF618074                    |                       | Taylor et al. (2014)          |
| soil, mineral horizon      |                            |                           |                             |                       | Baba et al. (2016)            |
| Vaccinium oldhamii         | Japan                      | S31                       | KU551020                    |                       | Bunch et al. (direct submission)|
| Root of Cypripedium acaule | USA                        | swc_1_1_2_L               | JX857285                    |                       |                               |
| Uncultured Helotiaceae     | Potentilla sp.             | China                     | Clone 17                    | FJ827181              | Gao & Yang (2010)             |
| Uncultured Leotiomyceta    | Soil                       | Australia                 | clone b5                    | JQ513892              | Drigo et al. (2012)           |

(continued on next page)
| Name                      | Substrate                  | Location             | Sequence source or specimen | GenBank accession no. | Reference                      |
|--------------------------|----------------------------|----------------------|----------------------------|-----------------------|--------------------------------|
| **Uncultured Phialocephala** | Root of Habenaria radiata | Japan                | clone 53_2                  | JQ684858              | Cowden & Shefferson (2013)      |
|                          |                            |                      |                            |                       |                                 |
|                          | Root of Habenaria radiata | Japan                | clone 60_2                  | JQ684862              | Cowden & Shefferson (2013)      |
|                          |                            |                      |                            |                       |                                 |
|                          | Root of Habenaria radiata | Japan                | clone 61                   | JQ684861              | Cowden & Shefferson (2013)      |
|                          |                            |                      |                            |                       |                                 |
|                          | Root system                | USA                  | single6.direct_27          | KF660561              | Hewitt et al. (2016)            |
|                          |                            |                      |                            |                       |                                 |
| **Uncultured Phialocephala clone** | Pinus sylvestris | Sweden              | 2P-26c                     | KF156281              | Stenström et al. (2014)         |
|                          |                            |                      |                            |                       |                                 |
|                          | Root of Habenaria radiata | Japan                | clone 34                   | JQ684857              | Cowden & Shefferson (2013)      |
|                          |                            |                      |                            |                       |                                 |
| **Uncultured Phialocephala isolate** | Kobresia sp. | China              | S8B_1                      | FJ378719              | Gao & Yang (2010)               |
|                          |                            |                      |                            |                       |                                 |
| **Variocladium giganteum** | Decaying submerged leaf of Crataegus monogyna | UK                | CBS:508.71T                | NR_111206             | Boonyuen et al. (direct submission) |
|                          |                            |                      |                            |                       |                                 |
| **Vibrissea brevistipitata** | Unspecified | Thailand            | MFLU 16-0597               | MK584980              | Ekanayaka et al. (2019)         |
|                          |                            |                      |                            |                       |                                 |
| **V. flilsporia**       | Unspecified                | USA: Oregon          | JLF2084                    | JX415338              | Frank (direct submission)       |
|                          |                            |                      |                            |                       |                                 |
|                          | Rachis of Juglans mandshurica | Korea            | KUS-F52561                 | JN033422              | Han et al. (2014)               |
|                          |                            |                      |                            |                       |                                 |
| **V. flavovirens**      | Salix alba twigs           | Germany              | CBS:121003                 | MT026430              | This study                      |
|                          | Unspecified                | New Zealand          | ICMP 19442                 | KF429257              | Johnston & Park (direct submission) |
|                          |                            |                      |                            |                       |                                 |
|                          | Unspecified                | Unspecified           | MBH39316                   | AY789427              | Wang et al. (2005)              |
|                          |                            |                      |                            |                       |                                 |
| **V. pezizoides**       | Quercus sp.                | USA: New York        | DAOX:159667                | MT026475              | This study                      |
|                          |                            |                      |                            |                       | Johnston & Park (direct submission) |
| **Vibrissea sp.**       | Unspecified                | UK: Wales            | FBOL:1726, AU_BD50         | JN995645              | Griffith (direct submission)    |
|                          |                            |                      |                            |                       |                                 |
|                          | Unspecified                | Chile                | PDD:99892                  | KF429259              | Johnston & Park (direct submission) |
|                          |                            |                      |                            |                       |                                 |
|                          | Unspecified                | Chile                | PDD:99893                  | KF429260              | Johnston & Park (direct submission) |
|                          |                            |                      |                            |                       |                                 |
| **V. truncorum**        | Alnus alnobetula           | Germany              | CBS:143.92                 | EU434855              | Gün et al. (2009)              |
|                          | Submerged Populus root     | Canada: Ontario      | CBS:258.91                 | MT026377              | This study                      |
To assess individual gene genealogies and apply the genealogical concordance phylogenetic species recognition concept (GCPSR; Taylor et al. 2000), phylogenetic analyses of single gene alignments (ITS, LSU, RPB1, LNS2, TOP1) were performed. Alignments consisting of 88 taxa represented by all genes were used to compare gene phylogenies. A phylogenetic analysis was also conducted with an alignment of ITS, LSU, RPB1, and TOP1 concatenated sequences. Besides the gene comparison, further phylogenetic analyses were conducted using additional sequences from GenBank and sequences generated from this study: (1) an expanded RPB1 alignment including 109 taxa; (2) a larger ITS alignment containing 204 taxa; (3) an ITS alignment containing 94 taxa focusing on the Phialocephala s.s. clade including relevant sequences from a GenBank BLAST search using Phialocephala dimorphospora DAOM 87232 as a seed; and (4) an ITS alignment containing 149 taxa exploring the biogeography of isolates and sequences within the Barrenia grass clade including relevant sequences from a GenBank BLAST search using Barrenia taeda (NR_164237) as seed.

The phylogenetic analyses exploring individual and concatenated gene genealogies (ITS, LSU, RPB1, LNS2, TOP1, and ITS-LSU-RPB1-TOP1) were conducted using both Bayesian inference (MrBayes) and Ultrafast Maximum Likelihood (IQ-TREE). Maximum likelihood trees were made with IQ-TREE (Trifinopoulos et al. 2016) using the automatic substitution model setting, 1 000 ultrafast bootstrap (BS) replications, and SH-aLRT branch test with 1 000 replicates. To calculate Bayesian posterior probabilities (PP) for branch support, MrBayes v. 3.2.7 (Ronquist et al. 2012) was used in the CIPRES Science Gateway portal (Miller et al. 2010) with two independent samplings with ten chains each and sampling every 1 000 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 kept and combined into one consensus tree with 50 % majority rule consensus. For the other phylogenetic analyses, maximum likelihood trees were created using IQ-TREE as stated above. Characteristics of alignments used for phylogenetic analyses are summarized in Table 3. Consensus trees were visualized in FigTree v. 1.4.2 (available at http://tree.bio.ed.ac.uk/software/figtree/) and exported as SVG vector graphics for assembly in Adobe Illustrator CC v. 23.9.1 (Adobe System, San Jose, CA, USA).

An LSU alignment consisting of an 843 bp intron region was used to investigate sequence similarity and quantitative taxonomic thresholds between 15 species placed in Acephala, Loramyces, Mollisia, cf. Niptera, Oblectodiscus, Phialocephala, Pyrenopeziza, and Vibrissea. A phylogenetic tree was constructed with MrBayes v. 3.2 using the GTR+I+G nucleotide substitution model and the identity and similarity of each sequence pair was calculated using Sequence Manipulation Suite: Ident and Sim (Stothard 2000).

RESULTS

Phylogenetic analyses

PCR amplification success for DNA obtained from cultures was 100 % for ITS, LSU, and RPB1, 97 % for LNS2, and 93 % for TOP1. A 56 °C annealing temperature significantly improved LNS2 and TOP1 amplification success compared to the
Touchdown PCR profile recommended by Stielow et al. (2015). The overall success rate of ITS amplification yielding usable sequences from herbarium specimen DNA was 50% (18/32 specimens), which enabled the sequencing of several species of mollisioid genera unreported in GenBank, including Hysteronea, Nipteraella (Fig. 3), and Trichobolium (=Mollisia sensu Richter & Baral 2008; Fig. 4). The addition of BSA significantly improved amplification of DNA from herbarium specimens (data not shown), although this also enhanced amplification of contaminating or co-occurring fungal DNA in some cases (e.g. Candida, Cladosporium, Malassezia, and Simplicitium spp.).

The oldest herbarium specimens successfully sequenced were Vibriissea vibriseoides (DAOM 120405; coll. 1933), Niptera discolor (DAOM 86811; coll. 1935), and Mollisia caesia (DAOM 86792; coll. 1936).

To compare phylogenies reconstructed from each gene, separate and concatenated analyses were conducted using the 88 taxa represented in all datasets (Figs 5–10). Discordance among clades and individual taxa were observed among the different gene phylogenies. Notably, Vibriissea is basal to Mollisiceae in the LSU, RPB1, and TOP1 phylogenies; however, ITS and LNS2 phylogenies place this genus sister to the Ph. fortinii sensu lato (s.l.)–Acephala planapata species complex (PAC). Phialocephala s.s., interpreted as the clade containing the type species P. dimorphospora, was sister to the PAC with weak support in the ITS, LNS2, and LSU phylogenies but placed elsewhere in the RPB1 and TOP1 phylogenies. The LNS2 phylogeny generated weakly supported polytomous clades and showed some discordance with the other genes, e.g. the placement of P. scopiformis and M. diesbachiana sp. nov. (described below). Whereas RPB1 showed no intraspecific variation, LNS2 SNPs were observed among strains of Phialocephala helenae sp. nov. (11 bp difference) and NB334-2C/DAOMC 251642 (4 bp difference; Clade A). The placement of the M. discolor-M. prismatica sp. nov. clade (Clade B), M. ligni var. ligni, M. rosea, and P. verruculata sp. nov. was inconsistent among the gene phylogenies. LSU indel motifs unique to the M. discolor-M. prismatica clade (Clade B), consisting of conspecific strains identified as Mollisia sp. (DAOMC 250743), M. caesia, M. discolor, M. fallens, and M. ventosa, resulted in their respective long branches. Mollisia s.s., interpreted here as the clade containing M. cf. cinerea, M. cinerea var. olivascens, and M. undulatodepressula, was strongly supported in all phylogenies (LSU, RPB1, TOP1: SH-aLRT = 100 %, BS = 100 %, PP = 1.0; ITS: SH-aLRT = 96 %, BS = 100 %, PP = 1.0; LNS2: SH-aLRT = 96 %, BS = 98 %, PP = 1.0). Phialocephala s.s. was also strongly supported in all phylogenies but to a lesser extent for LSU (SH-aLRT = 96 %, BS = 97 %, PP = 0.53). The morphologically divergent, semi-aquatic clade comprising Loramyces, Obtecdiscus, and Ombrophila hemiamyloidia, was strongly supported (ITS, RPB1: SH-aLRT = 100 %, BS = 100 %, PP = 1.0; LSU: SH-aLRT = 98 %, BS = 98 %, PP = 1.0; TOP1: SH-aLRT = 97 %, BS = 99 %, PP = 1.0) in all phylogenies except LNS2 (SH-aLRT = 96 %, BS = 100 %, PP = 1.0). Mollisia diesbachiana was strongly supported as the basal species in the semi-aquatic clade in the RPB1, TOP1, and concatenated phylogenies, but the LSU and ITS phylogenies placed M. diesbachiana sister to Om. hemiamyloidia and Ob. aquaticus with weak (LSU) or strong (ITS) support and the LNS2 phylogeny placed M. diesbachiana basal to clades A and B with low support.

The larger RPB1 phylogeny (Fig. 11) overall showed moderate-to-highly supported branches, placing Vibriissea outside of the main Mollisiceae lineage. Mollisia sensu lato is polyphyletic, with species in genera including Acephala, Acidomelania, Barrenia, Cystodendron, Loramyces, Niptera, Obtecdiscus, Ombrophila, and Phialocephala dispersed throughout the lineage. Acidomelania is part of a subclade within Clade I containing M. melaleuca, M. rava sp. nov., and an unidentified conifer endophyte species (DAOMC 251652, NB-334-2C); this subclade is in turn sister to M. novo brunsvicensis sp. nov. Given the close relationship between the monotypic genus Acidomelania and Mollisia s.s., Acidomelania is synonymised with Mollisia below. The terrestrial mollisioid species Mollisia diesbachiana is basal in the strongly supported semi-aquatic clade, which is placed within Mollisiceae, closely related to Mollisia s.s. and other Mollisia species producing typical mollisioid apothecia. The recently described asexual root endophyte genus Barrenia comprises two polyphyletic species (Clade IV), the type species B. panicia, sister to strains identified as M. hydrophila and Tapesia hydrophila, and B. taeda, sister to the clade containing B. panicia, M. hydrophila, T. hydrophila, and M. nigrecens. Phialocephala s.s. forms a strongly supported (SH-aLRT = 100 %, BS = 100 %, PP = 1.0) clade (Clade V) sister to

Table 3. Overview of phylogenetic analyses.

| Alignment          | No. of taxa | Characters | Best-fit evolutionary model (AIC) | Outgroup            |
|--------------------|-------------|------------|-----------------------------------|---------------------|
| ITS                | 88          | 304        | 57                                | GTR+H+G             | Mollisia dextrinospora |
| LNS2               | 88          | 131        | 9                                 | HKY+G               | M. dextrinospora       |
| LSU                | 88          | 274        | 51                                | GTR+H               | M. dextrinospora       |
| RPB1               | 88          | 323        | 33                                | HKY+G               | M. dextrinospora       |
| TOP1               | 88          | 398        | 49                                | GTR+H               | M. dextrinospora       |
| ITS-LSU-RPB1-TOP1  | 88          | 1 541      | 187                               | GTR+H               | M. dextrinospora       |
| RPB1 expanded      | 109         | 346        | 51                                | GTR+H               | M. dextrinospora       |
| ITS expanded       | 207         | 423        | 87                                | SYM+H+G             | C. versiformis         |
| ITS Barrenia-grass clade | 149    | 145        | 138                               | SYM+H+G             | M. dextrinospora       |
| ITS P. dimorphospora clade | 95 | 73 | 109 | 428 | TNe+H+G | M. dextrinospora |

320
**P. vermiculata** and **M. ligni** var. **olivascens** (Clade VI) and **Niptera** sp. (Clade VII), although with weak–moderate support. These taxa are sister to the PAC with moderate support (SH-aLRT = 91 %, BS = 90 %, PP = 0.77; Clade VIII).

**Phialocephala scopiformis** (Clade III) is distinct from **Phialocephala s.s.** and sister to a clade (Clade II) comprising endophytic isolates from *Fagus sylvatica* leaves (“**P. dimorphospora** CBS 112411) and conifer needles (**Cystodendron dryophilum** CBS 295.81, **Mollisia monilioides** sp. nov.) and apothecial isolates from decaying wood (“**M. cinerea**” CBS 122029, DAOMC 252005, DAOMC 251578). **Acephala** contains two species and is polyphyletic with the type species, **A. applanata**, basal to the PAC (with low support; Clade VIII) while **A. macrosclerotiorum** is basal (SH-aLRT = 92 %, BS = 99 %, PP = 1.0) in a clade containing **P. amethystea** sp. nov. and **P. compacta** (Clade IX).

**Mollisia cinerella** (CBS 312.61) and the ex-epitype of **Mollisia dextrinospora** (CBS 401.78) are congeneric or closely related to **Pyrenopeziza** (**Ploettnerulaceae**, **Helotiales**). A preliminary phylogenetic analysis placed **Strossmayeria basitricha** (Fig. 12) and **Variocladium giganteum** within the lineage based on weakly supported long branches; their placement within the lineage is probably a result of long-branch attraction and resulted in their exclusion from other phylogenetic analyses (data not shown).

The large ITS phylogeny contained 204 taxa including sequences derived from herbarium specimens identified as **Hysteroneevia scirpina** (DAOM 147320), **Nipterella parksi** (DAOM 56610; Fig. 3), **M. benesuada** (DAOM 56135), **M. caesia** (DAOM 86792; Fig. 13), **M. epitypha** (DAOM 150777), **M. subcornea** (DAOM 107202), **Niptera discolor** (DAOM 86811; Fig. 14), **Niptera ramincola** (DAOM 86812; Fig. 15), **Obtectodiscus aquaticus** (DAOM 189114, DAOM 172427 holotype), **Mollisia obscurum** (as **Trichobelonium obscurum**; DAOM 56173; Fig. 4), **Vibrissea pezizoides** (DAOM 159667), **V. truncorum** (DAOM 190457), and **V. vibrisseoides** (DAOM 46436, 120405) (Fig. 16). **Mollisia s.s.** belongs to a strongly supported (SH-aLRT 100 %, BS 100 %) larger clade (Clade 1) also containing **M. novobrunsvicensis** sp. nov. and **M. rava** sp. nov. and the ex-type of **Neomollisia gelatinosa** (=**M. solidaginis**; Baral et al. 2019). These taxa are subsequently placed sister to a subclade including **Mollisia melaleuca** and the ex-type of **Acidomelania panicolica**. Clade 1 is strongly supported (SH-aLRT 99 %, BS 100 %) and includes **Mollisia s.s.** with a subclade composed of morphologically
Fig. 5. A 50 % majority-rule consensus tree obtained from the maximum likelihood analysis based on ITS sequences containing representative *Mollisia* and allied taxa for comparing ITS, LSU, LNS2, RPBI, and TOPI phylogenies. Culture collection (DAOMC, CBS) or JB Tanney personal collection accession numbers (NB) follow the species name (type strains in bold). Significant branch support values, SH-aLRT support (>70 %), ultrafast bootstrap (BS) support (>70 %), Bayesian posterior probabilities (>0.95), are presented at the nodes with lower supports indicated by an en dash (−) and full support (100 % SH-aLRT, 100 % BS, 1.0 PP) indicated by an asterisk (*). Truncated branches are designated by a broken line, which is a 2× reduction unless indicated. The tree rooted with the ex-type of *Mollisia dextrinospora* (CBS 401.78).
Phialocephala hiberna CBS 110521
Mollisia sp. DAOMC 250/47

Phialocephala hiberna CBS 112688
CBS443 Phialocephala tortrix CBS 443.86
Phialocephala obtenuisis CBS 119277
Phialocephala subalpinna CBS 114313
Phialocephala europae CBS 119271
Phialocephala helvetica CBS 119273
Phialocephala turcensis CBS 112324
Acephala applanata CBS 109321
Phialocephala helenae NB-457B
Phialocephala helenae DAOMC 251553
Phialocephala helenae DAOMC 250756
Phialocephala helenae NB-365-10N
Phialocephala piceae DAOMC 250101
Phialocephala piceae DAOMC 2501013
Acephala macroscerotorum CBS 123555
Phialocephala amethystea NB-382-4F
Phialocephala amethystea DAOMC 251552
Phialocephala compacta CBS 937.94

Phialocephala vermiculata DAOMC 228535
Phialocephala dimorphospora DAOM 87232
Phialocephala dimorphospora JAOMC 229111
Phialocephala collarifera DAOMC 250735
Phialocephala biguttulata DAOMC 250745
Phialocephala aylmerensis DAOMC 250106
Phialocephala aylmerensis CBS 297.99
Phialocephala heterosperma CBS 292.59
Phialocephala obtonga UAOMC 250117
Phialocephala obtonga DAOMC 250119
Phialocephala obtonga DAOMC 250118
Phialocephala catenosa DAOMC 250109
Phialocephala catenosa DAOMC 250110
Phialocephala catenosa ADAMC 250108
Phialocephala nodosa DAOMC 250115
Phialocephala nodosa NB-452
Phialocephala mallochi DAOMC 250112

Mollisia rosea CBS 230.71
Vibrissea flavovirens CBS 215303
Vibrissea truncorum DAOMC 251541

Pyrenopezia sp. UAOMC 225130
Mollisia dextrinospora CBS 401.78

LSU

Mollisia malaleuca CBS 589.64
Mollisia ct. malaleuca DAOMC 250733
Mollisia ct. malaleuca DAOMC 250744
Mollisia sp. UAOMC 201042
Mollisia sp. NB-334-2C
Mollisia ct. cinerea UAOM 251729
Mollisia ct. cinerea DAOMC 251576
Mollisia ct. cinerea DAOMC 252029
Mollisia cinerea var. olavsens CBS 553.63
Mollisia undulatocephala CBS 559.63
Mollisia novobrunvicen시스 JAOMC 251538
Mollisia novobrunvicen시스 DAOM 867422
Mollisia novobrunvicen시스 NB-579
Mollisia rava DAOMC 252014
Mollisia rava DAOMC 251622

Ombrophila hemianthyofila DAOMC 251536
Loramyces macrosporus CBS 235.53
Loramyces macrosporus CBS 235.53

0.05

Phialocephala scopiformis CBS 489.94
Phialocephala scopiformis DAOMC 250126
Mollisia moniloides DAOMC 250734
Mollisia moniloides DAOMC 250735
Cystodendron dryophilum CBS 295.81
Mollisia sp. DAOMC 25047
Mollisia sp. DAOMC 250053
Phialocephala dimorphospora CBS 112411
Mollisia sp. DAOMC 251578
Mollisia nigrescens DAOMC 250739
Mollisia ct. nigrescens DAOMC 250738
Mollisia var. nigrescens NB-579

-85/-

Fig. 6. A 50% majority-rule consensus tree obtained from the maximum likelihood analysis based on LSU sequences containing representative Mollisia and allied taxa for comparing ITS, LSU, LSU2, RPB1, and TOP1 phylogenies. Culture collection (DAOMC, CBS) or J.B. Tanney personal collection accession numbers (NB) follow the species name (type strains in bold). Significant branch support values, SH-aLRT support (≥70 %), ultrafast bootstrap (BS) support (≥70 %), Bayesian posterior probabilities (≥0.95), are presented at the nodes with lower supports indicated by an en dash (–) and full support (100 % SH-aLRT, 100 % BS, 1.0 PP) indicated by an asterisk (*). Truncated branches are designated by a broken line, which is a 2× reduction unless indicated. Clades are labelled for convenience. The tree is rooted with the ex-type of Mollisia dextrinospora (CBS 401.78).
Fig. 7. A 50 % majority-rule consensus tree obtained from the maximum likelihood analysis based on RPB1 sequences containing representative Mollisia and allied taxa for comparing ITS, LSU, LNS2, RPB1, and TOP1 phylogenies. Culture collection (DAOMC, CBS) or JB Tanney personal collection accession numbers (NB) follow the species name (type strains in bold). Significant branch support values, SH-aLRT support (>70 %), ultrafast bootstrap (BS) support (>70 %), Bayesian posterior probabilities (>0.95), are presented at the nodes with lower supports indicated by an en dash (−) and full support (100 % SH-aLRT, 100 % BS, 1.0 PP) indicated by an asterisk (*). Truncated branches are designated by a broken line, which is a 2× reduction unless indicated. Clades are labelled for convenience. The tree is rooted with the ex-type of Mollisia dextrinospora (CBS 401.78).
dissimilar to P. dimorphospora, however the basal species *V. flavovirens* shows a significantly higher similarity to *P. dimorphospora* and *cf. Niptera* (96.88%), and *Vibrissea truncorum* is highly dissimilar to *P. compacta* (<95.7%). *Mollisia dextrinospora* CBS 401.78 is congeneric with *Pyrenopeziza* sp. (DAOMC 251530) and both species show a low similarity to the other taxa, consequently placing them outside *Mollisiaceae* and even *Helotiales* based on the threshold values for family (96.2 %) and class (94.7 %). There are plans to transfer *Mollisia dextrinospora* to *Pyrenopeziza* (B. Douglas, pers. comm.), thus *M. dextrinospora* is not considered further in this study.

