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Mollisiaceae: An overlooked lineage of diverse endophytes

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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 antiinsectan 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 nuc internal transcribed spacer rDNA (ITS) barcode and partial sequences of the 28S nuc 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 *Acephala*, *Acidomelania*, *Barrenia*, *Bispora*, *Cheirospora*, *Cystodendron*, *Fuscosclera*, *Hysteronaevia, Loramyces, Mollisia, Neopyrenopeziza*, *Obtectodiscus, 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*, *Acidomelania* with *Mollisia*, and *Loramycetaceae* 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 lignicola* (Hern.-Restr., J. Mena & Gené) J.B. Tanney & K.A. Seifert, *Mollisia panicicola* (E. Walsh & N. Zhang) J.B. Tanney & K.A. Seifert. **New species:** *Mollisia diesbachiana* Tanney & Seifert, *M. monilioides* Tanney & Seifert, *M. novobrunsvicensis* Tanney & Seifert, *M. prismatica* Tanney & Seifert, *M. rava* Tanney & Seifert, *Phialocephala amethystea* Tanney & Seifert, *P. biguttulata* Tanney & Seifert, *P. collarifera* Tanney & Seifert, *P. helenae* Tanney & Seifert, *P. vermiculata* Tanney & Seifert.

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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 vacuolar bodies, and ascospores that are usually 0(−1)-septate, elliptic-fusoid to fusiform, hyaline, and borne in 8-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*, *Haglundia*, *Hysteronaevia*, *Hysteropezizella*, *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 (*Haglundia*), 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 multiseptate spores (*Trichobelonium*) (Nannfeldt 1976, Nauta & Spooner 2000a, b). The name *Mollisia* has also been applied to phylogenetically distant but sometimes morphologically similar taxa, including *Cistella* (*Hyaloscyphaceae*), *Leptotrochila* (*Drepanopezizaceae*), *Mniaecia* (*Mniaeciaceae*), *Orbilia* (*Orbiliaceae, Orbiliales*), and especially *Pyrenopeziza* (*Ploettnerulaceae*) species, which can be quite similar to *Mollisiaceae* species but lack refractive vacuolar 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

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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), *Paradidymobotryum 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 *P. 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

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

Taxa	Morphological description	References
Acephala macrosclerotiorum	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 scolecoconidia	Webster (1961)
Bispora betulina	Monoblastic, brown (1–)2(–3)-septate didymospores in unbranched and branched acropetal chains with phialocephala-like synasexual morphs	Wang (1989)
Casaresia sphagnorum	Monoblastic stauroconidia with 5–6 recurved arms	Webster *et al.* (1993)
Cheirospora botryospora	Bulbils composed of brown globose cells surrounded in a gelatinous sheath arising from sporodochia or acervuli	Crous *et al.* (2015)
Cystodendron dryophilum	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)
Fuscosclera lignicola	Multiseptate, 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 scolecoconidia	Digby & Goos (1987)
Mollisia ligni	Black, synnema-like, arising from black stroma with sterile inflated vesicles interspersed among cystodendron-like conidiophores ("*forme Pycnidiella*" *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 phialides with 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 salicis	Brown meristematic 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" saprotrophs, 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* (*Loramycetaceae*, *Helotiales*) and *Vibrissea* (*Vibrisseaceae*, *Helotiales*) (Wang *et al.* 2006b), along with reports of conidia 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 to does the list of potential taxonomic and nomenclature issues. Even the consensus remains unclear on which family to place *Mollisia* and related genera, for example the inconsistent placement of *Mollisia*, *Phialocephala*, and other genera within *Dermateaceae*, *Loramycetaceae*, *Mollisiaceae*, and *Vibrisseaceae* (*e.g.* Adhikari *et al.* 2016, Robicheau *et al.* 2017, Wijayawardene *et al.* 2018). Johnston *et al.* (2019) presented a multigene phylogeny of *Leotiomycetes* that showed a "mollisioid" clade (*i.e.* taxa typically with mollisioid apothecia) comprising *Mollisiaceae*, *Ploettnerulaceae*, *Drepanopezizaceae*, and *Godroniaceae*. In the same study, a strongly supported clade comprising *Mollisiaceae*, *Loramycetaceae*, *Strossmayeria bakeriana*, *Vibrisseaceae*, and *Chlorosplenium chlora* was identified, although the boundaries of *Mollisiaceae* were not clear. Despite being nested within *Mollisiaceae*, *Loramycetaceae* is still recognized as a separate family solely because of its distinctive apothecial morphology (enclosed, perithecioid apothecia).

Given the ubiquity and ecological plasticity of *Mollisia* species, the rising research interest in endophytes of woody plants, and the increasing use of high-throughput next generation sequencing technology (NGS) to detect and characterise endophyte communities, there is a growing need to confront this neglected genus to develop an effective taxonomic framework permitting identification and classification. Taxonomic research on *Mollisia* 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

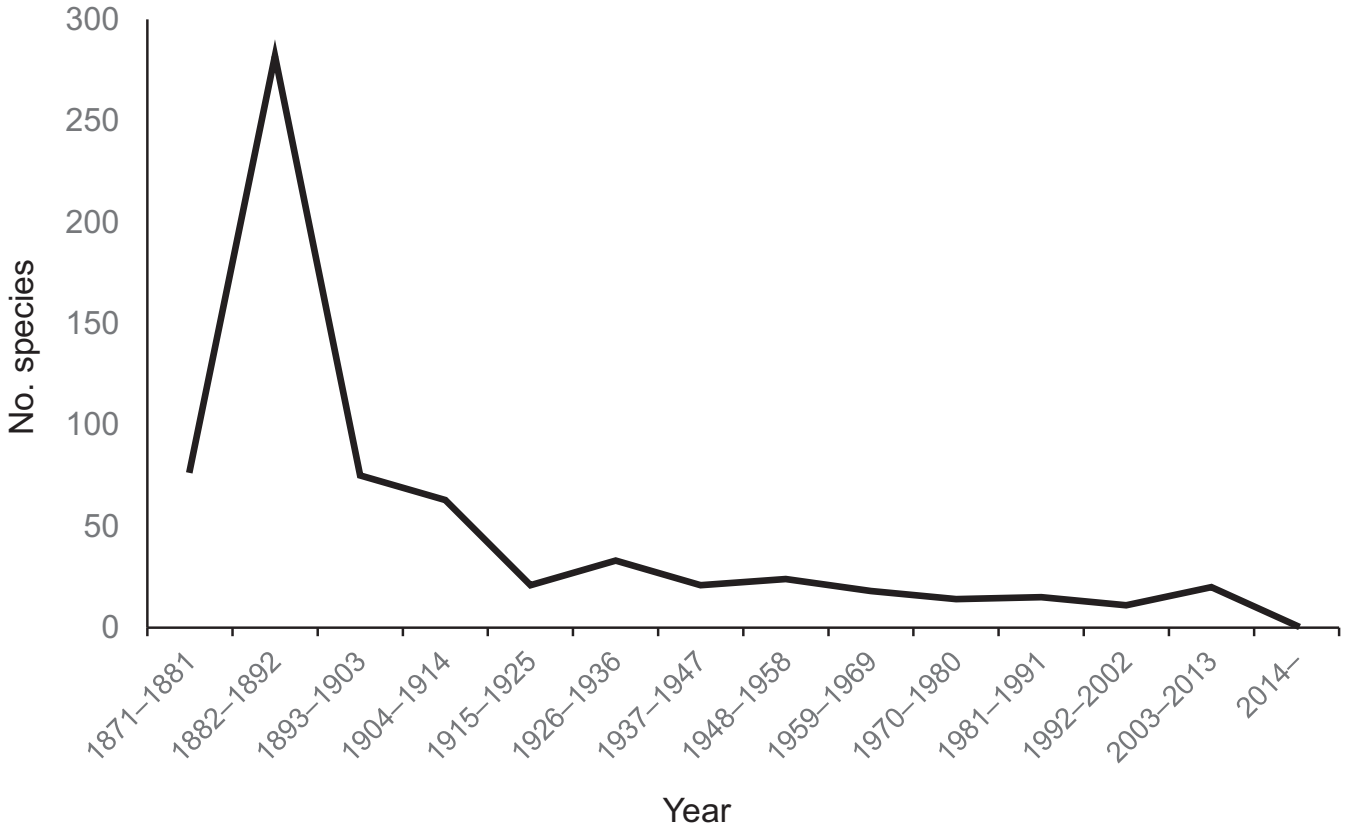

Fig. 1. Taxonomic and nomenclatural activity within *Mollisia* since its inception (1871) based on a query in Index Fungorum.

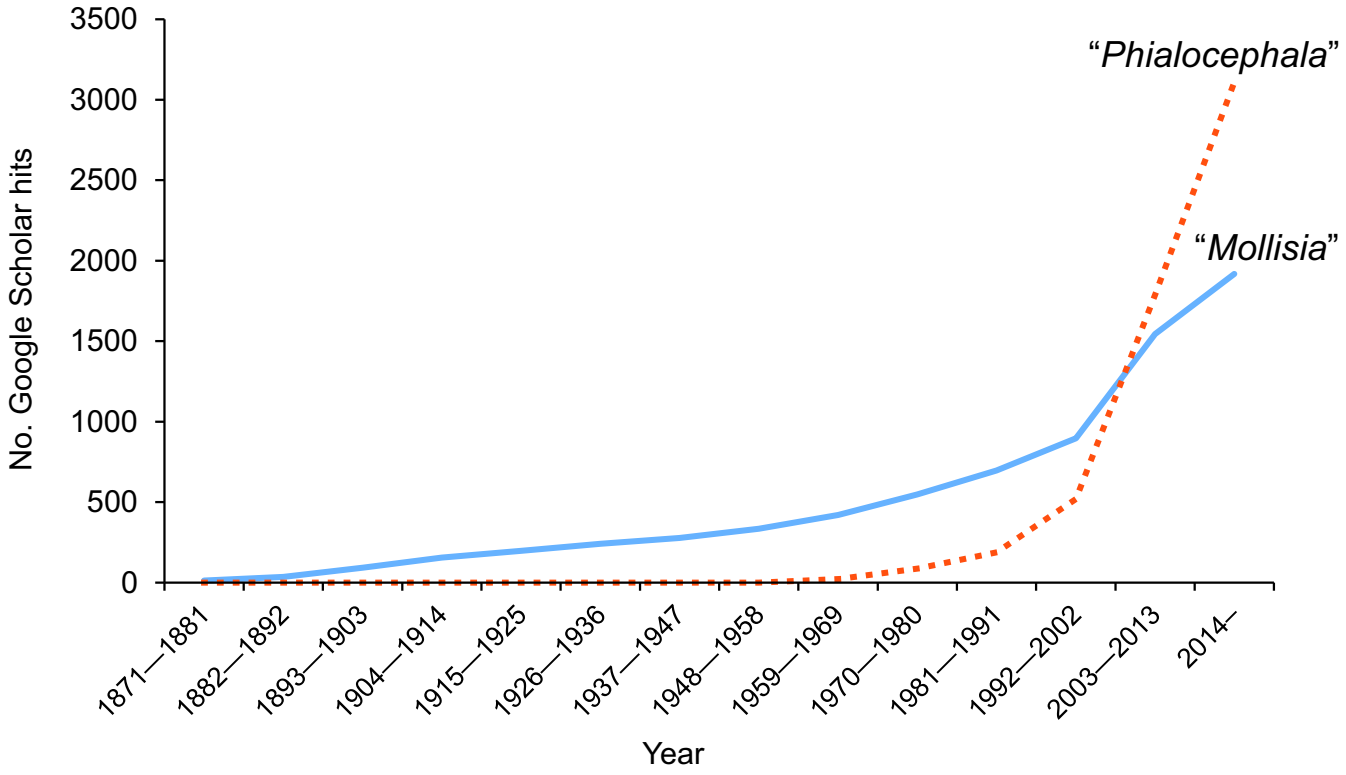

Fig. 2. Cumulative number of Google Scholar citations over time using "*Mollisia*" and "*Phialocephala*" keywords. The y-axis shows the number of Google Scholar hits and the x-axis shows ten-year intervals from 1871–2019. *Mollisia* hits are plotted by the solid blue line and *Phialocephala* hits by the red broken line.

study may serve as a prodromus for the impending and necessary revision of *Mollisiaceae*. 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 lignicolous 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 ascomata 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 drop of 5 % KOH on a microscope slide and observed any macroscopic reaction. Colony colors were described using the alphanumeric codes of Kornerup & Wanscher (1978). Microscopic measurements were taken from material mounted in water and are presented as ranges calculated from the mean ± standard deviation of each measured value, with outliers in brackets. Observations were made using an Olympus BX50 light microscope and micrographs were captured using an Evolution MP Color Camera (Media Cybernetics, Silver Spring, California) with Image-Pro Plus v. 6.0 (Media Cybernetics) or an InfinityX-32 camera (Lumenera Corp., Ottawa, Canada) with Infinity Analyze (Lumenera Corp.) software. Colony macrophotographs were captured with a Nikon Coolpix P5000 (Nikon Inc., Tokyo, Japan) and photographic plates were assembled using Adobe Photoshop CC (Adobe Systems, San Jose, California).

Attempts to induce sporulation in sterile cultures were made in accordance with Tanney *et al.* (2016a). In addition, for selected cultures, 5–10 mm agar blocks containing mycelia were floated in 10 cm Petri dishes containing *ca.* 30 mL of sterile distilled water and incubated for up to 18 mo (Tanney *et al.* 2018b). Cardinal temperatures were determined by assessing diameters of cultures growing from single-point inoculations on MEA and incubated at 5 °C intervals from 5 to 40 °C. Temperature treatments were conducted in triplicate for each isolate and colony measurements were made weekly for 1 mo.

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 Axygen 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 taxomic 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 *Helotiales* 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, Whaltman, 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.

Table 2. Substrate, provenance, and GenBank accession numbers for taxa included in phylogenetic analyses.

Name	Substrate	Location	Sequence source or specimen	GenBank accession no.					Reference
				ITS	LSU	*RPB1*	*TOP1*	*LNS2*	
Acephala applanata	Endophyte of *Picea abies* root	Switzerland	CBS:109321T	NR_119482	MT026532	MT018410	MT039071	MT009550	Grünig *et al.* (2002), this study
A. macrosclerotiorum	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	EU434830	—	—	—	—	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-1	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	—	—	—	—	Grünig & 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	MN559787	—	—	—	—	Yuan (direct submission)

Table 2. (Continued).

Name	Substrate	Location	Sequence source or specimen	GenBank accession no.					Reference
				ITS	LSU	*RPB1*	*TOP1*	*LNS2*	
	Needle of *Picea abies*	Switzerland	CdV_6-4.4b	EU434832	—	—	—	—	Grünig *et al.* (2009)
Acidomelania panicicola	Roots of *Panicum virgatum*	USA	CBS:137156T	KF874619	—	KT591690	—	—	Walsh *et al.* (2015)
Apostemidium vibrisseoides	Sticks in water	Canada: Ontario	DAOM:120405	MT026442	—	—	—	—	This study
	Twigs in running stream	Canada: Quebec	DAOM:46436	MT026443	—	—	—	—	This study
Ascomycete sp.	Unspecified	Norway	HK-S236	AM084465	—	—	—	—	Kauserud *et al.* (2005)
	Thuja plicata fence material	Canada	WRCF-A3	AY618688	—	—	—	—	Lim *et al.* (2005)
	Unspecified	China	ZGZII04159	DQ124146	—	—	—	—	Zhao *et al.* (direct submission)
	Unspecified	Norway	HK-S252	AM084466	—	—	—	—	Kauserud *et al.* (2005)
	Stump of *Picea abies*	Finland	A352	MG190540	—	—	—	—	Mueller (direct submission)
	Stump of *Picea abies*	Finland	Luke_A212	MG190489	—	—	—	—	Mueller (direct submission)
	Stump of *Picea abies*	Finland	Luke_A360	MG190544	—	—	—	—	Mueller (direct submission)
Barrenia panicia	Roots of *Digitaria*sp.	USA	AL5M2	—	—	KT591693	—	—	Walsh *et al.* (2015)
	Roots of *Coix lacryma-jobi*	USA	CM11M2	—	—	KT591694	—	—	Walsh *et al.* (2015)
	Roots of *Panicum virgatum*	USA	RUTPP:WSF1R37T	NR_164236	—	KT591692	—	—	Walsh *et al.* (2015)
B. taeda	Roots of *Pinus rigida*	USA	CM14P64	—	—	KT591695	—	—	Walsh *et al.* (2015)
	Roots of *Pinus rigida*	USA	RUTPP:WSF14P22T	NR_164237	—	KT591696	—	—	Walsh *et al.* (2015)
	Roots of *Pinus rigida*	USA	WSF14P13	—	—	KT591697	—	—	Walsh *et al.* (2015)
Belonium excelsior	Unspecified	UK	CBS:140.52	MH856965	—	—	—	—	Vu *et al.* (2019)
Bispora betulina	Unspecified	Sweden	CBS:136.49	MH856465	—	—	—	—	Vu *et al.* (2019)
	Unspecified	Canada: Quebec	CBS:141.61	MH858001	—	—	—	—	Vu *et al.* (2019)
Cadophora hiberna	*Robinia pseudoacacia* wood	USA: New Jersey	GB5129	AF530461	—	—	—	—	Bills (2004)
Calluna vulgaris root associated fungus	*Calluna vulgaris*	Germany	agrKH075	FM172774	—	—	—	—	Pietrowski (direct submission)

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Table 2. (Continued).

Name	Substrate	Location	Sequence source or specimen	GenBank accession no.					Reference
				ITS	LSU	*RPB1*	*TOP1*	*LNS2*	
	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	DAOMC:250748	MT026433	—	MT025200	—	—	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)
Chlorencoelia versiformis	Rotten hardwood log	Canada: Quebec	DAOMC:251598	MH457140	—	MT025212	—	—	McMullin *et al.* (2019), this study
Chlorosplenium chlora	Fallen branch	USA: Massachusetts	FH:BHI-F736	MG553993	—	—	—	—	Haelewaters *et al.* (2018)
	Wood	USA: Massachusetts	FH:BHI-F737	MG553994	—	—	—	—	Haelewaters *et al.* (2018)
Cystodendron dryophilum	*Juniperus communis* needle	Switzerland	CBS:295.81	MT026425	MT026557	MT018424	MT039096	MT009575	This study
Cystodendron sp.	*Abies alba*	Switzerland: Alptal	TS_90_233	EU434835	—	—	—	—	Grünig *et al.* (2009)
	Castanea sativa	Switzerland: Bellinzona	UAMH 10850	EU434834	—	—	—	—	Grünig *et al.* (2009)
Durella connivens	Broken hardwood stick	USA: Massachusetts	FH:BHI-F627	MF161306	—	—	—	—	Haelewaters *et al.* (2018)
	Branch of *Populus tremula*	Luxembourg	G.M. 2014-08-12	KY462810	—	—	—	—	Hermant (direct submission)
	Branch of *Salix*	Luxembourg	GM-2015-05-16	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	GU581235	—	—	—	—	Jensen *et al.* (2011)

Table 2. (Continued).

Name	Substrate	Location	Sequence source or specimen	GenBank accession no.					Reference
				ITS	LSU	*RPB1*	*TOP1*	*LNS2*	
	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 *Taxodium distichum*	USA	SV1702	MK036902	—	—	—	—	Kimbrough *et al.* (2019)
Fungal sp.	Root tips of *Pinus sylvestris*	Finland	2.2.4C	KM068396	—	—	—	—	Sarjala *et al.* (direct submission)
	Root tips of *Pinus sylvestris*	Finland	2.2.4D	KM068397	—	—	—	—	Sarjala *et al.* (direct submission)
	Root tips of *Pinus sylvestris*	Finland	3.12.4B	KM068428	—	—	—	—	Sarjala *et al.* (direct submission)
	Root tips of *Pinus sylvestris*	Finland	3.16.3A	KM068431	—	—	—	—	Sarjala *et al.* (direct submission)
	Root tips of *Pinus sylvestris*	Finland	3.44.4D	KM068438	—	—	—	—	Sarjala *et al.* (direct submission)
	Root tips of *Pinus sylvestris*	Finland	3.46.4A	KM068442	—	—	—	—	Sarjala *et al.* (direct submission)
	Withered plant material	Norway	A4-3	AM231338	—	—	—	—	Mysterud *et al.* (2007)
	Wood	Antarctica	AB45	FJ235978	—	—	—	—	Arenz & Blanchette (2009)
	Myotis septentrionalis wing	USA	APA-2013 clone LJ76MstMg10w	KF212280	—	—	—	—	Johnson *et al.* (2013)
	Myotis septentrionalis wing	USA	APA-2013 clone LJ81MstMg10w	KF212281	—	—	—	—	Johnson *et al.* (2013)
	Thuja koraiensis	South Korea: Gyeonggi	JE-2	LC163521	—	—	—	—	Eo *et al.* (2016)
	Carex bigelowii	Finland	K7T2J2 AR-2014	KF527816	—	—	—	—	Ruotsalainen *et al.* (direct submission)
	Phragmites australis var. *australis*	USA	OTU26	KT923245	—	—	—	—	Clay *et al.* (2016)
	Phragmites australis var. *australis*	USA	OTU5	KT923252	—	—	—	—	Clay *et al.* (2016)

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Table 2. (Continued).

Name	Substrate	Location	Sequence source or specimen	GenBank accession no.					Reference
				ITS	LSU	*RPB1*	*TOP1*	*LNS2*	
	Phragmites australis var. *australis*	USA	OTU54	KT923222	—	—	—	—	Clay *et al.* (2016)
	Tetrastigma hemsleyanum	China	TH11	KY607743	—	—	—	—	Song *et al.* (2017)
Fuscosclera lignicola	Dead wood	Spain	CBS:142287T	NR_164252	—	—	—	—	Hernández-Restrepo *et al.* (2017)
Grass root mycorrhizal sp.	*Deschampsia flexuosa*	Netherlands	PPO-1	AY599235	—	—	—	—	Baar *et al.* (direct submission)
Helgardia anguioides	Unspecified	Germany	CBS:496.80	MH861290	—	—	—	—	Vu *et al.* (2019)
Helotiales sp.	Bottom sediment of bog	Russia	65 OA-2013	JX507656	—	—	—	—	Grum-Grzhimaylo *et al.* (direct submission)
	Bottom sediment of bog	Russia	73 OA-2013	JX507664	—	—	—	—	Grum-Grzhimaylo *et al.* (direct submission)
	Bottom sediment of bog	Russia	74 OA-2013	JX507666	—	—	—	—	Grum-Grzhimaylo *et al.* (2016)
	Black sclerotium	USA: Florida	BA4b011	AB986446	—	—	—	—	Obase *et al.* (direct submission)
	Hair roots of *Phyllodoce aleutica*	Japan	EF804	LC130995	—	—	—	—	Shimono & Hirose (direct submission)
	Green herbarized *Populus euphratica* leaves	China	POPeuph60	FR865028	—	—	—	—	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)
Loramyces juncicola	*Eleocharis palustris*	UK	CBS:293.52	MH857043	MT026562	MT018376	MT039101	MT009580	Vu *et al.* (2019), this study
L. macrosporus	Submerged dead internode of *Equisetum limosum*	UK	CBS:235.53T	MH857170	MT026502	MT018375	MT039041	MT009520	Vu *et al.* (2019), this study

Table 2. (Continued).

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 lof 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 rubens*needle	Canada: New Brunswick	DAOMC:250738	MT026414	MT026535	MT018415	MT039074	MT009553	This study
M. cf. undulatodepressula	Decaying *Betula papyrifera* branch on ground	Canada: New Brunswick	DAOMC:250746	MT026449	—	—	—	—	This study
	Decaying decorticated 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. cinerella	*Calluna vulgaris*	Morocco	ER36M	KU986786	—	—	—	—	Hamim *et al.* (2017)
M. cinereo-olivascens	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

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Table 2. (Continued).

Name	Substrate	Location	Sequence source or specimen	GenBank accession no.					Reference
				ITS	LSU	*RPB1*	*TOP1*	*LNS2*	
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 2/10055T	MK088059	—	—	—	—	Matocec *et al.* (direct submission)
M. epitypha	*Typha latifolia*	Canada: Ontario	DAOM:150777	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	—	—	—	—	Haelewaters *et al.* (2018)
	Fagus sylvatica	Switzerland	CBS:234.71	AY259138	—	—	—	—	Crous *et al.* (2003)
	Gate post (*Quercus sp.*)	France	CBS:555.63	MT026435	—	MT025205	—	—	This study
M. fuscoparaphysata	Unspecified	Czech Republic	JI106_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	—	MT025208	—	—	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	—	MT025201	—	—	This study
M. lividofusca	*Lonicera coerulea*	Switzerland	CBS:231.71	MT026438	—	MT025206	—	—	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	JA85	DQ008242	—	—	—	—	Allmer *et al.* (2006)
	Ledum palustre	China	X12	KJ817294	—	—	—	—	Yang & Yan (direct submission)
M. monilioides	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:558.63	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	—	MT025207	—	—	This study

Table 2. (Continued).

Name	Substrate	Location	Sequence source or specimen	GenBank accession no.					Reference
				ITS	LSU	*RPB1*	*TOP1*	*LNS2*	
	Rotten wood of *Betula alleghaniensis*	Canada: New Brunswick	DAOMC:251495	MT026453	—	—	—	—	This study
	Decaying log	Canada: New Brunswick	DAOMC:251538	MT026383	MT026494	MT018360	MT039033	MT009512	This study
	Decaying lof of *Betula alleghaniensis*	Canada: New Brunswick	DAOMC:251631	MT026452	—	—	—	—	This study
	Old decaying mossy log of *Betula papyrifera*	Canada: New Brunswick	DAOMC:252263T	MT026382	MT026493	MT018359	MT039032	MT009511	This study
	Decaying *Betula alleghaniensis* branch on ground	Canada: New Brunswick	NB579	MT026384	MT026495	MT018361	MT039034	MT009513	This study
M. olivascens	Unspecified	France	CBS:293.59	MT026440	—	MT025202	—	—	This study
M. prismatica	Decaying stick of *Acer saccharum*	Canada: Quebec	DAOMC:250740	MT026394	MT026508	MT018371	MT039047	MT009526	This study
	Rotten hardwood	Canada: Ontario	DAOMC:251496	MT026432	MT021458	—	—	—	This study
	Rotten wood of *Acer saccharum*	Canada: Quebec	DAOMC:251599T	MT026395	MT026509	MT018372	MT039048	MT009527	This study
M. rava	Hardwood on ground (*Betula papyrifera*?)	Canada: New Brunswick	DAOMC:250737	MT026406	MT026522	MT018357	MT039061	MT009540	This study
	Rotten branch of *Betula alleghaniensis*	Canada: New Brunswick	DAOMC:251562T	MT026407	MT026523	MT018358	MT039062	MT009541	This study
M. rosae	*Rosa canina*	Italy	CBS:230.71	MH860088	MT026518	MT018429	MT039057	MT009536	Vu *et al.* (2019), this study
Mollisia sp.	Endophyte of *Picea rubens* needle	Canada: New Brunswick	DAOMC:250742	MT026417	MT026539	MT018419	MT039078	MT009557	This study
	Decaying hardwood branch submerged in stream	Canada: New Brunswick	DAOMC:250743	MT026393	MT026507	MT018370	MT039046	MT009525	This study
	Endophyte of *Picea rubens* needle	Canada: New Brunswick	DAOMC:250744	MT026385	MT026496	MT018362	MT039035	MT009514	This study
	Decaying hardwood stick on ground	Canada: Quebec	DAOMC:250745	MT026454	—	—	—	—	This study
	Endophyte of *Picea rubens* needle	Canada: New Brunswick	DAOMC:250747	MT026431	MT026564	MT018425	MT039103	MT009582	This study
	Decaying wood	Canada: Ontario	DAOMC:251578	MT026418	MT026540	MT018422	MT039079	MT009558	This study

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Table 2. (Continued).

Name	Substrate	Location	Sequence source or specimen	GenBank accession no.					Reference
				ITS	LSU	*RPB1*	*TOP1*	*LNS2*	
	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	NB3342C	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)

Table 2. (Continued).

