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Cones, needles and wood: *Micraspis* (*Micraspidaceae*, *Micraspidales fam. et ord. nov.*) speciation segregates by host plant tissues

L. Quijada[1]*, J.B. Tanney[2], E. Popov[3], P.R. Johnston[4], D.H. Pfister[1]

[1]Department of Organismic and Evolutionary Biology, The Farlow Reference Library and Herbarium of Cryptogamic Botany. Harvard University Herbaria. 20 Divinity Avenue, Cambridge, Massachusetts 02138, USA
[2]Pacific Forestry Centre, Canadian Forest Service, Natural Resources Canada, 506 West Burnside Road, Victoria, British Columbia V8Z 1M5, Canada
[3]Komarov Botanical Institute of the Russian Academy of Sciences, Laboratory of Systematics and Geography of Fungi, Professora Popova Street 2, Saint-Petersburg 197376, Russia
[4]Manaaki Whenua Landcare Research, Private Bag 92170, Auckland 1072, New Zealand

*Corresponding author: luis_quijada@fas.harvard.edu

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Abstract: *Micraspis acicola* was described more than 50 years ago to accommodate a phacidium-like fungus that caused a foliar disease of *Picea mariana*. After its publication, two more species were added, *M. strobilina* and *M. tetraspora*, all of them growing on *Pinaceae* in the Northern Hemisphere, but each species occupying a unique type of host tissue (needles, cones or wood). *Micraspis* is considered to be a member of class *Leotiomycetes*, but was originally placed in *Phacidiaceae* (*Phacidiales*), later transferred to *Helotiaceae* (*Helotiales*) and recently returned to *Phacidiales* but in a different family (*Tympanidaceae*). The genus remains poorly sampled, and hence poorly understood both taxonomically and ecologically. Here, we use morphology, cultures and sequences to provide insights into its systematic position in *Leotiomycetes* and its ecology. Our results show that the genus should not be included in *Tympanidaceae* or *Phacidiaceae*, and support the erection of a new family and order with a unique combination of morphological features supported by molecular data.

INTRODUCTION

In 1963, Grant D. Darker discovered a fungus causing an unusual foliar disease of *Picea mariana* in Northeastern Ontario, Canada (Darker 1963). The disease symptoms superficially resembled Phacidium snow-blight and affected the lower branches beneath the winter snow line; however, the color of the affected needles ("dingy grayish straw color") was different from *Phacidiaceae* pathogens such as *Phacidium* or *Lophophacidium hyperboreum*. Both the sexual and asexual morphs were found in the same infected needles and Darker used this material to describe a novel monotypic genus, *Micraspis*, typified by *M. acicola* (*op. cit.*). According to Darker (1963), *M. acicola* apothecia were scattered on the needles, shining black, elliptical in outline, subcuticular, and opened by a longitudinal slit. The inamyloid asci bore eight three-septate, elliptical to obovate, hyaline ascospores. The asexual morph, described as *Periperidium acicola*, was macroscopically almost indistinguishable from the apothecia, but gave rise to (1–)2(–3)-septate, slightly curved, filiform conidia.

Darker (1963) hesitantly placed *Micraspis acicola* within *Phacidiaceae*, noting similarities between *M. acicola* and *Phacidium* but also pointing out resemblances with distantly related genera including *Sphaeropezia* and *Eupropolella*. These genera produce minute subcuticular, intra- or subepidermal apothecia, which are blackish, circular or elongate and open by one or several radiate fissures (Korf 1973, Nauta & Spooner 2000, Baloch *et al.* 2013). *Sphaeropezia* is currently placed in *Stictidaceae* (*Ostropales*; Baral 2016) and differs by its amyloid hymenium (Baloch *et al.* 2013). *Eupropolella* ascospores are brownish at maturity and the paraphyses are brownish because of their contents and collectively form a pseudoepithecium (Nauta & Spooner 2000). *Phacidium* apothecia differ because the covering layer opens by several irregular teeth (Korf 1973). Darker (1963) also recognized that the inamyloid asci, phragmospores and asexual morph of *M. acicola* were distinct from *Phacidiaceae*. In his discussion, Darker compared *Micraspis* mostly with genera in the families *Dermateaceae* and *Phacidiaceae*, and concluded its placement was in the family "*Cryptomyceten*" *sensu* von Höhnel (1917) which was in *Phacidiales* (Darker 1963). Ten years after its description, *Micrapis* remained in the same family, *Cryptomycetaceae* (*Phacidiales*), in the classification of *Leotiomycetes* provided by Korf (1973). Later, DiCosmo *et al.* (1983, 1984) were undecided on the placement of *Micraspis* but excluded it from *Phacidiaceae* because it lacked two diagnostic characters for the family: (1) vertically-oriented cells comprising the covering layer, and (2) an amyloid ascal apex. Consequently, DiCosmo *et al.* (1984) suggested the retention of *Micraspis* within *Tympaneae sensu* Korf (1973). In the first Outline of Ascomycota, Eriksson (1999) changed the placement of the genus to the family *Helotiaceae*, where it remained until recently (Eriksson *et al.* 2001, Eriksson *et al.* 2003, Eriksson

2005, 2006, Lumbsch & Huhndorf 2009). Finally, Baral (2016) tentatively placed *Micraspis* in *Tympanidaceae* (*Phacidiales*) based on its blastic-phialidic microconidia produced by phialides arising from ascospores (*i.e*., microcyclic conidiation or iterative germination) and septate macroconidia.

The family *Tympanidaceae* is currently composed of nine genera: *Claussenomyces* (asexual morph *Dendrostilbella*), *Collophorina*, *Durandiella* (*Chondropodium*, *Gelatinosporium*), *Grovesiella* (*Pitostroma*), *Holwaya* (*Crinula*), *Micraspis* (*Periperidium*, *Sporonema*), *Myrio-discus*, *Pragmopora* (*Pragmopycnis*) and *Tympanis* (*Sirodothis*) (Baral 2016). In this wide concept the family is morphologically rather heterogeneous and consists mostly of tree pathogens (*Tympanis*, *Durandiella*, *Pragmopora*), but also saprobes (*Claussenomyces p.p.* and *Holwaya*). Macroscopically, most species in *Tympanidaceae* are discoid or irregularly elongate, have black melanized tissues and develop inside the host and become erumpent at maturity (Quijada 2015). Some species are morphologically similar to *Micraspis*, sharing several microscopic features such as: dark ectal excipulum with thick-walled cells, hyaline plectenchymous medulla, cylindrical inamyloid asci with thick-walled apices and cylindrical-clavate spores with transverse septa (*op. cit.*). *Tympanidaceae* was placed in *Phacidiales* by Baral (2016), but in the last published multigene phylogeny of *Leotiomycetes*, which include several genera of *Tympanidaceae*, the family is placed in a supported clade related to *Leotiaceae* and *Mniaeciaceae*, all of these in *Leotiales* rather than *Phacidiales* (Johnston *et al.* 2019). However, this phylogeny did not include sequences of *Micraspis*.