**Phylogenetic position of some remarkable endophytic taxa**

ITS BLAST queries provide several examples of *Mollisiaceae* strains that are conspecific or very closely related but were isolated from very diverse hosts and geographic areas. A *Picea rubens* endophyte isolated in this study (DAOMC 250744) appears to be conspecific with endophytes of *Spinulina anatominum* in Poland (*JX981467*; identities = 530/532 i.e. 99 %, gaps = 2/532), *Notothagus solandri* in New Zealand (*JN225881*; identities = 544/531 i.e. 99 %, no gaps), and *Picea abies* in Finland (*EF592102*; identities = 529/530 i.e. 99 %, gaps = 1/530). Similarly, DAOMC 250738, a *Picea mariana* needle endophyte closely related to *M. nigrescens*, is conspecific with a *Vaccinium vitis-idaea* root endophyte isolated in northern China (*KJ817299*; identities = 779/779 i.e. 100 %, no gaps) (*Fig. 18*). Six endophyte strains isolated from the stems of *Vaccinium angustifolium* and
V. corymbosum in eastern Canada share identical ITS sequences with DAOMC 250738 (M. Sumarah, pers. comm.). Several closely-related GenBank ITS sequences attributed to M. minutella were detected from a variety of hosts, host tissues, and locations, including Picea abies needles in the Czech Republic (FR837920), Luedum palustre roots in China (KJ817294; FR837920; identities = 521/522 i.e. 99 %, gaps = 1/522), Picea abies wood in Sweden (DQ008242; FR837920; identities = 483/483 i.e. 100 %, no gaps), and Pinus sylvestris roots in Finland (KMO08419; FR837920; identities = 489/490 i.e. 99 %, gaps = 1/490).

Phialocephala scopiformis, reported only from P. abies, is closely related to a strain isolated as a leaf endophyte of Calluna vulgaris (FM200586; identities = 493/495 i.e. 99 %, no gaps). Mollisia fusca (CBS 234.71), isolated from apothecia on Lonicera caerulea (CBS 231.71), isolated from apothecia occurring on Phialocephala strain isolated as a bark endophyte also from F. sylvatica in Switzerland (EU434850; identities = 476/481 i.e. 99 %, gaps = 2/481).

Connections between endophytes, apothecia collections, and named species were also inferred by comparison with unidentified GenBank sequence accessions. For example, Mollisia nigrescens was isolated from apothecia on decaying wood in France (CBS 558.63) and Canada (DAOMC 250739) and isolated from North Carolina both as an endolichenic fungus of Flavoparmelia caperata (JQ761691; CBS 558.63; identities = 858/858 i.e. 100 %, no gaps) and from a senescent Tsuga canadensis needle (KX908506; CBS 558.63; identities = 870/870 i.e. 100 %, no gaps). A strain identified as Mollisia olivascens (CBS 293.59) shares an identical ITS sequence with the Phialocephala urceolata ex-type (UAMH 10827), isolated from commercial heparin solution (Wang et al. 2009). Mollisia lividofusca (CBS 231.71), isolated from apothecia occurring on Lonicer caerulea in Switzerland, shares similar ITS sequences with a Picea abies needle endophyte from the Czech Republic (FR837926; identities = 785/787 i.e. 99 %, gaps = 1/787) and a Picea glauca needle endophyte from Canada (AY561210; identities = 595/595 i.e. 100 %, no gaps). Phialocephala amebthystea shares an almost identical ITS sequence with an unidentified endolichenic fungus isolated from Diploschistes scruposus in North Carolina (JQ761327; identities = 1 044/1 045 i.e. 99 %, no gaps).

ITS sequences place M. obscura within the Barrenia clade in Mollisia s.l., with its closest relatives being an unidentified Epacris microphylla (Ericaceae) root endophyte from Australia (AY288211; identities = 495/505 i.e. 98 %, gaps = 2/505) and apothecial collections from Nothofagus wood in New Zealand (MG195532; identities = 534/544 i.e. 98 %, gaps = 2/544) (Fig. 18). Mollisia obscura, the former type species of the synonymised mollisiosid genus Trichobolaniun (Aebi 1972, Richter & Baral 2008), is found on dead Calluna roots or stems and is morphologically distinct, characterized by fusiform 3–7-septate ascospores and a well-developed dark disc (Fig. 4). A Mollisia epitypha herbarium specimen (DAOM 150777) collected from dead Typha latifolia stems in Ontario, Canada is closely related to the recently described Australian turf grass pathogen Phialocephala bamuru (KJ877719; identities = 501/507 i.e. 99 %, gaps = 2/507). Currently, there is no described sexual state for P. bamuru; however, collections of apothecia from dead leaves of Baumea sp. in New Zealand appear to be conspecific with P. bamuru (e.g., KJ877719 and MG19553; identities = 509/510 i.e. 99 %, no gaps). Overall, sequences in this clade originate from diverse areas, including North America, Brazil, Northern and Central Europe, Australia, New Zealand, China, Korea, and Japan.

Inducing sporulation in vitro

Many strains were sterile despite repeated attempts to induce sporulation, including different media and substrates and incubation at various temperatures over prolonged periods of time. Sporulation was successfully induced in recalcitrant cultures, but this usually required prolonged incubation time at low temperature. For example, sporulation in cultures of Mollisia sp. (DAOMC 252005) was observed in water agar amended with trace elements (Visagie et al. 2014) incubated at 5 °C for 3 years (Fig. 21). Mollisia nigrescens (CBS 558.63) sporulated on MEA after 3 years incubation initially at 20 °C for the first 6 mo then 5 °C for ca. 2.5 years (Fig. 22). The phialidic anamorphs of Vibrissea flavovirens, Mollisia diesbacha sp. nov., and a previously unreported asexual morph of O. hemiamyloidea, described below, were induced by floating pieces of MEA or oatmeal agar (OA) for ca. 1 mo in Petri dishes containing sterile water.

TAXONOMY

Newly observed asexual morph of Ombrophila hemiamyloidea

Ombrophila hemiamyloidea (DAOMC 251536) produced an unreported phialidic asexual morph ca. 1 mo after floating pieces of MEA or OA cultures in water (Fig. 23), described here: Conidiophores monomoramic to macromoramic, arising vertically or laterally from mycelium submerged in water, hyaline to subhyaline or light brown, becoming darker with maturity, smooth, cylindrical, thin-walled, 2–3.5 μm diam, up to indeterminate length, with several septa, unbranched or indeterminately branched, forming dense globose conidiogeneous heads up to 400 μm diam. Conidiogenous cells phialidic, terminal, sometimes intercalary, cylindrical to ampuliform or subglobose phialides, (9–)11–14.5(–17.5) × 3–4(–4.5) μm, with deep, cylindrical, hyaline to subhyaline collarettes, (4–)4.5–5.5(–) × 2.5–3 μm, occurring singly or in whorls of 2–3(–4) from metulae. Metulae hyaline to subhyaline or pale brown, cylindrical to doliform, (4.5–)5–6.5(–7.5) × 3–4(–5) μm. Conidia oblong to oblong-ellipsoidal, hyaline, (4.5–)
ITS-LSU-RPB1-TOP1

| Mollisia s.s. | | |
| Clade A | Loramyces-Obectodiscus semi-aquatic clade | |
| Clade B | M. ligni var. ligni | |
| Clade C | P. scopiformis | |
| Clade D | Phialocephala s.s. | |
| PAC | | |
| Clade E | | |
| Clade F | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
5–6(–6.5) × (1.5–)2 μm; secondary conidia globose to sub-globose, hyaline, 2–2.5(–3) × (1.5–)2(–2.5) μm.

*Mollisia diesbachiana* Tanney & Seifert *sp.* nov. MycoBank MB833619. Fig. 24

**Etymology:** Named for the characteristic colour (Prussian blue) of the hymenium, in honour of Johann Jacob Diesbach, the chemist who first synthesized Prussian blue.

**Typs:** *Canada,* New Brunswick, Albert County, Alma, Fundy National Park, East Branch Trail, 45.64335 –65.11563, from decaying *Betula alleghaniensis* wood, 25 Sep. 2014, J.B. Tanney (holotype DAOM 745767a, isotype DAOM 745757b, culture ex-type culture DAO MC 250732 = NB-546).

Conidiophores micronematous to macronematous, arising vertically or laterally from mycelium submerged in water, pale to dark brown, smooth, cylindrical, thin- or thick-walled, unbranched or with 1–4 series of branches, 2–3.5 μm diam, 20 μm to indeterminate length, with several septa. *Conidiogenous cells* phialidic, terminal, sometimes intercalary, ampulliform, (7–) 9–13.5(–17.5) × (2.5–)3–4(–4.5) μm, collarettes cylindrical to doliform, (3–)4–5(–6) × 2–2.5(–3) μm, hyaline to pale brown, often appearing concolorous with the darker conidiophore, occurring singly or in whorls of 2–5 from metulae. *Metulae* hyaline to pale brown, cylindrical to obovoid, apices frequently clavate, 6.5–11(–14) × (2.5–)3–4(–4.5) μm. *Conidia* dimorphic; primary conidia ellipsoid to oblong, hyaline, (3–)4–5(–6) × 2–2.5(–3) μm; secondary conidia globose, hyaline, 2.5(–3) × 2–2.5 μm.

Apothecia scattered to gregarious in small groups (2–6), sessile, urceolate to cup-shaped when young, disc planar to concave at base, 20–50 μm thick towards margin, composed of globose to isodiametric cells with thin to slightly thickened walls, (14–)15.5–25.5(–34) × (6.5–)9–13(–14.5) μm; at upper flank and margin *textura angularis* to *prismatic,* composed of globose to obovoid cells with ± thin walls, (9–)9.5–11.5(–12) × 7–9 μm; marginal cells globose to obovoid-clavate, (9–)10–14(–15) × 5–6.5(–7) μm; pale to brown (SE4) around margin and becoming greyish brown (6F3) toward base, not gelatinized, crystals or exudates absent; tissue becoming dark green (27F5) when mounted in ≥KOH. *Subicular hyphae* sparse, 2.5–3.5 μm diam, thick-walled (0.5–1 μm), hyaline to brownish grey (5E2). *Medullary excipulum* hyaline, *textura intricata,* 20–70 μm thick. *Paraphyses* cylindrical with rounded apices, septate, simple, thin-walled, 2.5–4 μm wide, containing large highly refractive vacuole bodies; not exceeding mature asc. *KOH reaction* negative. Ascii arising from crosiers, cylindrical-clavate, 8-spored, (62–)65–71(–74) × (5–)5.5–7 μm, *parasporifera* 25–29 μm, pore amyloid in Melzer’s reagent or Lugol’s solution with 5 % KOH pretreatment, protoplasm turning brick red (7D7) in Lugol’s solution. Ascospores biseriate to obliquely uniseriate, (7–)7.5–8(–9) × 2 μm, cylindrical-oblong to cylindrical-fusiform, apices rounded, aseptate, thin-walled, 4–6(–8) guttules (up to 1 μm diam).

**Culture characteristics:** Colonies after 14 d in the dark at 20 °C on MEA 26–28 mm diam, flat, stellate, with white woolly aerial hyphae toward centre; margin filamentous, diffuse, wide, hyaline; surface white, olive brown in centre (4E6–4F4); reverse white, olive brown in centre (4F6–4F3). Exudates and soluble pigments absent. Mycelium consisting of hyaline to brown, smooth, septeate, branched, hyphae 1.5–3 μm diam, sometimes covered with gelatinous sheaths 1–2 μm diam.

**Cardinal temperatures:** Range 5–35 °C, optimum 20 °C, minimum <5 °C, maximum slightly >35 °C.

**Host range:** Associated with decaying *Betula alleghaniensis* wood.

**Distribution:** Canada (New Brunswick).

**Notes:** *Mollisia diesbachiana* forms apothecia with a characteristic dull to dark blue hymenium and narrow, cylindrical-oblong to cylindrical-fusiform guttulate ascospores. The phialidic, phialocephala-like asexual morph was only observed when agar culture blocks were floated in sterile water. *Mollisia diesbachiana* is basal to a clade containing semi-aquatic species including *Loramyces juncicola,* *L. macrosporus,* *Obectodiscus aquaticus,* and *Omphrolila hemiariaeloidea.*

*Mollisia monilioides* Tanney & Seifert *sp.* nov. MycoBank MB833620. Fig. 25

**Etymology:** Latin, *monilioides,* referring to the monilioid conidiophores.

**Typs:** *Canada,* New Brunswick, Northumberland County, Doaktown, 46.480353 –66.058096, isolated as an endophyte from healthy *Picea rubens* needles, 19 Jul. 2014, J.B. Tanney (holotype DAOM 745763, culture ex-type DAO MC 250734 = NB-625-6C).

Conidiophores micronematous to macronematous, arising vertically or laterally from mycelium submerged in water, pale to dark brown, smooth, cylindrical, thin- or thick-walled, frequently composed of monilioid cells, unbranched or 1–5 series of branches, branching angle usually acute, penicillate or sympodially branched, 2–4 μm diam, up to indeterminate length, with several septa; giving rise to globose or inverted cone-shaped conidiogenous heads or persisting as non-functional co-conidiophores. *Conidiogenous cells* phialidic, terminal, sometimes intercalary; ampulliform, (7.5–)9.5–12(–13) × (2–)2.5–4 μm; collarettes cylindrical to doliform or ovoid, 3–4(–4.5) × 2–2.5(–3) μm; hyaline to pale brown, often appearing concolorous with the darker conidiophore; occurring singly, alternately branched from metulae, or in whorls of 2–4 from metulae; phialides sometimes developing percurrent from aperture of proximal phialides or converting into cylindrical non-functional phialides in the sense of *Day et al.* (2012). *Metulae*
**RPB1**

Fig. 11. A 50 % majority-rule consensus tree obtained from the maximum likelihood analysis based on the expanded RPB1 sequence dataset containing representative *Mollisia* and allied taxa. Culture collection (DAOMC, CBS) or JB Tanney personal collection accession numbers (NB) follow the species name (type strains in bold). Significant branch support values, SH-aLRT support (>70 %)/ultrafast bootstrap (BS) support (>70 %)/Bayesian posterior probabilities (>0.95), are presented at the nodes with lower supports indicated by an en dash (–) and full support (100 % SH-aLRT, 100 % BS, 1.0 PP) indicated by an asterisk (*). Truncated branches are designated by a broken line, which is a 2× reduction unless indicated. Clades are labelled I–X for convenience. The tree is rooted with *Chlorencoelia versiformis* (DAOMC 251598).
hyaline to pale brown, cylindrical to obovoid, apices frequently clavate with tapered base, (4–)4.5–7(–8) × 3–3.5(–4) μm. Conidia dimorphic; primary conidia ellipsoidal to oblong or ossiform, hyaline, 3–4(–5) × 2–2.5(–3) μm; secondary conidia globose to ellipsoidal, hyaline, (2–)3(–3.5) × (1.5–)2–2.5 μm; primary conidium is preceded by secondary conidia, forming false chains that collapse into persistent slimy heads. Sexual morph not observed.

**Culture characteristics:** Colonies after 14 d in the dark at 20 °C on MEA 15–17 mm diam, flat with moderately abundant woolly aerial hyphae, fasciculare hyphae aggregated in centre; margin entire to undulate, diffuse and white; surface dark blond to olive brown (5D4–4D5); reverse brownish grey (5D3). Exudates and soluble pigments absent. Mycelium consisting of hyaline to brown, smooth, septeate, branched, hyphae 1.5–3.5 μm diam, sometimes covered with gelatinous sheaths 1–2 μm diam.

**Cardinal temperatures:** Range 5–35 °C, optimum 20 °C, minimum <5 °C, maximum slightly >35 °C.

**Host range:** Endophyte of healthy *Picea rubens* needles.

**Distribution:** Canada (New Brunswick).

Additional materials examined: Canada. New Brunswick, Northumberland County, Doaktown, 46.480353 –66.058096, isolated as an endophyte from asymptomatic *Picea rubens* needle, 19 Jul. 2014, J.B. Tanney, DAOMC 250735.

**Notes:** *Mollisia monilioides* is described based on its asexual morph and phylogenetic placement within *Mollisia* s.l. Based on sequence data, *M. monilioides* is closely related to other conifer needle endophytes (DAOM 250747 from a *Picea rubens* needle and *Cystodendron dryophilum* CBS 295.81 from a *Juniperus*...
communis needle) and Mollisia spp. producing apothecia on decaying wood (Mollisia cinerea CBS 122029, NB-655, DAOMC 252005). Conidiophores were only observed when MEA culture blocks were floated in sterile water. Structures interpreted as apothecial initials were observed but never formed immature or mature apothecioid structures after 18 mo.

*Mollisia novobrunsvicensis* Tanney & Seifert sp. nov. Myco-Bank MB833621. Fig. 26

*Etymology:* Named for the province of New Brunswick, where the fungus was collected.

*Typus:* Canada. New Brunswick, Albert County, Alma, Fundy National Park, Coppermine trail, 45.5493 -65.01878, decaying *Betula papyrifera* wood, 27 Sep. 2014, J.B. Tanney (holotype DAOM 867422, culture ex-type DAOMC 252263 = NB-580).

*Asexual morph* not observed. *Apothecia* scattered to confluent, sessile, subiculum not evident, urceolate to cup-shaped when young, disc planar to concave at maturity, outline entire to undulate or lobate, greyish to dull blue (22B4–23D4), outer surface grey toward base (23E1), 1–2.5 mm diam, 0.2–0.4 mm high, margin frequently paler color, smooth. *Ectal excipulum* at base and mid flanks textura globulosa to angularis, 60–175 μm thick near base, 20–48 μm thick towards margin, composed of globose to isodiametric cells with thin to slightly thickened walls, (16.5–)17–23(–25.5) × (7–)11–15.5(–16.5) μm; at upper flank and margin textura angularis to prismatica, composed of globose...
to subglobose or obovoid cells with ± thin walls, 9–12.5(–16.5) × (4–)6–8(–9) μm; marginal cells subglobose to obovoid or clavate, 8–14(–15) × (4–)5–7.5(–8) μm; pale to yellowish brown (5D5) around margin and becoming greyish brown (5F3) toward base, not gelatinized, crystals or exudates absent; tissue becoming dark green (27F5) when mounted in KOH. Subiculum cells in water. Exudates and soluble pigments absent. Mycelium consisting of yellowish brown (5E5) concentric rings with age; reverse white. Medullary excipulum cells in KOH. Subicular hyphae absent; tissue becoming dark green (27F5) when mounted in KOH. Narrowly ellipsoidal to oblong, apices rounded, aseptate, thin-walled, small (2.5–3.5 μm thick. Paraphyses cylindrical with rounded apices, septate, simple, thin-walled, (2.5–)3–4 μm wide, containing large highly refractive vacuole bodies; not exceeding mature asci. KOH reaction negative. Ascii arising from croziers, cylindrical-clavate, 8-spored, 50–58(–65) × (5–)6–8(–9) μm, pars spongia 20–24 μm, pore amyloid in Melzer’s reagent or Lugol’s solution with 5 % KOH pretreatment, protoplasm turning brick red (7D7) in Lugol’s solution. Ascosporae biseriate to obliquely uniseriate, (6–)7–9(–9.5) × 2–3 μm, ellipsoidal to oblong, apices rounded, asceptate, thin-walled, small (<1 μm) guttules sparsely present.

**Cardinal temperatures:** Range 5–35 °C, optimum 20–25 °C, minimum <5 °C, maximum slightly >35 °C.

**Host range:** Associated with decaying *Betula alleghaniensis* and *B. papyrifera* wood and healthy *Abies balsamea* needles.

**Distribution:** Canada (New Brunswick).

**Notes:** Apothecia of *Mollisia novobrunsvicensis* are common on decaying *Betula* wood in New Brunswick and characterized macroscopically by a greyish to dull blue hymenium. This species is probably closely related to *Mollisia cinerea* s.s. and shares similar (but probably variable and taxonomically insignificant) features described by Batsch (1786), including apothecia that become somewhat pulvinate with age, and a sinuate-lobate and crisped margin (at least when immature). Unlike *M. cinerea*, the hymenium of *M. novobrunsvicensis* is bluish versus cinereous, probably a subjective and variable character, and does not dry to a dirty white colour. The margin of *M. novobrunsvicensis* often appears white or pale in colour in younger specimens and some older specimens; although other authors such as Persoon (1799) and Karsten (1871) described a white margin for *Mollisia cinerea* s.s., this feature is absent from Batsch’s description. According to Karsten (1871), the ascospore dimensions of *M. cinerea* occupy a large range (5–12 × 1–2.5 μm), shorter than reported here for *M. novobrunsvicensis*.

**Additional materials examined:** Canada, New Brunswick, Albert County, Alma, Fundy National Park, rotten hardwood log, 24 Sep. 2013, J.B. Tanney, DAO/M 251538 = DAO/M 745741; ibid., decaying fallen branch of *Betula alleghaniensis*, 27 Sep. 2014, J.B. Tanney, NB-579; ibid., decaying log of *Betula alleghaniensis*, 23 Sep. 2015, J.B. Tanney DAO/M 251495; ibid., DAO/M 251631; Charlotte County, Little Lepreau, decaying fallen branch of *Betula papyrifera*, 12 Jul. 2014, J.B. Tanney, DAO/M 251048; Northumberland County, Doaktown, 46.480353, -66.058096, isolated as an endophyte from asymptomatic *Abies balsamea* needles, 18 Jun. 2013, J.B. Tanney, DAO/M 250736.

*Mollisia prismatica* Tanney & Seifert sp. nov. MycoBank MB833622. Fig. 27

**Etymology:** Latin, *prismatica*, referring to the large crystals present in the inner ectal excipulum and medullary excipulum.

**Typus:** Canada, Quebec, Gatineau (Aylmer), Forêt Boucher, 45.418969, -75.834870, decaying *Acer saccharum* wood, 14 Sep.
2015, J.B. Tanney & B. Tanney (holotype DAOM 696477, culture ex-type DAOMC 251599 = NB-688).

Asexual morph not observed. 

Apothecia scattered to gregarious in small groups (2–4), sessile, subiculum not evident, urceolate to cup-shaped when young, disc planar to pulvinate at maturity, outline entire, waxy to pale yellow (4A3), outer surface greyish yellow toward base (4B3), 0.7–1.75 mm diam, 0.2–0.5 mm high, margin frequently paler color, smooth. 