Name	Substrate	Location	Sequence source or specimen	GenBank accession no.					Reference
				ITS	LSU	*RPB1*	*TOP1*	*LNS2*	
	Dead wood of *Ulex europaeus*	New Zealand	TTT2238	MG195464	—	—	—	—	Johnston & Park (direct submission)
M. subcornea	*Oplopanax horridus*	Canada: British Columbia	DAOM:107202	MT026455	—	—	—	—	This study
M. undulatodepressula	Half submerged twig	France	CBS:559.63	MT026400	MT026514	MT018351	MT039053	MT009532	This study
M. ventosa	Branch of angiosperm tree	Netherlands	CBS:322.77	MT026392	MT026506	MT018369	MT039045	MT009524	This study
	Wood	Korea	KUS-F52181	JN033397	—	—	—	—	Han *et al.* (2014)
Neomollisia gelatinosa	Unspecified	Thailand	MFLU 18-0701T	NR_163788	—	—	—	—	Ekanayaka *et al.* (2019)
Neopyrenopeziza nigripigmentata	Unspecified	Thailand	MFLU 16-0599T	NR_163783	—	—	—	—	Ekanayaka *et al.* (2019)
Niptera discolor	On dead sticks in wet bog	Canada: Ontario	DAOM:86811	MT026456	—	—	—	—	This study
N. pulla	Unspecified	UK	CBS:271.53	MH857193	—	—	—	—	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
	On wood	Canada: British Columbia	DAOM:91022	MT026459	—	—	—	—	This study
Obtectodiscus aquaticus	*Carex rostrata*	Switzerland	CBS:553.79	MH872998	MT026501	MT018373	MT039040	MT009519	Vu *et al.* (2019), this study
	Carex rostrata	Switzerland	DAOM:172427T	MT026460	—	—	—	—	This study
	Carex rostrata	Switzerland	DAOM:189114T	MT026461	—	—	—	—	This study
Ombrophila hemiamyloidea	Decorticated branch in stream	Canada: New Brunswick	DAOMC:251536	MT026429	MT026561	MT018374	MT039100	MT009579	This study
Patellariopsis atrovinosa	Branch of *Cornus sanguinea*	Luxembourg	G.M. 2014-06-15-1	KY462814	—	—	—	—	Hermant (direct submission)
	Branch of *Carpinus betulus*	Luxembourg	G.M. 2016-05-04.1	KY970066	—	—	—	—	Hermant & Marson (direct submission)
P. dennisii	Wood of *Eucalyptus* sp.	France	G.M.2017-09-04.3	MK120898	—	—	—	—	Marson (direct submission)
Phialocephala amethystea	Fallen branch of *Acer saccharum*	Canada: New Brunswick	DAOMC:251552T	MT026387	MT026499	MT018412	MT039038	MT009517	This study

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Table 2. (Continued).

Name	Substrate	Location	Sequence source or specimen	GenBank accession no.					Reference
				ITS	LSU	*RPB1*	*TOP1*	*LNS2*	
	Endophyte of *Picea rubens* needle	Canada: New Brunswick	NB3824F	MT026388	MT026500	MT018413	MT039039	MT009518	This study
P. aylmerensis	Decaying hardwood on ground	Canada: Quebec	DAOMC:250106T	NR_136124	MT026489	MT018394	MT039028	MT009507	Tanney *et al.* (2016a), this study
	Decaying hardwood on ground	Canada: Quebec	DAOMC:250107	MT026379	MT026490	MT018395	MT039029	MT009508	Tanney *et al.* (2016a), this study
	Decaying log	Canada: Quebec	DAOMC: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)

Table 2. (Continued).

Name	Substrate	Location	Sequence source or specimen	GenBank accession no.					Reference
				ITS	LSU	*RPB1*	*TOP1*	*LNS2*	
	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	MT026482	MT018383	MT039021	MT009500	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	MT026546	MT018386	MT039085	MT009564	Tanney *et al.* (2016a), this study
	Dead *Acer saccharum* branch on ground	Canada: New Brunswick	DAOMC:250109	KP768359	MT026549	MT018387	MT039088	MT009567	Tanney *et al.* (2016a), this study
	Decaying *Betula alleghaniensis* branch on ground	Canada: New Brunswick	DAOMC:250110	KP768361	MT026550	MT018388	MT039089	MT009568	Tanney *et al.* (2016a), this study
	Rotten wood of *Fagus grandifolia*	Canada: Ontario	DAOMC:251593	MT026464	—	—	—	—	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	MT026372	MT026480	MT018381	MT039019	MT009498	This study
P. compacta	Living bark of *Alnus glutinosa*	Germany	CBS:507.94T	MH862480	MT026498	MT018411	MT039037	MT009516	Vu *et al.* (2019), this study
P. dimorphospora	Parchment	Portugal	a209	KT898775	—	—	—	—	de Carvalho *et al.* (2016)
	Pear tree	Greece	AXL1SP1	KX881592	—	—	—	—	Markakis *et al.* (2017)
	Fagus sylvatica leaves	Italy	CBS:112411	MT026413	MT026534	MT018421	MT039073	MT009552	This study
	Decaying hardwood on ground	Canada: Ontario	DAOMC:250111	KP768360	MT026478	MT018379	MT039017	MT009496	Tanney *et al.* (2016a), this study
	Pulp mill slime	Canada: New Brunswick	DAOMC:87232T	KP972464	MT026479	MT018380	MT039018	MT009497	Tanney *et al.* (2016a), this study
	Creosote-treated crosstie	Korea	KUC5023	GQ241290	—	—	—	—	Kim *et al.* (2010)

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Table 2. (Continued).

Name	Substrate	Location	Sequence source or specimen	GenBank accession no.					Reference
				ITS	LSU	*RPB1*	*TOP1*	*LNS2*	
	Picea abies 7-yr-old stump	Sweden	aurim1051	AY606307	—	—	—	—	Menkis *et al.* (2004)
	Picea abies 6-yr-old stump	Sweden	aurim1061	AY606305	—	—	—	—	Menkis *et al.* (2004)
	Picea abies 4-yr-old stump	Sweden	aurim1067	AY606302	—	—	—	—	Menkis *et al.* (2004)
	Picea abies 5-yr-old stump	Sweden	aurim1068	AY606308	—	—	—	—	Menkis *et al.* (2004)
	*Picea abies*trunk felled 7 years ago	Sweden	aurim107	AY606303	—	—	—	—	Menkis *et al.* (2004)
	Picea abies 5-yr-old stump	Sweden	olrim301	AY606304	—	—	—	—	Menkis *et al.* (2004)
	Picea abies 5-yr-old stump	Sweden	olrim315	AY606306	—	—	—	—	Menkis *et al.* (2004)
	Picea abies 7-yr-old stump	Sweden	olrim380	AY606309	—	—	—	—	Menkis *et al.* (2004)
	Unspecified	Unspecified	P59	AY249075	—	—	—	—	Harrington & McNew (2003)
P. europaea	Endophyte of *Picea abies* root	Switzerland	CBS:119271T	AY347399	MT026526	MT018406	MT039065	MT009544	Grünig *et al.* (2004), this study
P. fortinii	*Pinus sylvestris* root	Finland	CBS:443.86T	NR_103577	MT026530	MT018405	MT039069	MT009548	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	MT026512	MT018399	MT039051	MT009530	This study
	Decaying *Betula alleghaniensis* branch along river	Canada: New Brunswick	DAOMC:251553	MT026380	MT026491	MT018397	MT039030	MT009509	This study
	Fallen log	Canada: Ontario	DAOMC:252040	MT026466	—	—	—	—	This study
	Endophyte of *Picea rubens* needle	Canada: New Brunswick	NB36510N	MT026381	MT026492	MT018398	MT039031	MT009510	This study
	Decaying *Betula cordifolia* branch on ground	Canada: New Brunswick	NB457B	MT026399	MT026513	MT018400	MT039052	MT009531	This study

Table 2. (Continued).

Name	Substrate	Location	Sequence source or specimen	GenBank accession no.					Reference
				ITS	LSU	*RPB1*	*TOP1*	*LNS2*	
P. helvetica	Endophyte of *Picea abies* root	Switzerland	CBS:119273T	MT026409	MT026525	MT018403	MT039064	MT009543	This study
P. hiberna	Decorticated wood of *Robinia pesudoacacia*	USA: Pennsylvania	CBS:110521T	NR_119465	MT026538	MT018418	MT039077	MT009556	Bills (2004), this study
P. lagerbergii	Unspecified	Unspecified	CBS:266.33	NR_119426	—	—	—	—	McKemy *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)
	Burned *Pinus mugo* tree	Lithuania	VL274	JF440608	—	—	—	—	Lygis *et al.* (2014)
P. letzii	Endophyte of *Picea abies* root	Switzerland	CBS:119268T	AY347391	MT026527	MT018407	MT039066	MT009545	Grünig *et al.* (2004), this study
P. mallochii	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. nodosa	Decaying log of *Acer rubrum*	Canada: New Brunswick	DAOMC:250114	KP768356	—	—	—	—	Tanney *et al.* (2016a)
	Decaying *Acer saccharum* branch on ground	Canada: New Brunswick	DAOMC:250115T	NR_136121	MT026548	MT018389	MT039087	MT009566	Tanney *et al.* (2016a), this study
	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)
P. oblonga	Downed hardwood log	New Zealand	BHI-F752a	MG553996	—	—	—	—	Haelewaters *et al.* (2018)

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Table 2. (Continued).

Name	Substrate	Location	Sequence source or specimen	GenBank accession no.					Reference
				ITS	LSU	*RPB1*	*TOP1*	*LNS2*	
	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-75-2	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	NB698	MT026472	—	—	—	—	This study
	Decorticated wood	New Zealand	PRJ D1117	MG195483	—	—	—	—	Johnston & Park (direct submission)
	Decorticated wood	New Zealand	PRJ D1391	MG195480	—	—	—	—	Johnston & Park (direct submission)
	Decorticated wood	New Zealand	PRJ D1657	MG195484	—	—	—	—	Johnston & Park (direct submission)

Table 2. (Continued).

Name	Substrate	Location	Sequence source or specimen	GenBank accession no.					Reference
				ITS	LSU	*RPB1*	*TOP1*	*LNS2*	
	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*	Canada: New Brunswick	DAOMC:250101	MT026396	MT026510	MT018401	MT039049	MT009528	This study
	Decaying fallen branch of *Acer saccharum*	Canada: New Brunswick	DAOMC:250103	MT026397	MT026511	MT018402	MT039050	MT009529	This study
	Needle of *Picea abies*	Switzerland	UAMH:10851T	NR_111319	—	—	—	—	Grünig *et al.* (2009)

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Table 2. (Continued).

Name	Substrate	Location	Sequence source or specimen	GenBank accession no.					Reference
				ITS	LSU	*RPB1*	*TOP1*	*LNS2*	
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* needle	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	FJ903314	—	—	—	—	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	Di276-1	KC514879	—	—	—	—	Held & Blanchette (2017)
	Wood	Antarctica	Di84-2	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	—	—	—	—	Martínez-Álvarez *et al.* (2016)
	Pinus pinea, forest nursery	Spain	HP094	KT323172	—	—	—	—	Martínez-Álvarez *et al.* (2016)
	Wood	Iceland	ICE2-C3	KX100389	—	—	—	—	Blanchette *et al.* (2016)
	Unspecified	New Zealand	ICMP 21725	MH682239	—	—	—	—	Johnston & Park (direct submission)

Table 2. (Continued).

Name	Substrate	Location	Sequence source or specimen	GenBank accession no.					Reference
				ITS	LSU	*RPB1*	*TOP1*	*LNS2*	
	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	JS622014	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	KM042204	—	—	—	—	Arneaud *et al.* (direct submission)
	Rhizome of *Podophyllum peltatum*	USA: Delaware	PPE7	KM042204	—	—	—	—	Arneaud & 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	MT026529	MT018404	MT039068	MT009547	This study

(*continued on next page*)

Table 2. (Continued).

Name	Substrate	Location	Sequence source or specimen	GenBank accession no.					Reference
				ITS	LSU	*RPB1*	*TOP1*	*LNS2*	
P. turicensis	Endophyte of *Picea abies* root	Switzerland	CBS:119234T	JN091488	MT026531	MT018409	MT039070	MT009549	Duò *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
	Decaying wood chip of *Fraxinus* sp.	Canada: Ontario	DAOMC:251597	MT026441	—	MT025210	—	—	This study
Pyrenopeziza velebitica	*Lonicera borbasiana*	Croatia	CNF 2/10097T	NR_158942	—	—	—	—	Jadan *et al.* (direct submission)
Rhexocercosporidium panacis	*Panax quinquefolius*	Canada	DAOM:235605T	NR_119568	—	—	—	—	Reeleder *et al.* (2006)
Septonema sp.	Driftwood on beach	Canada: British Columbia	DAOMC:226875	MT026473	—	—	—	—	This study
Tapesia cinerella	Apothecium on unspecified substrate	Norway	ARON3188.H	AJ430228	—	—	—	—	Vrålstad *et al.* (2002)
	Fagus sylvatica timber	France	CBS:312.61	MH858062	—	MT025211	—	—	Vu *et al.* (2019), this study
T. fusca	Decaying twig/bark	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 alnobetula*	Switzerland	CBS:228.71	MH860087	—	MT025203	—	—	This study
Trichobelonium obscurum	*Calluna vulgaris*	Sweden	DAOM:56173	MT026474	—	—	—	—	This study
Trimmatostroma betulinum	Unspecified	Thailand	MFLU 15-2991	MK584993	—	—	—	—	Ekanayaka *et al.* (2019)
	Betula verrucosa	Netherlands	CBS:282.74	EU019299	—	—	—	—	Crous *et al.* (2007)
T. salicis	*Salix alba*	Germany	CPC 13571	EU019300	—	—	—	—	Crous *et al.* (2007)
T. salicis	Unspecified	Thailand	MFLU 18-0702	MK584996	—	—	—	—	Ekanayaka *et al.* (2019)
Trimmatostroma sp.	*Rhodiola sachalinensis*	Unspecified	CJL-2014, Rs-R-18	KJ542320	—	—	—	—	Cui *et al.* (2015)
	Rhodiola sachalinensis	Unspecified	CJL-2014, ZPRs-R-11	KJ542345	—	—	—	—	Cui *et al.* (2015)

Table 2. (Continued).

Name	Substrate	Location	Sequence source or specimen	GenBank accession no.					Reference
				ITS	LSU	*RPB1*	*TOP1*	*LNS2*	
	Unspecified	China	SGSF217	MK192902	—	—	—	—	Li & Xu (direct submission)
Uncultured *Acephala*	*Kobresia* sp.	China	C128_5	FJ378718	—	—	—	—	Gao & Yang (2010)
	Root of *Pseudorchis albida*	Czech Republic	PA A2 14	JN655570	—	—	—	—	Kohout *et al.* (2013)
Uncultured *Atheliaceae* clone	Roots of *Kobresia* sp.	China	d109c_3_1	JQ346839	—	—	—	—	Gao & Yang (direct submission)
Uncultured fungus	*Ophiocordyceps sinensis*	China	197T-2	HQ446078	—	—	—	—	Zhang *et al.* (2010)
	Wood stump	Finland	3_56	KF274391	—	—	—	—	Terhonen *et al.* (2014)
	Picea mariana forest soil, mineral horizon	USA: Alaska	3312I16	KF617331	—	—	—	—	Taylor *et al.* (2014)
	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	C82	AM260808	—	—	—	—	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	KU534827	—	—	—	—	Li *et al.* (2017)
	Quercus fabri, 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, mineral horizon	USA: Alaska	OTU501/3323D1	KF618074	—	—	—	—	Taylor *et al.* (2014)
	Vaccinium oldhamii	Japan	S31	KU551020	—	—	—	—	Baba *et al.* (2016)
	Root of *Cypripedium acaule*	USA	swc_1_1_2_L	JX857285	—	—	—	—	Bunch *et al.* (direct submission)
Uncultured *Helotiales*	*Potentilla* sp.	China	Clone 17	FJ827181	—	—	—	—	Gao & Yang (2010)
Uncultured Leotiomyceta clone	Soil	Australia	clone b5	JQ513892	—	—	—	—	Drigo *et al.* (2012)

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Table 2. (Continued).

Name	Substrate	Location	Sequence source or specimen	GenBank accession no.					Reference
				ITS	LSU	*RPB1*	*TOP1*	*LNS2*	
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	singlet6.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. filisporia	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	MT026563	MT018435	MT039102	MT009581	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	DAOM:159667	MT026475	—	—	—	—	This study
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	—	—	—	—	Grünig *et al.* (2009)
	Submerged *Populus* root	Canada: Ontario	CBS:258.91	MT026377	MT026486	MT018434	MT039025	MT009504	This study

Table 2. (Continued).

Name	Substrate	Location	Sequence source or specimen	GenBank accession no.					Reference
				ITS	LSU	*RPB1*	*TOP1*	*LNS2*	
	On twig in wet depression	Canada: Ontario	DAOM:190457	MT026476	—	—	—	—	This study
	Decaying *Alnus* branch in stream	Canada: New Brunswick	DAOMC:251541	MT026376	MT026485	MT018433	MT039024	MT009503	This study
	Unspecified	USA: Oregon	JLF2113	JX415337	—	—	—	—	Frank (direct submission)
	Unspecified	Unspecified	CUP 62562	AY789403	—	—	—	—	Wang *et al.* (2005)
V. filisporia	Unspecified	USA: North Carolina	ANM2064, ILLS60499	JQ256431	—	—	—	—	Hustad & Miller (2011)
Woollsia root associated fungus	*Woollsia pungens*	Australia	XV	AY230785	—	—	—	—	Midgley *et al.* (direct submission)

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 conducting 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*, *Obtectodiscus*, *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

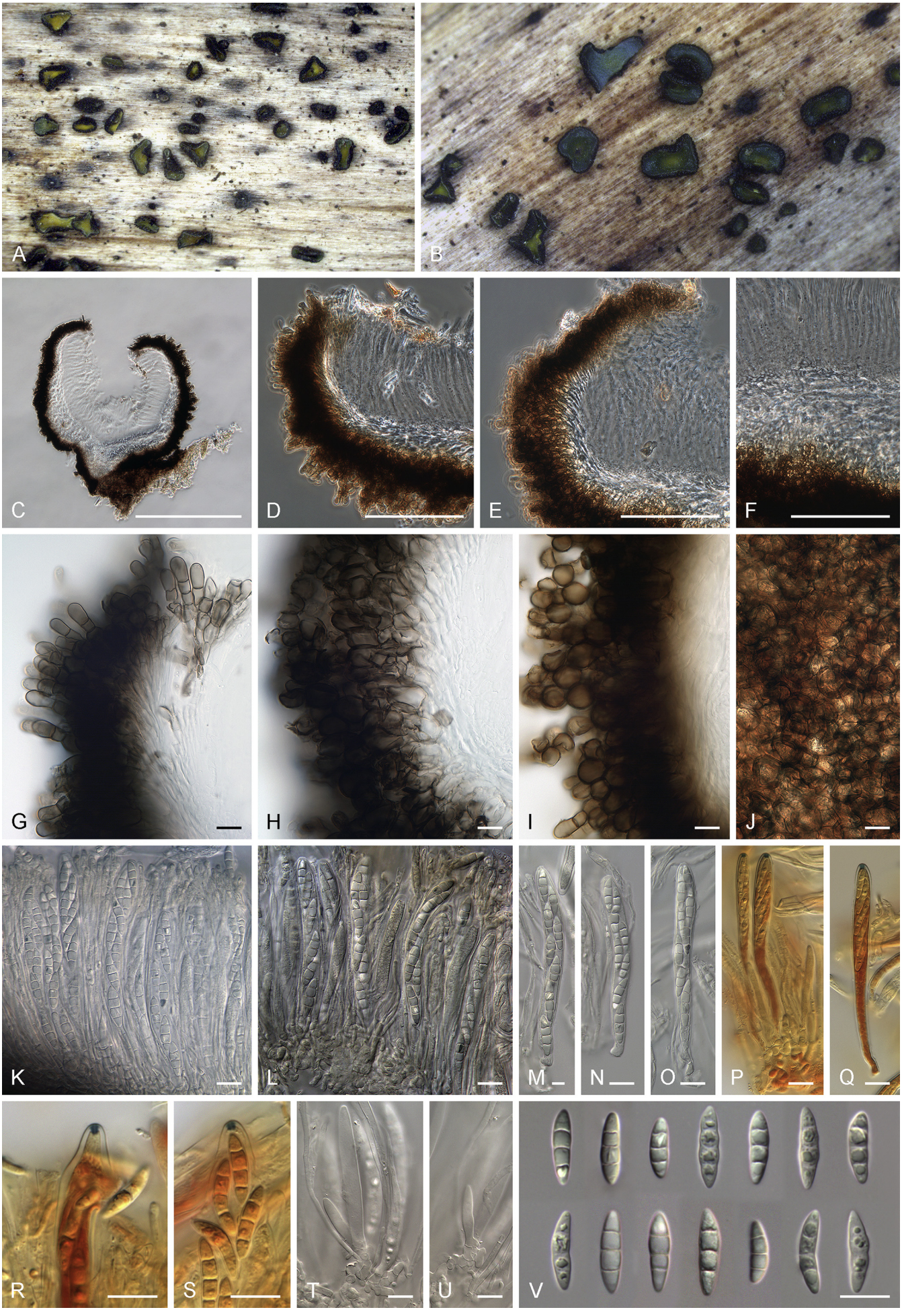
Touchdown PCR profile recommended by Stielow *et al.* (2015). The overall success rate of ITS amplification yielding usable sequences from herbarium specimen DNA was 50 % (16/32 specimens), which enabled the sequencing of several species of mollisioid genera unrepresented in GenBank, including *Hysteronaevia*, *Nipterella* (Fig. 3), and *Trichobelonium* (=*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 *Simplicillium* spp.). The oldest herbarium specimens successfully sequenced were *Vibrissea vibrisseoides* (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, *Vibrissea* is basal to *Mollisiaceae* 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 applanata* 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 weaklier 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. rosae*, and *P. vermiculata 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 *Molisia* 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*, *Obtectodiscus*, and *Ombrophila hemiamyloidea*, 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. hemiamyloidea* 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 *Vibrissea* outside of the main *Mollisiaceae* lineage. *Mollisia sensu lato* is polyphyletic, with species in genera including *Acephala*, *Acidomelania*, *Barrenia*, *Cystodendron*, *Loramyces*, *Niptera*, *Obtectodiscus*, *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. novobrunsvicensis 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 *Mollisiaceae*, 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. nigrescens*. *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
		Parsimony-informative	Singleton	Constant	Total		
ITS	88	304	57	412	773	GTR+I+G	*Mollisia dextrinospora*
LNS2	88	131	9	141	281	HKY+G	*M. dextrinospora*
LSU	88	274	51	1 649	1 974	GTR+I+G	*M. dextrinospora*
RPB1	88	323	33	466	822	GTR+I+G	*M. dextrinospora*
TOP1	88	398	49	423	870	GTR+I+G	*M. dextrinospora*
ITS-LSU-RPB1-TOP1	88	1 541	187	2 492	4 220	GTR+I+G	*M. dextrinospora*
RPB1 expanded	109	346	51	426	823	GTR+I+G	*Chlorencoelia versiformis*
ITS expanded	207	423	87	388	898	SYM+I+G4	*C. versiformis*
ITS *Barrenia*-grass clade	149	145	138	314	597	SYM+I+G4	*M. dextrinospora*
ITS *P. dimorphospora* clade	95	73	109	428	610	TNe+I+G4	*M. dextrinospora*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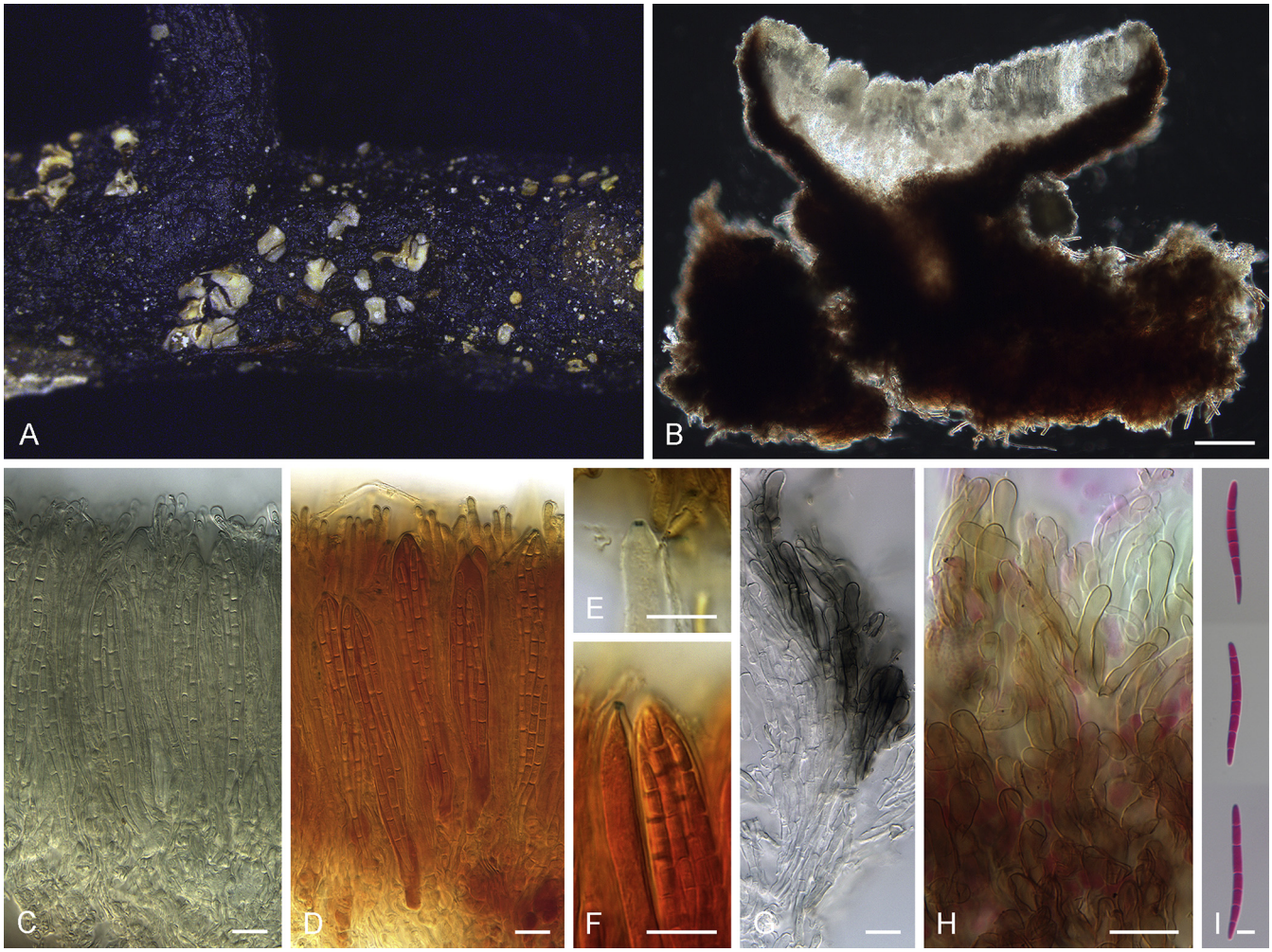


Fig. 4. *Mollisia obscura* DAOM 51673. **A.** Rehydrated apothecia on *Calluna vulgaris* root. **B.** Vertical section of apothecium. **C.** Asci and paraphyses. **D–F.** Ascus with amyloid tip in Lugol's solution after KOH pretreatment. **G.** Marginal cells. **H.** Margin in lactofuchsin mount. **I.** Ascospores in lactofuchsin mount. Scale bars: B = 100 µm, C–H = 10 µm, I = 5 µm.

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 *Hysteronaevia scirpina* (DAOM 147320), *Nipterella parksii* (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 panicicola*. Clade 1 is strongly supported (SH-aLRT 99 %, BS 100 %) and includes *Mollisia s.s.* with a subclade composed of morphologically

Fig. 3. *Nipterella parksii* DAOM 56610. **A, B.** Apothecia on *Alnus rubra* wood. **C.** Vertical section of apothecium. **D, E.** Margin of apothecia. **F.** Ectal and medullary excipula. **G–I.** Marginal cells. **J.** Ectal excipulum cells. **K, L.** Asci and paraphyses. **M–O.** Asci. **P–S.** Asci with amyloid tips in Lugol's solution after KOH pretreatment. **T, U.** Croziers at base of asci. **V.** Ascospores. Scale bars: C = 500 µm, D–F = 100 µm, G–V = 10 µm.