Currently, *Micraspis* comprises only three species: *M. acicola*, *M. strobilina* and *M. tetraspora* (Index Fungorum 2019). All species grow on *Pinaceae* (*Picea* or *Pinus*) hosts in the Holarctic regions and species seems to be segregated by host plant species and tissue (needles, cones or wood). *Micraspis* species are known only from distinct substrates of conifer trees: *M. acicola* from *Picea* needles (Darker 1963, Davis & Myren 1990, Tanney 2017), *M. strobilina* from *Pinus sylvestris* cone scales (Dennis 1971), and *M. tetraspora* from decorticated wood of *Picea sitchensis* (Graddon 1984). There are few scattered reports from North America (Canada, USA) and Europe (Denmark, Ireland, Russia and UK), with fewer than 50 reports in total, mostly before the 21st century and primarily for *M. acicola* and *M. strobilina* (Darker 1963, Dennis 1971, Graddon 1984, Davis & Myren 1990, Popov 2007, Tanney 2017, GBIF 2019). *Micraspis tetraspora* appears to be known only from the type locality, Dymock Woods in the UK, growing on decorticated wood of introduced *Picea sitchensis* (Graddon 1984).

Since its inception more than 50 years ago, *Micraspis* has remained an obscure genus lacking modern taxonomic treatment. In this study, we provide a multigene phylogenetic analysis and morphological evidence to resolve the placement of *Micraspis* within *Leotiomycetes*. We also provide detailed observations on its distinct morphology based on type specimens and cultures and discuss the ecology of these rarely collected species.

MATERIAL AND METHODS

Specimens studied

Nine specimens were studied for morphological comparison, including the holotypes for each *Micraspis* species, which were requested from the Canadian National Mycological Herbarium (DAOM), Museum of Evolution Herbarium, Uppsala University (UPS) and the Royal Botanic Garden Kew Fungarium (K). The type locality for *M. tetraspora* was surveyed by Brian Douglas and collaborators, but trees of its reported host were not found and the search was unsuccessful. Two new collections of *Micraspis acicola* and *M. strobilina* were used in this study to generate cultures and DNA sequences. These are deposited in DAOM and Komarov Botanical Institute (LE) herbaria.

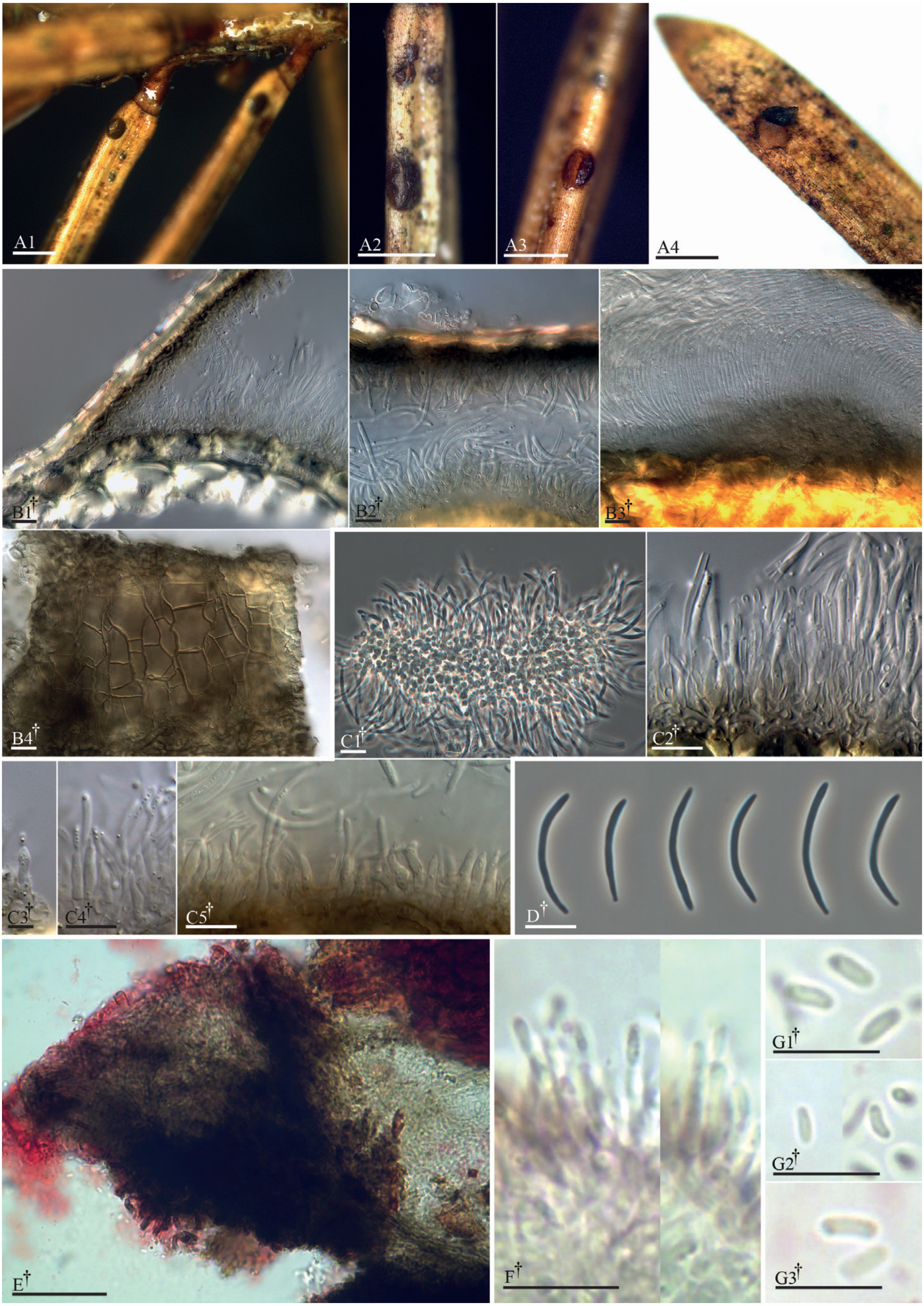
Morphology

Macro-photography of apothecia on the substrate were taken with fresh and dry samples. Herbarium specimens were hydrated with a spray bottle containing tap water before taking photographs. Micro-photography was done using a Motic B1 (MoticEurope S.L.U., Spain) compound light microscope with a USB Moticam 2500 camera. Motic Images Plus v. 2.0 processing software, calibrated for the optical devices of Motic B1, was used to carry out the biometry of each microscopic feature. Hand-sections for anatomic examination were made using a safety razor blade. Microscopic features were described from dry samples rehydrated in 85 % lactic acid (LA) or 3 % potassium hydroxide (KOH), and then stained with Congo Red (CR) or Melzer's reagent (MLZ) prior to observing morphological features. For the images of *M. acicola* in Fig. 1, fresh collections were used and observations were made from sections mounted in tap water, KOH, or LA using an Olympus BX50F4 light microscope (Olympus, Tokyo, Japan) and an Olympus SZX12 dissecting microscope; images were captured with an InfinityX-32 camera (Lumenera Corp., Ottawa, Canada) with Infinity Analyze (Lumenera Corp.) software. Photographic plates were assembled using Adobe Photoshop CC 2017 or Illustrator CC (Adobe Systems, San Jose, CA). Several transverse sections were studied to provide details about conidiomata, conidiophores, conidiogenous cells and conidia for the asexual morph, and excipula, paraphyses, asci, ascospores and associated conidia for the sexual morph. Species, genus, family and order descriptions were written with the new information gathered from our own observations based on the new collections and the types studies.