Ectal excipulum at base and mid flanks textura globulosa to angularis, 60–100 μm thick near base, 25–42 μm thick towards margin; at upper flank and

Fig. 15. Niptera ramincola DAOM 86812. A–D. Apothecia from dead sticks on moist ground in forest. E. Vertical section of apothecium. F. Margin. G. Medullary excipulum and hymenium. H–J. Marginal cells. K. Marginal cells in KOH. L. Ectal and medullary excipula. M, N. Asci and paraphyses. O. Ascus. P. Ascus with amyloid tip in Lugol’s solution after KOH pretreatment. Q. Ascospores. Scale bars: E = 1 000 μm, F, G = 100 μm, J–S = 10 μm.
A 50 % majority-rule consensus tree obtained from the maximum likelihood analysis based on the expanded ITS sequence dataset containing representative Mollisia and allied taxa including unidentified GenBank sequences, Culture collection (DAOMC, CBS), JB Tanney personal collection accession numbers (JBT, NB), or GenBank accession numbers follow the species name (type strains in bold). Significant branch support values, SH-aLRT support (≥70 %), ultrafast bootstrap (BS) support (≥70 %), are presented at the nodes with lower supports indicated by an en dash (−) and full support (100 % SH-aLRT; 100 % BS) indicated by an asterisk (*). Truncated branches are designated by a broken line, which is a 2× reduction unless indicated. The Bamia-grass clade and Phialocephala s.s. are collapsed and shown in Figs 18 and 19, respectively. Clades are numbered 1–14 for convenience. The tree is rooted with Chlorocenella versiformis (DAOMC 251598).
Fig. 16. (Continued).

margin textura angularis to prismatica, composed of globose to elongated clavate cells with ± thin walls, (8-)8.5–10.5(-11.5) × 6–8 μm; marginal cells globose to obovoid, (9–)10–15 × (5–)6–8.5–(11) μm; pale to greyish yellow (4B3) around margin and becoming brownish orange (5C5) toward base, not gelatinized, abundant crystals in inner ectal and medullary excipula, rhomboidal to amorphous, 3.5–16 μm diam; tissue becoming dark green (27F5) when mounted in KOH.

Subicular hyphae sparse, 2.5–3.5(-4) μm diam, thick-walled (0.5–1 μm), hyaline to light brown (5D4). Medullary excipulum hyaline, textura intricata, 80–200 μm thick. Paraphyses cylindrical with rounded apices, septate, simple, thin-walled, 3–4.5 μm wide, containing large highly refractive vacuole bodies; not exceeding mature asci. KOH reaction negative. Asci arising from croziers, cylindrical-clavate, 8-spored, (65–)87–90 × (5–)6–8(-9) μm, pars sporifera 24–45 μm, pore amyloid in Melzer’s reagent or Lugol’s solution with 5% KOH pretreatment, protoplasm turning brick red (7D7) in Lugol’s solution. Ascospores biseriate to obliquely uniseriate, 10–11.5–(12.5) × 3.5–4(-4.5) μm, ellipsoidal-fusiform to cylindrical-clavate, apices rounded, aseptate, thin-walled, small (up to 1.5 μm diam) guttules sparsely present.
Colony characteristics: Colonies after 14 d in the dark at 20 °C on MEA 7–10 mm diam, flat to slightly convex, moderately abundant woolly aerial hyphae; margin entire, reddish grey (7B2); surface dark brown to dark ruby (7F4–12F3); reverse reddish grey (12F2). Abundant light yellow (3A5), up to 2 mm diam, flat, dendritic or acicular crystals on colony surface and surface of surrounding agar, faint pale yellow (3A3) soluble pigment present in surrounding agar. Mycelium consisting of hyaline to pale brown, smooth, septate, cylindrical but sometimes sinuous in outline, often constricted at septa, branched, hyphae 1.5–4 μm diam, containing abundant oily guttules.

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

Host range: Associated with decaying Acer saccharum wood.

Distribution: Canada (Quebec, Ontario).

Additional materials examined: Canada, Quebec, Gatineau (Aylmer), Forêt Boucher, 45.418699–75.834870, decaying hardwood stick on ground, 16 Sep. 2014, J.B. Tanney & B. Tanney, DAOMC 250740; Ontario, Nepean, rotten hardwood, 17 Sep 2015, J. Mack, DAOMC 251496.

Notes: Mollisia prismatica is distinguished by its white to pale yellow hymenium, thick medullary excipulum, abundant crystals in the inner ectal and medullary excipulum, and broad ascospores. Several lignicolous Mollisia species with white to pale hymenia have been described, for example: (i) Mollisia discolor and M. melaleuca differ by their ascospore dimensions (8 × 2 μm; Phillips 1887) and dark ectal excipula; (ii) M. sublivida differs by the smaller asci (32–42 × 4.5–5.5 μm) and ascospores (4–7 × 1.5 μm); (iii) M. glenospora differs by its diminutive apothecia (0.25–0.5 mm), marginal hairs, and larger ascospores (12–15 × 7–8 μm); (iv) M. caespitica differs by its smaller asci (30–40 × 3–4.5 μm) and ascospores (4–6 × 1–1.5 μm); (v) Pyrenopeziza benesuadad, transferred to Pyrenopeziza by Greemmen (1958) but undoubtedly a Mollisia species, is similar in hymenium color and young subhemispherical ascomata, but differs by its apparent preference for Alnus wood, erumpent apothecia, and narrower ascospores (9–10 × 2–2.5 μm; Phillips 1887). Mollisia prismatica is macroscopically very similar to M. uda sensu auctorum and has similar ascospores (12 × 3–3.5 μm with a very low guttule content) although the ascospores of M. prismatica are more broadly cylindrical and M. uda occurs on submerged wood and shows a yellow reaction to KOH. Mollisia endocrystallina is closely related to, but distinct from, M. prismatica based on ITS sequences. The former, recently described from coarse woody debris of Piceaabies in humid conditions in Croatia, shares some characters with M. prismatica including pale grey apothecia that become subpulvinate with maturity; ascospores lacking guttules, and the absence of a KOH reaction. However, ascospores of M. endocrystallina are smaller (7–11 × 3.5–4.5) and the free-floating, rossetiform crystalloid bodies described in the ectal excipular and marginal cells are unlike those of M. prismatica (Crous et al. 2019). Mollisia prismatica would morphologically be referable to the previous or current concept of Belonopsis because of its white to pale yellow hymenium, less pigmented or pale ectal excipulum, presence of crystals in the inner ectal and medullary excipulum, and pulvinate apothecia. However, most Belonopsis species are graminicolous with longer ascospores that are frequently multiseptate when young. Trichobolium kneiffii (=Belonopsis reticula) is graminicolous (on Phragmites) with apothecia that contain abundant excipular crystals, long (12.5–28 × 2–3 μm), guttulate ascospores, and frequently occur on a well-developed subiculum.

Mollisia rava Tanney & Seifert sp. nov. MycoBank MB833623. Fig. 28

Etymology: Latin, rava, for the greyish colour of the hymenium.

Typus: Canada, New Brunswick, Albert County, Alma, Fundy National Park, Coppermine trail, 45.5493 -65.01878, decaying Betula alleghaniensis wood, 27 Sep. 2014, J.B. Tanney (holotype DAO 745742; culture ex-type DAOMC 251562 = NB-584).

Asexual morph not observed. Apothecia scattered to gregarious or caespitose, sessile, subiculum not evident, urceolate to cup-shaped when young, disc planar to concave or at maturity, outline entire unto undulate, dull blue to bluish grey (21D–21F2), outer surface darker base, 1–2 mm diam, 0.2–0.3 mm high, margin smooth, sometimes appearing crenulate. Ectal excipulum at base and mid flanks textura globulosa to angularis, 60–130 μm thick near base, 25–50 μm thick towards margin, composed of globose to isodiametric cells with to slightly thickened walls, (13–14)–18.5(–19.5) × (10–) 10.5–12(–13) μm; at upper flanks and margin textura angularis to prismatica, composed of globose to subglobose or obovoid cells with ± thick walls, (6–)7–9(–11.5) × (4.5–)5–7(–8) μm; marginal cells subglobose to obovate or clavate, 8.5–12(–13.5) × 4–6(–7) μm; brownish orange (5C3) around margin and becoming brown (5F5) toward base, not gelatinized, crystals or exudates absent; tissue becoming greenish grey (28F2) when mounted in KOH. Subicular hyphae sparse to moderately abundant, (2.5–)3–3.5(–4) μm diam, thick-walled (0.5–1 μm), dark brown (6F4). Medullary excipulum hyaline, textura intricate, 18–40 μm thick. Paraphyses cylindrical with rounded apices, septate, simple, thin-walled, 3–4 μm wide, containing large highly refractive vacuole bodies when living; not exceeding mature asci. KOH reaction negative. Ascii arising from croziers, cylindrical-clavate, 8-spored, (54–) 56–62(–65) × (4.5–)5–6 μm, pars sporifera 25–35 μm, pore amyloid in Melzer’s reagent or Lugol’s solution with 5 % KOH pretreatment, protoplasm turning brick red (7D7) in Lugol’s solution. Ascospores biseriate to obliquely uniseriate, (6.5–)7–9(–10) × 2–2.5(–3) μm, oblong, allantoid to slightly sigmoidal, one end sometimes more tapered or curbed, apices rounded, aseptate, thin-walled, (1–)2–4(–7) small (up to 1 μm diam) guttules present. All measurements made from rehydrated specimens.

Colony characteristics: Colonies after 14 d in the dark at 20 °C on MEA 11–14 mm diam, flat with sparse aerial hyphae; margin wide, undulate, hyaline; surface greyish orange (5B5) and yellowish brown (5F4), occasionally sectoring; reverse brownish orange to brown (5C4–5E4). Excudates and soluble pigments absent. Mycelium consisting of pale brown to brown, smooth, septeate, branched, hyphae 1.5–3.5 μm diam, sometimes...
Fig. 18. A 50 % majority-rule consensus tree obtained from the maximum likelihood analysis based on the ITS sequence dataset of the Barrenia-grass clade. Culture collection (DAOMC, CBS), GenBank number, collection accession number (CBS, DAOM, DAOMC), or JB Tanney personal collection accession number (NB) precedes the sequence metadata (taxon or sequence ID, host and substrate, country of origin). Major host groups (grass, conifer, orchid, Ericaceae) and sequences derived from apothecia collections are indicated by symbols defined in the upper right box. Significant branch support values, SH-aLRT support (>70 %)/ultrafast bootstrap (BS) support (>70 %), are presented at the nodes with lower supports indicated by an en dash (--) and full support (100 %) indicated by a asterisk (*). Truncated branches are designated by a broken line, which is a 2x reduction unless indicated. The tree is rooted with the ex-type of Mollisia dextrinospora (CBS 40178).
covered with thin (1 μm) yellow to deep orange (4A8–6A8) crystalline sheath or dark brown (5F8) exudate up to 5 μm diam.

**Cardinal temperatures**: Range 5–35 °C, optimum 20 °C, minimum <5 °C, maximum slightly >35 °C.

**Host range**: Associated with decaying Betula alleghaniensis wood.

**Distribution**: Canada (New Brunswick).

**Additional specimens and cultures examined**: Canada, New Brunswick, Albert County, Alma, Fundy National Park, Dickson's Falls, decaying Betula papyrifera wood, 23 Sep. 2013, G.J. Samuels, DAOMC 250737.

**Notes**: Mollisia rava is characterized by apothecia with dull blue to bluish grey hymenia. Based on the current phylogenetic analyses, it is closest related to Mollisia melaleuca CBS 589.84 and unidentified Mollisia endophytes of *Picea rubens* needles (DAOMC 252032, NB-334-2C) and is related to the *Mollisia cinerea* s.s. clade.

**Phialocephala amethystea** Tanney & Seifert *sp. nov*. MycoBank MB833624. Fig. 29

**Etymology**: Latin, *amethystea*, named for the purple colour of the large crystals produced abundantly on the surface and below the agar in cultures grown on MEA.

**Typus**: Canada, New Brunswick, Albert County, Alma, Fundy National Park, Maple Grove trail, 45.58178 –64.98633, fallen Acer saccharum branch, 16 Jul. 2014, J.B. Tanney (holotype DAOM 867431, culture ex-type DAOMC 251552 = NB-469).
Fig. 19. A 50 % majority-rule consensus tree obtained from the maximum likelihood analysis based on the ITS sequence dataset of *Phialocephala* s.s. Culture collection (DAOMC, CBS), GenBank number, collection accession number (CBS, DAOM, DAOMC), or JB Tanney personal collection accession number (NB) precedes the species metadata (taxon or sequence ID, host and substrate, country of origin). Significant branch support values, SH-aLRT support (>70 %)/ultrafast bootstrap (BS) support (>70 %), are presented at the nodes with lower supports indicated by an en dash (–) and full support (100 % SH-aLRT, 100 % BS) indicated by an asterisk (*). Trimmed branches are designated by a broken line, which is a 2× reduction unless indicated. Clades are labelled I–VI for convenience. The tree is rooted with the ex-type of *Mollisia dextrinospora* (CBS 40178).
Conidiophores micronematous to macronematous, occasionally reduced to conidiogenous cells, arising vertically or laterally from mycelium, hyaline to dark brown, smooth, cylindrical, thin- or thick-walled, older conidiophores sometimes covered in reduced to conidiogenous cells, arising vertically or laterally from mycelium. Conidiogenous cells phialidic; terminal, sometimes intercalary; ampulliform to ellipsoidal, (7.5–)10–13(–14.5) × (2.5–)3–4(–4.5) μm; collarettes deep, cylindrical to doliform with apex sometimes flaring, (2.5–)3–4(–7) × (2–)2.5–3(–4) μm; hyaline to pale brown or brown, becoming thick-walled, darker, septate, and swollen with age; occasionally occurring singly, usually in whorls of 3–5(–7) from metulae. Metulae pale to brown, cylindrical to broadly clavate, (3.5–)4–7(–10) × (2.5–)3–4(–5) μm. Conidia dimorphic; primary conidia bullet-shaped to elongate-pyriform or oissiform, base often truncate, hyaline, (3–)3.5–4.5(–6) × 1.5–2 μm; secondary conidia globose, base often protruding and truncate, hyaline, 2.5 × 2–2.5 μm; primary conidium is seceded by secondary conidia, forming false chains that collapse into persisting slimy heads. Sexual morph not observed.

Colony characteristics: Colonies after 14 d in the dark at 20 °C on MEA 30–35 mm diam, flat, slightly convex with sparse woolly aerial hyphae toward centre; margin filamentous, diffuse, wide, hyaline; surface olive brown (5F5); reverse greyish brown (5F3). Exudates and soluble pigments absent. Abundant cherry red to ruby (10B8–12D12), up to 400 μm diam, acicular crystals on colony surface, surface of surrounding agar, and submerged in agar. Mycelium consisting of hyaline to subhyaline or brown, smooth, septate, branched, guttulate, hyphae 2–4 μm diam, thin-walled or thick-walled (up to 1 μm thick), sometimes covered with entire or sinuate gelatinous sheaths 1–3 μm diam or encrusted with cherry red to ruby (10B8–12D12) thin crystalline sheath or acicular crystals.

Cardinal temperatures: Range 5–35 °C, optimum 25 °C, minimum <5 °C, maximum slightly >35 °C.

Host range: Associated with decaying Acer saccharum wood and healthy Picea rubens needles.

Distribution: Canada (New Brunswick).

Additional specimens and cultures examined Canada, New Brunswick, Albert County, Alma, Fundy National Park, Maple Grove trail, 45.58178 -64.98633, endophyte of asymptomatic Picea rubens needle, 24 Sep. 2013, J.B. Tanney, NB-382-4F.

Notes: Phialocephala amethystea is most closely related to P. compacta based on sequence data and shares some morphological similarities including phialides, collarettes, conidia, and conidiogenous heads of comparable dimensions and conidiogenous heads that become sclerotized with age. Kowalski & Kehr (1995) also mentioned the occasional formation of crystals in P. compacta cultures but did not provide details. Phialocephala dimorphospora and P. compacta share 95 % similarity for RP1 sequences and 94 % similarity for ITS sequences.

Phialocephala biguttulata Tanney & Seifert sp. nov. MycoBank MB833625. Fig. 30

Etymology: Latin, biguttulata, referring to the two large guttules in the ascospores.

Typus: Canada, Ontario, Ottawa, Saddlebrook Estates, South of John Aselford Drive, 45.375583 –76.04995, decaying stem of large wind-fallen Pinus strobus, 17 Jun. 2014, K.A. Seifert (holotype DAOM 867440, culture ex-type DAOMC 250754 = NB-649).

Asexual morph not observed. Apothecia scattered to gregarious, sessile, subiculum not evident, urceolate to cup-shaped when young, disc planar to concave at maturity, outline entire, brownish grey to bluish grey (5D2–20D3), outer surface darker; 0.7–2 mm diam, 0.2–0.3 mm high; margin frequently paler color,
smooth. *Ectal excipulum* at base and mid flanks *textura globulosa* to *angularis*, 40–95 μm thick near base, 19–34 μm thick towards margin, composed of globose to isodiametric cells with thin to slightly thickened walls, (13–) 14–20(–23) × (7–) 9–12.5(–13) μm; at upper flank and margin *textura angularis* to *prismatic*; composed of globose or cylindrical to elongated clavate cells with ± thick walls, (13–) 14–20(–22.5) × (5.5–) 7–9 μm; marginal cells cylindrical to obvoid or clavate, (10–) 11–17(–) 20 μm long, maximum width towards apex 6–8.5(–) 10 μm, minimum width at base (4–) 4.5–6 μm; brownish orange to brown (5D4–6E4) around margin and becoming dark brown (6F7) toward base, not gelatinized, crystals or exudates absent; tissue becoming dark green (27F5) when mounted in KOH. *Subicular hyphae* sparse, 2.5–4 μm diam, thick-walled (0.5–1 μm), dark brown (5F8). *Medullary excipulum* hyaline, *textura intricata*, 20–34 μm thick. Paraphyses cylindrical with rounded apices, septate, simple, thin-walled, 3–3.5 μm wide, containing large highly refractive vacuole bodies; not exceeding mature asci. KOH reaction negative. *Asci* arising from croziers, cylindrical-clavate, 8-spored, (53–) 58–72(–76) × (6–) 6.5–7.5 × 7.5 μm, *pars spuria* 18–27 μm, pore amyloid in Melzer’s reagent or Lugol’s solution with 5 % KOH pretreatment, protoplasting turning brick red (7D7) in Lugol’s solution. Ascospores biseriate to obliquely uniseriate, (7.5–) 8–(9–) 10 × 3–3.5(–4) μm, ellipsoidal-fusiform to oblong, apices rounded, aseptate, thin-walled, frequently guttulate with two polar guttules (1.5–2 μm diam).

**Colony characteristics**: Colonies after 14 d in the dark at 20 °C on MEA 23–25 mm diam, convex with woolly aerial hyphae; margin diffuse, hyaline; surface soot brown to dark brown (5F5–6F3); reverse brownish grey (7F2). Exudates and soluble pigments absent. Mycelium consisting of subhyaline to brown, smooth, septate, branched, hyphae 2–5.5 μm diam, thin- or 1–1.5(–2) μm thick-walled, aerial mycelia friable, sometimes covered with exudate layer 2–4(–6.5) μm diam.

**Cardinal temperatures**: Range 5–35 °C, optimum 25–30 °C, minimum <5 °C, maximum slightly >35 °C.

**Host range**: Associated with decaying *Pinus strobus* wood.

**Distribution**: Canada (Ontario).

**Notes**: *Phialocephala biguttulata* is morphologically distinguished from other species in the *Phialocephala dimorphospora* s.s. species complex by the two large (1.5–2 μm diam) guttules that occur towards both poles of the ascospores.

**Phialocephala collarifera** Tanney & Seifert sp. nov. MycoBank MB833626. Fig. 31

**Etymology**: Latin, *collarifera*, bearing collars, to describe the deep collarettes.

**Typus**: Canada, Quebec, Gatineau (Aylmer), Forêt Boucher, 45.418969 -75.834870, decaying hardwood stick on ground, 22 Jul. 2014, J.B. Tanney & B. Tanney, holotype DAOM 867927, culture ex-type DAOMC 250755 = NB-683.

*Conidiophores* micronematous to macronematous, arising vertically or laterally from mycelium, pale to dark brown, smooth, cylindrical, thin- or thick-walled, older conidiophores sometimes covered in 1–2.5 μm wide gelatinous sheath, unbranched or 1–2 series of branches, branching angle usually acute, (2.5–) 3–4 μm diam, (30–) 39–88(–125) μm tall, with several septa; giving rise to globose conidigenous heads. *Conidiogenous cells* phialidic; terminal, sometimes intercalary; ampulliform, (13.5–) 16.5–21.5(–24) × (2.5–) 3–3.5 μm; collarettes deep, cylindrical with slightly flaring apex, (5–) 6.5–8(–9) × (2–) 2.5–3 μm; hyaline to pale brown, often appearing concolorous with the darker conidiophore; occasionally occurring singly, usually in whorls of 2–4(–5) from metulae. *Meletes* pale brown to cylindrical to clavate, (8–) 9.5–13.5(–15) × (2.5–) 3–4 μm. *Conidia* dimorphic; primary conidia elongate-ellipsoid to elongate-yriform, hyaline, (6.5–) 7.5–8.5(–9.5) × 2–2.5 μm; secondary conidia ellipsoidal to oblong or obvoid, one end sometimes more tapered or subtruncate, hyaline, (3–) 3.5–4.5(–5.5) × 2–2.5(–3) μm; primary conidium is seceded by secondary conidia, forming false chains that collapse into persisting slimy heads. *Sexual morph* not observed.

**Colony characteristics**: Colonies after 14 d in the dark at 20 °C on MEA 28–30 mm diam, flat to slightly convex with moderately abundant woolly aerial hyphae; margin diffuse, wide, hyaline; surface olive brown to greyish brown (4F4–6F3); reverse dark brown to brownish grey (6F4–6F2). Exudates and soluble pigments absent. Mycelium consisting of subhyaline to brown, smooth, septate, branched, hyphae 2.5–5 μm diam, thin- or thick-(1 μm) walled, sometimes covered with gelatinous sheaths 1.5–4.5 μm diam. Conidiophores very abundant among aerial hyphae.

**Cardinal temperatures**: Range 5–35 °C, optimum 25 °C, minimum <5 °C, maximum slightly >35 °C.

**Host range**: On decaying *Betula papyrifera* wood.

**Distribution**: Canada (Quebec).

**Additional specimens and cultures examined**: Canada. Quebec, Gatineau (Aylmer), Forêt Boucher, 45.418969 -75.834870, decaying hardwood stick on ground, 22 Jul. 2014, J.B. Tanney & B. Tanney, NB-424.