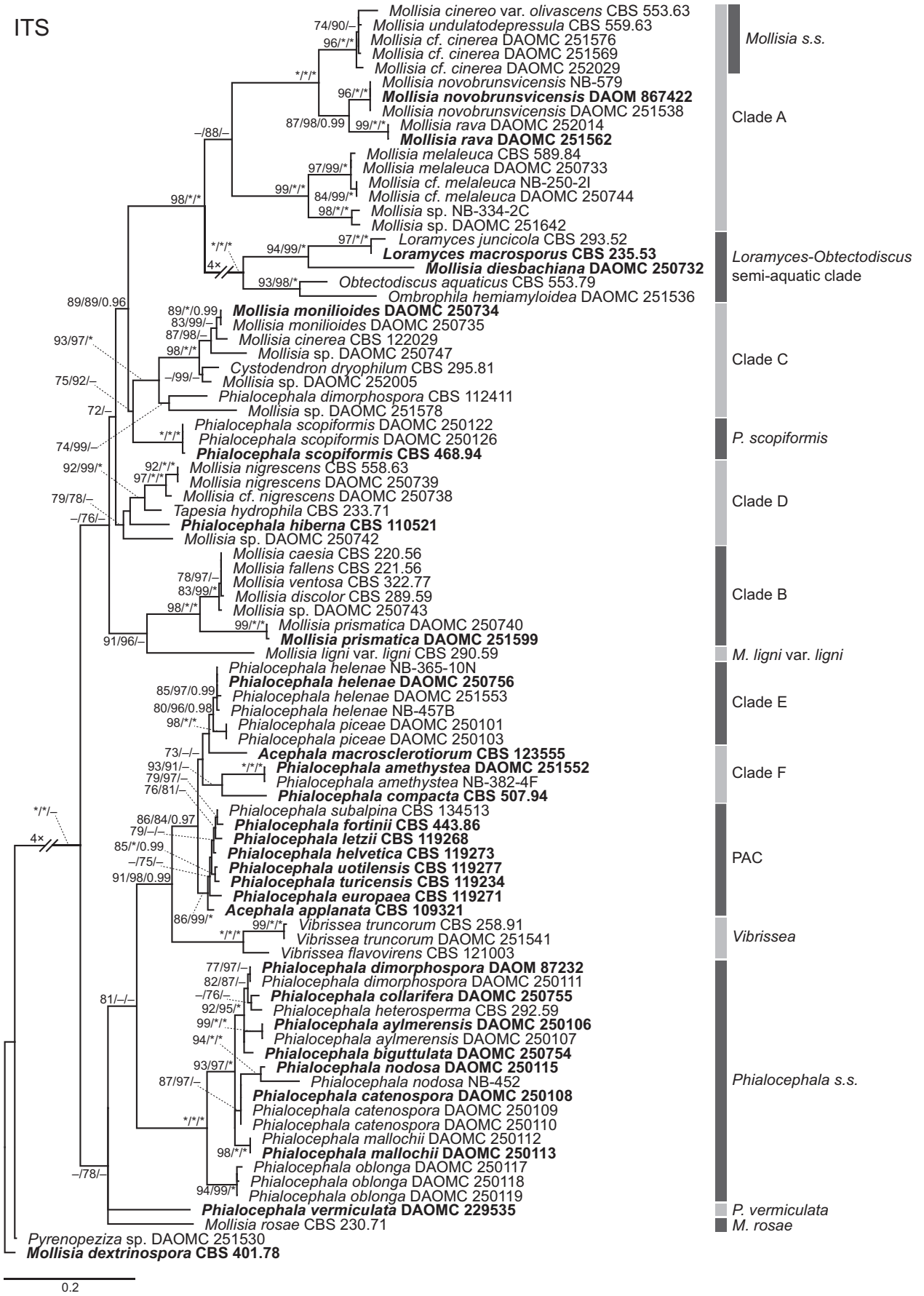


Fig. 5. A 50 % majority-rule consensus tree obtained from the maximum likelihood analysis based on ITS sequences containing representative *Mollisia* and allied taxa for comparing ITS, LSU, *LNS2*, *RPB1*, and *TOP1* phylogenies. Culture collection (DAOMC, CBS) or JB Tanney personal collection accession 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. The tree is rooted with the ex-type of *Mollisia dextrinospora* (CBS 401.78).

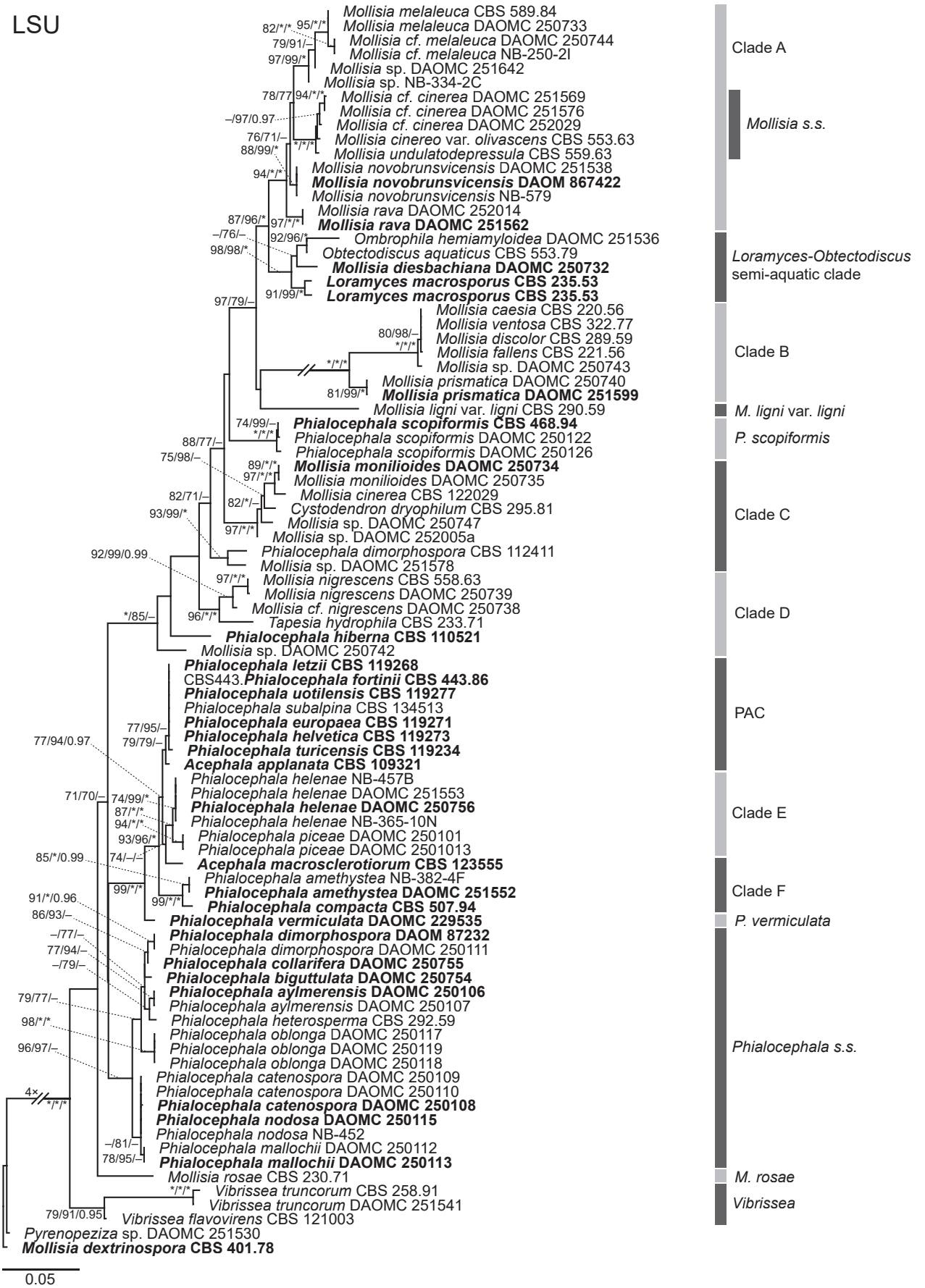


Fig. 6. A 50 % majority-rule consensus tree obtained from the maximum likelihood analysis based on LSU sequences containing representative *Mollisia* and allied taxa for comparing ITS, LSU, *LNS2*, *RPB1*, and *TOP1* phylogenies. Culture collection (DAOMC, CBS) or JB Tanney personal collection accession 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).

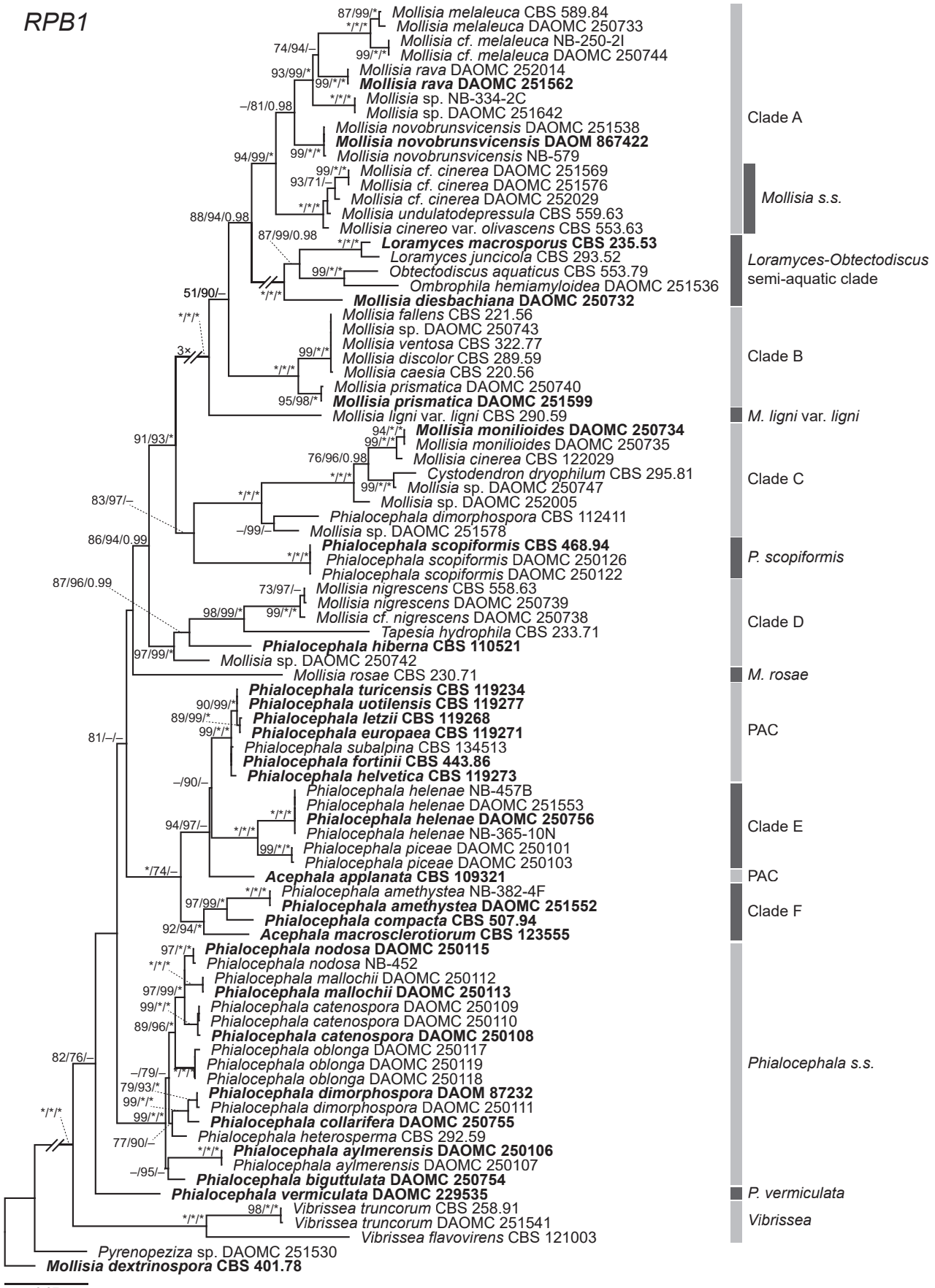
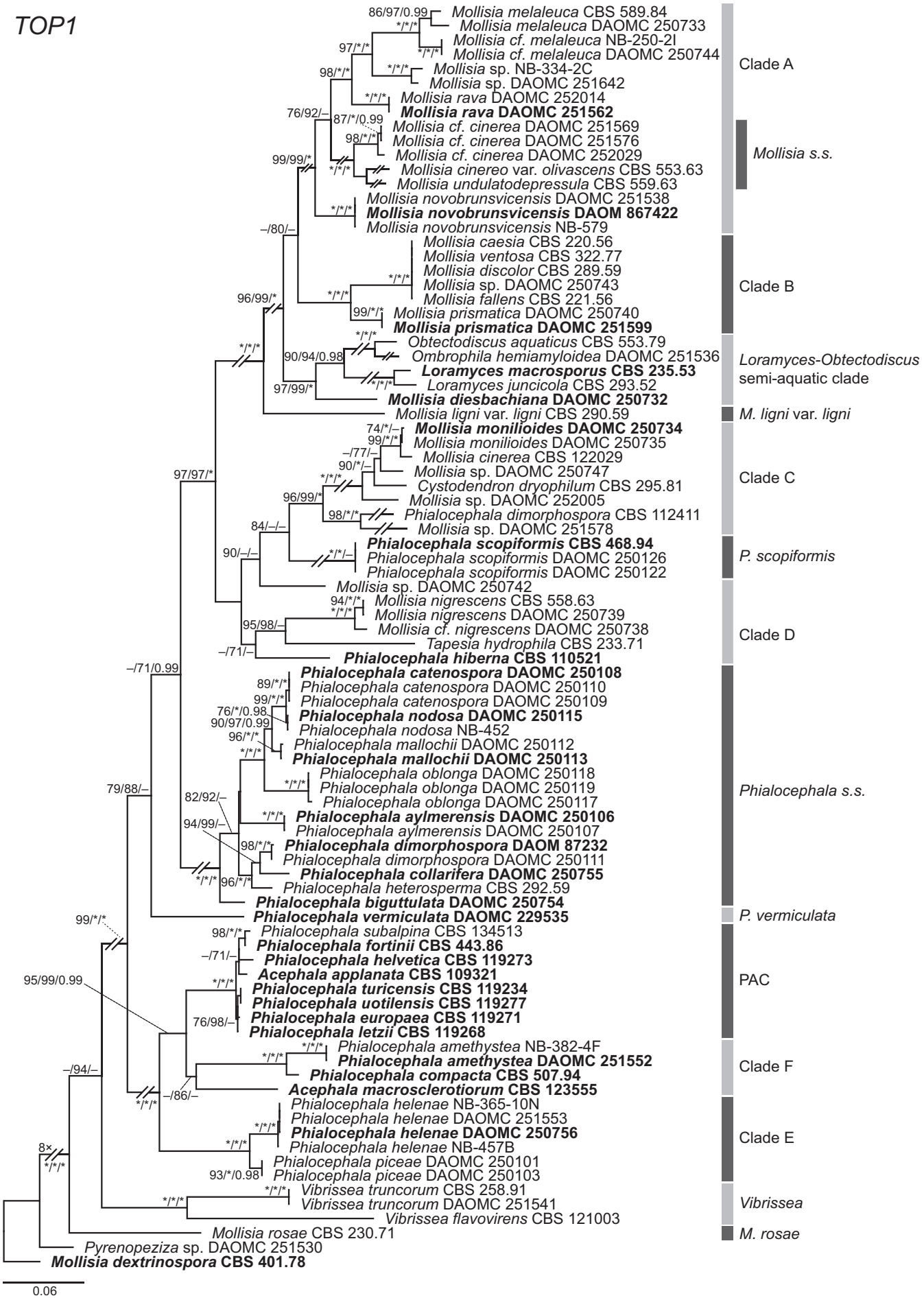


Fig. 7. A 50 % majority-rule consensus tree obtained from the maximum likelihood analysis based on *RPB1* sequences containing representative *Mollisia* and allied taxa for comparing ITS, LSU, *LNS2*, *RPB1*, and *TOP1* phylogenies. Culture collection (DAOMC, CBS) or JB Tanney personal collection accession 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).


TOP1
Mollisia melaleuca CBS 589.84
Mollisia melaleuca DAOMC 250733
Mollisia cf. melaleuca NB-250-2I
Mollisia cf. melaleuca DAOMC 250744
Mollisia sp. NB-334-2C
Mollisia sp. DAOMC 251642
Mollisia rava DAOMC 252014
Mollisia rava DAOMC 251562
Mollisia cf. cinerea DAOMC 251569
Mollisia cf. cinerea DAOMC 251576
Mollisia cf. cinerea DAOMC 252029
Mollisia cinereo var. olivascens CBS 553.63
Mollisia undulatodepressula CBS 559.63
Mollisia novobrunsvicensis DAOMC 251538
Mollisia novobrunsvicensis DAOM 867422
Mollisia novobrunsvicensis NB-579
Mollisia caesia CBS 220.56
Mollisia ventosa CBS 322.77
Mollisia discolor CBS 289.59
Mollisia sp. DAOMC 250743
Mollisia fallens CBS 221.56
Mollisia prismatica DAOMC 250740
Mollisia prismatica DAOMC 251599
Obtectodiscus aquaticus CBS 553.79
Ombrophila hemiamyloidea DAOMC 251536
Loramyces macrosporus CBS 235.53
Loramyces juncicola CBS 293.52
Mollisia diesbachiana DAOMC 250732
Mollisia ligni var. ligni CBS 290.59
Mollisia monilioides DAOMC 250734
Mollisia monilioides DAOMC 250735
Mollisia cinerea CBS 122029
Mollisia sp. DAOMC 250747
Cystodendron dryophilum CBS 295.81
Mollisia sp. DAOMC 252005
Phialocephala dimorphospora CBS 112411
Mollisia sp. DAOMC 251578
Phialocephala scopiformis CBS 468.94
Phialocephala scopiformis DAOMC 250126
Phialocephala scopiformis DAOMC 250122
Mollisia sp. DAOMC 250742
Mollisia nigrescens CBS 558.63
Mollisia nigrescens DAOMC 250739
Mollisia cf. nigrescens DAOMC 250738
Tapesia hydrophila CBS 233.71
Phialocephala hiberna CBS 110521
Phialocephala catenospora DAOMC 250108
Phialocephala catenospora DAOMC 250110
Phialocephala catenospora DAOMC 250109
Phialocephala nodosa DAOMC 250115
Phialocephala nodosa NB-452
Phialocephala mallochii DAOMC 250112
Phialocephala mallochii DAOMC 250113
Phialocephala oblonga DAOMC 250118
Phialocephala oblonga DAOMC 250119
Phialocephala oblonga DAOMC 250117
Phialocephala aylmerensis DAOMC 250106
Phialocephala aylmerensis DAOMC 250107
Phialocephala dimorphospora DAOM 87232
Phialocephala dimorphospora DAOMC 250111
Phialocephala collarifera DAOMC 250755
Phialocephala heterosperma CBS 292.59
Phialocephala biguttulata DAOMC 250754
Phialocephala vermiculata DAOMC 229535
Phialocephala subalpina CBS 134513
Phialocephala fortinii CBS 443.86
Phialocephala helvetica CBS 119273
Acephala applanata CBS 109321
Phialocephala turicensis CBS 119234
Phialocephala uotilensis CBS 119277
Phialocephala europaea CBS 119271
Phialocephala letzii CBS 119268
Phialocephala amethystea NB-382-4F
Phialocephala amethystea DAOMC 251552
Phialocephala compacta CBS 507.94
Acephala macrosclerotiorum CBS 123555
Phialocephala helenae NB-365-10N
Phialocephala helenae DAOMC 251553
Phialocephala helenae DAOMC 250756
Phialocephala helenae NB-457B
Phialocephala piceae DAOMC 250101
Phialocephala piceae DAOMC 250103
Vibrissea truncorum CBS 258.91
Vibrissea truncorum DAOMC 251541
Vibrissea flavovirens CBS 121003
Mollisia rosae CBS 230.71
Pyrenopeziza sp. DAOMC 251530
Mollisia dextrinospora CBS 401.78
0.06
Clade A
Mollisia s.s.
Clade B
Loramyces-Obtectodiscus semi-aquatic clade
M. ligni var. ligni
Clade C
P. scopiformis
Clade D
Phialocephala s.s.
P. vermiculata
PAC
Clade F
Clade E
Vibrissea
M. rosae

divergent species associated with aquatic or moist environments (*Obtectodiscus aquaticus*, *Hysteronaevia scirpina*, *Ombrophila hemiamyloidea*, *Pulvinata tomentosa*, *Loramyces*, and *M. fuscoparaphysata*). *Vibrissea* forms a well-supported (SH-aLRT 100 %, BS 95 %) clade (Clade 6) including *V. brevistipitata* (=*V. cesatii*; Baral *et al.* 2019), *V. filisporia*, *V. flavovirens*, *V. pezizoides*, *V. truncorum*, *V. vibrisseoides*, and unidentified *Vibrissia* species. Based on ITS, *Vibrissea* is placed within *Mollisiaceae*, sister to Clade 7 consisting of species currently or previously placed in *Niptera* (*N. discolor*, *N ramincola*, *M. caesia*, *M. ventosa*). The PAC, *Cheirospora botryospora*, *P. compacta*, *P. piceae*, *P. amethystea*, *P. helenae sp. nov.*, and *A. macrosclerotiorum* form Clade 8 (SH-aLRT 92 %, BS 100 %). A strain identified as *Septonema* sp. (DAOMC 226875; Fig. 17) that produces unbranched and simply branched acropetal chains of brown, cylindrical, (1–)2–3(–5)-septate conidia with flat, protruding hila is a *Mollisiaceae* sp. placed near *P. vermiculata* with low support (SH-aLRT = 82 %, BS = 80 %; Clade 10). *Neopyrenopeziza nigripigmentata*, the recently described type species of the monotypic genus, is closely related to *Patellariopsis dennisii* (NR_163783, MK120898; identities = 539/546 *i.e.* 99 %, no gaps; Clade 2). *Mollisia rosae*, which was variously placed within or basal to *Mollisiaceae* in the gene comparison phylogenies, is closest related to *Pyrenopeziza velebitica* (NR_158942; identities = 513/539 *i.e.* 95 %, gaps = 8/539 *i.e.* 1 %); both species are apparently outside both *Mollisia* and *Pyrenopeziza* (Clade 11).

In the ITS phylogenetic tree (Fig. 16), the *Barrenia*-grass clade and *Phialocephala s.s.* were collapsed. A focused ITS phylogeny of the *Barrenia* clade consisted of 149 sequences derived from diverse hosts and geographic locations (Fig. 18). Most sequences originated from unidentified strains isolated as endophytes from grasses and sedges (*Poales*), conifers, orchids (*Orchidaceae*), and *Ericaceae*. Identified sequences were typically from apothecial collections, for example *Mollisia epitypha* from a dead stem of *Typha latifolia* (*Typhaceae*, *Poales*) collected in Canada, *M. nigrescens* from decaying hardwood in Canada and France, *Mollisia obscurum* from *Calluna vulgaris* (*Ericaceae*), and *Mollisia hydrophila* from *Phragmites australis* (*Poaceae*) in France. The clade includes ex-types of *Phialocephala bamuru* from *Cynodon dactylon* (*Poaceae*) root in Australia, *Phialocephala urceolata* from heparin solution in the USA, *Barrenia taeda* isolated as a root endophyte from *Pinus rigida* (*Pinaceae*) in the USA, and *B. panicia* isolated as a root endophyte of *Panicum virgatum* (*Poaceae*) in the USA. Based on ITS sequences, it appears that the unknown sexual state of *P. bamuru* was discovered from collections of apothecia on dead leaves of *Baumea* sp. in New Zealand.

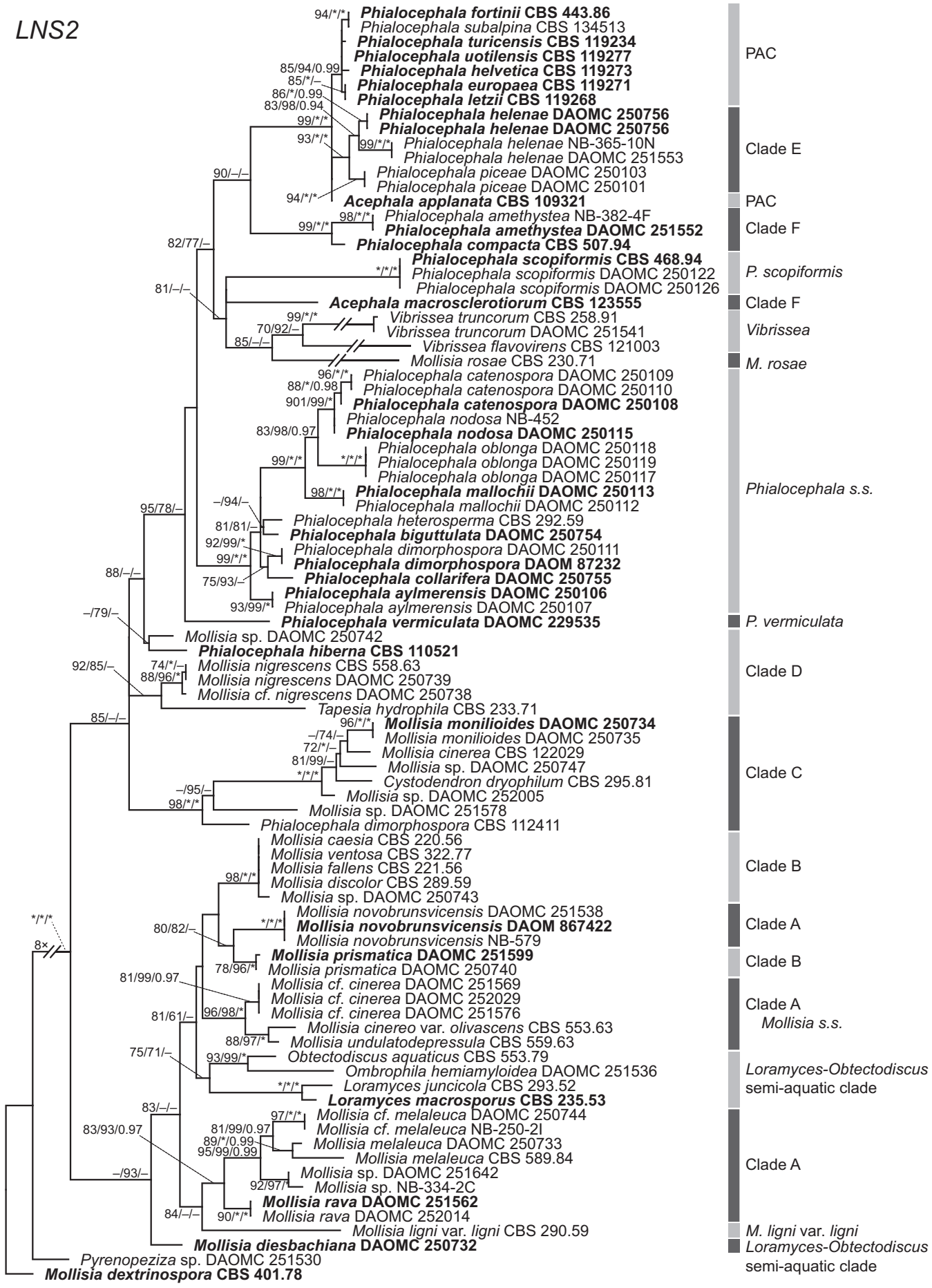
The ITS phylogeny of *Phialocephala s.s.* included 95 sequences, most of which belonged to *P. oblonga* (Fig. 19, Clade VI). *Phialocephala oblonga* shows a wide geographic range, represented by apothecial collections from decaying wood including *Nothofagus* and unidentified hosts in New Zealand, hardwoods such as *Betula* spp. and *Acer saccharum* in Canada, *Picea abies* stumps in Sweden, and dead attached branches of *Fagus sylvatica* in Germany. Overall, most sequences derive from strains associated with decomposing wood, which differentiates *P. dimorphospora s.s.* from other *Mollisiaceae* clades containing diverse endophytes. Based on phylogeny results and morphology, we synonymise *Fuscosclera* with *Phialocephala* and make the new combination *P. heterosperma* below. Results from the ITS phylogenies of the *Barrenia* grass clade and *P. dimorphospora s.s.* are discussed in further detail below.