Cultures

Cultures of *Micraspis acicola* were obtained by using a flame-sterilized electrolytically-sharpened tungsten needle to collect conidia from conidiomata and then streaking them onto the surface of Petri dishes containing either 2 % malt extract agar (MEA; 20 g Bacto malt extract, Difco Laboratories, Sparks,

Fig. 1. Asexual morphs of *Micraspis*: **A–D.** *Micraspis acicola*. **E–G.** *Micraspis strobilina*. A1–A4. Conidiomata on dead attached *Picea rubens* needles. B1. Conidioma embedded in host tissue showing host cuticle firmly attached to covering layer. B2–B3. Palisade of conidiophores lining inner layers of conidioma and covering layer. B4, E. Covering layer composed of *textura epidermoidea* cells. C1. Conidiophores and conidiogenous cells with attached conidia. C2–C5, F. Conidiophores and conidiogenous cells. D, G1–G3. Conidia. Mounted media: KOH = B1–B4, C2–C5; KOH + CR = E, F, G1–G3; LA = C1, D. Scale bars: E = 50 µm; B1–C2, C4, D, F, G1–G3 = 10 µm; C3 = 5 µm. † denotes photo from dead material (following Baral 1992).


A1
A2
A3
A4
B1†
B2†
B3†
B4†
C1†
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C3†
C4†
C5†
D†
E†
F†
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G2†
G3†

MD; 15 g agar, EMD Chemicals Inc., NJ; 1 L distilled water) or cornmeal agar (CMA; Acumedia Manufacturers Inc., Lansing, MI). Inoculated Petri dishes were incubated upside down at 20 °C in the dark. Endophytic cultures of *M. acicola* were obtained from surface-sterilized needles of *Picea rubens*, as described by Tanney *et al.* (2016). In an effort to induce taxonomically-informative morphological characters (*i.e.*, sporulation) in sterile cultures, endophytic cultures were inoculated on a variety of media including MEA, oatmeal agar (OA; Crous *et al.* 2019), and CMA and water agar (WA; 1.5 % water agar with 1 mL trace metal solution; Visagie *et al.* 2014) with or without the addition of sterile *P. rubens* needles on the surface, and incubated for prolonged periods (more than 2 yr) at 16 °C in the dark or 12:12 h fluorescent light/dark cycle or at 4 °C in the dark. Additionally, WA blocks containing *M. acicola* mycelia were floated in Petri dishes containing sterile water (Tanney *et al.* 2018b). Representative strains were deposited in the Canadian Collection of Fungal Cultures (DAOMC; Agriculture & Agri-Food Canada, Ottawa, Ontario, Canada).

DNA extraction, sequencing and phylogenetic analyses

Total genomic DNA was extracted from 8–12-wk-old *M. acicola* cultures using the UltraClean Microbial DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA) and from herbarium material of *M. strobilina* (LE 236400) using the NucleoSpin® Plant II DNA Isolation Kit (Macherey-Nagel GmbH & Co. KG, Germany) following the manufacturers' protocols. The nuclear ribosomal internal transcribed spacer region (ITS) was amplified and sequenced using primers ITS1F and ITS4 as described by White *et al.* (1990) and Larena *et al.* (1999), partial large subunit of the nuclear ribosomal DNA (LSU) was amplified using primers LR0R and LR5 and sequenced using primers LR0R, LR3, LR3R, and LR5 (Vilgalys & Hester 1990, Rehner & Samuels 1994), and partial nuclear small subunit of the ribosomal DNA (SSU) was amplified and sequenced using primers NS1 and NS4 (White *et al.* 1990). Part of the second largest subunit of RNA polymerase II (*RPB2*) was amplified and sequenced using primers RPB2-5f and RPB2-7Cr according to Liu *et al.* (1999), translation elongation factor 1-alpha (*TEF1*) regions were amplified and sequenced using the primer pair EF1-983F and EF1-1567R (Carbone & Kohn 1999, Rehner & Buckley 2005), and part of the minichromosome maintenance complex component 7 (*MCM7*) gene was amplified and sequenced using the primer pair MCM7-709for and MCM7-1348rev (Schmitt *et al.* 2009). PCR products were verified by agarose gel electrophoresis and then sequenced with the BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. PCR products of *M. strobilina* were purified with the Fermentas Genomic DNA Purification Kit (Thermo Scientific, Thermo Fisher Scientific Inc., MA, USA) according to the manufacturer's instructions. Purified PCR products were sequenced on an ABI model 3130 Genetic Analyzer (Applied Biosystems, CA, USA). Resulting sequence contigs were assembled, trimmed, and manually checked using Geneious Prime 2019.0.4 (http://www.geneious.com). ITS sequences of *M. acicola* were used to query for related sequences in J.B. Tanney's personal *Picea* endophyte sequence database.

A phylogeny was constructed using the newly generated sequences from *Micraspis* spp. (Table 1) incorporated into the 15-gene DNA sequence alignment from Johnston *et al.* (2019; data available from doi.org/10.7931/T5YV-BE95), together

Table 1. New sequences generated and used in the phylogenetic analysis in this study.