**Notes**: *Phialocephala collarifera* is a member of the *Phialocephala dimorphospora* s.s. clade and is closely related to *P. dimorphospora*. It differs morphologically from *P. dimorphospora* by its longer phialides, (13.5–) 16.5–21.5(–24) × (2.5–) 3–3.5 μm vs. 11–17.5 × 2.5–3 μm, longer collarettes, (5–) 6.5–8(–9) × (2–) 2.5–3 μm vs. 3.5–5 × 2.5–3 μm, larger primary conidia, (6.5–) 7–8.5(–9.5) × 2–2.5 μm vs. 3.5–5.5 × 2.5–3 μm, and larger secondary conidia (3–) 3.5–4.5(–5.5) × 2–2.5(–3) μm vs. 2–2.5 × 2–2.5 μm.

**Phialocephala helenae** Tanney & Seifert sp. nov. MycoBank MB833627. Figs 32 and 33

**Etymology**: Named for the collector of the type specimen, Helena Spizarsky.

**Typus**: Canada, New Brunswick, Albert County, Alma, Fundy National Park, Maple Grove trail, 45.58178 -64.98633, decaying *Acer saccharum* branch, 16 Jul. 2014, H.M. Spizarsky & J.B. Tanney (holotype DAOM 867437, culture ex-type DAOMC 250756 = NB-467).

*Conidiophores* micronematous to macronematous, arising vertically or laterally from mycelium, subhyaline to dark brown, smooth, cylindrical, thin- or thick-walled, older conidiophores sometimes covered in 1–3 μm wide gelatinous sheath, unbranched or 1–3 series of branches, 2.5–3(–3.5) μm diam, 25–110 μm tall, with several septa; giving rise to inverted cone-
shaped conidiogenous heads. *Conidiogenous cells* phialidic; terminal, sometimes intercalary; amyloid to ellipsoidal with age, (11–)13–18–(21.5) × (2–)2.5–3.5–(4) μm; collarettes deep, cylindrical sometimes with slightly flaring apex, (3–)4–7–(8) × 2–3 μm; hyaline to brown; occasionally occurring singly, usually in whorls of 2–4 from metulae. *Metulae* hyaline to brown, cylindrical to clavate, (6.5–)7.5–11(–12) × (2–)2.5–3.5(–4) μm. *Conidia* dimorphic; primary conidia elongate-ellipsoidal to elongate-pyriform, hyaline, 3.5–4.5(–5.5) × 1.5–2(–2.5) μm; secondary conidia globose, base rounded to protruding and truncate, hyaline, 2(–2.5) × 2(–2.5) μm; primary conidium is seceded by secondary conidia, forming false chains that collapse into persisting slimy heads.

*Apothecia* scattered to gregarious in small groups, sessile, subiculum not evident, urceolate to cup-shaped when young, disc planar to concave at maturity, outline entire to undulate or lobate, pale blue to dull blue (21A3–21D4) when young, becoming greyish blue (21E5), hymenium often white in centre or patches, outer surface dark brown toward base (7F4); 1–2 mm diam, 0.25–0.4 mm high, margin frequently paler color, smooth. *Ectal excipulum* at base and mid flanks textura globulosa to angularis, 50–200 μm thick near base, 20–40 μm thick towards margin, composed of globose to isodiametric cells with thin to slightly thickened walls, (7–)9–13(–15) × (5.5–)7–10(–11) μm; at upper flank and margin *textura angularis* to *prismatic*, composed of globose to elongated clavate cells with ± thin walls, (7–)9.5–15(–18) × (7–)8–11(–13) μm; marginal cells globose to elongate-obovoid or clavate, (7–)12–20 × (5–)6–8(–9) μm; pale to greyish yellow or greyish red (4B5–8D5) around margin and becoming dark brown (7F4) toward base, not gelatinized, crystals or exudates absent; tissue becoming dark green (2F5) when mounted in KOH. *Subicular hypae* sparse to moderately abundant, 2.5–3.5 μm diam, sometimes thick-walled (0.5–1 μm), light to dark brown (5D4–7F4). *Medul- lary excipulum* hyaline, textura intricata, 27–72 μm thick. *Paraphyses* cylindrical with rounded apices, septate, simple, thin-walled, 3–4(–4.5) μm wide, containing large highly refractive vacuole bodies; not exceeding mature asci. KOH reaction strong, paraphyses turning yellow (3A6), visible with unaided eye. Asci arising from croziers, cylindrical-clavate, 8-spored, (55–)64–76(–76.5) × (6–)7–8(–9) μm, *pars sponleri* 21–29 μm, pore amyloid in Melzer’s reagent or Lugol’s solution with 5 % KOH pretreatment, protoplasm turning brick red (7D7) in Lugol’s solution. Ascospores biseriate to obliquely uniseriate, (9.5–)11–13.5(–15.5) × (2.5–)3–3.5(–4) μm, oblong to oblong-fusiform, straight, occasionally curved or clavate on one end, apices rounded, aseptate, thin-walled, up to 1.5 μm diam guttules aggregated at both poles.

![Fig. 20. Testing the taxonomic thresholds of Vu et al. (2019) to select Mollisiaceae taxa using the LSU barcode. The 50 % majority-rule consensus tree obtained from the Bayesian inference of phylogeny analysis is presented on the left, with posterior probability values for branch support shown at the nodes. The tree is rooted with the ex-type of *Mollisia* destruxena (CBS 401.78) and the broken branch indicates its reduction by 4×. The matrix on the right shows the identity and similarity of each sequence pair, with values colour-coded according to the taxonomic threshold values predicted by Vu et al. (2019) for the LSU barcode: 98.2 %, 96.2 %, 94.7 % and 92.7 % for genus, family, order, and class levels, respectively.](image-url)
Colony characteristics: Colonies after 14 d in the dark at 20 °C on MEA 20–28 mm diam, flat, sparse aerial hyphae, fascicular hyphae aggregated in centre; margin entire, hyaline; surface brown to greyish brown (5E4–6F3); reverse greyish brown to brownish grey (5F3–5F2). Exudates absent, greyish yellow (4B4) soluble pigment sometimes present in surrounding agar. Mycelium consisting of subhyaline to brown, smooth, septate, branched, hyphae 1.5–4 μm diam, thin- or thick- (1 μm) walled, sometimes covered with gelatinous sheaths 1–3 μm diam.

Cardinal temperatures: Range 5–35 °C, optimum 25 °C, minimum <5 °C, maximum slightly >35 °C.

Host range: Endophyte of healthy Picea mariana and P. rubens needles and associated with decaying Acer saccharum and Betula alleghaniensis wood.

Distribution: Canada (Ontario and New Brunswick).

Additional specimens and cultures examined: Canada, New Brunswick, Albert County, Alma, Fundy National Park, Dickson’s Falls, endophyte of asymptomatic Picea rubens needle, 23 Sep. 2013, J.B. Tanney, NB-365-10N; Maple Grove trail, decaying fallen branch of Betula alleghaniensis along river, 16 Jul. 2014, J.B. Tanney, DAOMC 251553; Charlotte County, Bethel, bark of fallen branch of Betula alleghaniensis, 14 Jul. 2014, J.B. Tanney, NB-457. Ontario, Nepean, near Nepean Sportsplex, fallen log, 13 Jun. 2015, J.B. Tanney, DAOMC 252040.

Notes: Phialocephala helenae differs from the closely related P. piceae by its greyish blue hymenium colour, longer ascospores (9.5–)11–13.5(–15.5) μm vs. (7.5–)9–12(–15) μm (P. piceae), and larger asci (55–)64–76(–76.5) × (6–)7–8(–9) μm vs. (33–)37–49(–53) × 4–7 (P. piceae). The conidiophores of P. helenae are longer and more complexly branched than those of P. piceae and the phialides of P. helenae have larger collarettes, (3–)4–7(–8) × 2–3 μm, than P. piceae (3–4 × 2–2.5 μm). Both P. helenae and P. piceae produce apothecia exhibiting a strong yellow KOH reaction (not reported in Tanney et al. 2016a) and are Picea needle endophytes also found in association with decaying hardwood.

Phialocephala vermiculata Tanney & Seifert sp. nov. MycoBank MB833628. Fig. 34

Etymology: Named for the production of the macrocyclic dilactone vermiculin by the type strain.

Typus: Canada, New Brunswick, Sunbury County, Acadia Research Forest, 45.996125 -66.303769, isolated as an endophyte from asymptomatic Picea glauca needle, Jun. 1985, J.A. Findlay & J.D. Miller (holotype DAOM 745759, culture ex-type CBS 120378 = DAOMC 229535 = 4GP4C2).

Sexual and asexual morphs not observed.

Colony characteristics: Colonies after 14 d in the dark at 20 °C on MEA 30–32 mm diam, flat to slightly convex with moderately abundant aerial hyphae; margin entire, wide, hyaline; surface yellowish brown to sepia (5E5–5F4); reverse yellowish brown to

Fig. 21. Mollisia sp. DAOMC 252005. A–F. Conidiophores, phialides, and conidia developing from 3.5-y-old WA + TE culture incubated at 5 °C, mounted in water. Scale bars = 10 μm.
brownish grey (5E5–6F2). White plumose crystals up to 12 mm long forming on surface or submerged below agar surface often at colony margin, composed of acicular crystals up to 5 μm diam. Exudates and soluble pigments absent. Mycelium consisting of subhyaline to brown, smooth, septate, branched, hyphae 2–4 μm diam, sometimes covered with exudates 1.5–4.5 μm diam.

Host range: Endophyte of asymptomatic *Picea glauca* needle.

Distribution: Canada (New Brunswick).

Notes: *Phialocephala vermiculata* is closest related to a strain identified as *Mollisia ligni* var. *olivascens* CBS 291.59 and is sister to the *Phialocephala dimorphospora* s.s. clade. This species is based on a single strain, DAOMC 229535, which forms large plumose crystals and has so far not been induced to sporulate despite long-term incubation (up to 24 mo) at 5–30 °C on CMA, MEA, OA, WA with or without the addition of sterile filter paper, and floating agar blocks containing mycelia in sterile water for up to 10 mo. *Phialocephala vermiculata* DAOMC 229535 produces the antiinsectan and antifungal macrocyclic dilaetone vermiculin *in vitro* and in inoculated *Picea glauca* needles (Findlay et al. 2003).

### New combinations for *Mollisia*

*Mollisia panicicola* (E. Walsh & N. Zhang) J.B. Tanney & K.A. Seifert, *comb. nov.* MycoBank MB833629.

Basionym: *Acidomelania panicicola* E. Walsh & N. Zhang, *Mycologia* 106(4): 857 (2014)

### New combinations for *Phialocephala*

*Phialocephala heterosperma* (Le Gal) J.B. Tanney & Seifert, *comb. nov.* MycoBank MB833758.

Basionym: *Mollisia heterosperma* Le Gal, *Revue Mycol.,* Paris 23: 46 (1958)
**Phialocephala lignicola** (Hern.-Restr., J. Mena & Gené) J.B. Tanney & Sefert, **comb. nov.** MycoBank MB833759. 
**Basionym:** Fuscosclera lignicola Hern.-Restr., J. Mena & Gené, Stud. Mycol. 86: 82 (2017)

**NOMENCLATURE**

**Mollisiaceae** Rehm [as 'Mollisiaceae'], in Winter, Rabenb. Krypt.-Fl., Edn 2 (Leipzig) 1.3(lief. 35): 503 (1891) [1896]

MycoBank MB81017; Index Fungorum IF81017

=Loramyctaceae Dennis ex Digby & Goos, Mycologia 79(6): 829 (1988) [1987]

**DISCUSSION**

**Phylogenetic markers and barcodes**

In this study, two standard rDNA genes (ITS and LSU) and three protein-coding genes (LNS2, RPB1, TOP1) were sequenced to explore phylogenies based on linked and unlinked gene genealogies, test species concepts through the application of the genealogical concordance phylogenetic species recognition (GCPSR) concept, assess potential secondary barcode markers, and generate reference sequences. The nominal attempts to sequence protein-coding genes from **Mollisiaceae** using standard primers included actin (Carbone & Kohn 1999), β-tubulin (Glass & Donaldson 1995, O’Donnell & Cigelnik 1997), phosphoglycerate kinase (Stielow et al. 2015), MCM7 and TSV1 (Schmitt et al. 2009), and RPB2 (Liu et al. 1999), were discouraging because of amplification failures (data not shown). The ITS barcode was readily amplified for taxa included in this study and sufficiently resolved most species, although variation among members of the PAC and between species such as **Phialocephala catenospora** and *P. nodosa* is low (e.g. 4 bp difference between *P. catenospora* and *P. nodosa*). RPB1 and TOP1 showed promising results as supplementary barcodes to ITS and while LNS2 differentiated species adequately, pronounced intraspecific variation was observed in some species (e.g., *P. helena*).

Using the high fidelity primer pair RPB1-Af and RPB1-6Rlasc, RPB1 amplified readily and provided unambiguous alignments, good species resolution, and phylogenetic signal. Stielow et al. (2015) identified LNS2, PGK, and TOP1 as promising supplementary barcodes with higher resolution than ITS. For example, PGK and TOP1 resolved related *Fusarium* and *Penicillium* species as well as partial β-tubulin II (TUB2) and translation elongation factor 1-α (TEF1α). PGK was abandoned early in this study during the preliminary screening of potential secondary barcodes after amplification failure, although testing of other primer sets should continue (e.g. PGK533; Stielow et al. 2015). TOP1 sufficiently delineated species and the resulting phylogeny had comparable topology and posterior probability support values to the RPB1 phylogeny. The phylogeny resulting from the LNS2 alignment was more weakly supported than the other gene phylogenies, with several clades forming polytomies. Significant discrepancies between LNS2 and other gene phylogenies include the placement of *P. scopiformis* sister to *Vibrissa* and *Mollisia* rosea placed within the *P. dimorphospora* s.s. and PAC clade. The well-supported placement of *Vibrissa* as sister to the PAC within *Mollisiaceae* in the ITS phylogenies, discussed in more detail below, is striking and exemplifies the potential shortfalls of this barcode as a phylogenetic marker. The presence of indel motifs, for example in the ITS1 and ITS2 regions of some species, may lead to conflicting results.

DNA analyses of **Mollisiaceae** specimens or isolates should at least include ITS, RPB1, and LSU for identification and phylogenetic reconstruction. Additional supplementary barcodes that were untested or unsuccessfully amplified in the present study but show promise in other fungal groups, such as TEF1α, should also be considered. Additionally, we did not test the five loci (pPF-018, pPF-061, pPF-076, TEF1α, TUB2) proposed by Grünig et al. (2007) to define cryptic species within the PAC, which may prove to be useful as taxonomic and phylogenetic markers in other **Mollisiaceae** clades. In GenBank, **Mollisiaceae** is best represented by the readily amplifiable rDNA genes, for example ITS, which provides good taxonomic resolution based on current species concepts. RPB1 was readily amplified, provided strongly supported phylogenies with good species resolution, and there are a growing number of reference sequences available of this gene for other fungi. While LSU is more conserved than ITS and RPB1, providing lower resolution at the species rank, LSU sequences are represented by abundant reference sequences, provide good generic or higher level taxonomic classification, and may be aligned across distantly related taxa, which is useful for estimating phylogenies of communities, placing new fungal lineages or analyzing basal lineages (Liu et al. 2012, Porter & Golding 2012). TOP1 performed well in terms of amplification, phylogenetic signal, and interspecific sequence divergence; however, there are few reference sequences available for this gene. Stielow et al. (2015) reported that LNS2 was a promising secondary barcode for basidiomycetes such as Pucciniomycotina, but performance of LNS2 among the Ascomycota was insufficiently tested and therefore could not be thoroughly assessed. While LNS2 was readily amplified and generally provided good interspecific variation, our data do not provide strong support for its use in phylogenetic analyses for this lineage. The short fragment length of LNS2 sequences may be desirable for barcoding herbarium specimens that contain degraded or fragmented DNA or for barcoding environmental samples; however, the poorly resolved polytomy and high intraspecific variation observed in this study warrant further inquiry for its use in phylogenetic reconstruction. It should be noted that LNS2 and TOP1 were proposed by Stielow et al. (2015) as barcodes and not phylogenetic markers. Species identification using LNS2 is currently infeasible because of a shortage of available reference sequences.
Endophytism occurs throughout *Mollisiaceae* but is seldom studied outside of the PAC. The lack of available reference sequences and overall taxonomic neglect of *Mollisiaceae* contribute to our inability to effectively identify and classify related endophytes. The genera *Acidomelania* and *Barrenia* were described for root endophytes from *Poaceae* spp. and *Pinus*.
rigida in the New Jersey Pine Barrens (Walsh et al. 2014, Walsh et al. 2015). Based on the RPB1 phylogeny, Barrenia, comprising two species, is polyphyletic and resides in a clade containing sequences of Mollisia, “Tapesia”, and the ex-type of Phialocephala hiberna. One of the rationales presented by Walsh et al. (2015) for proposing Barrenia was that its endophytic trophic mode distinguishes it from Mollisia, but unfortunately this argument is flawed. While there have been previous reports of Mollisia endophytes (Sieber 1989, Barklund & Kowalski 1996, Shamoun & Sieber 2000, Kowalski & Andrule 2012), endophytes in this lineage are usually reported as Phialocephala spp. because of a lack of reference sequences, the absence of endophyte cultures correlated with apothecial states (see Tanney et al. 2018a), the preponderance of phialocephala-like asexual morphs in vitro, and the lack of apothecial production in vitro. In the absence of a working taxonomic framework, the taxonomic classification of endophytes within this lineage remains uncertain, leading to reports of unidentified taxa or species placed in arbitrary genera or genera described for convenience, often lacking support for monophyly.

**Host and host tissue preferences**

Mollisiaceae comprises a diverse lineage with many apparently facultative endophytes isolated from the roots, foliage, and branches of various plant hosts worldwide. Current sampling is too scant to allow recognition of overall host preferences or biogeographical patterns among endophyte taxa; however, the co-occurrence of the same or closely related OTUs in diverse hosts and disjunct ranges is striking. A primary example includes the connection between the Picea rubens endophyte DAOMC 250744 with endophytes isolated from Picea abies in Finland, Spinulum annotinum in Poland, and Nothofagus solandri in New Zealand. Broad host and geographic ranges are well documented for the PAC root endophytes (Addy et al. 2000, Grünig et al. 2008); however, while the PAC appears to be restricted to roots, other Mollisiaceae species have been detected as endophytes in both roots and above-ground plant tissues.

In addition to root endophytes, species within the encompassing Phialocephala s.s. and PAC clade include endophytes isolated from cambium (P. compacta) and conifer needles (P. amethystea, P. helenae, P. vermiculata, P. piceae). Phialocephala scopiformis is an endophyte of Picea needles and cambium, with evidence suggesting that it systemically infects above-ground host tissues (Kowalski & Kehr 1995, Tanney et al. 2016a). Phialocephala, and, to a lesser extent, Mollisia spp., are reported as endophytes of cambium, xylem, and bark in hardwood and conifer trees (Butin & Kowalski 1990, Kowalski 1991, Kowalski & Kehr 1992, Kowalski & Kehr 1995, Barklund & Kowalski 1996, Kowalski & Gajosek 1998). Kowalski & Kehr (1992) observed that many fungi, including Phialocephala and Mollisia strains, isolated from living tree branches were also the most frequent colonizers of dead branches, hypothesizing that this latent endophytic phase was associated with self-pruning. Nipetella tsgae, described by Funk (1978) from dead and dying lower branches of Tsuga heterophylla, is probably involved in self-pruning as a branch endophyte that switches to a saprotrophic phase when the lower branches are shaded out or otherwise dying.

The ITS phylogeny places within the Barrenia clade many unnamed isolates or sequences from grasses and sedges (Poales) such as Carex spp., Deschampsia flexuosa, Elymus mollis, and Saccharum sp., but also root and foliar endophytes from diverse hosts including Ericaceae (e.g., Calluna vulgaris, Eapris pulchella, Vaccinium vitis-idaea, Wooliilsia pungens), Orchidaceae (e.g., Cymbidium insigne and Pseudorchis albida), Pinaceae (e.g., Picea abies, Pinus pinea, P. rigida, and P. sylvestris), and other diverse host plants (e.g. Podophyllum peltatum, Tetragastris hemsleyanum, Vochysia divergens) (Fig. 18). This clade comprises several grass-inhabiting species such as Barrenia panicia, M. epitypha (=M. palustris, sensu Dennis 1950), Phialocephala bamuru, and two isolates named M. hydrophila and T. hydrophila that are not conspecific (CBS 233.71, 556.63). Several named apothecial species within this clade have well-developed and melanized subicula, such as M. hydrophila, M. nigrescens (Fig. 35), M. obscura, and Tapesia villosa; however, this is probably a poor delineating character above the species rank and may be unstable even within species (Aebi 1972). Several other potential synapomorphies appear throughout this clade and, taken together, suggest some morphological cohesion warranting further study. Examined cultures of M. nigrescens (CBS 558.63, DAO MC 250739) and M. cf. nigrescens (DAO MC 250738) produced a distinct red pigment soluble in the agar, which Le Gal & Mangenot (1961) also noted in cultures of M. nigrescens and M. hydrophila. Le Gal & Mangenot (1961) also described and illustrated hypophodio-like hyphal structures in older cultures of M. hydrophila (“…sur les cultures âgées, en tube, il se forme des éléments brunâtres, bifurqués ou étoilés, d’un aspect très particulier”). Interestingly, in their description of P. bamuru, Wong et al. (2015) described similar dark brown, branching or unlobed hyphal structures at the agar-polyesterine interface in the bottom of Petri dish cultures and interpreted them as appressoria with conspicuous infection pegs. Similar hypophodia were observed on switchgrass (Panicum virgatum) seedling roots inoculated with Barrenia panicia (Walsh et al. 2015). Additional graminicolous species, for example M. chionea, M. phragmitis, M. phalardis, M. retincola, and Tapesia eriophori, probably belong in this clade but require sequences to confirm their phylogenetic placement.