The comparison of LSU sequences between selected species placed within *Acephala*, *Loramyces*, *Mollisia*, *cf. Niptera*, *Obtectodiscus*, *Phialocephala*, *Pyrenopeziza*, and *Vibrissea* using an 843 bp intron region of the LSU provides an exercise in delineating generic and family boundaries using the threshold values of Vu *et al.* (2019) as a guide (Fig. 20). Applying these criteria, *Phialocephala subalpina*, *Acephala macrosclerotiorum*, and *P. piceae* are congeric and constitute the PAC, with *P. compacta* showing conflicting placement based on a below-threshold similarity with *P. piceae* (98.09 %). *Phialocephala dimorphospora* and *cf. Niptera* sp. (DAOMC 250748) are congeneric (98.8 %) and distinct from the PAC and *Mollisia s.s.*, *Loramyces juncicola*, *Obtectodiscus aquaticus*, and *Mollisia diesbachiana* are congeneric and placed within a family distinct from the PAC-*P. compacta* clade. *Mollisia diesbachiana* and *M. cinereo* var. *olivascens* show some conflict with the *Loramyces* clade by their closer similarity to *P. dimorphospora*. *Phialocephala scopiformis* appears to be in its own distinct genus. *Vibrissea* is outside *Mollisiaceae*, however the basal species *V. flavovirens* shows a significantly higher similarity to *P. dimorphospora* and *cf. Niptera* sp. (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 *Spinulum annotinum* in Poland (JX981467; identities = 530/532 *i.e.* 99 %, gaps = 2/532), *Nothofagus solandri* in New Zealand (JN225881; identities = 529/531 *i.e.* 99 %, no gaps), and *Picea abies* in Finland (EF592102; identities = 526/530 *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

Fig. 8. A 50 % majority-rule consensus tree obtained from the maximum likelihood analysis based on *TOP1* 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).


LNS2
Phialocephala fortinii CBS 443.86
Phialocephala subalpina CBS 134513
Phialocephala turicensis CBS 119234
Phialocephala uotilensis CBS 119277
Phialocephala helvetica CBS 119273
Phialocephala europaea CBS 119271
Phialocephala letzii CBS 119268
Phialocephala helenae DAOMC 250756
Phialocephala helenae DAOMC 250756
Phialocephala helenae NB-365-10N
Phialocephala helenae DAOMC 251553
Phialocephala piceae DAOMC 250103
Phialocephala piceae DAOMC 250101
Acephala applanata CBS 109321
Phialocephala amethystea NB-382-4F
Phialocephala amethystea DAOMC 251552
Phialocephala compacta CBS 507.94
Phialocephala scopiformis CBS 468.94
Phialocephala scopiformis DAOMC 250122
Phialocephala scopiformis DAOMC 250126
Acephala macrosclerotiorum CBS 123555
Vibrissea truncorum CBS 258.91
Vibrissea truncorum DAOMC 251541
Vibrissea flavovirens CBS 121003
Mollisia rosae CBS 230.71
Phialocephala catenospora DAOMC 250109
Phialocephala catenospora DAOMC 250110
Phialocephala catenospora DAOMC 250108
Phialocephala nodosa NB-452
Phialocephala nodosa DAOMC 250115
Phialocephala oblonga DAOMC 250118
Phialocephala oblonga DAOMC 250119
Phialocephala oblonga DAOMC 250117
Phialocephala mallochii DAOMC 250113
Phialocephala mallochii DAOMC 250112
Phialocephala heterosperma CBS 292.59
Phialocephala biguttulata DAOMC 250754
Phialocephala dimorphospora DAOMC 250111
Phialocephala dimorphospora DAOM 87232
Phialocephala collarifera DAOMC 250755
Phialocephala aylmerensis DAOMC 250106
Phialocephala aylmerensis DAOMC 250107
Phialocephala vermiculata DAOMC 229535
Mollisia sp. DAOMC 250742
Phialocephala hiberna CBS 110521
Mollisia nigrescens CBS 558.63
Mollisia nigrescens DAOMC 250739
Mollisia cf. nigrescens DAOMC 250738
Tapesia hydrophila CBS 233.71
Mollisia monilioides DAOMC 250734
Mollisia monilioides DAOMC 250735
Mollisia cinerea CBS 122029
Mollisia sp. DAOMC 250747
Cystodendron dryophilum CBS 295.81
Mollisia sp. DAOMC 252005
Mollisia sp. DAOMC 251578
Phialocephala dimorphospora CBS 112411
Mollisia caesia CBS 220.56
Mollisia ventosa CBS 322.77
Mollisia fallens CBS 221.56
Mollisia discolor CBS 289.59
Mollisia sp. DAOMC 250743
Mollisia novobrunsvicensis DAOMC 251538
Mollisia novobrunsvicensis DAOM 867422
Mollisia novobrunsvicensis NB-579
Mollisia prismatica DAOMC 251599
Mollisia prismatica DAOMC 250740
Mollisia cf. cinerea DAOMC 251569
Mollisia cf. cinerea DAOMC 252029
Mollisia cf. cinerea DAOMC 251576
Mollisia cinereo var. olivascens CBS 553.63
Mollisia undulatodepressula CBS 559.63
Obtectodiscus aquaticus CBS 553.79
Ombrophila hemiamyloidea DAOMC 251536
Loramyces juncicola CBS 293.52
Loramyces macrosporus CBS 235.53
Mollisia cf. melaleuca DAOMC 250744
Mollisia cf. melaleuca NB-250-2I
Mollisia melaleuca DAOMC 250733
Mollisia melaleuca CBS 589.84
Mollisia sp. DAOMC 251642
Mollisia sp. NB-334-2C
Mollisia rava DAOMC 251562
Mollisia rava DAOMC 252014
Mollisia ligni var. ligni CBS 290.59
Mollisia diesbachiana DAOMC 250732
Pyrenopeziza sp. DAOMC 251530
Mollisia dextrinospora CBS 401.78
0.07
PAC
Clade E
PAC
Clade F
P. scopiformis
Clade F
Vibrissea
M. rosae
Phialocephala s.s.
P. vermiculata
Clade D
Clade C
Clade B
Clade A
Clade B
Clade A
Mollisia s.s.
Loramyces-Obtectodiscus
semi-aquatic clade
Clade A
M. ligni var. ligni
Loramyces-Obtectodiscus
semi-aquatic clade

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), *Ledum palustre* roots in China (KJ817294; *cf.* FR837920: identities = 521/522 i.e. 99 %, gaps = 1/522), *Picea abies* wood in Sweden (DQ008242; *cf.* FR837920: identities = 483/483 *i.e.* 100 %, no gaps), and *Pinus sylvestris* roots in Finland (KM068419; *cf.* FR837920: identities = 489/490 *i.e.* 99 %, gaps = 1/490).

Phialocephala scopiformis, reported only from *Picea*, is closely related to a strain isolated as a leaf endophyte of *Calluna vulgaria* (FM200586; identities = 493/495 *i.e.* 99 %, no gaps). *Mollisia fusca* (CBS 234.71), isolated from apothecia on *Fagus sylvatica* in Switzerland, shares a similar ITS sequence with a *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; *cf.* CBS 558.63: identities = 858/858 *i.e.* 100 %, no gaps) and from a senescent *Tsuga canadensis* needle (KX908506; *cf.* 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 *Lonicera 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 amethystea* 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 (AY268211; 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 mollisioid genus *Trichobelonium* (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 subiculum (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* (KJ877190; 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.*, KJ877190 and MG195533; 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*

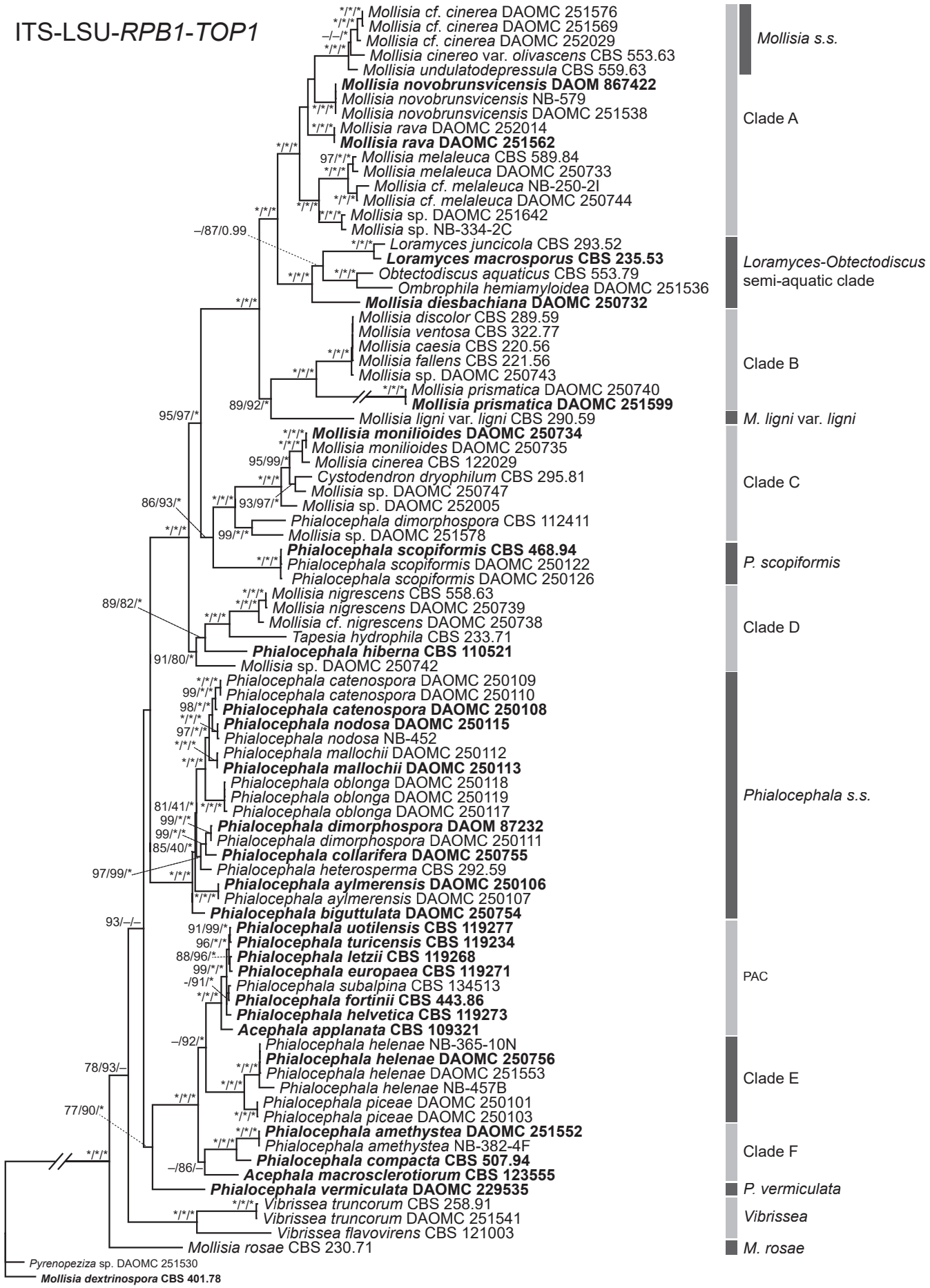
Many strains were sterile despite repeated attempts to induce sporulation, including different media and substrates and incubation at various temperatures over prolonged periods of time. Sporulation was successfully induced in recalcitrant cultures, but this usually required prolonged incubation time 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* micronematous to macronematous, 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(–6) × 2.5–3 µm, occurring singly or in whorls of 2–3(–4) from metulae. *Metulae* hyaline to subhyaline or pale brown, cylindrical to doliiform, (4.5–)5–6.5(–7.5) × 3–4(–5) µm. *Conidia* dimorphic; primary conidia oblong to oblong-ellipsoidal, hyaline, (4.5–)

Fig. 9. A 50 % majority-rule consensus tree obtained from the maximum likelihood analysis based on *LNS2* 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).


ITS-LSU-*RPB1*-*TOP1*
Mollisia cf. cinerea DAOMC 251576
Mollisia cf. cinerea DAOMC 251569
Mollisia cf. cinerea DAOMC 252029
Mollisia cinereo var. *olivascens* CBS 553.63
Mollisia undulatodepressula CBS 559.63
***Mollisia novobrunsvicensis* DAOM 867422**
Mollisia novobrunsvicensis NB-579
Mollisia novobrunsvicensis DAOMC 251538
Mollisia rava DAOMC 252014
***Mollisia rava* DAOMC 251562**
Mollisia melaleuca CBS 589.84
Mollisia melaleuca DAOMC 250733
Mollisia cf. melaleuca NB-250-2I
Mollisia cf. melaleuca DAOMC 250744
Mollisia sp. DAOMC 251642
Mollisia sp. NB-334-2C
Loramyces juncicola CBS 293.52
***Loramyces macrosporus* CBS 235.53**
Obtectodiscus aquaticus CBS 553.79
Ombrophila hemiamyloidea DAOMC 251536
***Mollisia diesbachiana* DAOMC 250732**
Mollisia discolor CBS 289.59
Mollisia ventosa CBS 322.77
Mollisia caesia CBS 220.56
Mollisia fallens CBS 221.56
Mollisia sp. DAOMC 250743
Mollisia prismatica DAOMC 250740
***Mollisia prismatica* DAOMC 251599**
Mollisia ligni var. *ligni* CBS 290.59
***Mollisia monilioides* DAOMC 250734**
Mollisia monilioides DAOMC 250735
Mollisia cinerea CBS 122029
Cystodendron dryophilum CBS 295.81
Mollisia sp. DAOMC 250747
Mollisia sp. DAOMC 252005
Phialocephala dimorphospora CBS 112411
Mollisia sp. DAOMC 251578
***Phialocephala scopiformis* CBS 468.94**
Phialocephala scopiformis DAOMC 250122
Phialocephala scopiformis DAOMC 250126
Mollisia nigrescens CBS 558.63
Mollisia nigrescens DAOMC 250739
Mollisia cf. nigrescens DAOMC 250738
Tapesia hydrophila CBS 233.71
***Phialocephala hiberna* CBS 110521**
Mollisia sp. DAOMC 250742
Phialocephala catenospora DAOMC 250109
Phialocephala catenospora DAOMC 250110
***Phialocephala catenospora* DAOMC 250108**
***Phialocephala nodosa* DAOMC 250115**
Phialocephala nodosa NB-452
Phialocephala mallochii DAOMC 250112
***Phialocephala mallochii* DAOMC 250113**
Phialocephala oblonga DAOMC 250118
Phialocephala oblonga DAOMC 250119
Phialocephala oblonga DAOMC 250117
***Phialocephala dimorphospora* DAOM 87232**
Phialocephala dimorphospora DAOMC 250111
***Phialocephala collarifera* DAOMC 250755**
Phialocephala heterosperma CBS 292.59
***Phialocephala aylmerensis* DAOMC 250106**
Phialocephala aylmerensis DAOMC 250107
***Phialocephala biguttulata* DAOMC 250754**
***Phialocephala uotilensis* CBS 119277**
***Phialocephala turicensis* CBS 119234**
***Phialocephala letzii* CBS 119268**
***Phialocephala europaea* CBS 119271**
Phialocephala subalpina CBS 134513
***Phialocephala fortinii* CBS 443.86**
***Phialocephala helvetica* CBS 119273**
***Acephala applanata* CBS 109321**
Phialocephala helenae NB-365-10N
***Phialocephala helenae* DAOMC 250756**
Phialocephala helenae DAOMC 251553
Phialocephala helenae NB-457B
Phialocephala piceae DAOMC 250101
Phialocephala piceae DAOMC 250103
***Phialocephala amethystea* DAOMC 251552**
Phialocephala amethystea NB-382-4F
***Phialocephala compacta* CBS 507.94**
***Acephala macrosclerotiorum* CBS 123555**
***Phialocephala vermiculata* DAOMC 229535**
Vibrissea truncorum CBS 258.91
Vibrissea truncorum DAOMC 251541
Vibrissea flavovirens CBS 121003
Mollisia rosae CBS 230.71
Pyrenopeziza sp. DAOMC 251530
***Mollisia dextrinospora* CBS 401.78**
0.1
Mollisia s.s.
Clade A
Loramyces-Obtectodiscus semi-aquatic clade
Clade B
M. ligni var. *ligni*
Clade C
P. scopiformis
Clade D
Phialocephala s.s.
PAC
Clade E
Clade F
P. vermiculata
Vibrissea
M. rosae

5–6(–6.5) × (1.5–)2 µm; secondary conidia globose to subglobose, 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.

Typus: **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 DAOMC 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, ampuliform, (7–)9–13.5(–17.5) × (2.5–)3–4(–4.5) µm, collarettes cylindrical to doliiform, (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 ellipsoidal 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 maturity, outline entire, dull to dark blue (21D5–21F4), outer surface bluish grey toward base (21F2), 1.5–2.5 mm diam, 0.2–0.4 mm high, margin frequently paler color to white when younger, smooth, subiculum not evident. *Ectal excipulum* at base and mid flanks *textura globulosa* to *angularis*, 60–180 µm thick near 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 *prismatica*, 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 (5E4) 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 asci. KOH reaction negative. *Asci* arising from croziers, cylindrical-clavate, 8-spored, (62–)65–71(–74) × (5–)5.5–7 µm, *pars sporifera* 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, septate, 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*, *Obtectodiscus aquaticus*, and *Ombrophila hemiamyloidea*.

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

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

Typus: **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 DAOMC 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 conidiophores. *Conidiogenous cells* phialidic, terminal, sometimes intercalary; ampuliform, (7.5–)9.5–12(–13) × (2–)2.5–4 µm; collarettes cylindrical to doliiform 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 percurrently from aperture of proximal phialides or converting into cylindrical non-functional phialides in the sense of Day *et al.* (2012). *Metulae*

Fig. 10. A 50 % majority-rule consensus tree obtained from the maximum likelihood analysis based on concatenated ITS, LSU, *RPB1*, and *TOP1* sequences 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. Clades are labelled for convenience. The tree is rooted with the ex-type of *Mollisia dextrinospora* (CBS 401.78).

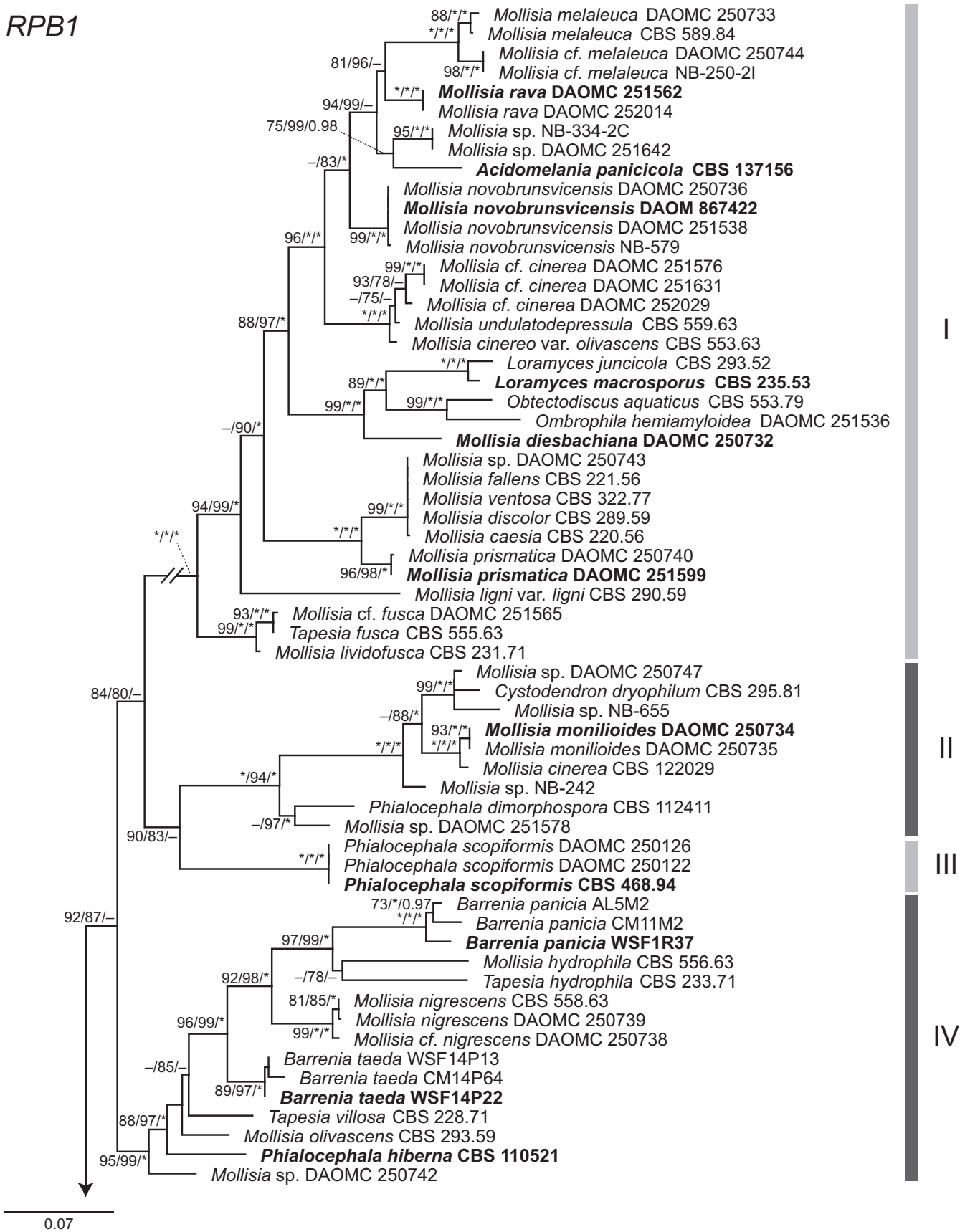


Fig. 11. A 50 % majority-rule consensus tree obtained from the maximum likelihood analysis based on the expanded *RPB1* sequence dataset containing representative *Mollisia* and allied taxa. Culture collection (DAOMC, CBS) or JB Tanney personal collection accession numbers (NB) follow the species name (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).

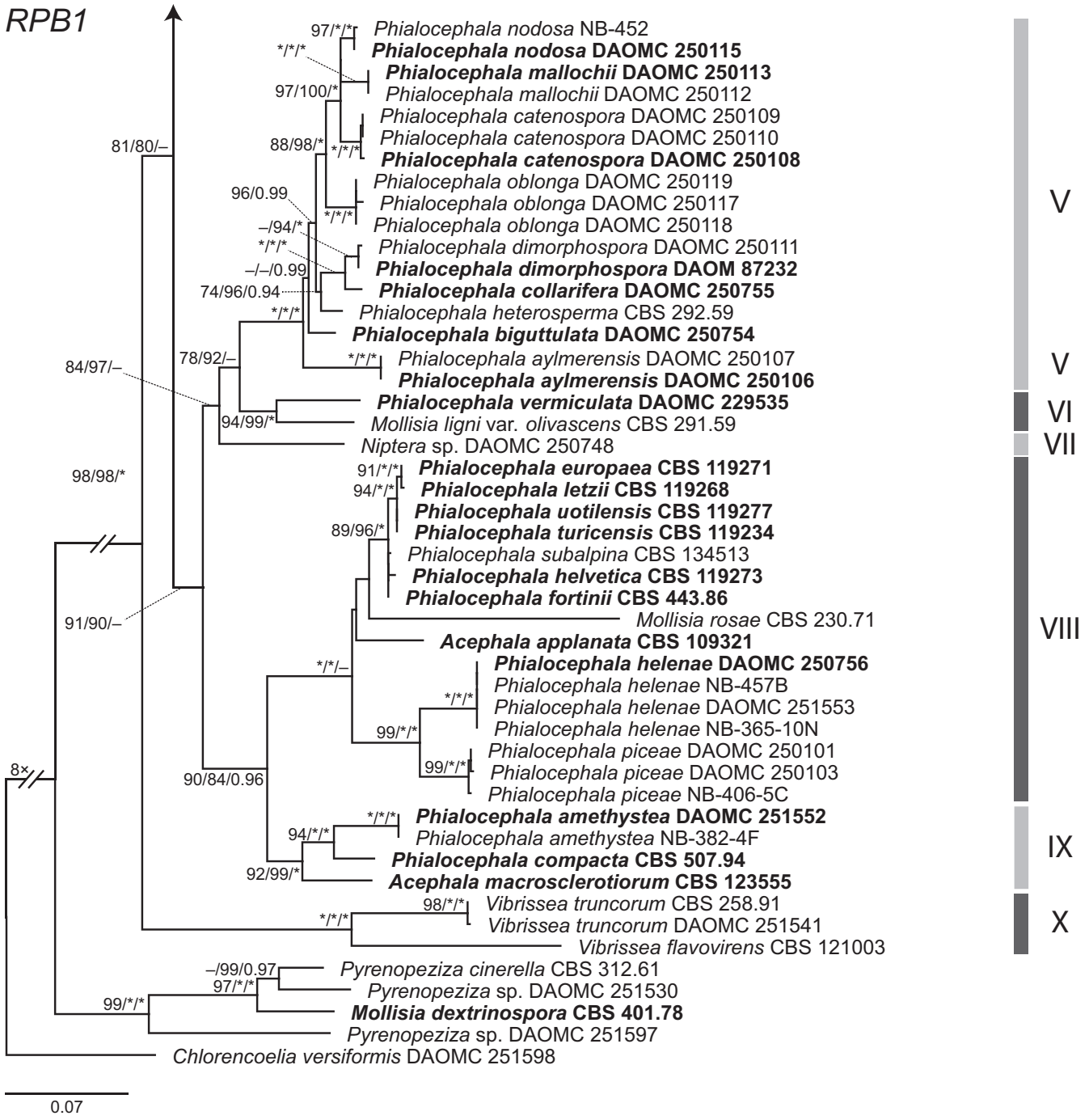


Fig. 11. (Continued).

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 seceded 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, fascicular 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, septate, 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*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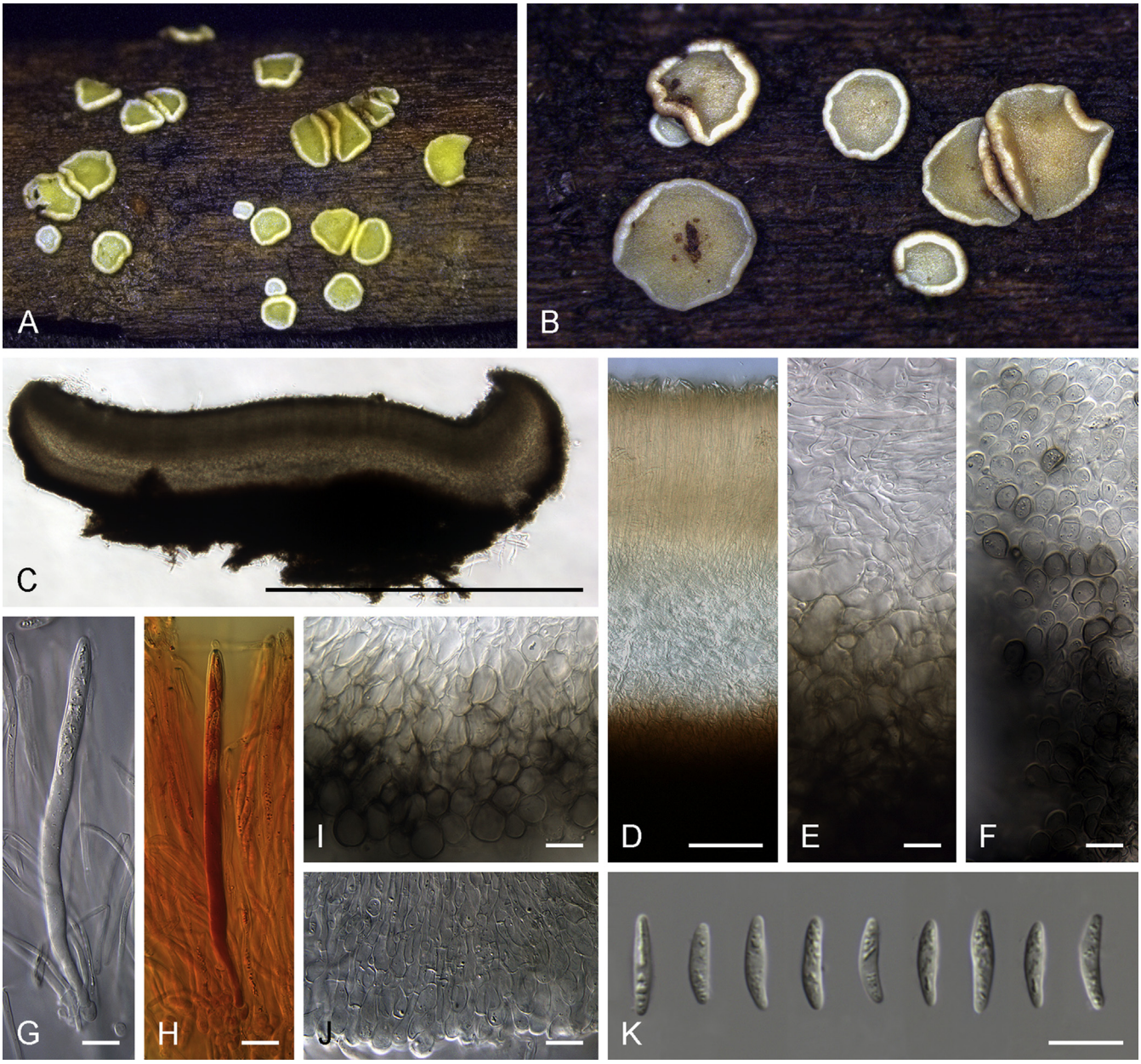


Fig. 13. *Mollisia caesia* DAOM 86792. **A, B.** Apothecia on sticks from wet holes in bog. **C.** Vertical section of apothecium. **D.** Ectal excipulum, medullary excipulum, and hymenium. **E.** Ectal and medullary excipula mounted in KOH. **F.** Ectal excipulum cells mounted in KOH. **G.** Ascus. **H.** Ascus with amyloid tip in Lugol's solution after KOH pretreatment. **I.** Ectal excipulum towards flanks mounted in KOH. **J.** Marginal cells mounted in KOH. **K.** Ascospores. Scale bars: C = 1 000 µm, E–K = 10 µm, D = 50 µm.