		GenBank Accession No.							
Species	**Voucher No.**	**ITS**	**LSU**	**SSU**	***TEF1α***	***RPB2***	***MCM7***	**Host/substrate**	**Location**
Micraspis acicola	DAOM 252164	KY633584	KY633624	MN037803	MN044025	MN044020	MN044017	*Picea rubens* needle endophyte	Canada, New Brunswick, Albert County, Alma, Fundy National Park, Coppermine Trail (45.5493 -65.01878)
Micraspis acicola	DAOM 252165	KY633587	MN043954	MN037802	MN044024	MN044021	MN044016	*Picea rubens* needle endophyte	Canada, New Brunswick, Charlotte County, Little Lepreau (45.13683 -66.48155)
Micraspis acicola	DAOMC 251614/ NB-778	KY633585	MN043953	MN037801	MN044023	MN044018	MN044013	Conidiomata on dead, attached *Picea rubens* needle	Canada, New Brunswick, Charlotte County, Little Lepreau (45.13683 -66.48155)
Micraspis acicola	DAOMC 252018/ NB-437-6B	KY633589	KY633625	MN037804	MN044026	MN044019	MN044015	*Picea rubens* needle endophyte	Canada, New Brunswick, Northumberland County, Doaktown (46.480353 -66.058096)
Micraspis acicola	DAOMC 251526/ NB-277-9C	KY633588	MN043957	MN037805	MN044027	MN044022	MN044014	*Picea rubens* needle endophyte	Canada, New Brunswick, Albert County, Alma, Fundy National Park, Dickson's Falls (45.586991 -64.969901)
Micraspis strobilina	LE 236400	MN043950	MN043959	n/a	n/a	n/a	n/a	Fallen cones of *Pinus sylvestris*	Russia, Leningrad Oblast, Vyborg District, Berezovye Ostrova Natural Sanctuary

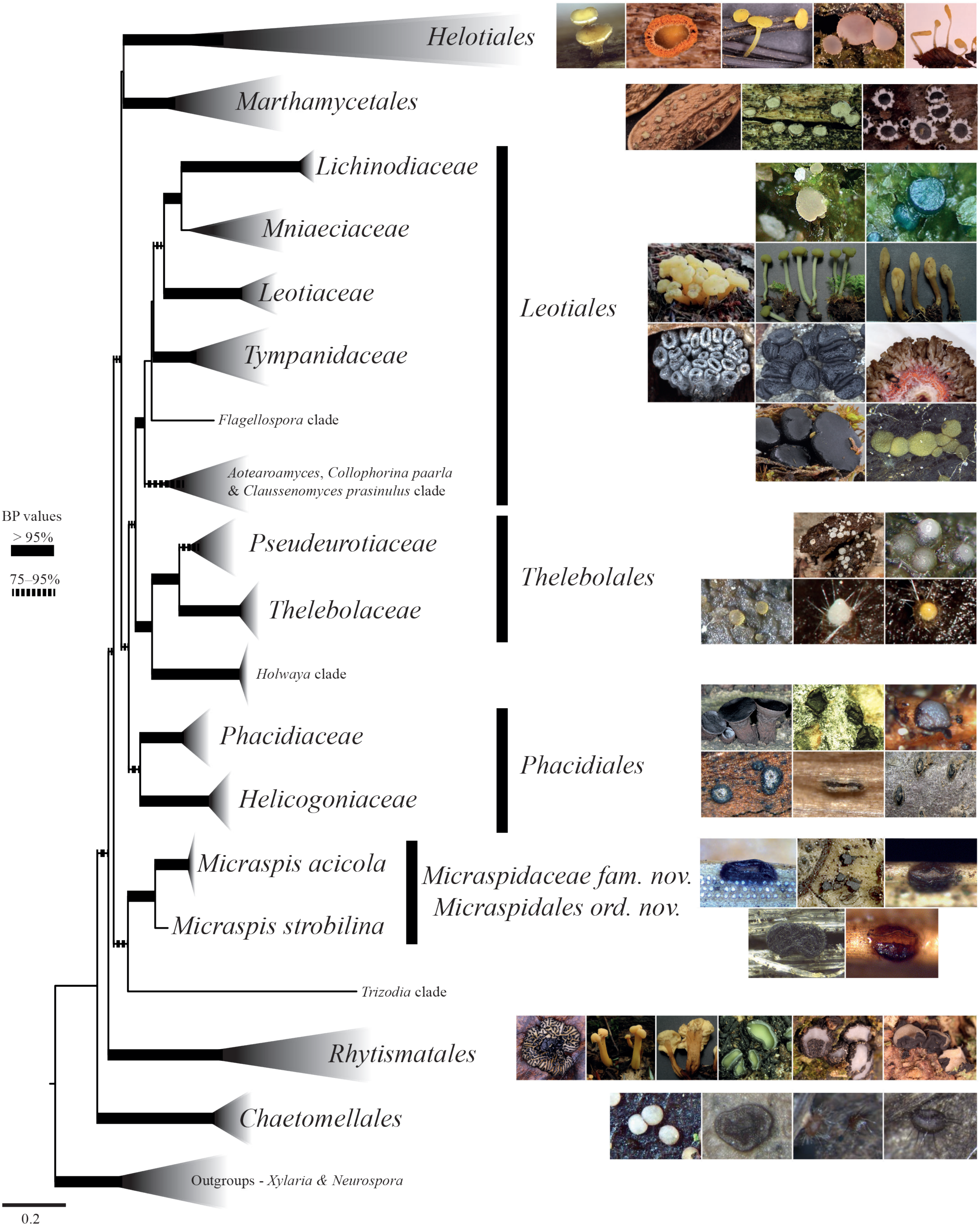


Fig. 2. ML tree based on concatenated DNA sequences for *Micraspis*, plus the taxa treated by Johnston *et al.* (2019) and *Lichinodiaceae* sequences from Prieto *et al.* (2019). The collapsed clades represent the strongly supported family-level and order-level clades accepted by Johnston *et al.* (2019), who provide data on the taxa used to represent each of these clades. The images to the right illustrate the macro-morphological diversity of the order-level clades. Thick branches have bootstrap support (BP) values > 95 % and the dashed branches bootstrap support values 75–95 %.

Table 2. Genes included in the 15 gene phylogeny, alignment information and models selected by ModelFinder (Kalyaanamoorthy 2017)**.**

Gene	Alignment length	Informative sites	Model
ribosomal DNA 5.8S region (5.8S)	157	40	TNe+R3
alpha-Tubulin	2015	1017	GTR+F+R6
beta-Tubulin	2154	993	TIM2+F+R6
ribosomal DNA large subunit (LSU)	985	395	TIM3e+R6
Minichromosome maintenance complex component 7 gene (*MCM7*)	720	375	GTR+F+R6
Mitochondrial small subunit gene (mtSSU)	772	338	TVM+F+R5
Replication protein A 70 KDa DNA-binding subunit gene (*RPA1*)	1069	642	GTR+F+R6
Replication protein A 32 KDa subunit gene (*RPA2*)	843	454	TIM3e+R6
DNA-directed RNA polymerase II subunit 1 gene (*RPB1*)	905	512	TIM3+F+I+G4
DNA-directed RNA polymerase II subunit 2 gene (*RPB2*)	3531	1988	GTR+F+R8
DNA-Directed RNA polymerase III 127.6 KDa polypeptide gene (*RPC2*)	1562	923	GTR+F+R7
Splicing factor 3B subunit 1 gene (*SF3B1*)	1096	481	GTR+F+R6
ribosomal DNA small subunit region (SSU)	1413	319	TIM+F+R4
General transcription and DNA repair factor IIH subunit gene (*TFB4*)	1280	476	GTR+F+R6
Elongation factor 1-alpha gene (*TEF1*)	1913	966	TIM2+F+R7