**Mollisiaceae host interactions**

The interactions between Mollisiaceae endophytes and their plant hosts are not well understood. PAC root endophytes may exhibit mutualism, neutralism, or pathogenicity with varying virulence, with such interactions apparently being strain-dependent and not correlated with species (Stoyke & Currah 1993, Vohnik et al. 2005, Newsham 2011, Tellenbach et al. 2011, Tellenbach et al. 2012). Results from a recent study provide evidence supporting both saprotrophic and pathogenic life history strategies in a strain of Phialocephala subalpina based on gene inventory and a comparative genome analysis with representative pathogens, saprotrophs, and ectomycorrhizae (Schlegel et al. 2016). Phialocephala subalpina also reduced mortality and disease intensity

![Fig. 26. Mollisia novobrunsvicensis DAO 867422. A–C. Apothecia on decaying Betula papyrifera wood. D. Vertical section of apothecium. E, H. Margin of apothecium. F. Ectal and medullary excipula. G. Green reaction of ectal excipulum in 10 % KOH. I. Asci and paraphyses with refractive vacuole bodies. J. Ectal excipulum cells. K. Ectal excipulum and marginal cells showing refractive contents. L. Ascos. M. Asci with amyloid tip in Lugol’s solution after KOH pretreatment. N. Ascospores. Scale bars: D = 500 μm, E–G = 100 μm, H–N = 10 μm.](www.studiesinmycology.org)
caused by the oomycete pathogens Phytophthora plurivora and Elongisporangium undulatum (Tellenbach & Sieber 2012). Five strains of P. fortinii s.l. isolated from roots of Rubus sp. and Chamaecyparis obtusa significantly inhibited the in vitro growth of the pathogen Fusarium oxysporum f. sp. asparagi and completely suppressed the disease in Asparagus officinalis grown under inorganic conditions (Surono & Narisawa 2018). In this same study, inoculation of A. officinalis by all P. fortinii strains significantly promoted plant growth.

Many Mollisiaceae strains produce secondary metabolites that may protect against plant pests and pathogens. For example, Gremmen (1956) described the pronounced antifungal properties of mollisin (as mollisine) toward the important tree pathogens Heterobasidion annosum and Chondrodiplosis populnea. Mollisin forms as yellow crystals in cultures of M. fallens; its structure, a dichloronaphthoquinone derivative, was elucidated by Van Der Kerk & Overeem (1957) and its total synthesis was achieved recently (Schlowol et al. 2013). A strain of the PAC species Phialocephala europaea also produces secondary metabolites (sclerin and sclerotin A) that significantly inhibit growth of Phytophthora citricola (=Pythium intermedium) (Breen et al. 1955). A P. scopiformis strain inoculated in Picea glauca seedlings produces rugulosin in needles at concentrations deleterious to the eastern spruce budworm (Choristoneura fumiferana), a major forest pest in eastern Canada (Sumarah et al. 2008, Miller 2011). A recent study showed the P. scopiformis strain significantly reduced the survival of budworm developing in the upper crown of endophyte-inoculated trees (Quiring et al. 2019). This P. scopiformis strain persists more than 10 years after inoculation and can spread through 40 % of uninoculated seedlings in the lower canopy within 3 years (Miller et al. 2009). Phialocephala vermiculata (DAOM 229535), isolated as a Picea glauca needle endophyte, produces the macrocyclic antibiotic vermiculin and several natural products, including 6,7-dihydroxy-2-propyl-2,4-octadien-4-olide, that are toxic to spruce budworm cells (Findlay et al. 2003).

Two strains initially reported as Phialocephala fortinii isolated from rhizomes of Podophyllum peltatum produce podophyllotoxin, a lignan well-studied for its antiviral and antineoplastic properties (Eyberger et al. 2006). The podophyllotoxin-producing strain PPE7 was referred to by the unpublished name Phialocephala podophylli (Arneaud & Porter 2015). However, based on ITS sequences, this strain appears conspecific with Barrenia taeda (KM042204 and KT598375; identities = 401/402 i.e. 99 %, no gaps) (Fig. 18). A Phialocephala cf. fortinii root endophyte strain of Rhodiola angusta produces high yields of the bioactive tyrosols salidroside and p-tyrosol, which are normally harvested from Rhodiola tissues (Cui et al. 2016). Production of podophyllotoxin and salidroside from Podophyllum and Rhodiola plant cell and tissue cultures indicates the mutual production of these bioactive metabolites by both the plant host and endophyte.

Finally, reports of pathogenic Mollisiaceae species are rare. Apart from pathogenic PAC strains, one notable example is Phialocephala bamuru, which was described as the causal agent of fairway patch, a serious emerging disease of golf course turf in Australia that appears to be resistant to chemical control measures (Wong et al. 2015). Cystodendron dryophilum, described from living leaves of Quercus pubescens in Italy (Bubák 1914), causes leaf spot disease in Quercus suber (Moricca et al. 2016) and is associated with brown spot and mummiﬁcation of Quercus acorns in Poland (Kwańska 1997). According to Gams (2000) illustration from the holotype, Cystodendron dryophilum produces dimorphic conidia from phialocephala-like conidiophores and phialides. If C. dryophilum is phylogenetically within Mollisiaceae, as its morphology suggests, its life history as a parasite causing leaf spots on Quercus leaves would be a striking deviation from that of other known Mollisiaceae species. The only available sequences of C. dryophilum are from the strain CBS 295.81; the identiﬁcation of this strain is questionable because it was isolated not from Quercus leaves but from a Juniperus communis needle in Switzerland.

Endophyte-saprotroph connections

Tanney et al. (2016a) described connections between saprotrophic and endophytic Phialocephala species with mollisoid apothecia in the field. In the present study, similar connections between unknown endophytes and field specimens include Mollisia nigrescens, Phialocephala amethystea, and P. helenea, detected as both needle endophytes and apothecia on decaying wood in the same forest stands. Phialocephala helenea and P. piceae are closely related and morphologically similar (apothecia often erumpent from bark, with oblong ascospores, and strong lemon-yellow KOH reaction). Both occur as Picea needle endophytes with apothecia often erumpent on nearby, fallen corticated hardwood branches (Acer saccharum and Betula alleghaniensis). An Abies balsamea endophyte (DAOMC 250733) is conspecific with Mollisia melaleuca (CBS 589.84; isolated as a foliar endophyte of Picea abies). Additional connections inferred from ITS sequences include M. nigrescens isolated from apothecia on decaying wood in France (CBS 558.63) and Canada (DAOMC 250739) and also isolated from North Carolina as both an endolichenic fungus of Phialocephala amethystea, described here, shares an almost identical ITS sequence with an unidentiﬁed leaf spot species. If Phialocephala amethystea is conspeciﬁc with Phialocephala urceolata (UAMH 10827), which was described as the causal agent of fairway patch, a serious emerging disease of golf course turf in Australia that appears to be resistant to chemical control measures (Wong et al. 2015). Cystodendron dryophilum, described from living leaves of Quercus pubescens in Italy (Bubák 1914), causes leaf spot disease in Quercus suber (Moricca et al. 2016) and is associated with brown spot and mummiﬁcation of Quercus acorns in Poland (Kwańska 1997). According to Gams (2000) illustration from the holotype, Cystodendron dryophilum produces dimorphic conidia from phialocephala-like conidiophores and phialides. If C. dryophilum is phylogenetically within Mollisiaceae, as its morphology suggests, its life history as a parasite causing leaf spots on Quercus leaves would be a striking deviation from that of other known Mollisiaceae species. The only available sequences of C. dryophilum are from the strain CBS 295.81; the identiﬁcation of this strain is questionable because it was isolated not from Quercus leaves but from a Juniperus communis needle in Switzerland.
dead leaves of *Baumea* sp. in New Zealand appear to be conspecific, thus presenting a straightforward opportunity to collect and discover the *P. bamuru* sexual state.

*Mollisia* s.l. can no longer be regarded simply as genus of saprotrophs associated with dead or decaying above-ground plant tissues (e.g. Walsh et al. 2015). The precise details of life cycles of species within the lineage may remain enigmatic; what role does endophytism play in the life histories of endophytic species? Observations by Tanney et al. (2016a) show that endophytism is facultative and the fungi are not necessarily restricted to a specific host substrate or narrow host range (e.g. *Phialocephala scopiformis* and *P. piceae*). Endophytism in *Mollisiaceae* might represent an alternative life history strategy that facilitates persistence and dispersal in the absence of primary substrates or in challenging environmental conditions, in the spirit of the foraging ascomycete theory (Carroll 1999; Thomas et al. 2016). Additional ecological strategies extend the impressive ecological plasticity of the taxa of this lineage even further, as discussed below.

**Divergent aquatic lineages**

**Vibrissaceae**

Earlier phylogenetic studies using rDNA sequences reported an unexpectedly close relationship between *Mollisia, Phialocephala*, and the aquatic genera *Loramycyes* and *Vibrissa* (Wang et al. 2006a, Raja et al. 2008). Consequently, *Phialocephala* is
Fig. 30. Phialocephala biguttulata DAOM 867440. A–C. Apothecia on decaying *Pinus strobus* log. D, E. Vertical sections of apothecia. F, G. Margin and flanks of apothecium. H. Ectal excipulum and medullary excipulum. I–L Ectal excipulum, medullary excipulum, and hymenia. M, N. Asci and paraphyses with refractive vacuole bodies. O. Mature asci with ascospores. P. Asci with amyloid tips in Lugol’s solution after KOH pretreatment. Q. Ascospores. Scale bars: D–F = 100 μm, G–R, S = 10 μm.

Fig. 31. Phialocephala collarifera DAOMC 250755. A. Conidiophore; arrow pointing to deep phialide collarette containing three conidia. B. Conidiophore branched at base. C. Conidiophore exhibiting slimy conidial head. D. Phialides with deep collarettes; arrow denoting primary conidium. E. Conidiophore and conidia. F, G. Close-up of phialides; arrow denoting primary conidium. H. Conidia; arrow denoting primary conidium. I. Hyphae. Scale bars = 10 μm.
Fig. 32. Phialocephala helenae DAOM 867437. A–D. Apothecia on decaying hardwood. E. Vertical section of apothecium in water. F, I. Margin and flanks of apothecium. G. Vertical section showing centre of apothecium. H. Vertical section showing yellow reaction in 10 % KOH. J. Asci and paraphyses with refractive vacuole bodies. K. Ascus with amyloid tip in Lugol’s solution after KOH pretreatment. L. Yellow reaction of apothecium placed in 10 % KOH under dissecting microscope. M. Ascospores. Scale bars: E, F = 500 μm, G–I = 100 μm, J–R, T = 10 μm.

Fig. 33. Phialocephala helenae DAOMC 250756. A, B, E–G. Conidiophores. C. Older conidiophore with hypha proliferating from phialides and encompassing conidial head. D. Dematiaceous hyphae with exudates. H, I. Phialides with deep collarettes. J. Hyphal coll. K. Conidia. Scale bars = 10 μm.

Fig. 34. Phialocephala vermiculata DAOMC 229535. A. Eight-week-old culture on MEA exhibiting surface and submerged plumose crystals. B–E. Hyphae showing guttules and exudates. F. Dendritic crystals on agar surface. G–H. Raphide crystals that form larger dendritic structures. Scale bars: B–E = 10 μm, F = 1000 μm, G, H = 100 μm.
Fig. 35. Mollisia nigrescens DAOMC 250739. A–D. Apothecia on decaying hardwood with well developed and melanized subicula. E. Vertical section of apothecium. F. Vertical section showing ectal excipulum, medullary excipulum, and paraphyses with refractive vacuole bodies. G. Subicular hyphae. H. Apothecium showing yellow reaction in KOH. I, J. Marginal cells. K. Ectal and medullary excipula. L. Textura globulosa of ectal excipulum. M, N. Paraphyses with refractive vacuole bodies. O. Asci with amyloid tips in Lugol’s solution after KOH pretreatment. P. Ascospores. Scale bars: F = 100 μm, G, K–P = 10 μm, I, J = 5 μm.
sometimes considered to belong to Vibrisseaceae (Adhikari et al. 2016, Robichau et al. 2017). In this study, the LSU, RPB1, and TOP1 phylogenies strongly support the placement of Vibrissea outside or basal to the main Mollisia lineage (i.e. Mollisiaceae), while the ITS and LNS2 gene phylogenies place Vibrissea close to the P. dimorphospora s.s. clade or the PAC, with varying support. Evidence showing the placement of Vibrissea within Mollisiaceae based on the LNS2 phylogeny is not compelling given the overall weakly supported branches and discrepancies from other genes.

Morphological characters distinguishing apothecia of Vibrissea from typical Mollisiaceae ascomata include stipes that are often several cm long and vivid yellow hymenia in some species, filiform, multi-septate ascospores often several hundred μm long that sometimes disarticulate into parts, and bluing reaction of the perihymenial medullary excipulum in iodine (Baral et al. 2019), and asci bearing distinct apical caps (“nasse apicale”; Bellemére 1977, Baral 1987a). Based on these morphological differences alone, it is likely that Vibrissea should be excluded from Mollisiaceae and that the discordance observed between the individual genes are a result of long-branch attraction artefacts and/or very highly conserved, less informative gene regions. Similar discrepancies between phylogenies using protein-coding genes (RPB1) and rDNA genes (SSU, LSU) also are reported in other groups, such as Lecanoromycetes (Hofstetter et al. 2007).

Conversely, gross morphological dissimilarities suggest a more recent evolutionary history between Vibrissea and Mollisiaceae, as Vibrissea spp. share some important mollisioid characters: paraphyses with refractive vacuolar bodies, anguillospora- and phialocephala-like asexual morphs, and a textura globulosa ectal excipulum comprised of pigmented, thin-walled, round cells. Some Vibrissea spp., such as those previously placed within Apostemidium (e.g. V. flavovires), are sessile and somewhat mollisioid. The divergent ascospore and ascus tip morphologies in Vibrissea may be autapomorphic characters resulting from adaptations to aquatic environments, similar to the divergent ascospore, ascus, and ascomatal characters observed in Loramyces and Oblectodiscus, genera that are strongly supported in Mollisiaceae. The association of Anavirga densidromorpha and its phialocephala-like synapsexual morph with Vibrissea flavovires cultures flooded with water (Hamad &
Webster 1988, as *Apostemidium torrenticola* is also compelling. The phialocephala-like synasexual morph has dimorphic conidia; however, conidia are often brown and may be roughened, unlike those of *Phialocephala* s.s. In our study, *Vibrissea flavovirens* CBS 121003 produced few sparsely-branched conidiophores bearing phialides similar to those described by Descals & Sutton (1976) when agar blocks containing mycelia were floated in sterile water. Additionally, the apical cap appears to be a homoplasic character also present in *Lachnum aeruginosum* (=*Belonidium aeruginosum*) and *Incrucipulum ciliare*, both Lachnaceae (Helotiales) species occurring on fallen *Quercus* leaves (Partel 2016).

Vu et al. (2019) suggested the optimal thresholds for discriminating families using ITS and LSU were 88.51 % and 96.21 %, respectively. The ITS and LSU similarities between *Phialocephala dimorphospora* DAOMC 87232 and *Vibrissea truncorum* CBS 258.91 are 87.71 % and 95.32 %, respectively, thus placing *Vibrissea* outside of *Mollisiaceae* following these criteria (Fig. 20). However, *Vibrissea flavovirens* CBS 121003, which produces sessile apothecia in contrast to the stipitate apothecia of *V. truncorum*, shows some conflict with the other taxa in the comparison. For example, based on the threshold values of Vu et al. (2019), *V. flavovirens* would be considered a distinct genus from *V. truncorum* and within the same family as *P. dimorphospora*, cf. *Niptera*, and *P. scopiformis*, but not other *Mollisiaceae* species. These conflicts probably arise from the perhaps overemphasis on the LSU region but also highlight that judgement should be applied when using formulaic approaches to estimate taxonomic boundaries. Despite that caution, the threshold values provided by Vu et al. provide a good reference point and will be discussed later. Based on phylogenetic and morphological evidence, we exclude *Vibrissea* from *Mollisiaceae*, a conclusion also supported by the recent multigene phylogenetic overview of *Leotiomycetes* by Johnston et al. (2019). Additional taxon sampling and sequencing is needed to determine the placement of possibly related genera such as *Leucovibrissea*. Recently, *Pocillum* was synonymized under *Vibrissea* based on study of the type species and an ITS phylogeny (Baral et al. 2019).

**Loramyces-Obtectodiscus semi-aquatic clade**

Loramyces, *Obtectodiscus aquaticus*, *Ombrophila hemi-amylolidea*, and *Mollisia diesbachiana* form a strongly supported clade within *Mollisiaceae* in all phylogenies except LNS2, which places these species in a moderately supported clade and excludes *M. diesbachiana*. All species except the basal *Mollisia*

![Fig. 38. Niptereilla parksi JBT-113. Apothecia on fallen dead stick of *Alnus rubra.*](image-url)
**Fig. 39.** Hypothetical graphical representation of three approaches to classifying *Mollisiaeae*, based on the expanded RP81 phylogeny in Fig. 11. Option 1 is to lump all taxa within the family into *Mollisia* to conserve this genus and recognize the prevalence and taxonomic importance of the typical mollisoid apothecium. Option 2 is to formally recognize a paraphyletic *Mollisia*, transferring most taxa into *Mollisia* but recognizing morphologically divergent genera such as *Loramycyes* and *Obtectodiscus* and important clades such as the PAC and *Phialocephala* s.s. Option 3 is to formally recognize and name monophyletic groups, consequently relegating *Mollisia* s.s. to a smaller genus and describing novel genera based on molecular phylogenetic evidence combined with a polyphasic approach.

diesbachiana are found in semi-aquatic habitats; *Loramycyes* and *Ob. aquaticus* occur on Poaceae spp., while *Om. hemiamyloidea* and *Mollisia diesbachiana* are found on decaying hardwood. Semi-aquatic species within this clade exhibit morphological characters that deviate remarkably from other mollisoid taxa. *Loramycyes* is characterized by perithecoid apothecia surrounded by gelatinous excipular hyphae and ascospores bearing gelatinous sheaths and long (100–140 μm in *L. macrosporus*) basal cellular appendages (Weston 1929, Ingold & Chapman 1952). The ascospores are forcibly ejected through a wide apical opening, which is unlike the ascus apex of *Mollisia*. The taxonomic placement of *Loramycyes* was uncertain based on various morphological interpretations; for example its assignment within Sphaeriaceae, Trichosphaeriaceae, Hypocreales, and eventually its own monotypic family, Loramycetaceae, was based primarily on ascus and ascospore morphology (Digby & Goos 1987). We propose combining *Loramycetaceae* with *Mollisiaeae* because of the strongly supported placement of *Loramycyes* within *Mollisiaeae* based on all phylogenetic analyses, which clearly show *Loramycetaceae sensu* Digby & Goos (1987) as a paraphyletic family (also see Johnston et al. 2019).

*Obtectodiscus aquaticus* has more or less perithecoid apothecia with long, filiform ascospores and was sampled here because of its resemblance to *Loramycyes*. Baral (1992) reported a sulphur-yellow reaction of refractive vacuolar bodies to KOH in *Ob. aquaticus*, a reaction also observed in many species of *Mollisia* and related genera (*e.g.* Nimborrhombollisia, *Phialocephala* s.l.). Baral (1999) also noted several typical *Mollisia* characters in *Ombrophiella hemiamyloidea*, such as the textura globulosa ecal excipulum and refractive vacuolar bodies in the paraphyses that display a yellow KOH reaction, as well as characters shared with *Niptera*, including similar spore morphology and the presence of a gelatinous ascospore sheath that turns red in IKI. Hemiamyloid reactions of ascospore sheaths are also described for *Loramycyes* and *Ob. aquaticus* (Baral 1987b). Baral (1999) initially considered *Om. hemiamyloidea* to be an undescribed genus with taxonomic affinities to *Vibrisseaeae/Mollisiaeae*, but divergent characters ultimately led instead to its description within *Ombrophiella*, namely a strongly gelatinized medullary excipulum and hyaline ecal excipulum. Verkleer (2003) considered the apical apparatus and its reactivity with annular periodic acid (PA)-thiocarbohydrazide (THC)-silver proteinate (PA-THC-SP) more indicative of *Pezicula* rather than of *Vibrissea* and *Mollisia* relatives. However, our molecular phylogenetic evidence strongly supports the placement of *Om. hemiamyloidea* within *Mollisiaeae*, supporting the initial taxonomic assessment of Baral (1999).

A herbarium specimen of *Hysteronoeavia scirpina* (DAOM 147320) is sister to *Obtectodiscus aquaticus* based on ITS sequences (Fig. 16, Clade 1). There are 13 described *Hysteronoeavia* spp., which are graminicolous, reported from terrestrial to partially-submerged or submerged substrates, and characterized morphologically by small (0.1–0.3(–0.8) μm diam apothecia immersed or erumpent on host culms or leaves, inamyloid asci, generally large (12.5–)20–30(–40) × (1.5–)2–4(–8) μm, 0–1-septate, ellipsoidal to fusiform to subcylindrical ascospores, and sometimes a fimbriate apothecial margin (Nannfeldt 1984, Dennis & Spooner 1993, Shearer 1993, Raitv...
2008). Saccardo (1889) transferred Peziza scirpina to Mollisia scirpina and Nannfeldt (1984) later combined M. scirpina, along with other species of Hysteroppeziza and Hysterostegiella, with Hysteronaevia. Nannfeldt (1984) considered Hysteronaevia related to Mollisia (“Dermateaceae-Mollisioidae”), which is supported by the placement of the H. scirpina specimen within Mollisiaceae. Similar genera containing species that have been classified in the Dermateaceae or considered relatives of Mollisia, such as Diplonaevia, Hysteroppeziza, Hysterostegiella, Micropeziza, Naevala, Naeviopsis, and Scutomollisia, are poorly represented by sequence data and may include species related to the Loramyces clade or other Mollisiaceae genera (Hein 1976, 1982). For example Hein (1976) described phialocephala-like conidiophores in cultures of Naevala minutissima and Naeviopsis epilobi.

The larger ITS phylogeny also includes sequences from a specimen identified as Mollisia fuscoparaphysata and the ex-type of Pulvinata tomentosa (Fig. 16, Clade 1). Mollisia fuscoparaphysata, placed sister to Loramyces, is somewhat typical of Mollisia but with diminutive (ca. 350 μm diam) apothecia and paraphyses that are forked and dark-pigmented at the apices, possibly from a pigmented gelatinous coating (Graddon 1977, Douglas 2015). Loramyces and Obiectodiscus apothecia are also surrounded in a gel secreted by the excipular hyphae (Müller et al. 1979, Digby & Goos 1987). Like other species within the semi-aquatic clade, M. fuscoparaphysata is graminnicolous, on dead leaves and culms of Trichophorum cespitosum s.l., in wet habitats. Pulvinata was recently described to accommodate the type species P. tomentosa, collected from an unidentified host (“herbaceous stem”) in the UK. The 700–800 μm diam, whitish to brownish apothecia are essentially mollisioid but differentiated from Obiectodiscus by its pulvinate form (Ekanayaka et al. 2019).

Mollisia diesbachiana, a more or less typical mollisioid species, was collected from decaying hardwood in a terrestrial habitat and is basal to the semi-aquatic species. The unidentified Mollisia sp. represented by JBT-36-1, a collection consisting of a hardwood branch containing hundreds of whitish mollisioid apothecia protruding from a wet culvert in Québec, Canada, is closely related to M. diesbachiana (identities = 538/547 i.e. 98 %, 4/547 gaps). Various mollisioid ascmycetes are reported from submerged and/or partially submerged substrata (Fisher & Webster 1983, Shearer 1993). While an Anguillospora asexual morph has been described for Loramyces juncicola, no asexual morphs have been attributed to L. macrosporus, Ob. aquaticus, or Om. hemiamyloidea. In this study, phialocephala-like asexual morphs were observed for Ombrophila hemiamyloidea and Mollisia diesbachiana after floating agar blocks containing mycelia for several weeks in water.