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.*** MycoBank 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

Fig. 12. *Strossmayeria basitricha* DAOM 696485. **A–E.** Apothecia on very moist decaying hardwood. **F.** Apothecium. **G.** Asci. **H.** Paraphyses showing refractive vacuole bodies. **I.** Ascus mounted in KOH. **J.** Ascus mounted in Lugol's solution. **K, L.** Asci and ascospores mounted in Lugol's solution after KOH pretreatment. **M.** Ascospores mounted in water. **N–S, X.** Conidiophores and conidia. **T–W.** Phialidic synasexual morph with flaring collarettes and globose conidia sometimes observed on ascus apices or ascospores. Scale bars: F = 100 µm, G–X = 10 µm.

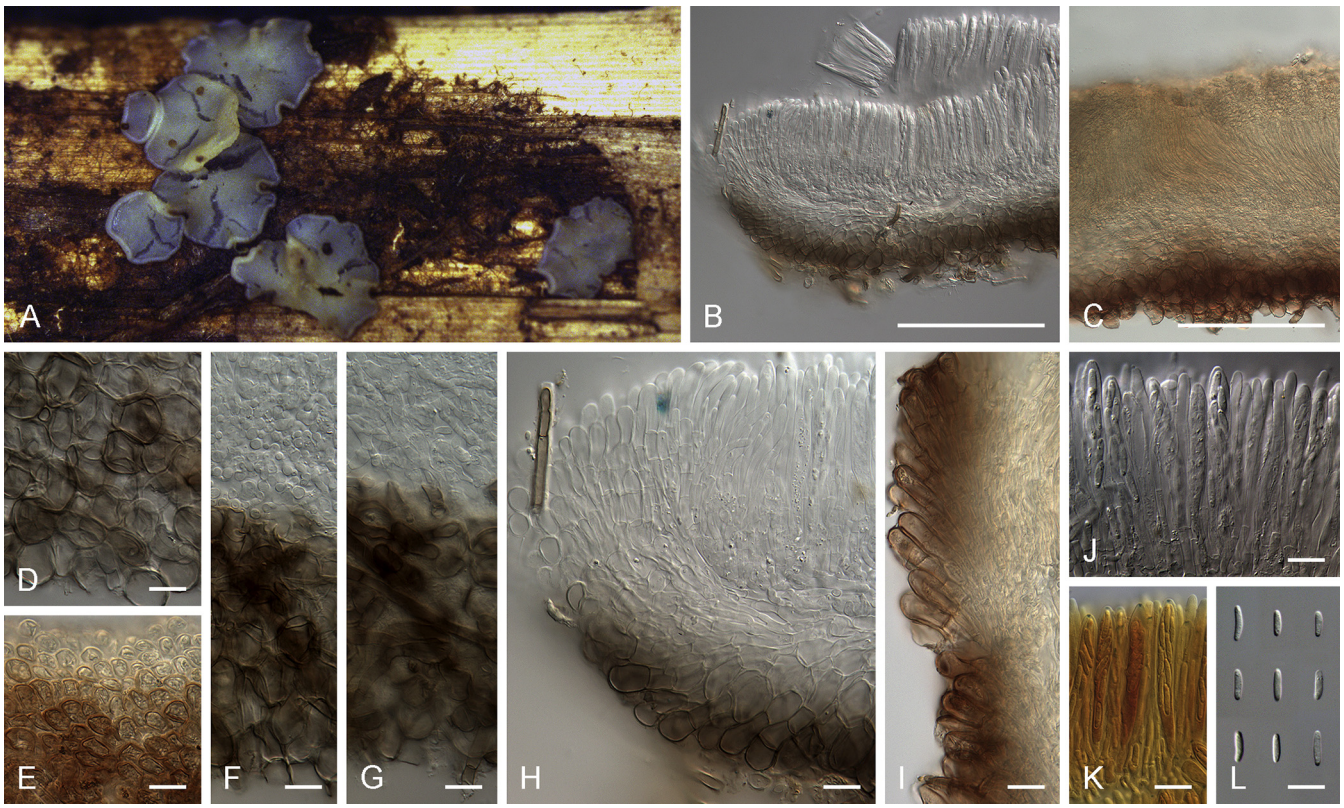


Fig. 14. *Niptera discolor* DAOM 86811. **A.** Apothecia on dead sticks from wet bog. **B, C.** Vertical sections of apothecia. **D, F, G.** Ectal excipulum mounted in KOH. **E.** Ectal excipulum cells in water. **H.** Apothecium margin in KOH. **I.** Apothecium margin in water. **J.** Ascospores and asci. **K.** Asci with amyloid tips in Lugol's solution after KOH pretreatment. **L.** Ascospores. Scale bars: B, C = 100 µm, D–L = 10 µm.

to subglose 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. *Subicular hyphae* sparse, 2.5–3.5 µm diam, thick-walled (0.5–1 µm), dark brown (6F4). *Medullary excipulum* hyaline, *textura intricata*, 30–60 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. *Asci* arising from croziers, cylindrical-clavate, 8-spored, 50–58(–65) × (5–)6–8(–9) µm, *pars sporifera* 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. *Ascospores* biseriate to obliquely uniseriate, (6–)7–9(–9.5) × 2–3 µm, ellipsoidal to oblong, apices rounded, aseptate, thin-walled, small (<1 µm) guttules sparsely present.

Colony characteristics: Colonies after 14 d in the dark at 20 °C on MEA 24–26 mm diam, flat with sparse aerial hyphae; margin diffuse, hyaline; surface white, occasionally sectoring or developing yellowish brown (5E5) concentric rings with age; reverse white. Exudates and soluble pigments absent. Mycelium consisting of hyaline to pale brown, smooth, septate, branched, hyphae 1.5–4 µm diam, sometimes covered with gelatinous sheaths 1–2 µm diam.

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, DAOMC 251538 = DAOM 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 DAOMC 251495; *ibid.,* DAOC 251631; Charlotte County, Little Lepreau, decaying fallen branch of *Betula papyrifera*, 12 Jul. 2014, J.B. Tanney, DAOMC 251548; Northumberland County, Doaktown, 46.480353 -66.058096, isolated as an endophyte from asymptomatic *Abies balsamea* needle, 18 Jun. 2013, J.B. Tanney, DAOMC 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.

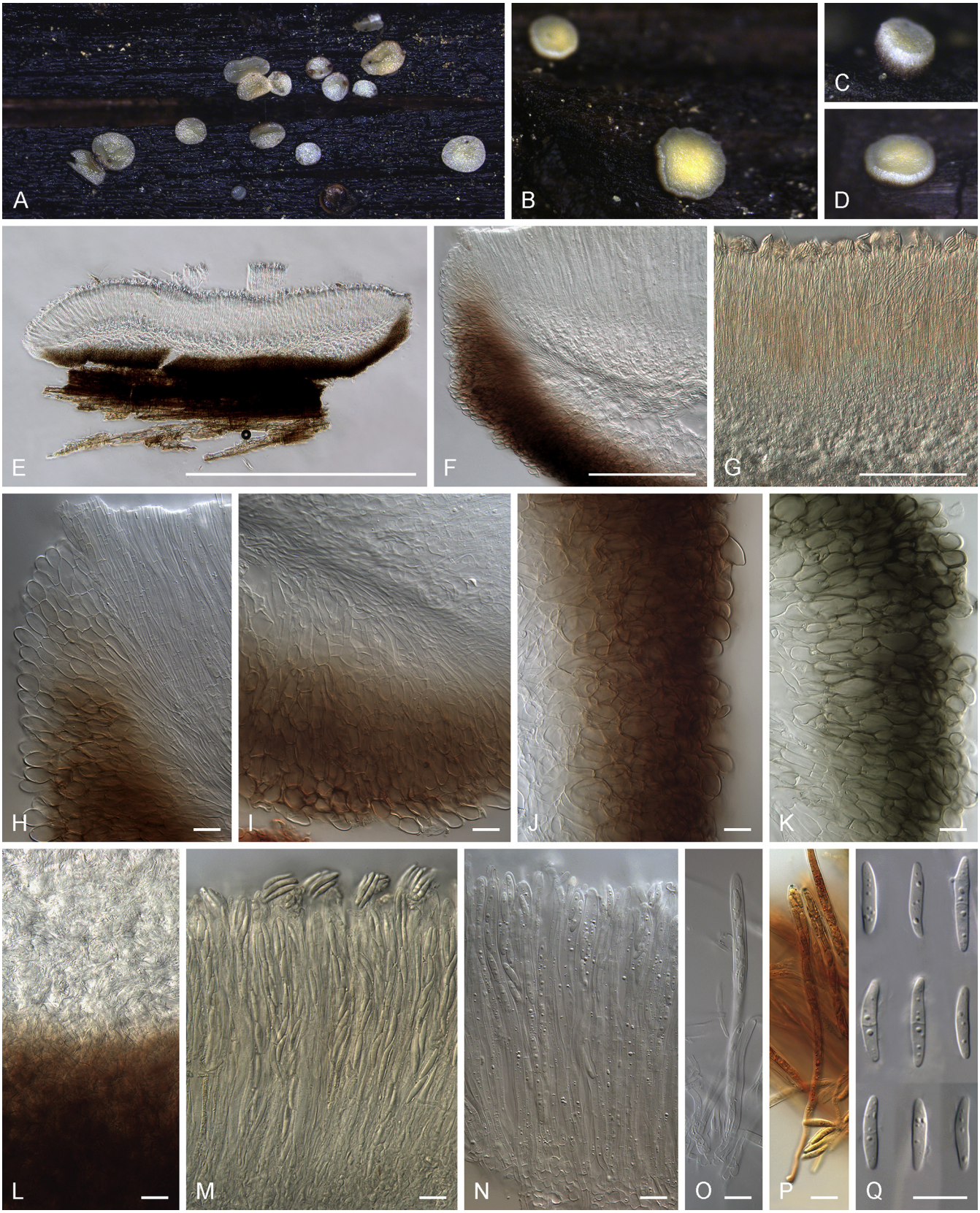


Fig. 15. *Niptera ramincola* DAOM 86812. **A–D.** Apothecia from dead sticks on moist ground in forest. **E.** Vertical section of apothecium. **F.** Margin. **G.** Medullary excipulum and hymenium. **H–J.** Marginal cells. **K.** Marginal cells in KOH. **L.** Ectal and medullary excipula. **M, N.** 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.

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

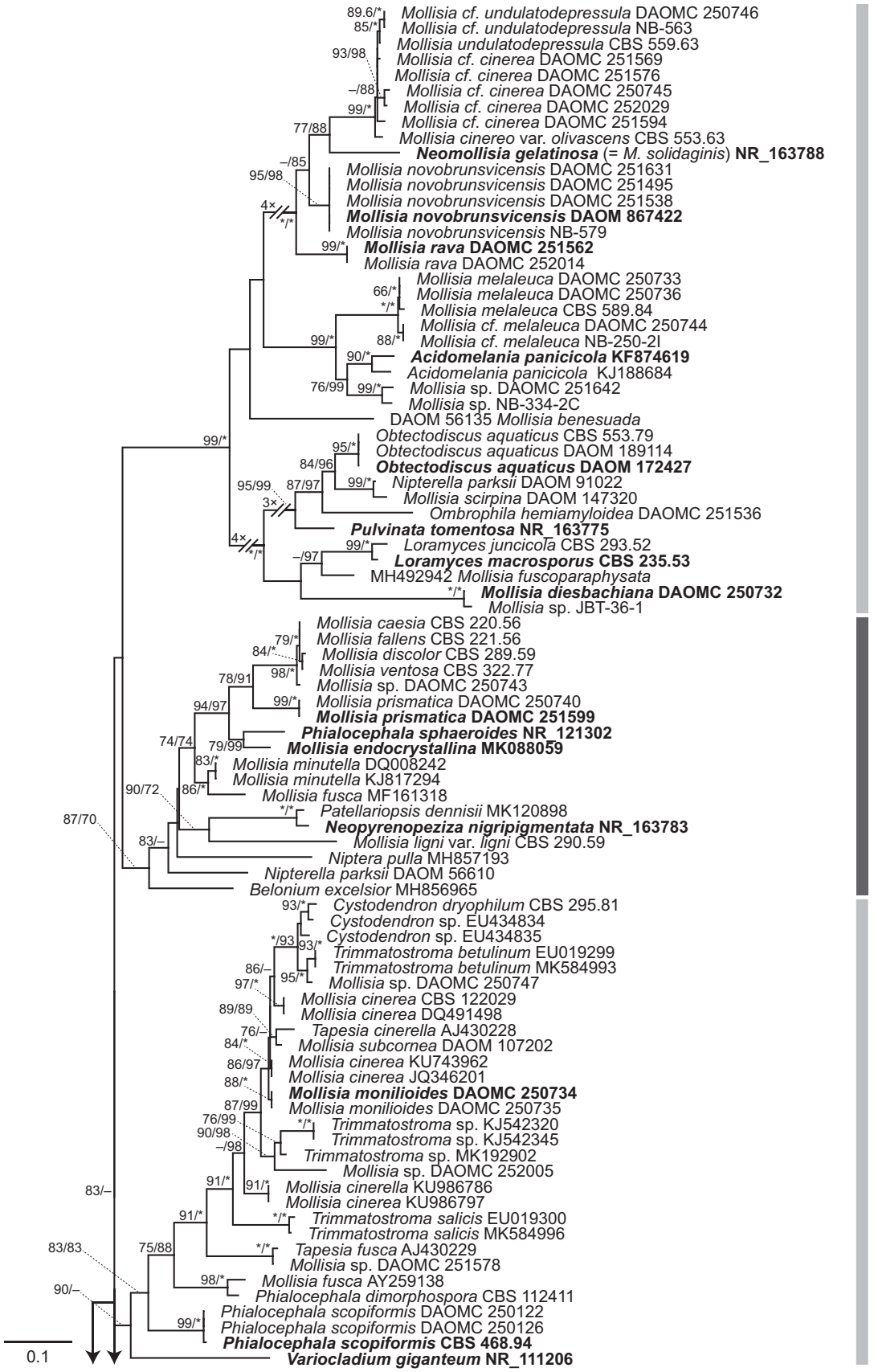


Fig. 16. A 50 % majority-rule consensus tree obtained from the maximum likelihood analysis based on the expanded ITS sequence dataset containing representative *Mollisia* and allied taxa including unidentified GenBank sequences. Culture collection (DAOMC, CBS), JB Tanney personal collection accession 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 *Barrenia*-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 *Chlorencoelia versiformis* (DAOMC 251598).

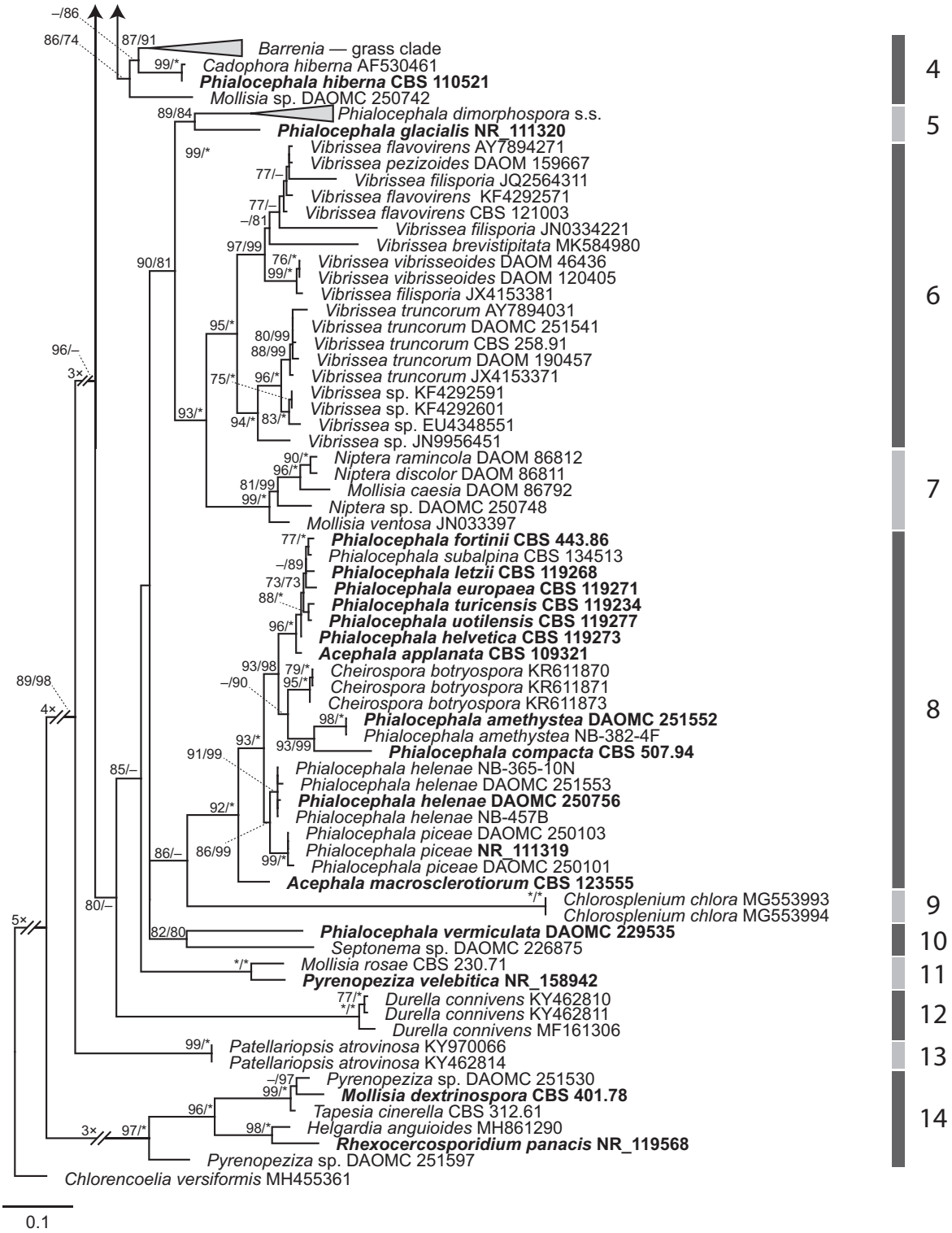
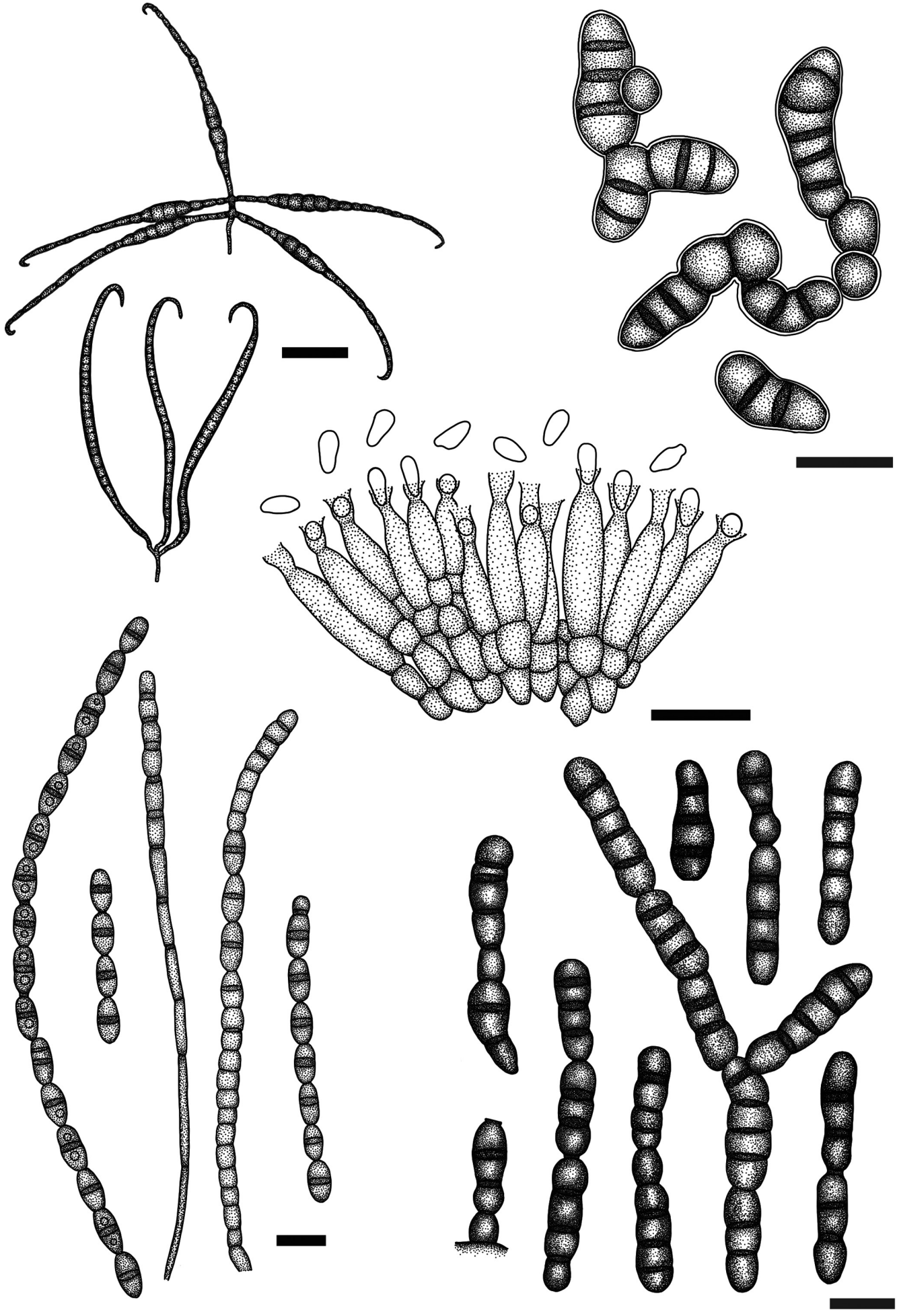


Fig. 16. (Continued).

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–) 67–80(–82) × (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, 45.418969 -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. sublividula* 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. caespiticia* differs by its smaller asci (30–40 × 3–4.5 µm) and ascospores (4–6 × 1–1.5 µm); (v) *Pyrenopeziza benesuada*, transferred to *Pyrenopeziza* by Gremmen (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 *Picea abies* 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, rosettiform 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. *Trichobelonium kneiffii* (=*Belonopsis retincola*) 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, *ravum*, 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** DAOM 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 to undulate, dull blue to bluish grey (21D4–21F2), 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 thin to slightly thickened walls, (13–)14–18.5(–19.5) × (10–)10.5–12(–13) µm; at upper flank and margin *textura angularis* to *prismatica*, composed of globose to subglose or obovoid cells with ± thin walls, (6–)7–9(–11.5) × (4.5–)5–7(–8) µm; marginal cells subglobose to obovoid 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 intricata*, 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. *Asci* 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 curved, 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). Exudates and soluble pigments absent. Mycelium consisting of pale brown to brown, smooth, septate, branched, hyphae 1.5–3.5 µm diam, sometimes

Fig. 17. Line drawings of *Casaresia sphagnorum* conidia (top left; redrawn from Fragoso 1920 and Webster & Descals 1975), *Trimmatostroma salicis* conidia (top right; KAS 5068), *Septonema* sp. conidia and conidiophores (bottom right; DAOM 226875 from MEA culture), *Bispora betulina* conidia and conidiophores (bottom left; DAOM 147525), and *Cystodendron* sp. conidia and conidiophores (middle; KAS 3224). Scale bars = 10 µm, except *C. sphagnorum* = 100 µm.

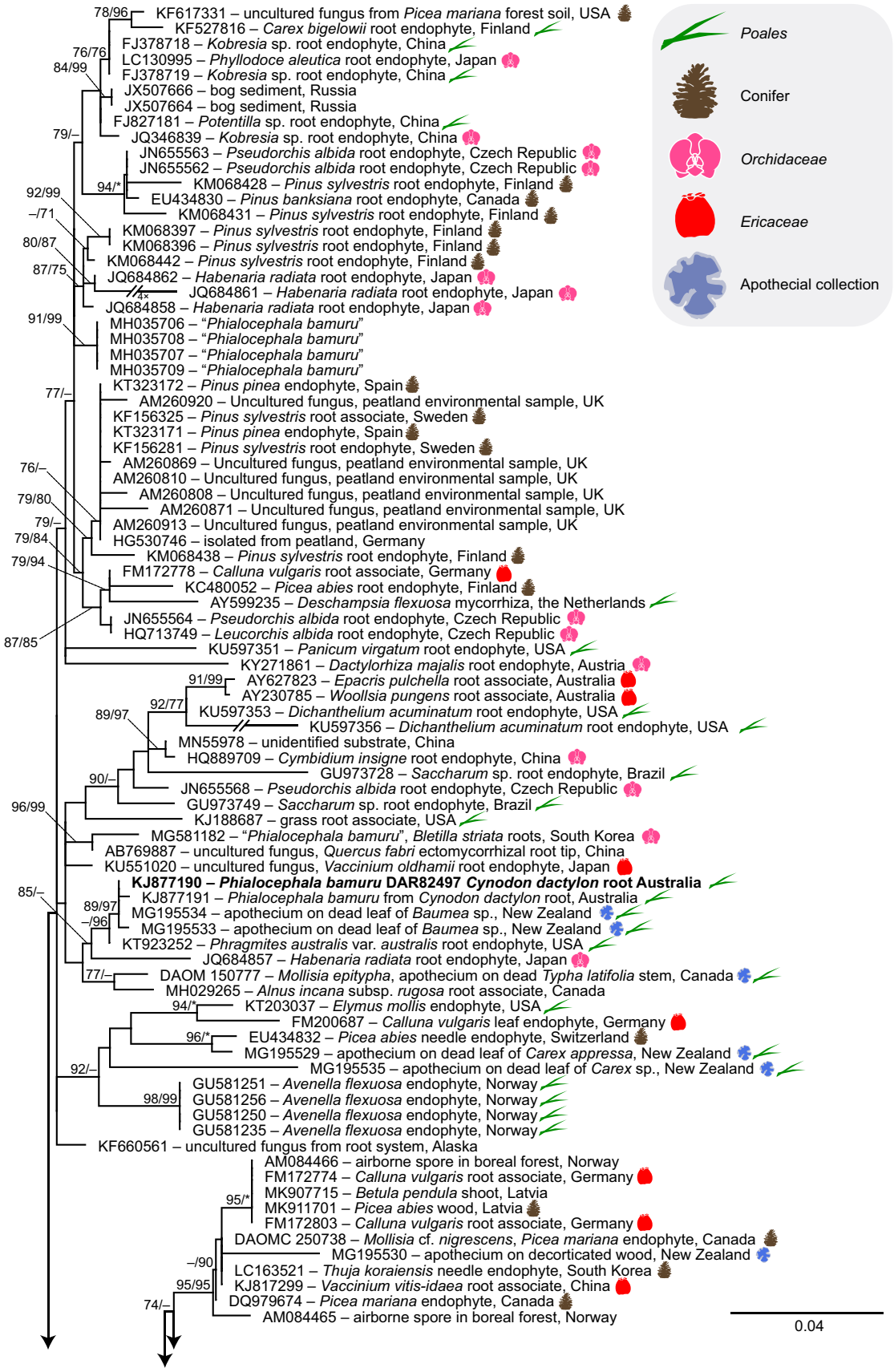


Fig. 18. A 50 % majority-rule consensus tree obtained from the maximum likelihood analysis based on the ITS sequence dataset of the *Barrenia*-grass clade. Culture collection (DAOMC, CBS), GenBank number, collection accession number (CBS, DAOM, DAOMC), or JB Tanney personal collection accession number (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 % SH-aLRT, 100 % BS) indicated by an asterisk (*). Truncated branches are designated by a broken line, which is a 2× reduction unless indicated. The tree is rooted with the ex-type of *Mollisia dextrinospora* (CBS 401.78).