with *Lichinodium sirosiphoideum* and *L. ahlneri* sequences of SSU, 5.8S, LSU, *MCM7*, *RPB1*, and *RPB2* from Prieto *et al.* (2019), representing *Lichinodiaceae*. The sequences available for each gene were aligned using MAFFT (Katoh & Standley 2013) as implemented in Geneious. The ends were manually trimmed, and introns were removed manually; all remaining data were then concatenated. Maximum likelihood (ML) analyses were run with IQ-TREE v. 1.6.6 (Nguyen *et al.* 2015, Chernomor *et al.* 2016), using models selected by ModelFinder (Kalyaanamoorthy 2017) for each partitioned gene (see Table 2); ultrafast bootstrap (BS) analysis with 1 000 replicates estimated branch support in the ML tree (Hoang *et al*. 2018). *Xylaria hypoxylon* (AFTOL-ID 51, isolate OSC 100004, JGI genome Xylhyp) and *Neurospora crassa* (isolate OR74A, JGI genome Neucr2) were used as outgroups.

RESULTS

Information about alignment and informative sites of the phylogenetic analysis are summarized in Table 2. The overall topology of the ML phylogeny (Fig. 2) is consistent with that presented by Johnston *et al.* (2019). The large *Helotiales* clade is collapsed in Fig. 2, as it does not inform the relationship of *Micraspis*, which is positioned amongst the basal lineages within *Leotiomycetes*. *Lichonidiaceae*, not treated by Johnston *et al.* (2019), is strongly supported as a member of the *Leotiales sensu* Johnston *et al.* (2019). *Micraspis*, represented here by specimens identified as *M. acicola* and *M. strobilina*, forms a strongly supported clade with no clear relationships to any existing family or order within *Leotiomycetes* (Fig. 2). The poorly supported sister relationship to a specimen identified as *Trizodia acrobia* features a long branch and this relationship requires testing with additional genes or additional phylogenetically closely related taxa. The five *Micrapis acicola* specimens sampled all had identical ITS sequences (unpubl. data); the *M. strobilina* ITS sequence had a 92 % similarity to *M. acicola*.

A query of J.B. Tanney's personal endophyte sequence database was made using ITS sequences of cultures obtained from *M. acicola* conidia. The results showed that sequences of seven unidentified *Picea rubens* endophyte cultures were identical to sequences of cultures from *M. acicola* conidia, *e.g.*: DAOMC 251614, DAOMC 251526; identities = 593/593 (100 %). Prior to the availability of ITS sequences from the *M. acicola* field collection, *M. acicola* endophyte strains were unidentifiable because of the absence of taxonomically informative morphological characters *in vitro* and relevant reference sequences. The seven endophyte cultures of *M. acicola* originated from five separate collections within New Brunswick, Canada (Table 1). The colony morphologies of endophyte and conidial isolates were congruent with one another and colonies remained sterile despite efforts to induce sporulation.

Taxonomy

Micraspidales Quijada & Tanney ***ord. nov.*** MycoBank MB831355.

Etymology: Named after the type genus *Micraspis*.

Diagnosis: Phylogenetically isolated within *Leotiomycetes*; conidiomata not distinct macroscopically from the ascomata, differs from the micro-morphologically similar *Leotiales*, *Phacidiales* and *Rhytismatales* by having tissues composed of *textura epidermoidea* in the ectal excipulum and covering layer, and ascospores producing conidia from their walls but also from germ tubes.

Type family: *Micraspidaceae* Quijada & Tanney

Classification: *Micraspidales*, *Leotiomycetes*, *Pezizomycotina*, *Ascomycota*.

Apothecia orbicular to elliptic, developing inside the host tissues, receptacle stromatic, shining black, irregularly opening by longitudinal slits that remain as rectangular lids or disappear in a smooth protruding margin, hymenium concolor or gray-orange. *Ectal excipulum* at flanks and margin composed of *textura epidermoidea* covered outside by a thick refractive smooth yellowish gel. *Medullary excipulum* plectenchymatous. *Asci* 4–8-spored, inamyloid with the apical wall strongly thickened with an ocular chamber. *Ascospores* cylindrical-fusoid to clavate-ellipsoid, septate, without sheaths, eguttulate or with tiny sparse guttules, germinating at both poles and producing cylindrical or sub-cylindrical conidia directly from the ascospores walls or germ tubes. Asexual morph: *Conidiomata* pycnidioid, macroscopically indistinguishable from apothecia, unilocular to multilocular, convoluted. *Conidiophores* simple or branched, hyaline, smooth, frequently reduced to conidiogenous cells. *Conidiogenous cells* enteroblastic, phialidic, hyaline, smooth, aperture minute, collarette inconspicuous. *Conidia* filiform to falcate or fusiform to allantoid, hyaline, smooth, guttulate or eguttulate.

Micraspidaceae Quijada & Tanney ***fam. nov.*** MycoBank MB831356.

Etymology: Named after the type genus *Micraspis*.

Diagnosis: Phylogenetically isolated within *Leotiomycetes*; differs from morphologically similar *Tympanidaceae* and *Phacidiaceae* because of the *textura epidermoidea* in the ectal excipulum and covering layers and the simultaneous production of conidia from ascospore walls and germ tubes.