Other purported aquatic hyphomycetes in Mollisiaceae

Asexual morphs have been induced in other aquatic mollisioid taxa by floating or flooding cultures with sterile water (Webster & Descals 1975, Descals & Sutton 1976, Fisher & Webster 1983, Digby & Goos 1987, Webster et al. 1993). It is unknown why this method induces sporulation in some Mollisiaceae species, e.g. cues resulting from changes in exposure to ambient gases, increased moisture, exposure to a nutrient-poor substrate, being subjected to small movements while suspended in water (i.e. thigmotropism), or the dilution of inhibitory factors that may otherwise locally accumulate in solid agar media. The induction of sporulation by means of mimicking aquatic conditions or observations of ingoldian conidia suggests a larger diversity of aquatic or aero-aquatic Mollisiaceae species than is perhaps now realized. However, these reported synasexual morphs should be investigated further. For example, the classification of the asexual morph of Anguillospora crassa in Mollisia is somewhat dubious based on the hyphal elements composing the excipulum as described by Webster (1961) and the placement of
A. crassa and A. furtiva sequences (e.g. AY204581, KC834038) in Hymenoscyphus s.l. (Cudoniella or Phaeohelotium) (Qiao et al. 2015). An Anguillospora crassa specimen occurring on wet wood yielded both Anguillospora and phialidic synasexual morphs when cultured on CMA (Fig. 36). The phialidic synasexual morphs was characterized by ampulliform phialides with deep, funnel-shaped collarettes, which developed from sparingly branched penicillate conidiophores or directly from Anguillospora conidia (i.e. microcyclic conidiation). This synasexual morph vaguely resembles Phialocephala (i.e. phialides with flaring collarettes); however, ITS sequences place this isolate in Hymenoscyphus s.l. Additionally, several collections were made of a species characterized by sessile mollisioid apothecia from wood partially submerged in stream water (Fig. 37). Apothecia were superficially similar to Mollisia, including paraphyses with refractive vacuole bodies and a thick medullary excipulum sometimes found in Mollisia spp. from wet environments, although the inamylloid asci and other microscopic characters contradicted this initial field identification. ITS sequences place this species in Hymenoscyphus s.l., likely conspecific with the previously mentioned collections of Anguillospora crassa (identities = 542/544 i.e. 99 %, no gaps), and morphologically it resembles a sessile species within the Hymenoscyphus cf. imberbis group. The Hymenoscyphus cf. imberbis ascosporae were similar to the Mollisia state of A. crassa described by Webster (1961), (7−) 7.5−10(−11) × 3−4 μm vs. 7.5−10 × 2.5−3 μm, and younger ascosporae also contained two prominent bipolar guttules similar to those of A. crassa. Small differences such as hymenium colour (white or cream to pale blue) vs. white to cream) and apothecium diameter (1.5−2 mm vs. 1 mm) may be a result of the A. crassa state developing in vitro or intraspecific variation. These observations suggest that the A. crassa sexual state described for Mollisia by Webster (1961) (referred to as Mollisia uda by Shearer 1993) belongs to H. cf. imberbis or another mollisioid species of Hymenoscyphus s.l. However, an anguillospora-like asexual morph was convincingly described from single ascospores isolates of Loramyces juncicola, so this conidial morphology is present in Mollisiaceae (Digby & Goos 1987).

A Mollisia sexual state is reported for Casaresia sphagnorum, an aquatic hyphomycete that produces impressive dematiaceous conidia (Fig. 17) and is reported to have a phialocephala-like synasexual morph and a Mollisia sexual state (Webster & Descals 1975; Webster et al. 1993). Repeated attempts by the first author to culture C. sphagnorum from conidia failed, possibly due to robust conidia remaining intact past viability. The phylogenetic placement of this distinct species within Mollisiaceae should be re-evaluated.

ITS and LSU sequences of the ex-type culture placed Variocladium giganteum, an aquatic hyphomycete, in Mollisiaceae (Baschien et al. 2013), a conclusion supported by the ITS phylogeny by Johnston et al. (2019); however, a preliminary RBP1 phylogeny in this study suggests this placement is misleading and a result of incomplete taxa sampling causing long-branch attraction (data not shown). Well-formed phialocephala-like asexual morphs have been reported in cultures of V. giganteum and V. rangiferinum (Willoughby & Minshall 1975, Descals & Webster 1982); sequences are unavailable for V. rangiferinum, which could conceivably belong in Mollisiaceae. Alternatively, it is possible that purported Variocladium cultures forming phialocephala-like synasexual morphs were misidentified and are undescribed aquatic species related to Mollisiaceae. The placement of Strossmayeria bastrichia within Mollisiaceae based on a LSU phylogeny by Hustad & Miller (2011) is also dubious because of branch length in the RBP1 phylogeny. Strossmayeria bastrichia produces two synasexual morphs: a pseudocolsoros-like dematiaceous asexual morph dissimilar to those known for Mollisiaceae species and a phialidic asexual morph with flaring collarettes somewhat reminiscent of Phialocephala phialides; however, unlike Phialocephala the phialides are produced directly from ascospores and asc (and paraphyses according to Iturriaga & Korf 1990) (Fig. 12). The multigene phylogenetic analysis by Johnston et al. (2019) places Strossmayeria bakeriana (and Chlorosplenium chlora) basal to Mollisiaceae and Vibrissea truncorum in the informal “mollisioid clade”.

**Niptera**

The ITS phylogeny depicts a strongly supported (SH-aLRT 100 %, BS 99 %) clade of lignicolous species preferring wet habitats that is distinct from the Loramyces clade (Fig. 16, Clade 1). This clade comprises herbarium specimens identified as Niptera discolor (Fig. 14), Niptera ramincola (Fig. 15) and Molliisa caesia (=Niptera caesia) (Fig. 13), a GenBank accession identified as Mollisia ventosa, and a species of Niptera s.l. collected from a decaying branch in a drying stream in New Brunswick (DAOMC 250748). The ITS phylogeny places the Niptera clade sister to Vibrissea and the PAC while the RBP1 phylogeny placed Niptera sp. (DAOMC 251628) basal to Phialocephala s.s. (Fig. 11, Clade V) and Mollisia ligni vari. oligosacens (CBS 291.59) and Phialocephala verniculata (SH-aLRT = 84 %, BS = 97 %, PP = N/A; Fig. 11, Clade VI).

These species are generally characterized by fusiform, 0−1(−3)-septate ascospores, long asci with narrow and elongated apical pores, and frequently well-developed and melanized subicula. Dennis (1972) noted that while Niptera was an exceptionally well-defined genus for its day (Fries 1849), interpretations by other authors obscured the generic concept by including terrestrial species that would be referred to Mollisia or similar genera, and not congeneric with the type species N. laeustris, on the basis of 1-sepate ascospores (De Notaris 1864, Rehm 1891). Consequently, the name Niptera has been applied to more than 150 species and species have been transferred from Niptera to Mollisia and other genera such as Belonium, Belonopsis, Nimbo-mollisia, and Scutomollisia. Given the nomenclatural priority of Niptera (1849) over Mollisia (1871) and Phialocephala (1961) and the existence of a coherent niptera-like clade that shares some morphological and ecological characters, as suggested in this study, the segregation of a Niptera s.s. clade within Mollisiaceae is feasible pending more sampling. It is also conceivable that Niptera s.l. comprises several phylogenetically distinct clades sharing morphological homoplasies resulting from adaptation to semi-aquatic habitats. For example, the cf. Niptera clade identified in Fig. 16 is comprised of lignicolous species with ascospores not surrounded in a gelatinous sheath, which is distinct from the graminicolous Niptera (s.s.)? species with ascospores surrounded in gelatinous sheaths. Morphological characters including gelatinous sheaths and septation of ascospores have been used to delineate semi-aquatic mollisioid genera such as Nimbowollisia and Niptera (Nannfeldt 1983). However, these genera remain mostly unsequenced and their
relationship with Mollisia s.l. and the phylogenetic resolution of these morphological characters remains unclear. Collecting, culturing, and sequencing unrepresented mollisioid species or attributed aquatic synasexual morphs from semi-aquatic habitats will fill taxonomic gaps and provide insight into the evolution of aquatic-adapted species and genera throughout the lineage.

**Phialocephala s.s.**

Phialocephala s.s. contains species occurring throughout Mollisioceae (e.g. P. hiberna and P. scopiformis) and in other orders (e.g. P. fluminis and P. virens; Day et al. 2012). Phialocephala s.s., defined as the clade containing the type species P. dimorphospora, includes P. alymerensis, P. biguttulata, P. botulispora, P. catenospora, P. cladophialophoroides, P. collarifera, P. heterosperma, P. lagerbergii, P. lignicola, P. mallowchi, P. nodosa, P. oblonga, and P. repens. All species except P. heterosperma and P. oblonga are identified by each taxonomic cultures and sequences.

Hernández-Restrepo et al. (2017) described a new Mollisioceae genus and species, Fuscosclera lignicola, which produces moniliform conidia and is probably P. nodosa based on morphology and ITS sequence similarity. In this study, we synonymize Fuscosclera with Phialocephala but defer synonymizing P. lignicola with P. nodosa.

According to arguments by Tanney et al. (2016a), Phialocephala s.s. is almost exclusively comprised of lenticular saprotophs except for rare reports of endophytism, for example P. nodosa isolated from Picea mariana and Pinus strobus foliage in Canada (Tanney et al. 2016a) and a Populus euphratica leaf in China (FRB865028; Unterseher et al. 2012). Sequences in GenBank corresponding to species within the P. dimorphospora clade confirm its overall lenticuloc, expanded biogeographic ranges, and indicate putatively novel and undescribed species (Fig. 19). Phialocephala oblonga apothecia appear to be very common on decaying hardwood in Eastern Canada (this study) and New Zealand (P. Johnston, pers. comm.) and this species is apparently not restricted to hardwood based on sequences from isolated from Picea abies stumps in Sweden (AY606308, AY606309; Menkis et al. 2004). Phialocephala cladophialophoroides is known only from a strain isolated from the toenail of an immunocompromised human patient (Crous et al. 2017); however, based on ITS sequences in GenBank, P. cladophialophoroides was also isolated from driftwood in Iceland (KX100398; Blanchette et al. 2016) and wood in Antarctica (KC514878; Held & Blanchette 2017) (Fig. 19, Clade I). Putatively novel species include one unidentified species represented by four strains isolated from apothecia occurring on dead wood, including wood from Notothofagus sp., in New Zealand (e.g., MG195462; Fig. 19, Clade I) and another unidentified species represented by two strains independently isolated from stumps and wood of Picea abies in Latvia (MK911655, FJ903314; Fig. 19, Clade I).

Several dematiaceous synsexual morphs are associated with species of Phialocephala s.s., including the diplococcium like asexual morph of P. catenospora and a synnematous asexual morph with similar conidiogenesis formerly placed in Parâ oppositorium (=P. oblonga) (Tanney et al. 2016a). Phialocephala catenospora is possibly conspecific with Bispora betulina based on morphology (see Wang 1989) and ITS sequences from two independently isolated strains identified as B. betulina (CBS 136.49, CBS 141.61; Fig. 19, Clade V). An available ITS sequence of B. attenuata (KY462800), the type species of Bispora, places it within Hymenoscyphus s.l. Strains identified as Diplococcium spicatum, the type species of Diplococcium, are also placed within Mollisioceae in the LSU phylogeny presented by Shenoy et al. (2010).

Phylogeny attributed to Phialocephala s.s. are typically greyish brown to greyish blue with ellipsoidal to oblong, typically aseptate, ascospores within the range of (7–8–10–12) x (2.5–3–4 μm). In this study, apothelial and asexual morph collections representing two distinct species were collected from decaying wood in Canada and described as P. biguttulata and P. collarifera. Overall, Phialocephala s.s. is a strongly supported clade of wood decaying species predominantly isolated from temperate climates worldwide, with evidence indicating additional undescribed species.

**Mollisia s.s.**

The delineation of Mollisia s.s. is dependent upon the epi- typification of the type species, M. cinerea. Current efforts are underway to designate an epitype collected close to the type locale in Jena, Germany (A. Gminder pers. comm.). Based on the RPB1 phylogenies and preliminary data, Mollisia s.s., interpreted as the clade containing M. cf. cinerea and M. undulatodepressula, is closest related to M. melaleuca, M. novobrunsvicensis, M. rava, and an unidentified conifer endophyte species (DAOMC 251642) (Fig. 7, Clade A; Fig. 11, Clade I). Unexpectedly, this clade is sister to the Loramycetes-Ombrophila hemiamyloidea clade consisting of mostly semi-aquatic species, which introduces some taxonomic conflicts described in detail later on.

**Nipterella**

Starbäck (1895) described Niptera duplex from Juniperus wood and while the author noted that the species probably warranted its own genus (“Sie scheint mir sogar zu verdienen, als eine besondere Gattung Nipterella unterschieden zu werden”), he did not formally propose the name Nipterella. Dennis (1962) validated Nipterella for N. duplex, the type species, and N. parksi, formerly Belonidium parksi (as “Belonidium parksii”; Cash 1936). The third Nipterella species, N. tsugae, occurs on dead attached branches of Tsuga heterophylla (Funk 1978). The three species share some morphological characters including hymenia ranging from yellow to bluish green, scurfy external appearance, and an involuted margin composed of dark moniliform cells, although the conidia of N. tsugae are shorter and asceptate. Dennis (1962) classified Nipterella within the Helotiaeaceae subfamily Encoelioidae, distinguished from other genera such as Encocellae by its septate ascospores and amyloid asci. Müller & Defago (1967) considered Nipterella a member of Dermatocaceae and synonymized it with Dibioneilla, while Korf (1973) referred to it within Helotiaeaceae-Encoelioidae. Starbäck (1989) noted that the ectal excipulum of N. duplex was like that of Mollisia.

A specimen of Nipterella parksi (DAOMC 56610) was available for study, showing the distinctive greenish yellow to greyish yellow (1A5–1B8) hymenium, darkly pigmented (10F3) ectal excipulum
composed of moniliform cells, giving the apothecia a scurfy appearance, an inrolled and plicate margin comprised of cylindrical moniliform, scale-like marginal cells, and 3-septate, fusiform-ellipsoidial ascospores (Fig. 3). The ITS sequence generated from N. parksi (DAOM 56610) places it with weak support within a generally poorly supported clade containing the ex-types of Phialocephala phaerooides, Mollisia endocryssata, and M. prismaticata, and strains or collections identified as Belonium excelsor, Mollisia fusca, M. ligni var. ligni, M. minutella, Neopyrenopeziza nigri pigmentata, Niptera pulla, Patellariopsis dennisi, and conspecific cultures attributed variously to M. caesia (CBS 220.56), M. discolor (CBS 289.59), M. fallens (CBS 221.56), and M. ventosa (CBS 322.77) (Fig. 16). As previously mentioned, Neopyrenopeziza nigri pigmentata shares a 99 % similar ITS sequence with Patellariopsis dennisi (MK120898). Based on ITS sequences and morphology, these taxa are conspecific and represent another divergent morphology among species adapted to xeric habitats (e.g., dead attached branches) within Mollisiaceae. Other examples of morphologically divergent Mollisiaceae species from xeric habitats include Nipterella spp., described above, and M. ligni, which produces peculiar apothecia that often fold into a triangular outline when slowly dried, possess a brown scurfy ectal excipulum with conspicuous multilacinal marginal cells, and, notably, inamyloid asc (Gminder 2012). An asexual morph was not described for N. nigri pigmentata, but Müller (1962) described a pycnidal asexual morph resembling glutinum in Patellariopsis dennisi cultures and on the dead stems bearing P. dennisi apothecia. However, their description was not illustrated or detailed enough to interpret its relationship with other known Mollisiaceae asexual morphs, for example the distinctive Pycriella (sensu von Höhnel 1918) asexual morph attributed to M. ligni by Tulasne & Tulasne (1865) and verified in culture by Le Gal & Mangenot (1956).

While Nipterella parksi is clearly in Mollisiaceae, its precise placement is unknown. An NCBI BLAST search yields a closest match with purported Mollisia minitella strains (e.g. KJ817294; identities = 415/443 i.e. 94 %, gaps = 6/443). Nipterella parksi is evidently common on dead attached branches of Alnus rubra in Vancouver Island, British Columbia, Canada (Fig. 38) and will be evidently common on dead attached branches of Alnus excelsior with purported placement is unknown. An NCBI BLAST search yields a closest RPB1 siaceae Mollisiaceae (Rehm 1891 [as Dermateaceae]) to xeric habitats (e.g. polyphyletic due to inclusion of Chlorovi-brissea and Myxoxephalia; Sandoval-Leiva et al. 2014, Nonaka et al. 2015). Loramyces is strongly supported within Mollisiaceae and recognition of Loramyces would result in a paraphyletic Mollisiaceae; therefore, we synonymized Loramyctaceae with Mollisiaceae to maintain monophyly and to better classify Loramyces and related taxa.

**Taxonomic issues and directions**

Priorities for users and incentives for developing a stable, predictive taxonomic framework include: (1) elucidating and describing unknown OTUs within a lineage presently spanning many poorly-characterized and unsequenced genera; (2) generating reference sequences from type or epitype material; (3) describing novel species (or genera) in the absence of proper study of historical species concepts; and (4) fixing the nomenclatural and taxonomic issues surrounding this entangled line-age, which includes a mixture of relatively old sexual morph and asexual morph based names.

Detection and identification of fungal OTUs relies increasingly on DNA sequence-based methods. Without authenticated reference sequences, preferably originating from type or epitype material, users will be unable to confidently identify related OTUs in their work. In biodiversity and ecology studies, the inability to assign specific or taxonomic qualifiers significantly diminishes insight into biological systems and reduces the overall value of data generated from high-throughput next generation sequencing technologies. Populating sequence databases with reference sequences will provide taxonomic benchmarks for users, increasing taxonomic resolution. However, effectively generating reference sequences requires dedicated and concerted sampling efforts from new collections and authenticated and type specimens.

Unlike other genera such as Penicillium, few authenticated or ex-type cultures of Mollisia species exist because previous workers did not prioritize culturing specimens or accessioning strains in public culture collections. Consequently, authenticated materials are restricted primarily to herbaria. Amplification of DNA from herbarium specimens is challenging because of DNA degradation resulting naturally or accelerated by post-collection practices, while contaminating or co-occurring fungi can result in amplification of non-target DNA. The latter can be at least partly solved by developing taxon-specific primers; however, this can be infeasible in cases where the phylogenetic placement of the targeted fungus is unknown and reference sequences to assist in primer design are unavailable. In some cases, sequencing attempts may be impractical because of specimens being in too poor condition, insubstantial, or even lost. Despite these pitfalls, we have had some success with obtaining DNA sequences from herbarium specimens; in some cases, even a single ITS sequence of a few hundred bases may be enough to link a type specimen to freshly collected material from which a full suite of phylogenetic data can be recovered.

Epitypification with new collections offers an alternative to destructive sampling of type specimens and can provide additional information, such as detailed morphological observations including vital taxonomic characters (Baral 1992; Van Nooren 2010). New collections can be used to generate cultures, which may in turn provide accessioned material for inoculation studies, characterisation of secondary metabolites, in vitro studies, whole genome sequencing, etc. Careful and proper epitypification of described Mollisia and allied taxa is encouraged; however, the large number of insufficiently described or apparently morphologically indistinct species is problematic. A pragmatic strategy must be adopted to assist progress.
sequence directly from type or authenticated material when possible, prioritize the epitypification of important or morphologically distinct species, and encourage the description of novel species. The high-quality description of novel species from apothecia or cultures (sterile or sporulating) will provide a wealth of data associated with reference sequences. The risk of describing a previously named species is real; however, we feel that propelling Mollisiaceae taxonomy into the 21st century at the expense of some novel species eventually being synonymized is a necessary risk. Hibbett et al. (2011) estimated the odds of an unidentified molecular operational taxonomic unit (MOTU) representing a species that is described but lacking reference sequences in GenBank as 18:1 or 44:1, depending on global biodiversity estimates. Taking into account these estimates of error, the historical difficulty in discerning Mollisia species from one another, lack of recent taxonomic work, and paucity of global surveying of Mollisiaceae species, we encourage our colleagues to accelerate the modern description of novel species and, while practicing due diligence, not to be further delayed over concerns of unintentionally redescribing named species. Additionally, unidentified sequences annotated with collection and morphological data can be shared and integrated into a repository, facilitating the incremental understanding of species concepts worldwide (Hosoya et al. 2015).

The polyphyletic nature of most genera presented in our phylogenies presents a dilemma when describing novel species: what generic name should one choose? Several nomenclatural and taxonomic options will to be evaluated: (1) the entire lineage is transferred to Mollisia to create a large all-encompassing generic concept (“lumping”) with hundreds of species with diverse morphology, especially of asexual morphs; (2) the bulk of mollisioid taxa are considered part of a paraphyletic Mollisia and morphologically divergent taxa are maintained as distinct genera, regardless of monophyly; or (3) the lineage is divided and genera are erected or maintained based principally on monophyly (“splitting”) (demonstrated in Fig. 39).

Option 1: lumping Mollisia to conserve its status within Mollisiaceae

The first option simplifies taxonomic decisions by favouring a broad generic concept based on the morphotaxonomic concept of Mollisia s.l. Lumpng the entire lineage into Mollisia acknowledges the history of the genus, the nomenclatural priority of Mollisia over most genera within the lineage (although not all, e.g. Bispora 1837, Trimmatostruma 1837, Niperta 1849, Cheirospora 1850, Micropeziza 1870), the number of species attributed to Mollisia versus other genera, and the characteristic mollisioid apothecial morphology found throughout the lineage.

A consequence of this approach is that the semi-aquatic Loramyces-Obtectodiscus-Ombrophila hemiamyloidea clade would be transferred to Mollisia to maintain a monophyletic generic concept. These transfers would dilute the morphotaxonomic concept of Mollisia and cause a loss of information, albeit for a small group of somewhat obscure species. Additionally, morphologically distinct species, such as those placed in Niperta and other mollisioid genera, are undersampled, poorly represented by DNA sequences, and could conceivably form monophyletic groups supported by morphological, biological, and ecological characters. Prematurely lumping these genera/clades into Mollisia could therefore conceal potentially robust and coherent taxonomic concepts. Similarly, additional sampling may support the recognition of patterns, such as the existence of clades containing species sharing corresponding life histories with narrower host-, biogeographic-, or substrate preferences. Introducing or maintaining genera to accommodate such clades may be more informative than subsuming such a broad array of diversity under the name Mollisia. A compromise might be the eventual recognition of taxonomic sections in Mollisia in the sense of Aspergillus and Penicillium, for example, although the use of sections in Mollisiaceae may be mysterious for non-taxonomists. Considering the current taxonomic confusion and relatively few field mycologists interested in Mollisia, relegating the name Mollisia to a moderate-sized clade (i.e. option 3, below) would not result in significant inconvenience. For example, most field mycologists are unable to confidently identify Mollisia species using morphological characters; therefore, using “mollisia” as a general descriptor for mollisioid apothecia would suffice.