Fig. 18. (Continued).

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).

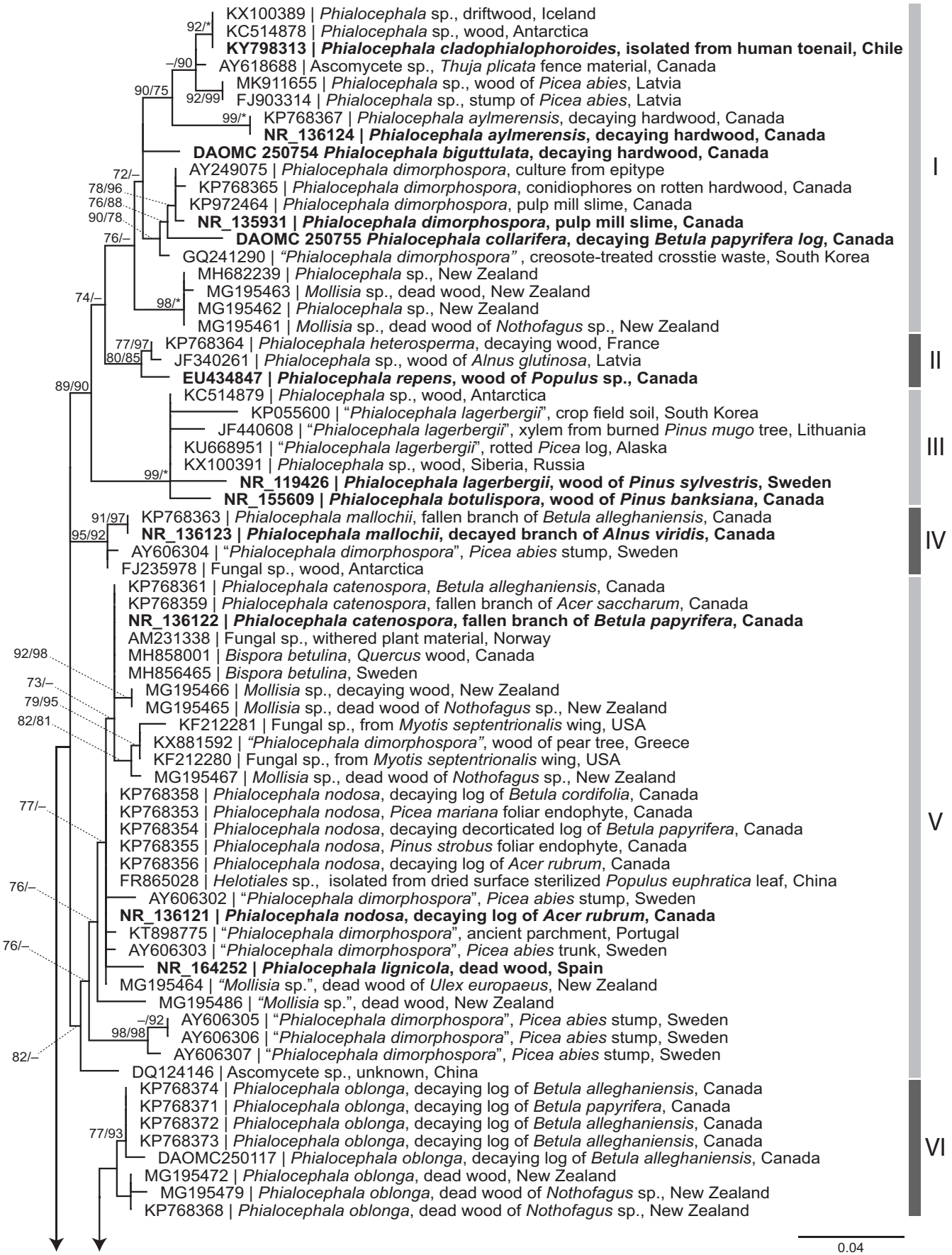


Fig. 19. A 50 % majority-rule consensus tree obtained from the maximum likelihood analysis based on the ITS sequence dataset of *Phialocephala s.s.* Culture collection (DAOMC, CBS), GenBank number, collection accession number (CBS, DAOM, DAOMC), or JB Tanney personal collection accession number (NB) precedes the sequence 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 (*). Truncated 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 401.78).

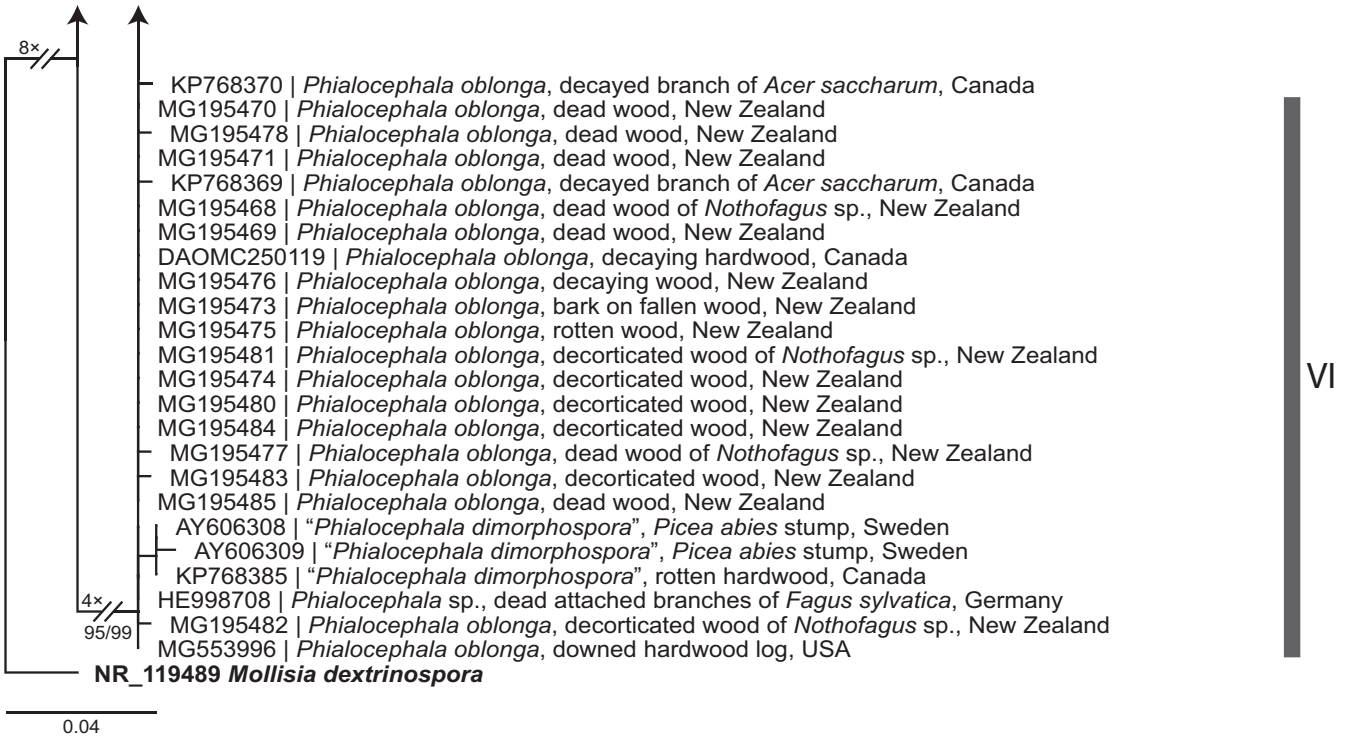


Fig. 19. (Continued).

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 0.5–2 µm wide gelatinous sheath, unbranched or 1–3 series of branches, often branching from base, (2.5–)3–4 µm diam, 25–46(–55) µm tall, with several septa; giving rise to flabellate to globose conidiogenous heads. *Conidiogenous cells* phialidic; terminal, sometimes intercalary; ampuliform to ellipsoidal, (7.5–)10–13(–14.5) × (2.5–)3–4(–4.5) µm; collarettes deep, cylindrical to doliiform 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 ossiform, base often truncate, hyaline, (3–)3.5–4.5(–6) × 1.5–2 µm; secondary conidia globose, base often 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. *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 amethystea* and *P. compacta* share 95 % similarity for *RPB1* 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 *prismatica*, composed of globose or cylindrical to elongated clavate cells with ± thin walls, 13–20(–22.5) × (5.5–)7–9 µm; marginal cells cylindrical to obovoid 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 µm, *pars sporifera* 18–27 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, (7.5–)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 *Betula papyrifera* log, 29 Aug. 2015, J.B. Tanney & B. Tanney (**holotype** DAOM 675858, 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 conidiogenous heads. *Conidiogenous cells* phialidic; terminal, sometimes intercalary; ampuliform, (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. *Metulae* pale to brown, cylindrical to clavate, (8–)9.5–13.5(–15) × (2.5–)3–4 µm. *Conidia* dimorphic; primary conidia elongate-ellipsoidal to elongate-pyriform, hyaline, (6.5–)7–8.5(–9.5) × 2–2.5 µm; secondary conidia ellipsoidal to oblong or obovoid, 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-

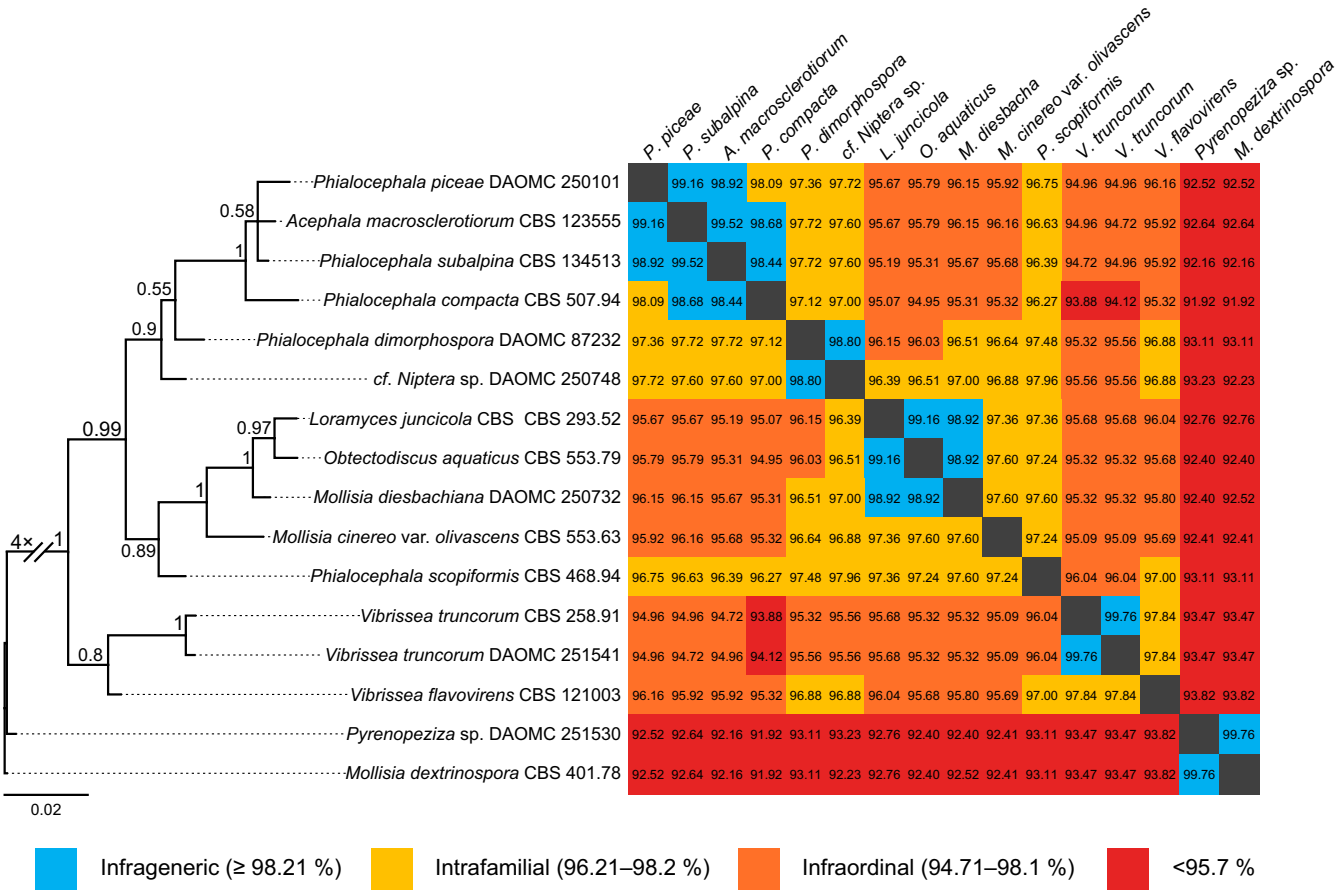


Fig. 20. Testing the taxonomic thresholds of Vu *et al.* (2019) to select *Mollisiaceae* taxa using the LSU barcode. The 50 % majority-rule consensus tree obtained from the Bayesian inference of phylogeny analysis is presented on the left, with posterior probability values for branch support shown at the nodes. The tree is rooted with the ex-type of *Mollisia dextrinospora* (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.

shaped conidiogenous heads. *Conidiogenous cells* phialidic; terminal, sometimes intercalary; ampuliform 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 *prismatica*, 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 (27F5) when mounted in KOH. *Subicular hyphae* sparse to moderately abundant, 2.5–3.5 µm diam, sometimes thick-walled (0.5–1 µm), light to dark brown (5D4–7F4). *Medullary 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 sporifera* 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.

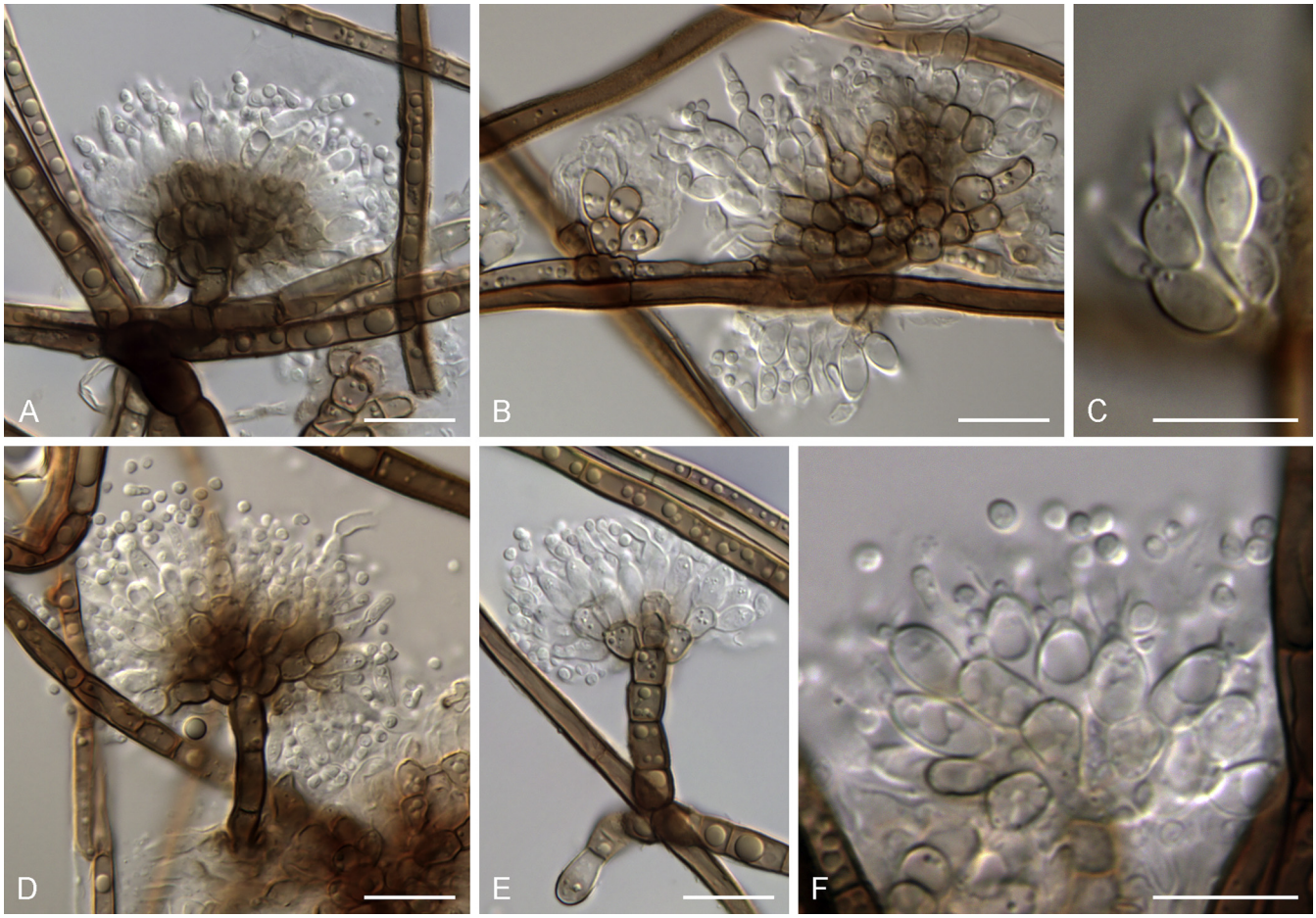


Fig. 21. *Mollisia* sp. DAOMC 252005. **A–F.** Conidiophores, phialides, and conidia developing from 3.5-y-old WA + TE culture incubated at 5 °C, mounted in water. Scale bars = 10 µm.

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 dilaetone vermiculin by the type strain.

Typus: **Canada**, New Brunswick, Sunbury County, Acadia Research Forest, 45.996125 -66.303769, isolated as an endophyte from a 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. 22. *Mollisia nigrescens* CBS 558.63. **A–D, F, G.** Conidiophores and phialides. **E.** Conidia. **H, I.** Phialides showing deep collarettes. All material mounted in water. Scale bars = A–G = 10 µm, H, I = 5 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)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Phialocephala lignicola **(**Hern.-Restr., J. Mena & Gené) J.B. Tanney & Seifert, ***comb. nov.*** MycoBank MB833759.

Basionym: *Fuscosclera lignicola* Hern.-Restr., J. Mena & Gené, Stud. Mycol. 86: 82 (2017)

NOMENCLATURE

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

MycoBank MB81017; Index Fungorum IF81017

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

DISCUSSION

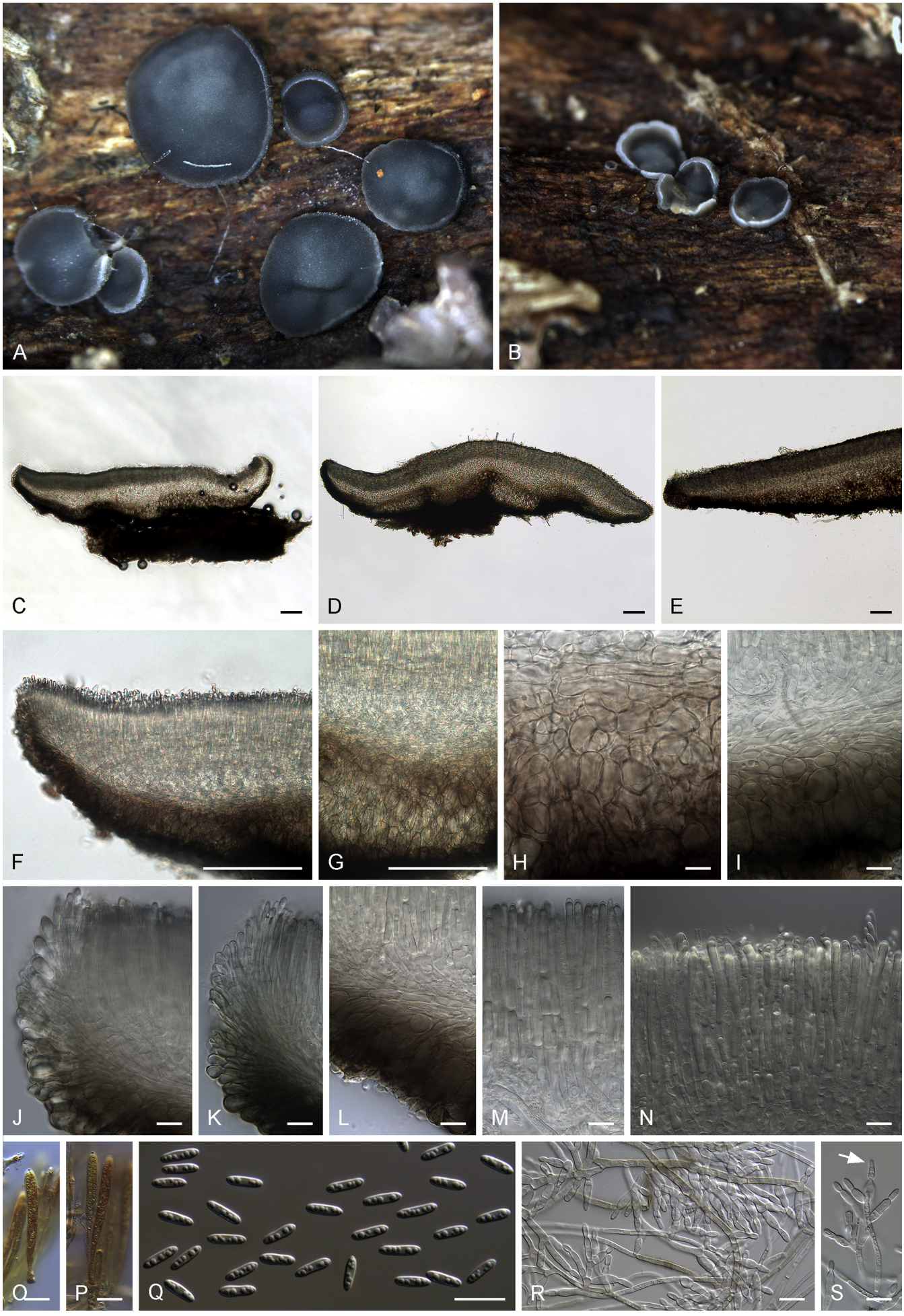
Phylogenetic markers and barcodes

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. Our preliminary 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 *TSR1* (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. helenae*).

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 polytomic groupings. Significant discrepancies between *LNS2* and other gene phylogenies include the placement of *P. scopiformis* sister to *Vibrissea* and *Mollisia rosae* placed within the *P. dimorphospora s.s.* and PAC clade. The well-supported placement of *Vibrissea* 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 polytomies and high infraspecific 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.

Fig. 23. *Ombrophila hemiamyloidea* DAOMC 251536. **A–D.** Apothecia on decaying hardwood. **E.** Vertical section of apothecium showing abundant crystals in medullary excipulum. **F.** Ectal and medullary excipulum showing crystals. **G.** Hymenium. **H.** Ectal excipulum, medullary excipulum, subhymenium, and hymenium towards margin. **I, J.** Ectal excipulum cells towards flanks. **K, L.** Crystals in medullary excipulum. **M.** Margin. **N, R.** Asci mounted in KOH. **O.** Ascus with amyloid tip in Lugol's solution after KOH pretreatment. **P, Q.** Ascus showing hemiamyloid tips in Lugol's solution without KOH pretreatment. **S.** Large globose conidiogenous head. **T, V.** Conidiophores bearing phialides with deep collarettes. **U.** Dimorphic conidia (arrows pointing to primary conidium). Scale bars: E = 1 000 µm, F–R, T–V = 10 µm, S = 100 µm.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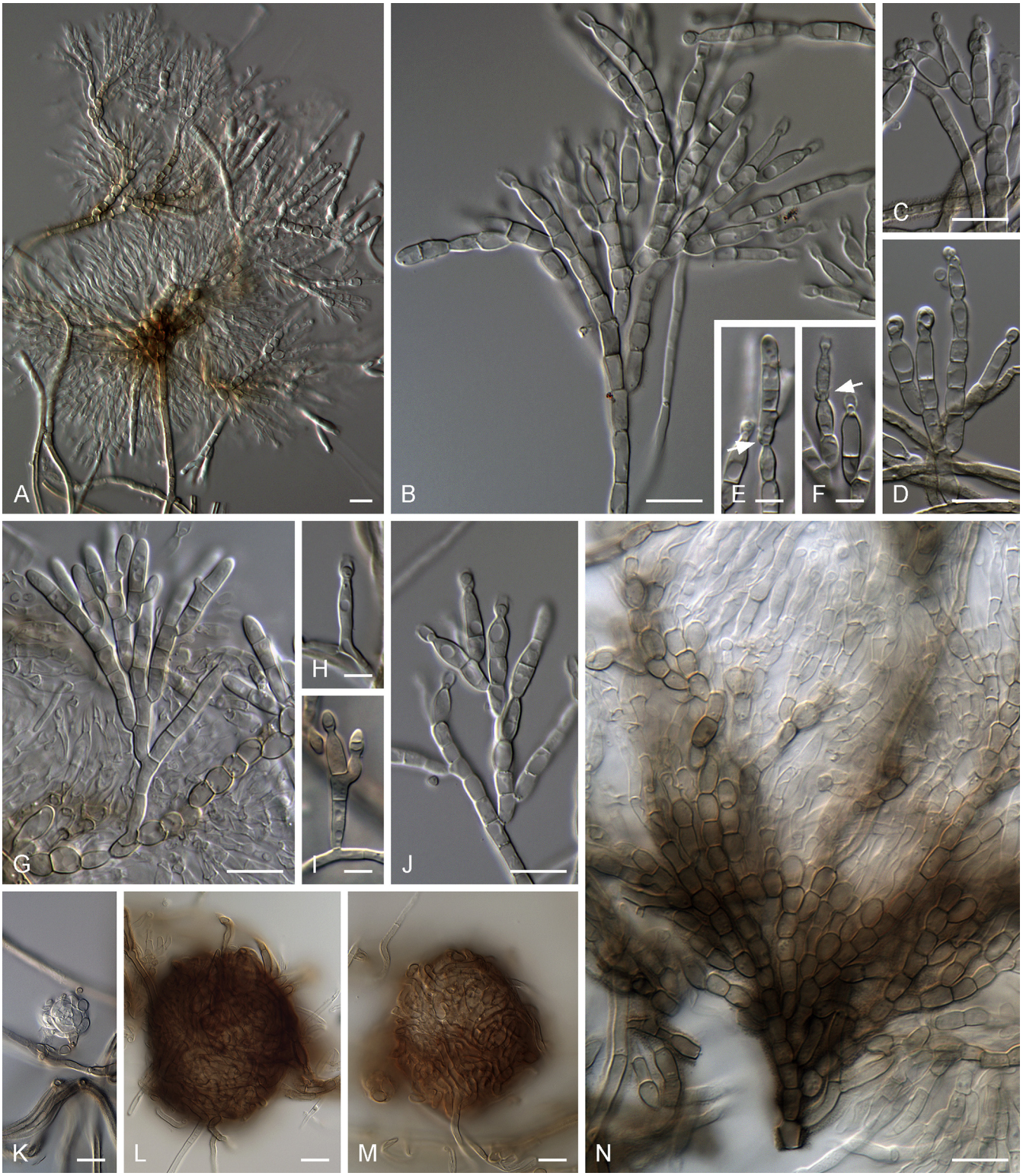
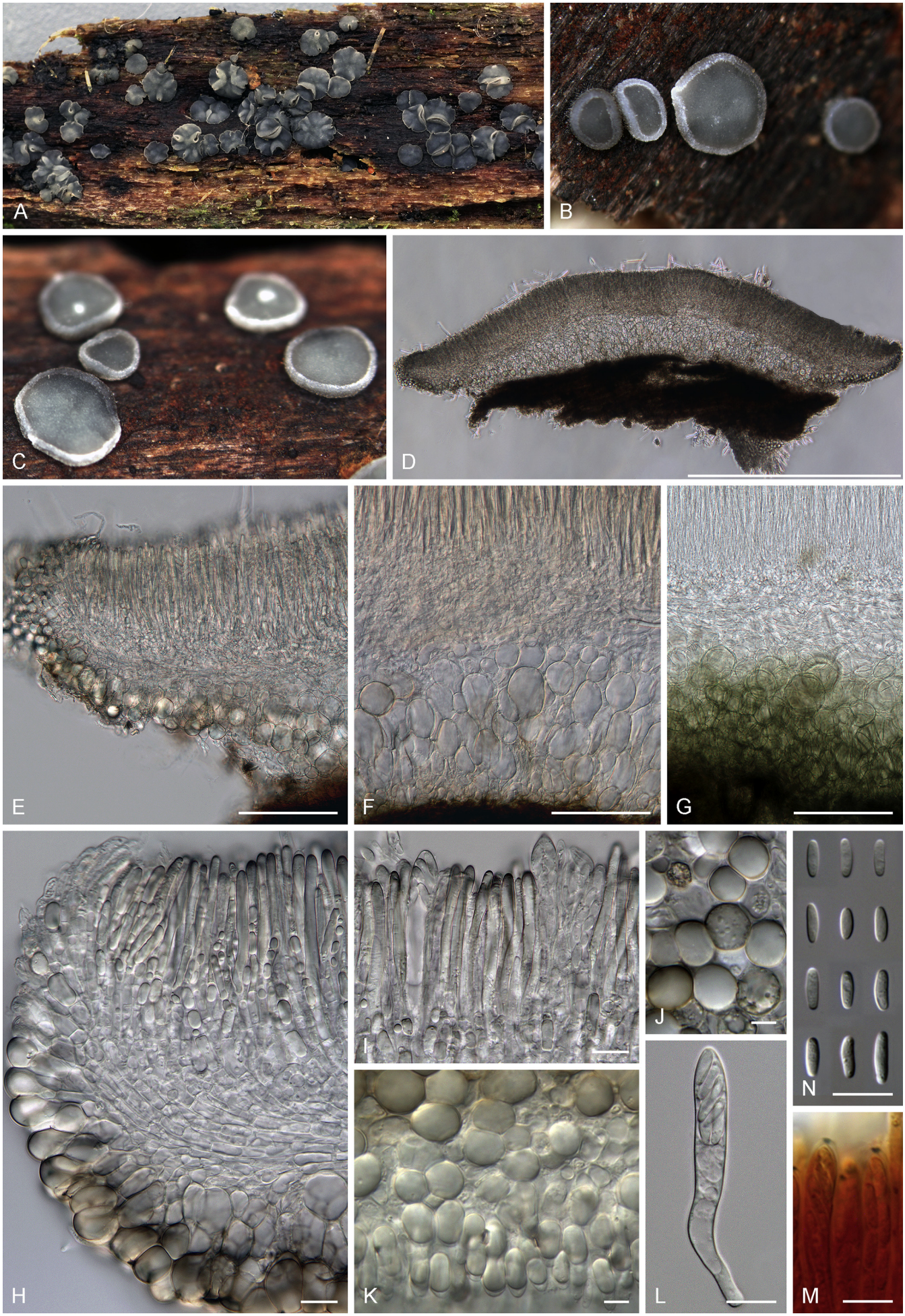


Fig. 25. *Mollisia monilioides* DAOMC 250734. **A.** Conidiophores showing monilioid form. **B–D, J.** Branching pattern of metulae and phialides, phialide collaretes, and conidia. **E, F.** Arrows denoting proliferation of new phialide or conidiophore initial from existing phialide. **G.** Sterile conidiophore. **H.** Intercalary phialide. **I.** Semi-micronematous conidophore. **K–M.** Development of structures interpreted as apothecia initials. **N.** Dematiaceous and monilioid condiophore. Scale bars = 10 µm.