Type genus: *Micraspis* Darker

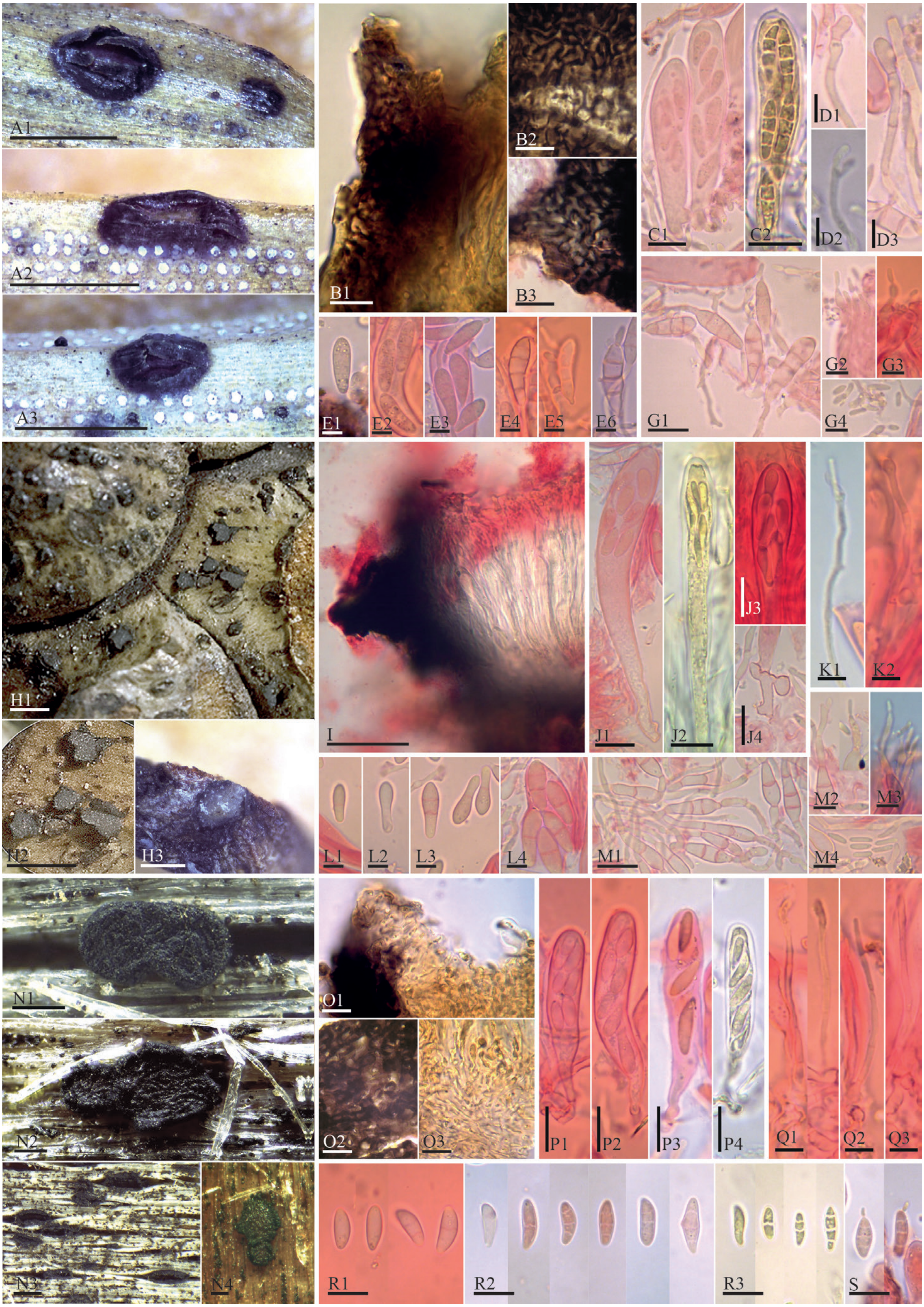
Micraspis Darker, *Canad. J. Bot*. **41**: 1390. 1963.

Sexual morph: *Apothecia* scattered or in small clusters, not confluent, orbicular to irregularly elongate, subcuticular or erumpent between fibers, primordia developing inside the superficial layers of the host tissues, closed at first, then opening by irregular lacerate longitudinal slits when mature, tearing open of the original stromatic covering layer, like rectangular lids that open when moist, outside dark, inside black or light gray (hyaline), or less differentiated, smooth and protruding slightly above the hymenium, receptacle highly melanized, shining black, contracted and hiding the hymenium when dry, when fresh exposing a pale gray-orange or black hymenium. *Ectal excipulum* poorly developed, similar thickness from lower flank to margin, 20–120 µm thick, thicker at upper flank and thinner near the margin or in the lower flanks, up to 30 µm thick or less at base; composed of *textura epidermoidea* at margin and flanks, hyphae thick-walled agglutinated and interwoven mostly in one plane, cells irregularly shaped, walls dark brown and lumen hyaline, cortical layer with cells covered by a thick refractive smooth yellowish gel, more abundant at upper and lower flanks; excipulum at base of *textura globosa–angularis*, cells smaller than at flanks and with thin dark walls and vertically arranged near medulla changing progressively in the medulla to hyaline cells. At the margin, covering layer lined on the inside with up to 5–20 µm of branched netlike interwoven colorless hyphae immersed in a yellowish gel (plectenchymatous tissue), merging at the periphery of the stroma with vertically oriented cells. *Medullary excipulum* mostly restricted beneath the hymenium, 20–100 µm thick, not developed to undifferentiated from the ectal excipulum at flanks, below the hymenium composed of a loose network of narrow hyphae (plectenchymatous tissue), then brownish to hyaline *textura globosa–angularis* in the transition with ectal excipulum. *Paraphyses* cylindrical uninflated to slightly-medium clavate at the apex, straight or slightly sinuous, no more than 5-septate, equidistantly spaced or with 1–2 cells apically closely septate and constricted, simple or branching dichotomously below the apical cell, rarely branched in the lower cells, covered or not by a thin brownish amorphous layer. *Asci* cylindric-clavate, 4–8-spored, bi- or triseriate inside the asci, in dead state entire wall of sporiferous part thick-walled, apex hemispherical, inamyloid, wall strongly thickened with an ocular chamber, arising from croziers. *Ascospores* cylindrical-fusoid to clavate-ellipsoid, hyaline, poles rounded to obtuse, sometimes with one extreme wider than the other, straight or slightly curved, with 1–3(–5) transverse septa at maturity, not or slightly constricted at septa, without sheaths, eguttulate or with tiny sparse guttules, germinating at both poles and producing cylindrical or sub-cylindrical conidia directly from the ascospores walls or germ tubes. *Asexual morph*: *Conidiomata* pycnidioid, scattered, dark brown to black, shining, outline elliptical to circular or irregular, raised to pulvinate, immersed, subcuticular to partly subepidermal, unilocular to multilocular or convoluted, inostiolate, opening by single longitudinal or several radial splits, covering layer *textura epidermoidea* composed of dark brown to black, septate, thick-walled, irregular hyphae, firmly attached to host cuticle or epidermis. *Conidiophores* lining all inner surfaces of conidioma, simple or branched, sometimes septate, subcylindrical, tapering toward apex, hyaline, smooth, frequently reduced to conidiogenous cells. *Conidiogenous cells* enteroblastic, phialidic, integrated or discrete, determinate or indeterminate, simple or unbranched, hyaline, smooth, aperture minute, collarette inconspicuous. *Conidia* filiform to falcate or fusiform to allantoid, 0–2(–3)-septate, hyaline, smooth, guttulate or eguttulate.

Micraspis Darker emended species descriptions

Micraspis acicola Darker, *Canad. J. Bot.* **41**: 1390. 1963. ***emend.*** Quijada & Tanney, Fig. 3A–G.
Synonym: *Periperidium acicola* Darker*, Canad. J. Bot.* **41**: 1392. 1963.