While accepting a broad Mollisia generic concept may be more convenient for field biologists in the short term, it is likely to be unstable and less informative in conveying biological and ecological traits for definable monophyletic groups (e.g. the Loramyces-Obtectodiscus-Ombrophila hemiamyloidea clade, Niperta, Phialocephala s.s., PAC). From a pragmatic standpoint, research within this lineage is primarily focused on species classified within Phialocephala s.l. (e.g. the PAC); therefore, this option would result in name changes that would affect the largest number of users (i.e. endophyte researchers). Based on a Google Scholar search, the keyword “Phialocephala” was used approximately four times more than the keyword “Mollisia” since 2015 and approximately 1.5 times more overall (Fig. 2). While Phialocephala s.l. is polyphyletic, with species occurring not only throughout Mollisiaceae but also in distant orders (Grüning et al. 2002, Grüning et al. 2009), Phialocephala s.s. is unambiguously defined based on the recent epitypification of the type species P. dimorphospora (Tanney et al. 2016a). This clade is comprised of species primarily isolated from wood that exhibit penicillate conidiophores consistent with the historical concept of Phialocephala, but also includes newly recognized connections with sometimes well-known dematiaceous hyphomycetes that were not previously known to be part of life cycles in this clade. While the identity of M. cinerea s.s. has been speculated upon for some time (e.g. Crossland 1898), this question still cannot be sufficiently answered because of a lack of diagnostic morphological characters to be garnered from descriptions of the authentic material (Batsch 1786, Karsten 1871, Dennis 1950) and the reported loss of the type specimen (A. Gminder, pers. comm.). Relegating the strongly supported Phialocephala s.s. concept to Mollisia, a genus typified by an ambiguous species lacking a type specimen and of unknown precise phylogenetic placement, would not conform with rigorous taxonomic practise. However, it must be noted that Cheirospora botryospora and Niperta spp. are tentatively placed within the Phialocephala dimorphosporapac clade based on some ITS analyses (this study, Crous et al. 2015), creating potential nomenclatural issues because of priority. Efforts to epitypify M. cinerea are underway (A. Gminder, pers. comm.) and are crucial for establishing generic boundaries.

Option 2: accepting a paraphyletic Mollisia and prioritizing sexual morphology

The second option compromises by encompassing most species within a large Mollisia genus and accepting select distinct species or clades as polyphyletic or paraphyletic genera. For
example, most species in the lineage presented in the expanded RPB1 phylogeny (Fig. 11) would be classified in Mollisia except for the divergent Loramyces-Obtectodiscus-Ornithophila hemi-
aryloidea clade and perhaps the PAC and P. dimorphospora s.s. clades. The consensus among most taxonomists is that pro-
posing paraphyletic and polyphyletic taxa is unacceptable (but see Vellinga et al. 2015, Lachance 2016). However, the Lor-
amyces-Obtectodiscus-Ornithophila hemiaryloidea clade clearly repre-
resents divergent taxa that should not be classified within Mollisia, thereby presenting a quintessential example for or
against recognizing paraphyletic genera.

As sampling increases and patterns emerge, a broad and
paraphyletic Mollisia concept would eventually give way to its
division to recognize more informative and monophyletic
generic concepts. Accepting a paraphyletic Mollisia concept
prioritizes its sexual morphology and subsequent recognition
in the field. However, the classic mollisioid apothecium may
eventually prove to be a less significant character for generic
delineation; therefore, maintaining a large Mollisia genus based
on the occurrence of uninformative homoplastic characters
throughout the lineage would be misleading and short-lived. For example, mycosphaerella-like sexual states are present in
ca. 50 genera occurring across several families in Capnodiales
(Dothideomycetes) (Crous 2009, Crous et al. 2009, Crous &
Groenewald 2013); upholding a taxonomic system that em-
phasizes highly conserved morphological characters of the
sexual state over other evidence (e.g. molecular, asexual
morphology) would result in less informative generic concepts.
Our historic inability to reliably identify and classify mollisioid
taxa based on apothecial morphology may indicate the limits of
taxonomic informativeness that these characters have across
the lineage.

Alternatively, asexual morphology may provide more taxo-
nomic insight, for example as seen in the asexual morphs of
Calonectria, Mycosphaerellaceae, and Teratosphaeriaceae
(Lombard et al. 2010). Despite early asexual morph connections
(Tulasne & Tulasne 1865), an apothecium-centric approach may
have resulted in the neglect of potentially taxonomically-
informative asexual morph characters, especially given the
infrequent cultural studies of Mollisia (but see Aebi 1972, Brefeld
1891, Le Gal & Mangenot 1956, 1960, 1961, 1966, Tanney et al.
2016a), Tanney et al. (2016a) described dematiaceous syna-
sexual morphs in Phialocephala s.s. that could be used to readily
distinguish known species, including microsclerotia composed of
moniliform cells (P. nodosa), diplococcium-like chains of didymo-
and phragmoconidia (P. calenospora), and didymoconidia pro-
duced in short acropetal chains from tall (up to 120 µm)
syn-
nemata (P. oblonga). As previously mentioned, strains attributed
to Diplococcium spicatum and Bispora betulina are placed within
Mollisiaceae and Phialocephala s.s. based on LSU and ITS
sequences, respectively. An isolate identified as Septonema sp.
(DAOM 226875; Fig. 17) is closest related to Phialocephala
vermiculata. Le Gal & Mangenot (1956) reported a sporodochial
cystodendron-like asexual morph (as Bloxamia) occurring in the
aerial mycelia or directly on the agar surface of 6–8-mo-old
cultures of Mollisia discolor var. longispora and M. benesuadu.

Bills (2004) described similar sporodochia produced by Phialo-
cephala hiberna in both cultures and from colonies on Robinia
pseudacacia wood in situ. Aebi (1972) considered Phialoce-
phala a synonym of Cystodendron and described this asexual
morph in cultures of Belonopsis excelsorum. As previously
mentioned, the placement of C. dryophilum should be confirmed
by molecular phylogenetic study given its atypical ecology as a
leaf pathogen of Quercus and its nomenclatural precedence over
genera such as Phialocephala.

Crous et al. (2015) reported an unexpected connection be-
tween Phialocephala and Cheirospora botryospora, a fungus
commonly found on dead branches of Fagus spp. in Europe and
North America. Cheirospora botryospora produces bulbils
composed of globose cells surrounded in a gelatinous sheath from
acervuli erumpent through the host bark (Fig. 40); on OA media,
C. botryospora produces both bulbils and a phialocephala-like
asexual morph. ITS and LSU sequences place C. botryospora
close to Phialocephala piceae and P. helenium within the PAC and
Phialocephala s.s. clade. Other peculiar asexual morphs reported
for Mollisiaceae species include the previously discussed Glut-
iunum morph of Patellariopsis denisii and Pycnidiaella coelemo-
ceteous morph attributed to M. ligni. Altogether, these
observations suggest that asexual morphs may provide more
taxonomic resolution than previously realized.

Whether formally accepted or not, the interim working solution
will probably be an informal recognition of a paraphyletic Mollisia
concept to facilitate the identification and classification of envi-
rmental DNA sequences and strains, and so encourage
increased sampling. The description of distinctive synasexual
morphs throughout Mollisiaceae may provide important ecolog-
cal and taxonomic information and warrants further study to
assess whether these morphological characters provide resolu-
tion to species or clades that might eventually be recognized as
genera (Table 1). To resolve nomenclatural priority of names
tentatively attributed to Mollisiaceae based on nuc rDNA se-
quences, reference strains of type species of dematiaceous
hyphomycete genera, such as Bispora and Trimmatostruma, are
required for study.

**Option 3: prioritizing monophyly throughout
Mollisiaceae**

The third option, to divide Mollisiaceae into genera based pri-
marily on monophyly inferred from DNA sequences, in addition to
biological data, is likely to be favoured in the future. Increased
sampling and a polyphasic taxonomic approach combining
characters including molecular phylogenetic data, morphology,
biology, ecology, chemotaxonomy, etc., will lead to more infor-
mative and elegant generic concepts within Mollisiaceae.
Phylogeny-based taxonomic systems may prove impractical for
users relying entirely on morphology for identification. However,
given the overall paucity of work being conducted on mollisioid
discomycetes and the significant challenges imposed by the
current taxonomic system regarding identification and classifi-
cation, such criticism hardly warrants the maintenance of an
artificial taxonomic system under the guise of serving the rela-
tively few users who actively collect mollisioid specimens.
A classification based on useful phylogenetic concepts will be
adopted by the much larger community of endophyte re-
searchers and other users and will encourage the accumulation
of relevant data and cumulatively improve our understanding of
Mollisiaceae biodiversity and ecology. However, this approach
will likely relegate Mollisia s.s. to a smaller genus, although this
might be unavoidable given the position of the Loramyces clade.

This third option requires effort and time for the development
of a stable system. Describing genera based solely on mono-
phyly inferred from phylogenetic data is premature given the
sparseness of sampling and data. The delineation of genera as
illustrated in Fig. 39, Option 3 would overall provide very little
taxonomic resolution and information and probably result in unstable generic concepts. However, waiting for complete sampling of Mollisiaceae before acting is infeasible and impractical; therefore, taxonomists must exercise due diligence when making taxonomic changes considering the large sampling gaps and known taxonomic issues. Epitypification of M. cinerea and type species of other mollisioid genera is the first critical step to delineating generic concepts and building a strong taxonomic framework. Continuing studies of type specimens and new collections are also supplementing our understanding of species concepts with detailed morphological study (e.g., Gminder 2006; 2012). Notably, the online database MollisBase was recently conceived to facilitate the connection of Mollisia sequences with apothecial morphology and collection data (Hosoya et al. 2015).

While waiting for additional data is prudent, some direction is required to assist users. In this study, the need to describe novel endophyte and/or apothecial isolates is emphasized. Deciding which genus to describe our novel species in was challenging considering the taxonomic shortcomings and risk of taxonomic instability. A parsimonious approach was chosen that effectively followed option 2 as described above: species occurring within the P dimorphospora s.s. and PAC lineage were described in Phialocephala and species outside this lineage were described in Mollisia. These generic designations are admittedly provisional but are probably the most pragmatic course of action. Until major taxonomic issues are addressed and sampling is adequate, the haphazard or unwarranted erection of novel genera within this lineage should be avoided in the pursuit of taxonomic stability and informativeness, or proceed only when sufficient supporting evidence exists, i.e. monophyletic groups comprising several species sharing distinct characters. If genera are erected in a piecemeal fashion based on current data, the results may be a taxonomic system characterized by many small, uninformative, and most likely unstable genera, exemplified by the recent description of ad hoc genera such as Acephala, Acidomelania, Barrenia, Neomollisia, and Pulvina.

Final thoughts

This study is part of a larger research program involving the identification of unknown and potentially bioactive conifer endophyte strains by connecting them with morphologically identifiable field specimens by inducing sporulation in vitro and with phylogenetic analyses (McMullin et al. 2019). Endophytes across diverse lineages including Botryosphaeriales (Tanney & Seifert 2019), Diaporthales (Tanney et al. 2016b), Phacidiiales (Tanney & Seifert 2018), Pleosporales (Richardson et al. 2015), Rhytismatales (McMullin et al. 2017; Tanney & Seifert 2017; McMullin et al. 2019), Xylariales (Richardson et al. 2014), and Mollisiaceae (Tanney et al. 2016a) have been studied so far. Mollisiaceae is among the most difficult family to characterize for reasons detailed above, yet the occurrence of enigmatic endophytes, pathogens, and producers of bioactive secondary metabolites within this family of mostly forgotten or ignored taxa offers exciting research opportunities not limited to taxonomy, ecology, evolutionary biology, comparative genomics, natural products chemistry, plant-fungus interactions, phytobiome-mediated plant improvement, and biological control. Reports of pathogenicity are limited, for example P. bamuru and some PAC strains, and there is little evidence suggesting Mollisiaceae species in general pose a significant phytosanitary risk. However, their host promiscuity, saprotroph-endophyte life histories, and ubiquity suggest that they may have been transported globally with international trade, for example as endophytes associated with plants for planting. More study is needed to determine the biogeography and host interactions of Mollisiaceae species worldwide, especially from a plant health and phytosanitary perspective.

Mollisiaceae has been a long-neglected and even maligned family, but the application of DNA sequence-based identification and phylogenetic methods allow for more accurate species delineation and progress towards an effective taxonomic framework, facilitating further research and communication of taxa. Major obstacles to this progress are a lack of participating taxonomists and a dearth of sampling — the latter issue can be ameliorated through the active characterisation and sequencing of specimens from fresh field collections, culture collections, and herbaria. A “collect, culture, and sequence” approach focusing on Mollisiaceae apothecia and potential dematiaceous synasexual morphs will provide further connections and insight. Collecting asexual morphs in situ will also circumvent the prolonged (and inconvenient) periods of incubation sometimes required to induce asexual morphs in vitro. For example, conidiophores and microsclerotia-like structures, as described by Le Gal & Mangenot (1961), were observed for M. nigrescens (CBS 558.63) three years after inoculation on MEA and incubation initially at 20 °C then 5 °C for ca. 2.5 years (Fig. 22). Prolonged incubation times and cold temperatures may be required to induce or at least promote sporulation in some Mollisiaceae species, for example P. fortinii (at least 1 year on MMN at 5 °C; Wang & Wilcox 1985), P. sphaeroides (8 mo at 5 °C; Wilson et al. 2004), P. urceolata (3 mo or more on OA at 5 °C; Wang et al. 2009), P. hiberna (exclusively sporulates in winter in E. USA; Bills 2004), P. glacialis and P. piceae (more than one year on MEA at 4 °C; Grünig et al. 2009), P. compacta and P. scopiformis (both species show more abundant sporulation after several mo at 4 °C; Kowalski & Kehr 1995). Floating agar plugs containing mycelia in water induces sporulation in diverse Mollisiaceae species and should be considered in future culture studies. Overall, focused field collecting efforts are crucial for progressing research in Mollisiaceae.

Taxonomic decisions ultimately rely on first clarifying the placement of type species of Mollisiaceae genera, importantly Niptera and Mollisia. Additionally, many nomenclatural issues require attention, for example while Tapesia is recognized as a synonym of Mollisia (Hawksworth & David 1989), many species remain classified in Tapesia and some nomenclatural conflicts exist that require individual attention (Gminder 2006). Some possibly congeneric names are also older than Mollisia and Phialocephala, which will require confirming their placement and deciding about nomenclatural priority and conserving names. A taxonomic upheaval in this lineage is probably on the horizon; being parsimonious with name changes and mindful of the guidelines for introducing new genera presented by Vellenga et al. (2015) will ensure a stabler, user-friendly taxonomic system. Much work is needed to elucidate species and genus boundaries within Mollisiaceae, resolve old species concepts lacking adequate type material, and deal with the accumulated nomenclatural issues. While these issues are beyond the scope of this study, the present work sheds some light on the remarkable depth of unnamed and undescribed biodiversity within Mollisiaceae. The convergence of
evidence suggests that Mollisiaceae species are much more important than previously realized and offers stimulating and pragmatic research opportunities.

ACKNOWLEDGEMENTS

J.B. Tanney thanks H.-O. Baral, B. Douglas, A. Gminder, T. Hosoya, and P. Johnston for their enlightening correspondence, observations, and insights into Mollisiaceae. We thank the Microbiology Molecular Technologies Laboratory (MMTL) group of the Ottawa Research and Development Centre (Agriculture and Agri-Food Canada) for processing DNA sequences, Tara Rintoul and Benoit Goulaf at the DAOMC for culture preservation, Robert Kowbel for assisting with GenBank accessions, and are especially grateful to R. Assabgui for his gracious assistance in sequencing and culture preservation. We also thank D.W. Malloch (New Brunswick Museum) for being a generous host during field collecting trips and S. Clayden and D. McAlpine (New Brunswick Museum) and G. Adams (JD Irving, Ltd) for organizing collecting trips in New Brunswick. This study was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) PGSD2-459312-2014 to J.B. Tanney and the NSERC CRDPJ 421782-11 to J.D. Miller, K.A. Seifert, and D.W. Malloch.

REFERENCES

Addy HD, Hambleton S, Currah RS (2000). Distribution and molecular character- ization of the root endophyte Phialocephala fortinii along an environmen- tal gradient in the boreal forest of Alberta. Mycological Research 104: 1213–1221.

Adhikari M, Kim S, Yadav DR, et al. (2016). Phialocephala lagerbergii: a new record from crop field soil in Korea. 44: 132–137.

Aebi B (1972). Untersuchungen über Discomyceten aus der Gruppe Heloti- alles. Nova Hedwigia 23: 49–112.

Alian-Boulé N, Lévesque C, Martínez C, et al. (2004). Identification of Pythium species associated with cavity-spot lesions on carrots in eastern Quebec. Canadian Journal of Plant Pathology 26: 365–370.

Allmer J, Vasilkauskas R, Ihmork K, et al. (2006). Wood-inhabiting fungal communities in woody debris of Norway spruce (Picea abies (L.) Karst.), as reflected by sporocarps, mycelial isolations and T-RFLP identifi- cation. FEMS Microbiology Ecology 55: 57–67.

Anderson Stewart CR, Dolom M, Taylor JE. (2019). Analysis of fungal endo- phytes in Scottish Sitka spruce plantations shows extensive infections, novel host partners and gives insights into origins. Forest Pathology 49, e12471.

Arenal BE, Blanchette RA (2009). Investigations of fungal diversity in wooden structures and soils at historic sites on the Antarctic Peninsula. Canadian Journal of Microbiology 45: 46–56.

Arhipova N, Galtietersk T, Donis J, et al. (2011). Butt rot incidence, causal fungi, and related yield loss in Picea abies stands of Latvia. Canadian Journal of Forest Research 41: 2337–2345.

Arhipova N, Galtietersk T, Donis J, et al. (2012). Heart-rot and associated fungi in Alnus glutinosa stands in Latvia. Scandinavian Journal of Forest Research 27: 327–336.

Arneaud SL, Porter JR (2015). Investigation and expression of the sesquisat- ural-coenzyme dehydrogenase gene involved in podophyllotoxin biosynthesis. Molecular Biotechnology 57: 961–973.

Artz RR, Anderson IC, Chapman SJ, et al. (2007). Changes in fungal community composition in response to vegetation succession during the natural regeneration of cutover peatlands. Microbial Ecology 54: 508–522.

Baba T, Hirose D, Sasaki N, et al. (2016). Mycorrhizal formation and diversity of endophytic fungi in hair roots of Vaccinium oxycoccos Miq. In Japan. Microbes and Environments 31: 186–189.

Baral HO (1987a). Der Apikalapparat der Heliotales. Eine lichtmikroskopische Studie über Arten mit Amyloidring. Zeitschrift für Mykolologie 53: 119–136.

Baral HO (1987b). Lugol’s solution/KI versus Melzer’s reagent: hemiamyloid, a universal feature of the ascus wall. Mycologia 29: 399–450.

Baral HO (1992). Vital versus herbarium taxonomy: morphological differences between living and dead cells of ascomycetes, and their taxonomic impli- cations. Mycotaxon 44: 333–390.

Baral HO (1999). Ombrophila hemiamyloidea, an aquatic discymycete. Myco- logia Bavarica 3: 50–63.

Baral HO, Lindemann U, Wischollek D (2019 (2017)). Vibrissea cataryta – a rare aquatic inoperculcate discymycete. Mycologia Montenegrina 20: 111–126.

Barklund P, Kowalski T (1996). Endophytic fungi in branches of Norwegian spruce with particular reference to Tryblidiopsis pinastri. Canadian Journal of Bot- any 74: 673–678.

Baschenis C, Tusi CK-M, Guis V, et al. (2013). The molecular phylogeny of aquatic hyphomycetes with affinity to the Leotiomycetes. Fungal Biology 117: 660–672.

Batsch AJGK (1786). Elenchus Fungorum. Continuatio prima. J.J. Gebauer, Germany.

Bellémère A (1977). L’appareil apical de l’asque chez quelques. Discomycetes: Étude ultrastructurale comparative. Revue de Mycologie 41: 233–264.

Bills G (2004). Codophora hiberna sp. nov., a winter-fertilizing helotialean anamorph from wood of Robinia pseudoacacia and forest soil. In: Fungi in forest ecosystems: systematics, diversity, and ecology (Cripps CL, ed). New York Botanical Garden, USA: 113–124.

Blanchette RA, Held BW, Hellmann L, et al. (2016). Arctic driftwood reveals unexpectedly rich fungal diversity. Fungal Ecology 23: 58–65.

Bouguere D, Cairney JW (2005). Assemblages of endocort mycorrhizal and other root-associated fungi from Epacris pulchella (Ericaceae) as deter- mined by culturing and direct DNA extraction from roots. Environmental Microbiology 7: 819–827.

Breen J, Dacre J, Raistick H, et al. (1955). Studies in the biochemistry of micro- organisms. 95. Rugulosin, a crystalline colouring matter of Penicillium rugulosum Thom. Biochemical Journal 60: 616–626.

Breield O (1891). Ascomyceten. II. Die Formen der Ascomyceten und ihre Culture in Nährlösungen. Untersuchungen aus dem Gesamtgebiete der Mykologie 10: 157–378.

Bubák Fr (1914). Ein Beitrag zur Pilzflora von Tirol und Istrien. Annales Mycologici 12: 205–220.

Butin H, Kowalski T (1990). Natural pruning of branches and its biological prerequisites. V. The fungal flora of spruce, pine and larch. European Journal of Forest Pathology 20: 44–54.

Carbone I, Kohn LM (1999). A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91: 553–556.

Carroll GC (1999). The foraging ascomycete. In: Abstracts from the XVI Inter- national Botanical Congress, St Louis, Mo., 1–7 August 1999. The Inter- national Botanical Congress, USA [Abstr. 98].

Cash EK (1936). Some ascomycetes new to California. Mycologia 28: 247–252.

Clay K, Shearin ZR, Bourke KA, et al. (2016). Diversity of fungal endophytes in non-native Phragmites australis in the Great Lakes. Biological Invasions 18: 2703–2716.

Cowden CC, Shefferson RP (2013). Diversity of root-associated fungi of mature Habenaria radiata and Epipactis thunbergii colonizing manmade wetlands in Hiroshima Prefecture, Japan. Mycoscience 54: 327–334.

Crossland C (1996). Mollisia cinerea and its varieties. Transactions of the British Mycological Society 1: 106–109.

Crous PW (2009). Taxonomy and phylogeny of the genus Mycosphaerella and its anamorphs. Fungal Diversity 38: 1–24.