Endophytism throughout *Mollisiaceae*

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*

Fig. 24. *Mollisia diesbachiana* DAOM 745767a. **A, B.** Apothecia on decaying *Betula alleghaniensis* wood. **C–E.** Vertical sections of apothecia. **F.** Margin and flanks of apothecium. **G.** Ectal excipulum and medullary excipulum. **H, I.** Ectal excipulum and medullary excipulum. **J.** Margin showing refractive vacuoles in marginal cells. **K.** Margin mounted in 10 % KOH. **L.** Ectal excipulum and medullary excipulum towards margin. **M, N.** Asci and paraphyses with refractive vacuole bodies. **O–P.** Asci with amyloid tips in Lugol's solution after KOH pretreatment. **Q.** Ascospores. **R, S.** Asexual state (DAOMC 250732) induced in cultures floating in sterile water; arrow indicates seceding primary conidium. Scale bars: C–G = 100 µm, H–S = 10 µm.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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 & Andruch 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. *Nipterella tsugae*, 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 saprotroph 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*, *Epacris pulchella*, *Vaccinium vitis-idaea*, *Woollsia 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*, *Tetrastigma 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, DAOMC 250739) and *M.* cf. *nigrescens* (DAOMC 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 hyphopodia-like hyphal structures in older cultures of *M. hydrophila* ("*…sur les cultures âgées, en tube, il se forme des élements brunàtres, 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-polystyrene interface in the bottom of Petri dish cultures and interpreted them as appressoria with conspicuous infection pegs. Similar hyphopodia 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. phalaridis*, *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, Vohník *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* DAOM 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.** Ascus. **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.


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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 *Chondroplea populea*. Mollisin forms as yellow crystals in cultures of *M. caesia* and *M. fallens*; its structure, a dichloronaphthoquinone derivative, was elucidated by Van Der Kerk & Overeem (1957) and its total synthesis was achieved recently (Schwolow *et al.* 2013). A strain of the PAC species *Phialocephala europaea* also produces secondary metabolites (sclerin and sclerotinin A) that significantly inhibit growth of *Phytophthora citricola s.l.*, which includes plant pathogens with broad host ranges (Tellenbach *et al.* 2012). Sclerin and related compounds are also phytotoxic to several cruciferous species *in vivo*, causing leaf chlorosis and necrosis (Pedras & Ahiahonu 2004). *Phialocephala scopiformis* strains produce rugulosin, a bis-anthraquinone pigment first described from *Talaromyces rugulosus*, which exhibits antibacterial activity against *Streptococcus aureus* and moderate activity against *Globisporangium intermedium* (=*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).

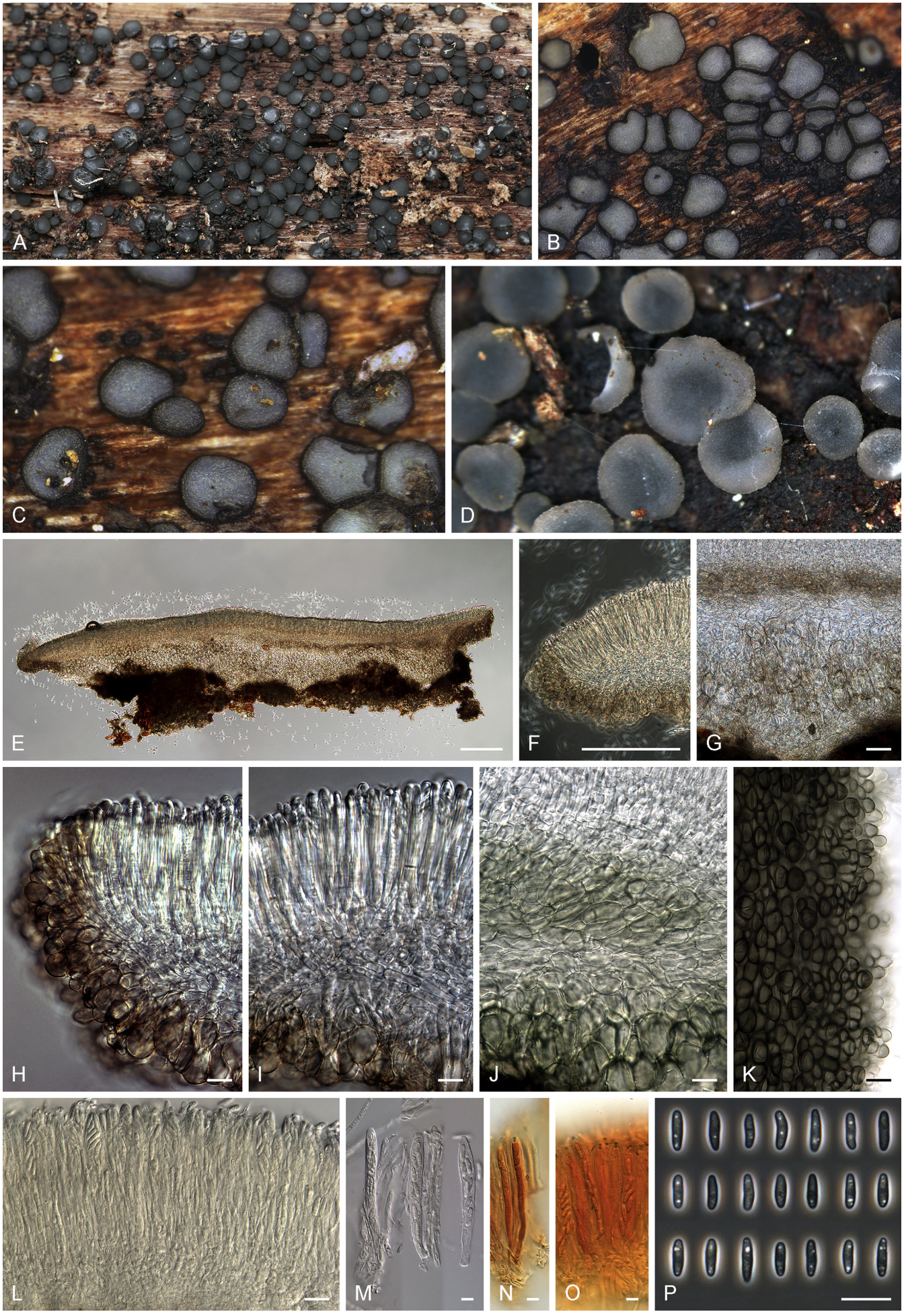
Two strains initially reported as *Phialocephala fortinii* isolated from rhizomes of *Podophyllum peltatum* produce podophyllotoxin, a lignan well-studied for its antiviral and antineoplastic properties (Eyberger *et al.* 2006). The podophyllotoxin-producing strain PPE7 was referred to by the unpublished 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 (Kwaśna 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 mollisioid apothecia in the field. In the present study, similar connections between unknown endophytes and field specimens include *Mollisia nigrescens*, *Phialocephala amethystea*, and *P. helenae*, detected as both needle endophytes and apothecia on decaying wood in the same forest stands. *Phialocephala helenae* 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 *Flavoparmelia caperata* and from a senescent *Tsuga canadensis* needle. *Phialocephala amethystea*, described here, shares an almost identical ITS sequence with an unidentified endolichenic fungus isolated from *Diploschistes scruposus* in North Carolina. *Mollisia olivascens* (CBS 293.59) shares an identical ITS sequence with the ex-type of *Phialocephala urceolata* (UAMH 10827), which should be investigated further to confirm whether or not *P. urceolata* is conspecific and therefore synonymous with *M. olivascens*. While a sexual state is unknown for the Australian turf grass pathogen *P. bamuru*, collections of apothecia from

Fig. 27. *Mollisia prismatica* DAOM 696477. **A–F.** Apothecia on decaying *Acer saccharum* wood. **G.** Vertical section of apothecium, arrow denoting crystals. **H–J, M.** Ectal and medullary excipula. **K.** Marginal cells. **L.** Ectal excipulum towards flanks. **N, O.** Crystals present in inner ectal and medullary excipula. **P, Q.** Asci and paraphyses with refractive vacuole bodies. **R, S.** Mature asci. **T.** Ascus with amyloid tip in Lugol's solution after KOH pretreatment. **U.** Ascospores under DIC. **V.** Ascospores under phase contrast. Scale bars: G = 1 000 µm, H–J = 100 µm, K–V = 10 µm.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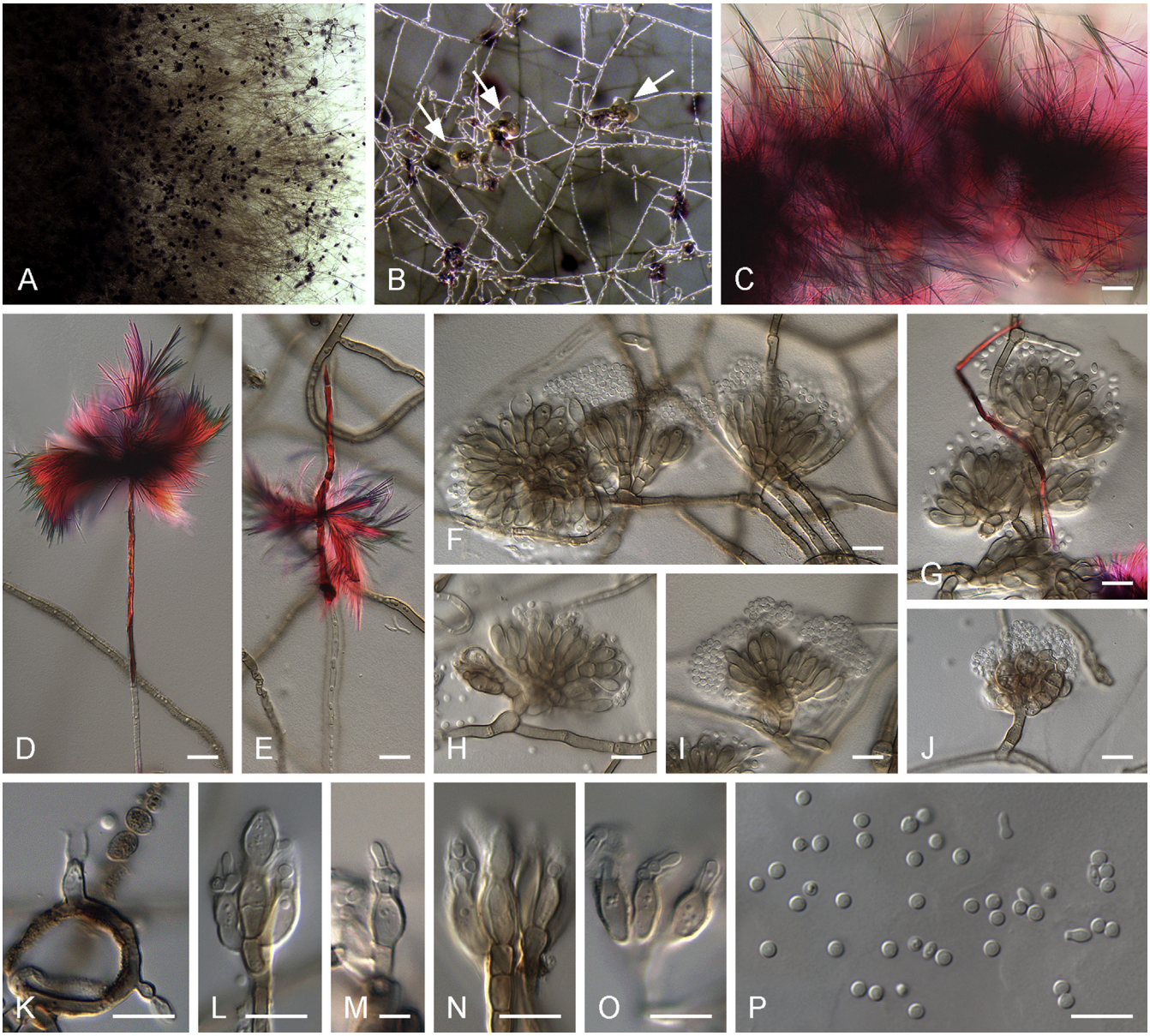


Fig. 29. *Phialocephala amethystea* DAOMC 251552. **A.** Dissecting microscope image with bottom light illumination showing abundant crystals on surface and below agar of 4-mo-old colony on MEA. **B.** Dissecting microscope image of colony surface of 4-mo-old colony on MEA; arrows denote collapsed conidiophores with slimy conidial heads. **C.** Large crystals under agar surface. **D**, **E.** Crystals and crystalline sheath encrusting hyphae. **F–J.** Conidiophores. **K.** Solitary intercalary phialide. **L–O.** Phialides bearing false chains or clusters of conidia within collarettes. **P.** Conidia. Scale bars = 10 µm.

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.

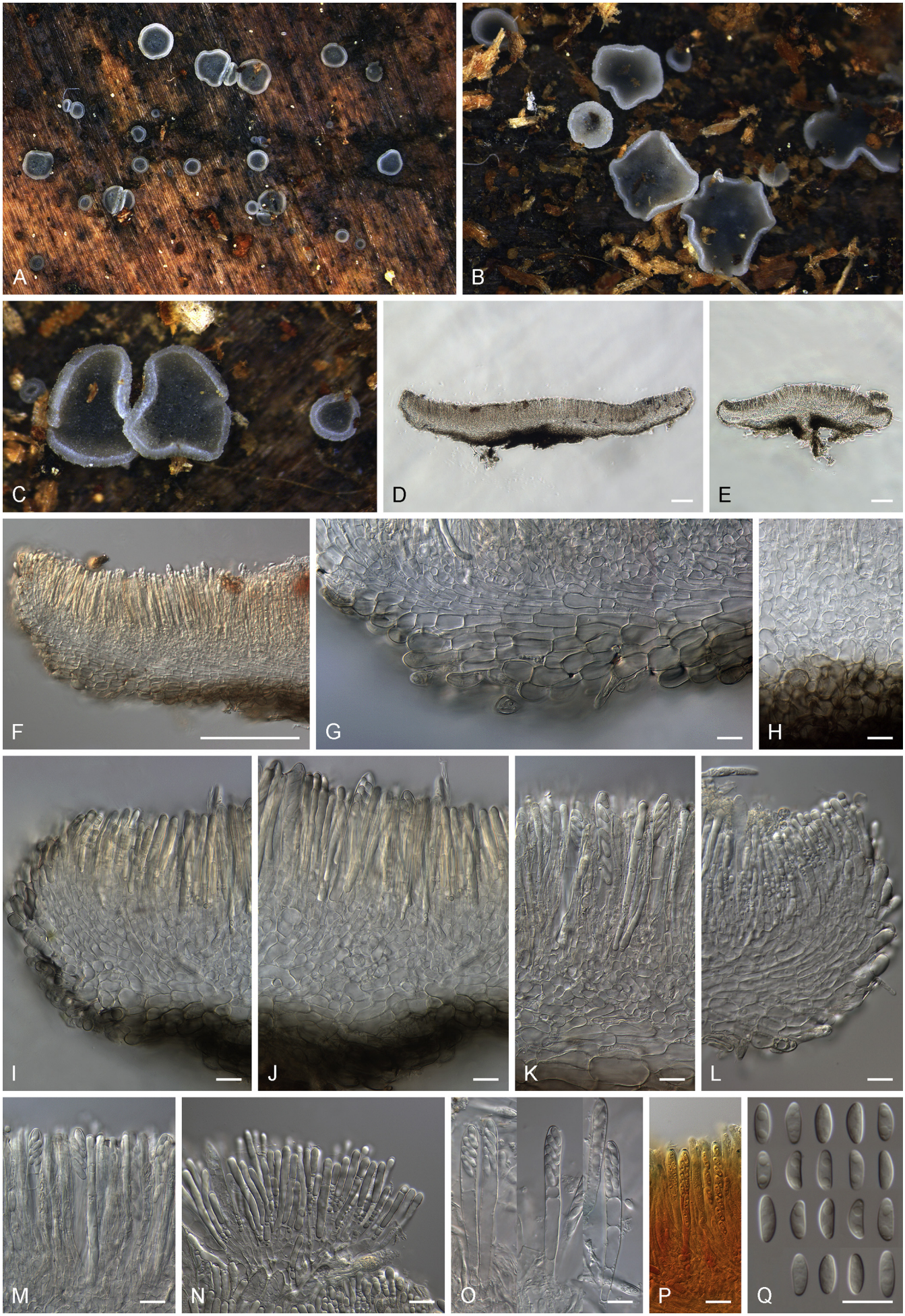
Mollisia s.l. can no longer be regarded simply as genus of saprotrophs associated with dead or decaying above-ground plant tissues (*e.g.* Walsh *et al.* 2015). The precise details of life cycles of species within the lineage may remain enigmatic; what role does endophytism play in the life histories 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

Vibrisseaceae

Earlier phylogenetic studies using rDNA sequences reported an unexpectedly close relationship between *Mollisia*, *Phialocephala*, and the aquatic genera *Loramyces* and *Vibrissea* (Wang *et al.* 2006a, Raja *et al.* 2008). Consequently, *Phialocephala* is

Fig. 28. *Mollisia rava* DAOM 745742. **A, D.** Apothecia on decaying *Betula alleghaniensis* wood. **B, C.** Rehydrated apothecia on decaying *Betula* wood. **E.** Vertical section of apothecium. **F, H.** Apothecia margins. **G.** Ectal and medullary excipula. **I.** Ectal excipulum, medullary excipulum, and hymenium towards margin. **J.** Ectal excipulum mounted in 10 % KOH. **K.** Ectal excipulum cells mounted in 10 % KOH. **L.** Asci and paraphyses. **M.** Asci. **N, O.** Asci with amyloid tips in Lugol's solution after KOH pretreatment. **P.** Ascospores. Scale bars: E, F = 100 µm, G–L, P = 10 µm, M–O = 5 µm.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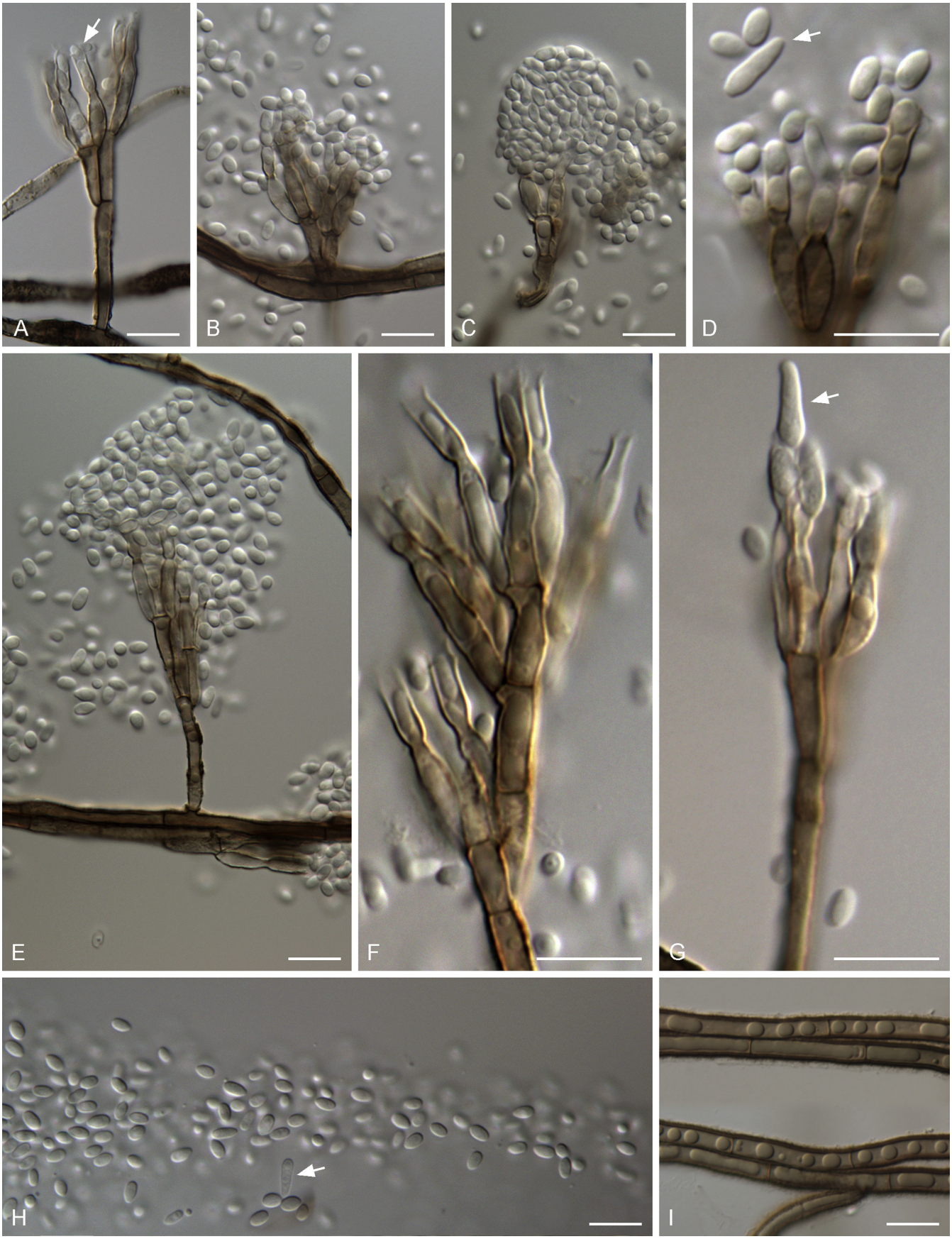

Fig. 31. *Phialocephala collarifera* DAOMC 250755. **A.** Conidiophore; arrow pointing to deep phialide collarette containing three conidia. **B.** Conidiophore branched at base. **C.** Conidiophore exhibiting slimy conidial head. **D.** Phialides with deep collarettes; arrow denoting primary conidium. **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. 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.


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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 coil. **K.** Conidia. Scale bars = 10 µm.

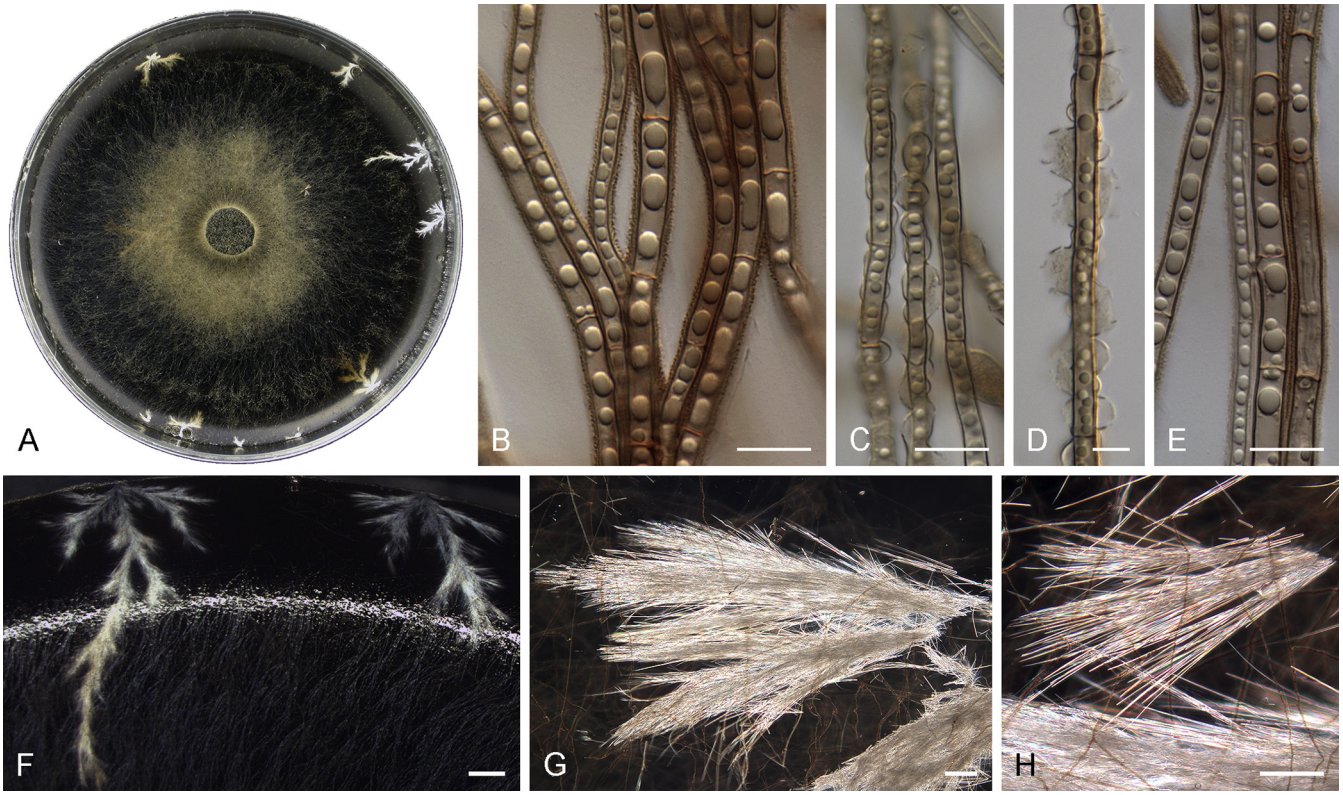

Fig. 34. *Phialocephala vermiculata* DAOMC 229535. **A.** Eight-week-old culture on MEA exhibiting surface and submerged plumose crystals. **B–E.** Hyphae showing guttules and exudates. **F.** Dendritic crystals on agar surface. **G–H.** Raphide crystals that form larger dendritic structures. Scale bars: B–E = 10 µm, F = 1 000 µm, G, H = 100 µm.