Apothecia up to 0.4–0.6 mm diam, black, subcuticular, elliptical, tearing openi, hymenium pale orange, margin lacerate with rectangular covering layers over the hymenium. *Ectal excipulum* brown-black of *textura epidermoidea* from margin to lower flank, 11–34 µm thick, at base poorly developed, hyaline and not differentiated from medullary excipulum, mostly *textura angularis*, 15–27 µm thick. Cells at margin and upper flank 3.5–8.5 × 1.5–4 µm, cells at base 3–6.5 × 1.5–4 µm, cell walls dark brown, up to 1.5 µm thick. Cortical layer from margin to lower flanks covered by a thick refractive golden colored gel, 2.5–5.5 µm thick. *Asci* (41–)50–55(–58.5) × (7–)8.5–10(–11) µm, 8-spored. *Ascospores* (10–)13–14.5(–18.5) × (3.3–)3.8–4.2(–5.2) µm, 1–3-septate, low to medium content of tiny guttules in each cell, germinating at both poles and producing cylindrical or sub-cylindrical conidia (3.4–4.7 × 1–1.6 µm) directly from the ascospore walls or germ tubes. *Paraphyses* cylindrical, slightly to medium clavate, 3–5-septate, apical cells shorter and constricted at the septa, simple or dichotomously branched below apical cell, slightly sinuous, without exudates or guttules, apical cells 3–7 × 3–4.5 µm, lower cells 5.5–9.5 × 1.5–2.5 µm, basal cells 10.5–19.5 × 1.5–2.5 µm. *Conidiomata* pycnidioid, amphigenous, immersed, subcuticular, scattered, up to 0.4–0.6 mm diam, dark brown to black, shining, outline elliptical to circular or irregular,


A1
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raised to pulvinate, unilocular, inostiolate, covering layer opening by a single longitudinal slit. *Covering layer* 10–16(–20) µm thick, of *textura epidermoidea*, composed of dark brown to black, septate, thick-walled, irregular hyphae agglutinated to form a compact integument that is firmly attached to the host cuticle above. *Conidiophores* simple or branched, 1–2-septate, subcylindrical and tapering towards apex, hyaline, smooth, frequently reduced to conidiogenous cells, in palisade lining all inner surfaces of conidioma, arising from a layer composed of hyaline to pale brown *textura angularis*, 2–4 cells in thickness and ca. 10 µm thick. *Conidiogenous cells* enteroblastic, phialidic, integrated or discrete, determinate, subcylindrical and tapering towards apex to ampulliform, widest +/- at middle, hyaline, smooth, aperture minute, collarette inconspicuous, (7–)7.5–11(–14) × 2–2.5(–3) µm. *Conidia* curved to falcate, apex and base obtuse, (1–)2(–3)-septate, hyaline, thin-walled, eguttulate, (18–)19–24(–26) × 1.5–2 µm.

Typus: **Canada**, Ontario, North of long portage into Gull Lake, Lake Temagami, Nipissing District, on needles of *Picea mariana*, 27 Jul. 1927, *G.D. Darker* (UPS-F-646094, **isotype**; DAOM 90961, **holotype**).

Additional materials examined: **Canada**, Newfoundland, 5 mi. N. of Port Aux Basques, on needles of *Picea rubens*, 5 Aug. 1963, *R.F. Cain* (DAOM 115204, DAOM 736596); 8 Aug. 1963, *R.F. Cain* (DAOM 158535); New Brunswick, Albert County, Alma, Fundy National Park, Coppermine Trail, endophyte isolated from surface-sterilized needle of *Picea rubens*, 17 Jul. 2014, *J.B. Tanney* (NB-505-2I); New Brunswick, Albert County, Alma, Fundy National Park, Coppermine Trail, endophyte isolated from surface-sterilized needle of *Picea rubens*, 17 Jul. 2014, *J.B. Tanney* (NB-505-12I); New Brunswick, Northumberland County, Doaktown, endophyte isolated from surface-sterilized needle of *Picea rubens*, 13 Jul. 2014, *J.B. Tanney* (DAOMC 252018); New Brunswick, Albert County, Alma, Fundy National Park, Dickson's Falls, endophyte isolated from surface-sterilized needle of *Picea rubens*, 24 Jun. 2013, *J.B. Tanney* (DAOMC 251526); New Brunswick, Albert County, Alma, Fundy National Park, Dickson's Falls, endophyte isolated from surface-sterilized needle of *Picea rubens*, 23 Sep. 2013, *J.B. Tanney* (NB-366-3A); New Brunswick, Albert County, Alma, Fundy National Park, East Branch Trail, endophyte isolated from surface-sterilized needle of *Picea rubens*, 25 Sep. 2013, *J.B. Tanney* (NB-392-3M2); New Brunswick, Charlotte County, Little Lepreau, on needles of *Picea rubens*, 17 Jun. 2016, *D. Malloch* (DAOM 745745, DAOMC 251614).

Micraspis strobilina Dennis, *Kew Bull*. **25**: 362. 1971. ***emend.*** Quijada & Tanney, Fig. 3H–M.
Synonym: *Sporonema diamandidis* Minter, *Trans. & Proc. Bot. Soc. Edinb*. **43**: 183. 1980.

Apothecia up to 0.6 mm diam, receptacle black, round to irregular ellipsoid, erumpent, covering layer opening one (rarely more) longitudinal or lateral slit, hymenium gray. *Ectal excipulum* dark brown, composed of *textura epidermoidea* from margin to lower flank, 11–66 µm thick, at base *textura epidermoidea* to *textura angularis*, 12–39 µm thick. Cells at margin and upper flank (3.5–)4.5–6(–8.5) × (1.5–)2–2.5(–3.5) µm, cells at base (2.5–)3–4.5(–6) × 1.5–2(–2.5) µm, cell walls dark brown, up to 1.5 µm thick. Cortical layer of the receptacle covered by a refractive golden gel up to 5 µm thick. *Medullary excipulum* light brownish, of *textura angularis* to plectenchymatous tissues, 45–65 µm thick. *Asci* (57.5–)61.5–76(–87.5) × (7.5–)8.5–10(–10.5) µm, 8-spored. *Ascospores* (6.7–)10.4–12.3(–14.5) × (2.7–)3.4–3.9(–4.8) µm, 1–3-septate, scattered tiny guttules mostly present in immature ascospores, germinating at both poles and producing cylindrical or sub-cylindrical conidia (3.7–6.1 × 0.9–1.7 µm) directly from the ascospore walls or germ tubes. *Paraphyses* cylindrical uninflated or slightly clavate, 3–5 equidistant septa, sometimes apical cells shorter, simple or dichotomously branched in the apical or lower cells, slightly sinuous, without exudates or guttules, apical cells 4–9.5 × 1.5–2.5 µm, lower cells 6.5–16 × 1.5–2 µm, basal cells 10–16 × 1–1.5 µm. *Conidiomata* pycnidioid, immersed in substrate, subcuticular or sometimes partly subepidermal, scattered, up to 1 mm diam, black, shining, outline +/- circular, pulvinate, convoluted locules, inostiolate, covering layer opening by a single longitudinal slit or several radial slits. *Covering layer ca*. 20 µm thick, *textura epidermoidea*, composed of dark brown to black, septate, thick-walled, irregular hyphae agglutinated to form a compact integument that is firmly attached to the host cuticle above and sometimes containing degraded epidermal cells. *Conidiophores* simple or branched, aseptate or septate, subcylindrical and tapering towards apex, hyaline, smooth, frequently reduced to conidiogenous cells, in palisade lining all inner surfaces of conidioma, arising from a layer composed of hyaline to subhyaline *textura angularis*. *Conidiogenous cells* enteroblastic, phialidic, integrated or discrete, indeterminate, subcylindrical and tapering towards apex to ampulliform, widest +/- at middle, hyaline, smooth, aperture minute, collarette inconspicuous, 7–9(–12) × 1.5–2(–2.5) µm. *Conidia* bacillar to allantoid, apex and base obtuse, aseptate, hyaline, thin-walled, eguttulate, (3–)4–6(–8) × 1.5–2 µm.