Crous P, Braun U, Groenewald J (2007). Mycosphaerella is polyphyletic. Studies in Mycology 58: 1–32.

Crous PW, Carnegie A, Wingfield M, et al. (2019). Fungal Planet description sheets: 868–950. Persoonia: Molecular Phylogeny and Evolution of Fungi 42: 291–473.

Crous PW, Groenewald JZ, Coppins M, et al. (2016). Opening the Pandora’s box called Mycosphaerella. Book of abstracts 10th International Congress of plant pathology. Beijing, China: 541.

Crous PW, Groenewald JZ, Gams G (2003). Eyespot of cereals revisited: ITS phylogeny reveals new species relationships. European Journal of Plant Pathology 109: 841–850.

Crous PW, Schumacher RR, Wingfield MJ, et al. (2015). Fungal Systematics and Evolution: FUSE 1. Sydowia 67: 81–118.

Crous PW, Summerell BA, Carnegie AJ, et al. (2009). Unravelling Mycos-phaerella: do you believe in genera? Persoonia: Molecular Phylogeny and Evolution of Fungi 23: 99–118.

Crous P, Wingfield M, Burgess T, et al. (2017). Fungal Planet description sheets: 558–624. Persoonia: Molecular Phylogeny and Evolution of Fungi 38: 240–384.

Cui J, Guo T, Chao J, et al. (2016). Potential of the endophytic fungus Phia-locorpha forini Rac56 found in Rhodiola plants to produce salidroside and p-Tyrosol. Molecules 21: 502.
Cui JL, Guo TT, Ren ZX, et al. (2015). Diversity and antioxidant activity of
culturable endophytic fungi from alpine plants of Rhodiola crenulata, R. angustula, and R. sachalinensis. PLoS One 10: e0118204.

Day MJ, Hall JC, Currah RS (2012). Phialide arrangement and character evo-
lution in the helotialean anamorph genera Cadophora and Phialocephala.
Mycologia 104: 371–381.

de Camarredo HE, Boaventura M, Trivelio J, et al. (2016). Diversity of fungal species in ancient parchments collections of the Archive of the University of Coimbra. International Biodeterioration & Biodegradation 108: 57–66.

De Notaris G (1964). Proposte di alcune rettificazioni al profilo dei Discornici. Commentario della Società Crittogamologica Italiana 1: 357–388.

Dennis R (1950). Karsten.

Dennis R (1952). A reassessment of
B Not. Pers. Person.: 159–177.

Dennis R (1956). A new, crystalline, antibiotic substance produced by Mollisia
species (Discomycetes). Antonie van Leeuwenhoek 22: 58–64.

Dennis R (1962). Niptera
Rehm. Kew Bulletin 26: 430–443.

Dennis RWG, Spooner BM (1993). The fungi of North Hoy, Orkney.

Gminder A (2006). Studies in the genus
Amphisphaeria. a Crittogamologica Italiana 64: 269–381.

Gminder A (2012). Studies in the genus
Mollisia s.l. III: Revision of some
Mollisia species described by J. Velenovský (part 2).

Findlay JA, Li G, Miller JD, Eyberger AL, Dondapati R, Porter JR (2006). Endophyte fungal isolates from
Thuja plicata and its toxin in the crown of a mature
hemlock. Studies in Mycology 281: 1121–1124.

Fisher PJ, Webster J (1983). The teleomorphs of
Phialocephala fortinii
strains of the
known as dark-septate endophyte Type 1. Mycologia 97: 628–640.

Grunig CR, Sieber TN (2005). Molecular and phenotypic description of the widespread root symbiont Acetaphora applanata gen. et sp. nov., formerly known as dark-septate endophyte Type 1. Mycologia 97: 628–640.

Grunig CR, Brunner PC, Du A, et al. (2007). Suitability of methods for species recognition in the Phialocephala fortinii–Acetaphora applanata species complex using DNA analysis. Fungal Genetics and Biology 44: 773–788.

Grunig CR, McDonald BA, Sieber TN, et al. (2004). Evidence for subdivision of the root-endophyte Phialocephala fortinii into cryptic species and recombi-
nation within species. Fungal Genetics and Biology 41: 676–687.

Grunig CR, Queloz V, Du A, et al. (2009). Phylogeny of Phaeomollisia piceae gen. sp. nov.: a dark, septate, conifer-needle endophyte and its relationships to Phialocephala and Acheplasma. Mycological Research 113: 207–221.

Grunig CR, Queloz V, Sieber TN, et al. (2008). Dark septate endophytes (DSE) of the Phialocephala fortinii s.l-Aceplasma applanata species complex in tree roots: classification, population biology, and ecology. Botany 86: 1355–1369.

Grunig CR, Sieber TN, Rogers SO, et al. (2002). Genetic variability among strains of Phialocephala fortinii and phylogenetic analysis of the genus Phialocephala based on rDNA ITS sequence comparisons. Canadian Journal of Botany 80: 1239–1249.

Grum-Grzhimaylo OA, Debets AJ, Bilanenko EN (2016). Diversity of microfungi in peatlands originated from the White Sea. Mycologia 108: 233–254.

Haeltewaters D, Dirks AC, Kappler LA, et al. (2018). A preliminary checklist of fungi at the Boston Harbor islands. Northeastern Naturalist 25: 45–77.

Hamad S, Webster J (1998). Anaerobic dendromorph, anamorph of Aposte-
midium torrenticola. Sydowia 40: 60–64.

Hamim A, Miche L, Douai A, et al. (2017). Diversity of fungal assemblages in roots of Ericaceae in two Mediterranean contrasting ecosystems. Comptes Rendus Biologies 340: 226–237.

Han JG, Hosiya T, Sung GH, et al. (2014). Phylogenetic reassessment of
Hyaloscyphaceae sensu lato (Helotiales, Leotiomycetes) based on multi-
genome analysis. Fungal Biology 118: 150–167.

Harrington TC, McNew DL (2003). Phylogenetic analysis places the Phial-
aphora-like anamorph genus Cadophora in the Helotiales. Mycologia 87: 141–152.

Hawksworth D, David J (1989). Proposal to conserve Mollisia (EM Fries) P. Karsten over Tapesia (Pers.: EM Fries) Fuctel (Fungl). Taxon 38: 496.

Hein B (1976). Revision der Gattung Laetiporus Nannf. (Ascomycetes) und Neuerordnung der Laetiporales. Wildenowia Beihel. 8: 1–136.

Hein B (1982). Zum Wert von Zeillmaßen für die Systematik des Hyster-
comycetaceae. a Crittogamologica Italiana 9: 136–147.

Hennebert GL, Bellemere A (1979). Les formes conditionnes des Discomycetes. Essai taxonomique. Revue des Mycologes 43: 259–315.

Hernández-Restrepo M, Gené J, Casiáñez-Ruiz R, et al. (2017). Phylogeny of saprobic microfungi from Southern Europe. Studies in Mycology 86: 53–97.

Hewitt RE, Hollingsworth TN, Chapin III FSIII, Taylor DL (2016). Fire-severity effects on plant–fungal interactions after a novel tundra wildfire disturbance: implications for arctic shrub and tree migration. BMC Ecology 16: 25.

Hibbett DS, Ohman A, Glotzer D, et al. (2017). Phylogenetic relationships, host affinity, and geographic structure of boreal and arctic endophytes from three major plant lineages. Molecular Phylogenetics and Evolution 42: 543–555.
Hofstetter V, Miadlikowska J, Kaff F, et al. (2007). Phylogenetic comparison of protein-coding versus ribosomal RNA-coding sequence data: a case study of the Lecanoromycetes (Ascomycota). Molecular Phylogenetics and Evolution 44: 412–426.

Hosoya T, Jinbo U, Tsujino N, et al. (2015). “MollBase”, a new sequence database including unidentified Mollisia and its allied genera. Ascomyceta 7: 311–314.

Huang J, Nara K, Zong K, et al. (2014). Ectomycorrhizal fungal communities associated with Masson pine (Pinus massoniana) and white oak (Quercus fabri) in a manganease mining region in Hunan Province, China. Fungal Ecology 9: 1–10.

Hustad VP, Miller AN (2011). Phylogenetic placement of four genera within the Leotiomycetes (Ascomycota). North American Fungi 6: 1–13.

Ingold CT, Chapman B (1952). Aquatic Ascomycetes: Loramyces junciola Wehner and L. macrospora n. sp. Transactions of the British Mycological Society 35: 268–272.

Itniama F, Korf RP (1990). A monograph of the discomycete genus Strossmayenia (Leotiales), with comments on its anamorph, Pseudosporites (Dermatiales). Mycotaxon 36: 383–454.

Jensen JB, González VT, Guevara DU, et al. (2011). Kit for detection of fungal endophytes of grasses yields inconsistent results. Methods in Ecology and Evolution 2: 197–201.

Johnston LJ, Miller AN, McCleery RA, et al. (2013). Psychrophilic and psychrotolerant fungi on bats and the presence of Geomyces spp. on bat wings prior to the arrival of white nose syndrome. Applied and Environmental Microbiology 79: 5465–5471.

Johnston PR, Quijada L, Smith CA, et al. (2019). A multigene phylogeny toward a new phylogenetic classification of Leotiomycetes. IMA Fungus 10: 1.

Karsten PA (1871). Mycologia Fennica. Pars prima. Discomycetes.

Karsten PA (1871). Mycologia Fennica. Pars prima. Discomycetes.

Kim MJ, Lee H, Choi YS, et al. (2010). Diversity of fungi in creosote-treated subtropical bamboo forests. Soil Biology and Biochemistry 40: 179–190.

Kumar YW, Kim JJ, Chedgy R, et al. (2005). Fungal diversity from western red-cedar fences and their resistance to B-thujaplicin. Antonie van Leeuwenhoek 87: 109–117.

Liu K-L, Porras-Alfaro A, Kuske CR, et al. (2012). Accurate, rapid taxonomic classification of fungal large-subunit rRNA genes. Applied and Environmental Microbiology 78: 1523–1533.

Liu YJ, Whalen S, Hall BD (1999). Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. Molecular Biology and Evolution 16: 1799–1808.

Lombard L, Crous PW, Wingfield BD, et al. (2010). Species concepts in Calonectria (Cylindrocladium). Studies in Mycology 66: 1–13.

Luo J, Waithi E, Naik A, et al. (2014). Temperate pine barren and tropical rain forests are both rich in undescribed fungi. PLoS One 9: e103753.

Lygis V, Vasilasikate I, Matelis A, et al. (2014). Fungi in living and dead stems and stumps of Pinus mugo on coastal dunes of the Baltic Sea. Plant Protection Science 50: 221–226.

Markakis EA, Kavvoulakis N, Ntougias S, et al. (2017). Characterization of fungi associated with wood decay of tree species and grapevine in Greece. Plant Disease 101: 1929–1940.

Martínez-Alvaro P, Fernández-González RA, Sanz-Ros AV, et al. (2016). Two fungal endophytes reduce the severity of pitch canker disease in Pinus radiata seedlings. Biological Control 94: 1–10.

McMullin DR, Green BD, Prince NC, et al. (2017). Natural products of Picea endophytes from the Acadian Forest. Journal of Natural Products 80: 1475–1483.

McMullin DR, Tenney JB, McDonald KP, et al. (2019). Phthalides produced by Coccycomyces strobi (Rhytismataceae, Rhytismatales) isolated from needles of Pinus strobus. Phycology Letters 29: 17–24.

Menkus A, Allmer J, Vasilasikas R, et al. (2004). Ecology and molecular characterization of different sepsis fungi from roots, living stems, and fine woody debris. Mycological Research 108: 965–973.

Miller JD (2011). Foliar endophytes of spruce species found in the Acadian forest: basis and potential for improving the tolerance of the forest to spruce budworm. In: Endophytes of forest trees (Pirilla A, Frank A, eds). Springer, the Netherlands: 237–249.

Miller JD, Cherid H, Sumarah MW, et al. (2009). Horizontal transmission of the Picea glauca foliar endophyte Phialocephala scottii CBS 120377. Fungal Ecology 2: 98–101.

Miller MA, Pfeiffer W, Schwartz T (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. IEEE: 1–8.

Moncira S, Linakieddu BT, Ginetti B, et al. (2016). Endemic and emerging pathogens threatening cork oak trees: management options for conserving a unique forest ecosystem. Plant Disease 100: 2184–2193.

Müller E (1962). Quelques discomycètes Méditéranéens. Revue de Mycologie 27: 69–75.

Müller E, Defago A (1967). Beloniiaca, Sacc., Boud. und Diboneliaceae Nannf., zwei wenig bekannte Discomycetengattungen. Sydowia 18: 157–168.

Müller E, Petini O, Sambles GJ (1979). Oblotectocidus aquaticus gen. nov. et sp. nov., ein neuer, wasserbewohnender Ascomycet aus den Alpen. Sydowia 32: 190–197.

Münzenberger B, Bubner B, Wölcke J, et al. (2009). The ectomycorrhizal morphotype Pinhirza sclerotina is formed by Acephala macroserolotrum sp. nov., a close relative of Phialocephala forini. Mycorrhiza 19: 481–492.

Mysterud I, Hestand K, Koller G, et al. (2007). Molecular characterization and evaluation of plant litter-associated fungi from the spring ‘grazing corridor’ of a sheep herd vulnerable to alveid disease. Mycopathologia 164: 201–215.

Nannfledt JBN (1976). Micropeziza Fuck, and Scudosporilia Nannf. nov. gen. (Discomycetes inoperculati). Botaniska Notiser 129: 323–340.
Nannfeldt JA (1983). Nimbomiltioila and Discurtisia: two new genera of mollisioid Discomycetes. Mycologia 75: 292–310.
Nannfeldt J (1984). Hysteronomia, a new genus of mollisioid Discomycetes. Nordic Journal of Botany 4: 225–247.
Nauta M (2010). Notes on Mollisioid Ascomycetes from the Beartooth Plateau, Rocky Mountains USA. North American Fungi 5: 181–186.
Nauta MM, Spooner B (2000a). British dermatideaceae: 4B. Dermateoidaceae genera B–E. Mycologist 14: 21–28.
Nauta MM, Spooner B (2000b). British dermatideaceae: 4B. Dermateoidaceae genera G–Z. Mycologist 14: 65–74.
Newsham KK (2011). A meta-analysis of plant responses to dark septate endophytes. New Phytologist 190: 783–793.
Nonaka K, Kaneta T, Osumu S, et al. (2015). Mariannaeae macrochlamydospora, a new huyphomycete (Nectriaceae) from soil in the Bonin Islands, Japan. Mycoscience 56: 29–33.
O’Donnell K, Cigelink E (1997). Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus Fusarium are nonorthologous. Molecular Phylogenetics and Evolution 10: 113–116.
Partel K (2016). Application of ultrastructural and molecular data in the taxonomy of helotialean fungi. Ph.D. dissertation. Department of Botany, University of Tartu, Estonia.
Partel K, Baral H-O, Tamm H, et al. (2017). Evidence for the polyphyly of Encoela and Encocoeilideae with reconsideration of respective families in Leotiomycetes. Fungal Diversity 82: 183–219.
Pedras MSC, Ahiahonu PW (2004). Phytotoxin production and phytoalexin elicitation by the phytopathogenic fungus Sclerotiorum sclerotiorum. Journal of Mycology 30: 2163–2179.
Persoon CH (1879). Observationes Mycologicae 2. Gesnus, Usterius & Wolfii, Germany.
Phillips W (1867). A manual of the British discomycetes: with descriptions of all the species of fungi hitherto found in Britain, included in the family and illustrations of the genera. Kegan Paul, Trench, Trübner & Co., UK.
Porter TM, Golding GB (2012). Factors that affect large subunit ribosomal DNA transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proceedings of the National Academy of Sciences 109: 6241–6246.
Schwolow S, Kunz H, Rheinheimer J, et al. (2013). Total synthesis of the antifungal natural product mollisin. European Journal of Organic Chemistry 29: 6519–6524.
Seifert K, Morgan-Jones G, Gams W, et al. (2011). The Genera of Hyphomycetes. CBS Biodiversity Series No. 9: 1–997. CBS-KNAW Fungal Biodiversity Centre, Netherlands.
Shamoun SF, Seibert TN (2000). Colonisation of leaves and twigs of Rubus parviflorus and R. spectabilis by endophytic fungi in a reforestation site in British Columbia. Mycological Research 104: 841–845.
Shearer CA (1993). The freshwasser ascomycetes. Nova Hedwigia 65: 1–33.
Shenoy BD, Jeewon R, Wang H, et al. (2010). Sequence data reveals phylogenetic affinities of fungal anamorphs Bahusutrabeeja, Diplomococcum, Natarajania, Paliphora, Polyscherna, Rattania and Spadicocides. Fungal Diversity 44: 161–169.
Sibert TN (1989). Endophytic fungi in twigs of healthy and diseased Norway spruce and white fir. Mycological Research 92: 322–326.
Singh S, Harms H, Schlosser D (2014). Screening of ecologically diverse fungi for their potential to pretreat lignocellulosic biomass feedstock. Applied Microbiology and Biotechnology 98: 3355–3370.
Song Y, Wu P, Li Y, et al. (2017). Effect of endophytic fungi on the host plant growth, expression of expansin gene and flavonoid content in Tetrastigma hemsleyanum Diels & Gilg ex Diels. Plant and Soil 417: 393–402.
Starback K (1895). Discomyceten Studien. Bihang till Kong Svenska vetenskaps-akademien handlings (Afd 3) 21: 1–42.
Stensröm E, Nöide NE, Jonsson M, et al. (2014). Root-associated fungi of healthy-looking Pinus sylvestris and Picea abies seedlings in Swedish forest nurseries. Scandinavian Journal of Forest Research 29: 12–21.
Stielsow J, Lévesque C, Seifert K, et al. (2015). One fungus, which genes? Development and assessment of universal primers for potential secondary fungal DNA barcodes. Persoonia: Molecular Phylogey and Evolution of Fungi 35: 242–263.
Stiller JW, Hall BD (1997). The origin of red algae: implications for plastid evolution. Proceedings of the National Academy of Sciences 94: 4520–4525.
Stothard P (2000). The sequence manipulation suite: JavaScript programs for analyzing and formatting protein and DNA sequences. Biotechniques 28: 1102–1104.
Stoyke G, Currah RS (1993). Resynthesis in pure culture of a common sub-alpine fungus-root association using Phialocephala fortinii and Menziesia ferruginia (Eriaceae). Arctic and Alpine Research 25: 189–193.
Strooher S, Dubach V, Queloz V, et al. (2018). Resilience of Phialocephala fortinii s.l.–Aschepha appianita communities–Effects of disturbance and strain introduction. Fungal Ecology 31: 19–28.
Sumarah WM, Adams GW, Berghout J, et al. (2008). Spread and persistence of a rugulosis-producing endophyte in Picea glauca seedlings. Mycological Research 112: 731–736.
Suroyo, Narisawa K (2018). The inhibitory role of dark septate endophytic fungus Phialocephala fortinii against Fusarium disease on the Asparagus officinalis growth in organic source conditions. Biological Control 121: 159–167.
Tanney JB, McMullin DR, Green BD, et al. (2016). Production of antifungal and antiseptic metabolites by the Picea endophyte Diaportha maritima sp. nov. Fungal Biology 120: 1448–1457.
Tanney JB, McMullin DR, Miller JD (2018a). Toxigenic foliar endophytes from the Acadian forest. In Endophytes of forest trees: biology and applications (Frank A, Pittilla AM, eds). Springer, Switzerland: 343–361.
Tanney JB, Nguyen HD, Pinzari F, et al. (2015). A century later: rediscovery, culturing and phylogenetic analysis of Diploloma rosea, a rare oenogaeanean endophyte. Antonie van Leeuwenhoek 108: 1023–1035.

Tanney JB, Renaud JB, Miller JD, et al. (2016b). New 1, 3-benzodioxin-4-ones from Synnematapeosteloides encaecarum sp. nov., a biosynthetic link to remarkable compounds within the Xylariales. Phytochemistry 131: e018832.

Tanney JB, Seifert KA (2017). Lophodermium resistans sp. nov. from red pine Pinus resinosa in Eastern Canada. Botany 95: 773–784.

Tanney JB, Seifert KA (2018). Phaciaceae endophytes of Picea rubens in Eastern Canada. Botany 96: 555–588.

Tanney JB, Seifert KA (2019). Filosepora piceae gen. et sp. nov. (Septorioidaeae, Botryosphaeriaceae) from Picea rubens. Mycological Progress 18: 163–174.

Taylor DL, Hollingsworth TN, McFarland JW, et al. (2014). A first comprehensive census of fungi in soil reveals both hyperdiversity and fine-scale niche partitioning. Ecological Monographs 84: 3–20.

Taylor JW, Jacobson DJ, Kroken S, et al. (2000). Phylogenetic species recognition and species concepts in fungi. Fungal Genetics and Biology 31: 21–32.

Tellenbach C, Grüning CR, Sieber TN (2011). Negative effects on survival and performance of Norway spruce seedlings colored by dark septate root endophytes are primarily isolate-dependent. Environmental Microbiology 13: 2508–2517.

Tellenbach C, Sieber TN (2012). Do colonization by dark septate endophytes and elevated temperature affect pathogenicity of oomycetes? FEMS Microbiology Ecology 82: 157–168.

Tellenbach C, Sumarah MW, Grüning CR, et al. (2012). Inhibition of Phytophthora species by secondary metabolites produced by the dark septate endophyte Phialophora europaea. Fungal Ecology 6: 12–18.

Terhorsten E, Karlı S, Sun H, et al. (2014). Endophytic fungi of Norway spruce roots in boreal pristine mire, drained peatland and mineral soil and their inhibitory effect on Heterobasidion parviporum in vitro. Fungal Ecology 9: 17–26.

Thomas DC, Vandegrift R, Ludden A, et al. (2016). Spatial ecology of the fungal genus Xylaria in a tropical cloud forest. Biotropica 48: 381–393.

Timling I, Walker DA, Nusbaum C, et al. (2018). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR protocols: a guide to methods and applications. Academic Press, Inc., USA: 315–322.

Tellenbach C, Sieber TN (2012). Do colonization by dark septate endophytes and elevated temperature affect pathogenicity of oomycetes? FEMS Microbiology Ecology 82: 157–168.

Tellenbach C, Sumarah MW, Grüning CR, et al. (2012). Inhibition of Phytophthora species by secondary metabolites produced by the dark septate endophyte Phialophora europaea. Fungal Ecology 6: 12–18.

Terhorsten E, Karlı S, Sun H, et al. (2014). Endophytic fungi of Norway spruce roots in boreal pristine mire, drained peatland and mineral soil and their inhibitory effect on Heterobasidion parviporum in vitro. Fungal Ecology 9: 17–26.

Unterseher M, Petzold A, Schnittler M (2012). Xerotolerant foliar endophytic Tri...