Fig. 32. *Phialocephala helenae* DAOM 867437. **A–D.** Apothecia on decaying hardwood. **E.** Vertical section of apothecium in water. **F, I.** Vertical sections of apothecium showing yellow reaction in 10 % KOH. **G.** Margin and flanks of apothecium. **H.** Vertical section showing centre of apothecium. **J.** Apothecium margin. **K.** Ectal and medullary excipula. **L, M.** Ectal excipulum towards flanks. **N, O.** Asci and paraphyses with refractive vacuole bodies. **P, Q.** Asci. **R.** Ascus with amyloid tip in Lugol's solution after KOH pretreatment. **S.** Yellow reaction of apothecium placed in 10 % KOH under dissecting microscope. **T.** Ascospores. Scale bars: E, F = 500 µm, G–I = 100 µm, J–R, T = 10 µm.

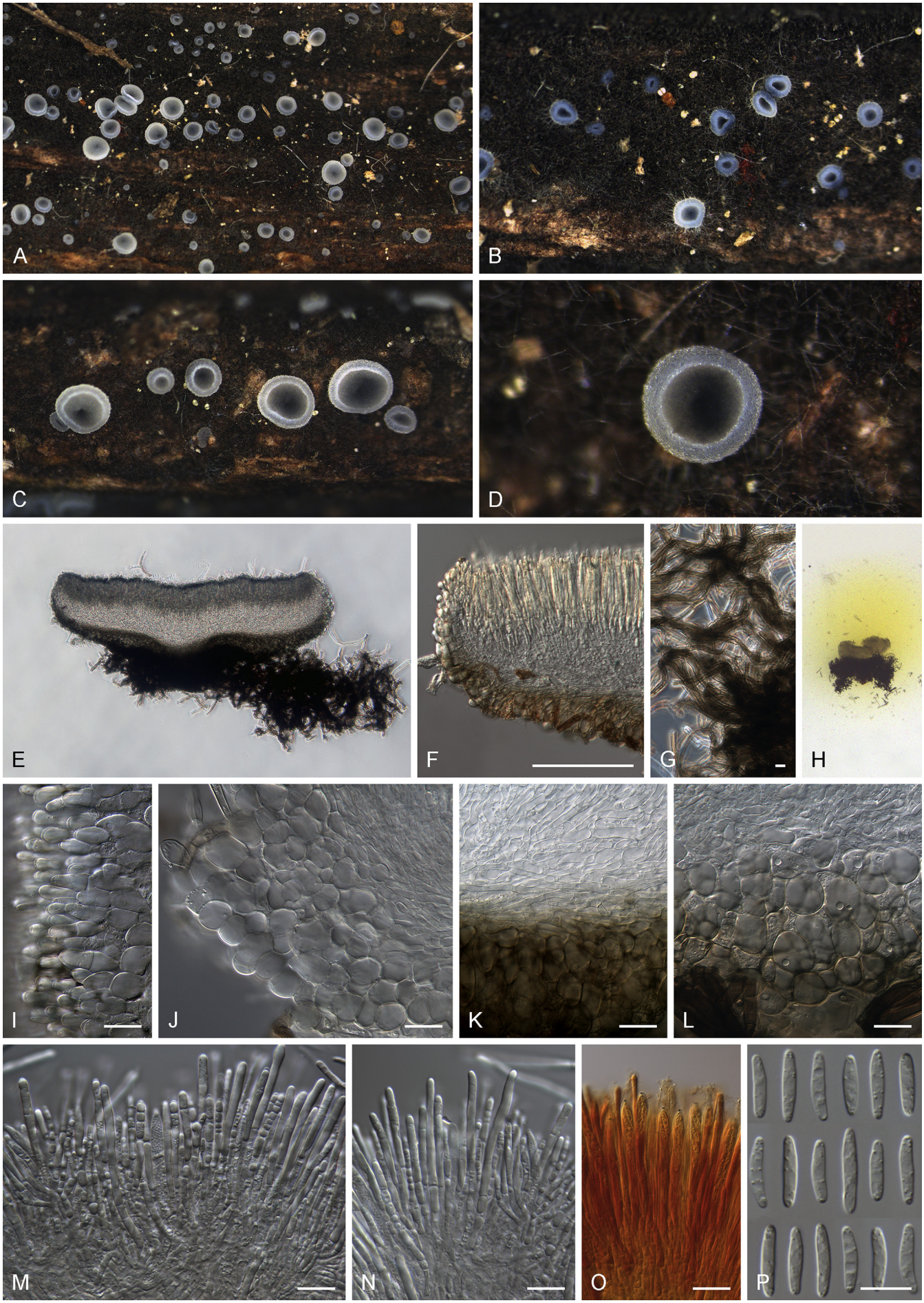

Fig. 35. *Mollisia nigrescens* DAOMC 250739. **A–D.** Apothecia on decaying hardwood with well developed and melanized subicula. **E**. Vertical section of apothecium. **F.** Vertical section showing ectal excipulum, medullary excipulum, and paraphyses with refractive vacuole bodies. **G.** Subicular 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.

Fig. 36. *Anguillospora crassa* NB-681. **A, B.** Macroconidia. **C.** Phialidic synasexual morph and microconidia. **D.** Macroconidial and microconidial synasexual morphs co-occuring. **E.** Phialides with deep, funnel-shaped collarettes. **F.** Phialides proliferating directly from macroconidium. Scale bars = 10 µm.

sometimes considered to belong to *Vibrisseaceae* (Adhikari *et al.* 2016, Robicheau *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 part-spores (Sánchez & Korf 1966), 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. flavovirens*), 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 *Obtectodiscus*, genera that are strongly supported in *Mollisiaceae*. The association of *Anavirga dendromorpha* and its phialocephala-like synasexual morph with *Vibrissea flavovirens* cultures flooded with water (Hamad &


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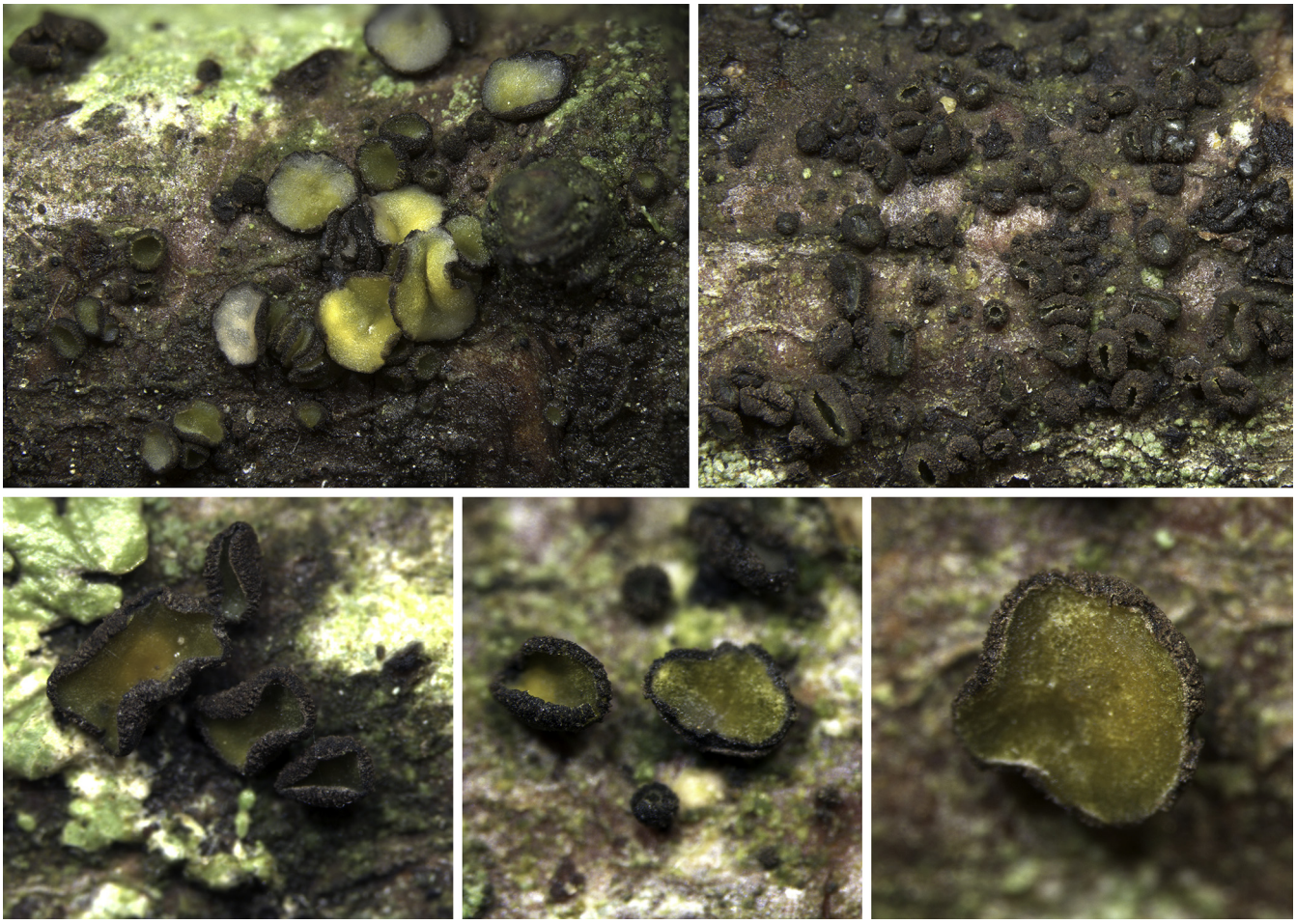

Fig. 38. *Nipterella parksii* JBT-113. Apothecia on fallen dead stick of *Alnus rubra*.

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 (Pärtel 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 stiptiate 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* sp., 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 hemiamyloidea*, 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. 37. *Hymenoscyphus cf. imberbis* DAOMC 251627. **A–C.** Apothecia on decaying hardwood partially submerged in stream. **D, H.** Vertical sections of apothecia. **E.** Medullary excipulum. **F, G.** Ectal excipulum. **I.** Ectal excipulum and margin. **J, K.** Asci and paraphyses with refractive vacuole bodies. **L, M.** Asci with inamyloid tips in Lugol's solution after KOH pretreatment. **N.** Ascus. **O.** Ascospores under DIC microscopy. **P.** Ascospores under phase contrast microscopy. **Q.** Croziers at bases of asci. Scale bars: D = 500 µm, E–G, I–Q = 10 µm, H = 1 000 µm.

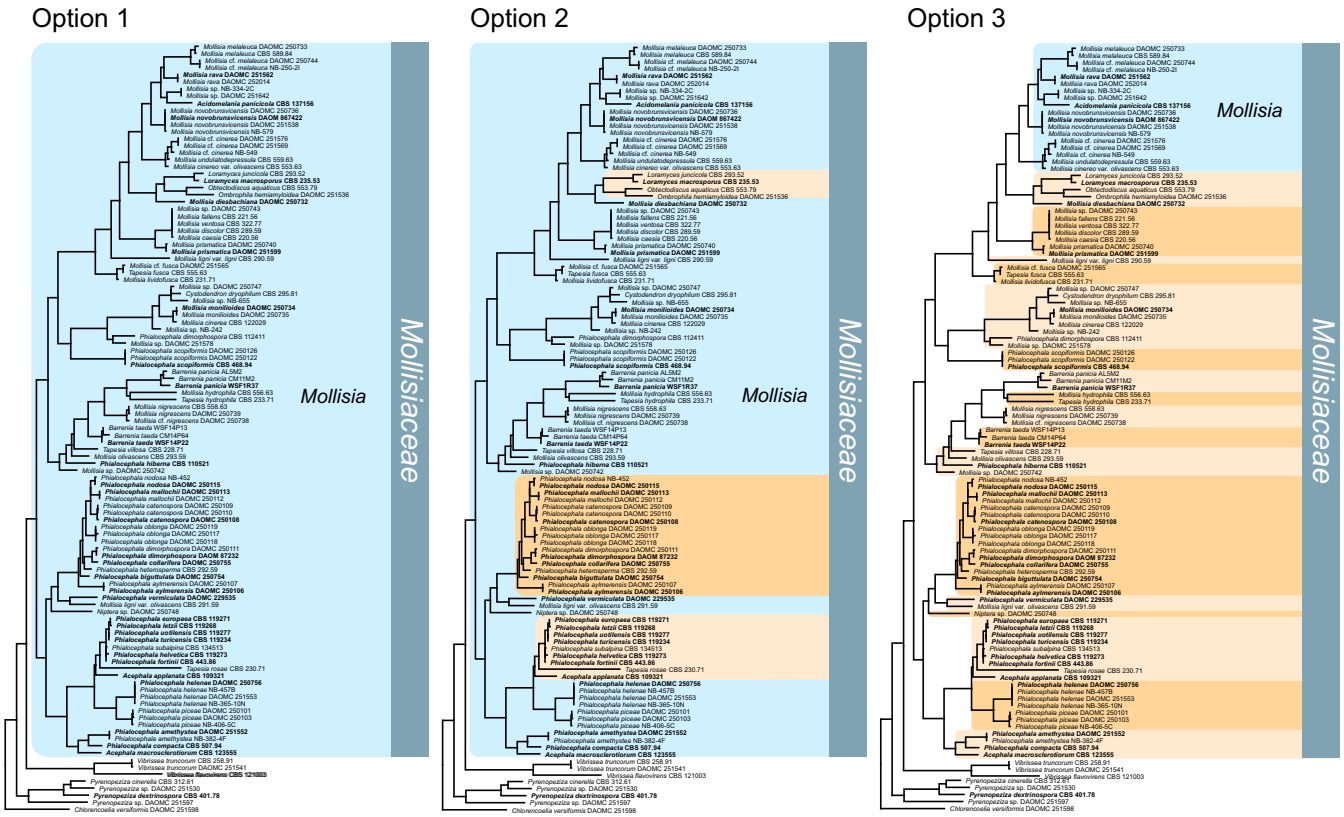

Fig. 39. Hypothetical graphical representation of three approaches to classifying *Mollisiaceae*, based on the expanded *RPB1* phylogeny in Fig. 11. Option 1 is to lump all taxa within the family into *Mollisia* to conserve this genus and recognize the prevalence and taxonomic importance of the typical mollisioid apothecium. Option 2 is to formally recognize a paraphyletic *Mollisia*, transferring most taxa into *Mollisia* but recognizing morphologically divergent genera such as *Loramyces* 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; *Loramyces* 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 mollisioid taxa. *Loramyces* is characterized by perithecioid 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 very wide apical opening, which is unlike the ascus apex of *Mollisia.* The taxonomic placement of *Loramyces* 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 *Mollisiaceae* because of the strongly supported placement of *Loramyces* within *Mollisiaceae* 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 perithecioid apothecia with long, filiform ascospores and was sampled here because of its resemblance to *Loramyces*. 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. Nimbomollisia*, *Phialocephala s.l.*). Baral (1999) also noted several typical *Mollisia* characters in *Ombrophila hemiamyloidea*, such as the *textura globulosa* ectal 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 *Loramyces* and *Ob. aquaticus* (Baral 1987b). Baral (1999) initially considered *Om. hemiamyloidea* to be an undescribed genus with taxonomic affinities to *Vibrisseaceae*/*Mollisiaceae*, but divergent characters ultimately led instead to its description within *Ombrophila*, namely a strongly gelatinized medullary excipulum and hyaline ectal excipulum. Verkley (2003) considered the apical apparatus and its reactivity with annular periodic acid (PA)-thiocarbohydrazide (TCH)-silver proteinate (PA-TCH-SP) more indicative of *Pezicula* rather than of *Vibrissea* and *Mollisia* relatives. However, our molecular phylogenetic evidence strongly supports the placement of *Om. hemiamyloidia* within *Mollisiaceae*, supporting the initial taxonomic assessment of Baral (1999).

A herbarium specimen of *Hysteronaevia scirpina* (DAOM 147320) is sister to *Obtectodiscus aquaticus* based on ITS sequences (Fig. 16, Clade 1). There are 13 described *Hysteronaevia* spp., which are graminicolous, reported from terrestrial to partially-submerged or submerged substrates, and characterized morphologically by small 0.1−0.3(−0.8) mm 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, Raitviir

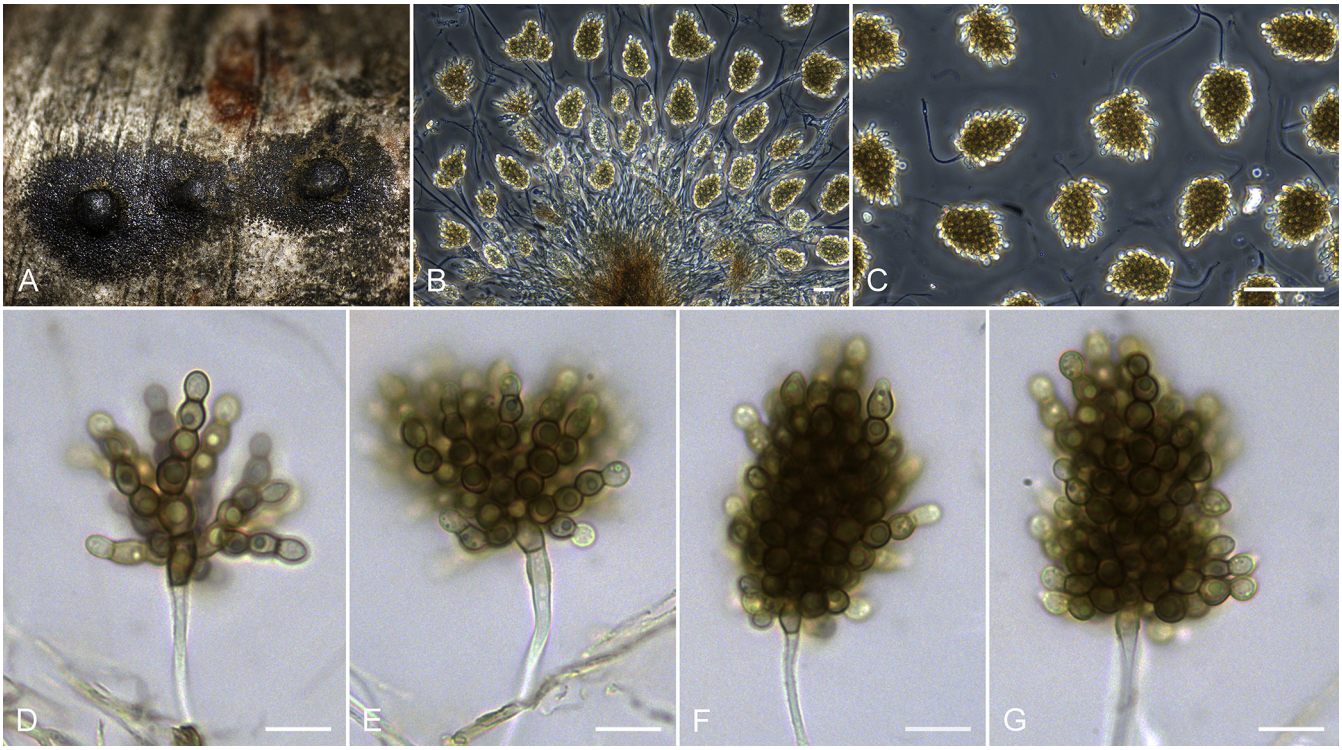

Fig. 40. *Cheirospora botryospora* JBT-41. **A.** Conidiomata surrounded by slimy conidial masses on dead attached branch of *Fagus grandifolia*. **B–G.** Conidia. Scale bars: B, C = 50 µm, D–G = 10 µm.

2008). Saccardo (1889) transferred *Peziza scirpina* to *Mollisia scirpina* and Nannfeldt (1984) later combined *M. scirpina*, along with other species of *Hysteropezizella* and *Hysterostegiella*, with *Hysteronaevia.* Nannfeldt (1984) considered *Hysteronaevia* related to *Mollisia* ("*Dermateaceae-Mollisioideae*"), 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, Hysteropezizella*, *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 condiophores in cultures of *Naevala minutissima* and *Naeviopsis epilobii*.

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 *Obtectodiscus* 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 graminicolous, 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 *Obtectodiscus* 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 mollisiod 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 ascomycetes 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 inamyloid 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* ascospores 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 ascospores 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 stauroconidia (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 *RPB1* 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 basitricha* within *Mollisiaceae* based on a LSU phylogeny by Hustad & Miller (2011) is also dubious because of branch length in the *RPB1* phylogeny. *Strossmayeria basitricha* produces two synasexual morphs: a pseudospiropes-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 asci (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 *Mollisia 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 *RPB1* phylogeny placed *Niptera* sp. (DAOMC 251628) basal to *Phialocephala s.s.* (Fig. 11, Clave V) and *Mollisia ligni* var. *olivascens* (CBS 291.59) and *Phialocephala vermiculata* (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. lacustris*, 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*, *Nimbomollisia*, 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 *Nimbomollisia* 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.l. contains species occurring throughout *Mollisiaceae* (*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. aylmerensis*, *P. biguttulata*, *P. botulispora*, *P. catenospora*, *P. cladophialophoroides*, *P. collarifera*, *P. heterosperma*, *P. lagerbergii*, *P. lignicola*, *P. mallochii*, *P. nodosa*, *P. oblonga*, and *P. repens*. All species except *P. heterosperma* and *P. oblonga* are represented by ex-type cultures and sequences.

Hernández-Restrepo *et al.* (2017) described a new *Mollisiaceae* 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 synonymise *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 lignicolous saprotrophs 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 (FR865028; Unterseher *et al.* 2012). Sequences in GenBank corresponding to species within the *P. dimorphospora* clade confirm its overall lignicolous habit, expand known 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 strains 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 (KX100389; 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 *Nothofagus* 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 synasexual 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 *Paradidymobotryum* (=*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 *Mollisiaceae* in the LSU phylogeny presented by Shenoy *et al.* (2010).

Apothecia 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) × (2.5−)3−4 µm. In this study, apothecial 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 epitypification 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 *Loramyces-Obtectodiscus-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 warden*"), he did not formally propose the name *Nipterella*. Dennis (1962) validated *Nipterella* for *N. duplex*, the type species, and *N. parksii*, formerly *Belonidium parksii* (as "*Belonidium parksi*"; 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 inrolled margin composed of dark moniliform cells, although the conidia of *N. tsugae* are shorter and aseptate. Dennis (1962) classified *Nipterella* within the *Helotiaceae* subfamily *Encoelioideae*, distinguished from other genera such as *Encoelia* by its septate ascospores and amyloid asci. Müller & Defago (1967) considered *Nipterella* a member of *Dermateaceae* and synonymized it with *Dibeloniella,* while Korf (1973) referred to it within *Helotiaceae-Encoelioideae*. Starbäck (1895) noted that the ectal excipulum of *N. duplex* was like that of *Mollisia*.

A specimen of *Nipterella parksii* (DAOM 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-ellipsoidal ascospores (Fig. 3). The ITS sequence generated from *N. parksii* (DAOM 56610) places it with weak support within a generally poorly supported clade containing the ex-types of *Phialocephala sphaeroides*, *Mollisia endocrystallina*, and *M. prismatica*, and strains or collections identified as *Belonium excelsior*, *Mollisia fusca, M. ligni* var. *ligni, M. minutella, Neopyrenopeziza nigripigmentata*, *Niptera pulla*, *Patellariopsis dennisii*, 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 nigripigmentata* shares a 99 % similar ITS sequence with *Patellariopsis dennisii* (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 multicellular marginal cells, and, notably, inamyloid asci (Gminder 2012). An asexual morph was not described for *N. nigripigmentata*, but Müller (1962) described a pycnidial asexual morph resembling *Glutinium* in *Patellariopsis dennisii* cultures and on the dead stems bearing *P. dennisii* apothecia. However, their description was not illustrated or detailed enough to interpret its relationship with other known *Mollisiaceae* asexual morphs, for example the distinctive *Pycnidiella* (*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 parksii* is clearly in *Mollisiaceae*, its precise placement is unknown. An NCBI BLAST search yields a closest match with purported *Mollisia minutella* strains (*e.g.* KJ817294; identities = 415/443 *i.e.* 94 %, gaps = 6/443). *Nipterella parksii* is evidently common on dead attached branches of *Alnus rubra* in Vancouver Island, British Columbia, Canada (Fig. 38) and will be the subject of further study along with *N. tsugae*. These results suggest that collecting *Mollisiaceae* from overlooked xeric habitats such as dead attached tree branches may yield diverse, morphologically distinct, and potentially unknown species, and possibly lend support to a more strongly supported clade including xeric species.

Family placement of *Mollisia* and related genera

Mollisia has been variously placed within *Dermateaceae* or *Mollisiaceae* (Rehm 1891 [as "*Mollisieae*"], Velenovsky 1934, Kirk *et al.* 2008). *Mollisia* and allied taxa clearly do not belong in *Dermateaceae* and should be considered in *Mollisiaceae* (Goodwin 2002). *Phialocephala*, frequently considered a member of *Vibrisseaceae*, should also be recognized within *Mollisiaceae*. *Vibrisseaceae* is considered distinct from *Mollisiaceae* based on *RPB1* and *TOP1* phylogenies and requires a modern circumscription (*e.g.* polyphyletic due to inclusion of *Chlorovibrissea* and *Myxocephala*; Sandoval-Leiva *et al.* 2014, Nonaka *et al.* 2015). *Loramyces* is strongly supported within *Mollisiaceae* and recognition of *Loramycetaceae* would result in a paraphyletic *Mollisiaceae*; therefore, we synonymized *Loramycetaceae* 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 lineage, 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 *Mollisiaceae* 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 may 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 impermissible 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.* Lumping 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, *Trimmatostroma* 1837, *Niptera* 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 *Niptera* 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, *Niptera*, *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 (Grünig *et al.* 2002, Grünig *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 1896), this question still cannot been 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 *Niptera* spp. are tentatively placed within the *Phialocephala dimorphospora*-PAC 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-Ombrophila hemiamyloidea* clade and perhaps the PAC and *P. dimorphospora s.s.* clades. The consensus among most taxonomists is that proposing paraphyletic and polyphyletic taxa is unacceptable (but see Vellinga *et al.* 2015, Lachance 2016). However, the *Loramyces-Obtectodiscus-Ombrophila hemiamyloidea* clade clearly represents 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 homoplasic 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 emphasizes 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 taxonomic 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 synasexual 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. catenospora*), and didymoconidia produced in short acropetal chains from tall (up to 120 µm) synnemata (*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. benesuada*. Bills (2004) described similar sporodochia produced by *Phialocephala hiberna* in both cultures and from colonies on *Robinia pseudoacacia* wood *in situ*. Aebi (1972) considered *Phialocephala* a synonym of *Cystodendron* and described this asexual morph in cultures of *Belonopsis excelsior*. 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 between *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. helenae* within the PAC and *Phialocephala s.s.* clade. Other peculiar asexual morphs reported for *Mollisiaceae* species include the previously discussed *Glutinium* morph of *Patellariopsis dennisii* and *Pycnidiella* coelomyceteous 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 environmental DNA sequences and strains, and so encourage increased sampling. The description of distinctive synasexual morphs throughout *Mollisiaceae* may provide important ecological and taxonomic information and warrants further study to assess whether these morphological characters provide resolution 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 sequences, reference strains of type species of dematiaceous hyphomycete genera, such as *Bispora* and *Trimmatostroma*, are required for study.

Option 3: prioritizing monophyly throughout Mollisiaceae

The third option, to divide *Mollisiaceae* into genera based primarily 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 informative 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 classification, such criticism hardly warrants the maintenance of an artificial taxonomic system under the guise of serving the relatively few users who actively collect mollisioid specimens. A classification based on useful phylogenetic concepts will be adopted by the much larger community of endophyte researchers 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 monophyly 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 MolliBase 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 *Pulvinata*.

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), *Phacidiales* (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 Vellinga *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.

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