Typus: **UK**, Isle of Mull, Lonch Bàa, on cones of *Pinus sylvestris*, 7 Aug. 1968, *R.W.G. Dennis* (K-M-31759, **holotype**).

Additional material examined: **Russia**, Leningrad Oblast, Vyborg District, Berezovye Ostrova Natural Sanctuary, Severny Berezovy Isl., on decaying cones of *Pinus sylvestris*, 19 Jul. 2005, *E.S. Popov* (LE 236400).

Micraspis tetraspora Graddon, *Trans. Br. mycol. Soc*. **83**: 379. 1984. ***emend.*** Quijada, Fig. 3N–S.

Apothecia up to 0.2–0.5 mm diam., black, elliptical, erumpent between fibers, margin differentiated, smooth to crenate or

Fig. 3. Sexual morphs of *Micraspis*: **A–G.** *Micraspis acicola*. **H–M.** *Micraspis strobilina*. **N–S.** *Micraspis tetraspora*. A1–A3, H1–H3, N1–N4. Rehydrated apothecia; B, H, O1–O3. Transverse section of the excipulum; C1–C2, J1–J4, P1–P4. Immature and mature asci with ascospores and base of the asci with croziers; D1–D3, K1, K2, Q1–Q3. Paraphyses; E1–E4, L1–L4, R1–R3. Immature and mature ascospores without conidia; E5, E6, S. Ascospores with conidia budding directly from walls; G1–G3, M1–M3. Germinated ascospores and conidia produced from the germ tube; G4, M4. Free conidia. Mounted media: KOH + MLZ = B1, C2, C3, D2, J2, P4, O1–O3, R3; KOH + CR = B2, B3, C1, D1, D3, E1–E6, G1–G4, I, J1–J4, K1, K2, L1–L4, M1–M4, P1–P3, Q1–Q3, R1, R2, S. Scale bars: 500 µm = A1–A3, H1–H3; 100 µm = N1–N4; 50 µm = I; 10 µm = B1–B3, C1, C2, G1, J1–J4, M1, P1–P4, R1–R3, S; 5 µm = D1–D3, E1–E6, G2–G4, K1, K2, L1–L4, M2–M4, O1–O3, Q1–Q3.

slightly lacerate, hymenium black to dark brownish and without covering layers above. *Ectal excipulum* dark brown, composed of *textura epidermoidea* from margin to lower flank, 21–48 µm thick, at base *textura epidermoidea* to *textura angularis*, 25–30 µm thick. Cells at margin and upper flank 3.5–6 × 1.5–3 µm, cells at base 3–7 × 1.5–4 µm, cells wall dark brown, up to 1.5 µm thick. Cortical layer of the margin outside covered by a thin refractive golden gel, up to 1 µm, mostly present in the upper flanks and margin of the receptacle. *Medullary excipulum* plectenchymatous, strongly differentiated from the ectal excipulum, 15–54 µm thick. *Asci* (33.5–)42–46(–51) × (6–)7–8(–9) µm, 4–8-spored. *Ascospores* (7.5–)9.5–10.5(–13.5) × (2–)3–4 µm, 1–3(–5)-septate, with groups of tiny guttules mostly at poles, conidia only formed directly from ascospores walls, 2–3 × 0.7–1.1 µm. *Paraphyses* cylindric, uninflated, 3–4 equidistant septa, not branched, straight or slightly sinuous, covered by a brownish thin smooth exudate, without guttules, apical cells (5–)9–13 × 1–1.5 µm, straight or slightly curved, lower cells 8.5–11.5 × 1–1.5 µm, basal cells (6–)9–12.5 × 1–1.5 µm. *Asexual morph* unknown.

Typus: **UK**, Dymock woods, Herefords, on wood of *Picea sitchensis,* 10 Nov. 1982, *W.D. Graddon* (K-M-44301, **holotype**).

Key to species based on sexual morphs

1a. Asci 4–8-spored (in mature asci some spores aborted), maximum length of asci ≤ 50 µm (mean length 42–46 µm), paraphyses equidistantly septate, not branched and covered by a brownish exudate, on *Picea* wood .... *M. tetraspora*
1b. Asci 8-spored (no aborted spores), maximum length of asci ≥ 50 µm, paraphyses with apical cells shorter than lower cells, simple or dichotomously branched, without brownish exudate, on *Pinus* cones or *Picea* needles 2

2a. Mean of the ascus length 50–55 µm, maximum ascus length ≤ 65 µm, growing on *Picea* needles .... *M. acicola*
2b. Mean of the ascus length 60–75 µm, maximum ascus length ≥ 65 µm, growing on *Pinus* cones .... *M. strobilina*

DISCUSSION

Previously, the taxonomic placement of the type species of *Micraspis*, *M. acicola*, was uncertain, with Darker (1963) originally placing it in *Phacidiaceae* and later DiCosmo *et al*. (1983) and Baral (2016) considering it in *Tympaneae* and *Tympanidaceae*, respectively. In this study, we present morphological and phylogenetic evidence supporting the description of a novel order and family to accommodate the genus *Micraspis*. From the phylogenetic insight presented in this study, it becomes obvious why previous morpho-taxonomic work attempting to place *Micraspis* into an existing family and order resulted in confounding taxonomic conclusions. Our understanding of *Micraspis* is almost entirely restricted to original species descriptions (Darker 1963, Dennis 1971, Minter 1980, Graddon 1984) and the DiCosmo *et al*. (1984) revision of *Phacidiaceae*. Therefore, we will present a detailed discussion of morphologically similar taxa and the biology and ecology of *Micraspis*.

It is understandable that *Micraspis* was previously considered in *Tympanidaceae* and *Phacidiaceae*. Morphologically, *Micraspis* resembles some *Tympanidaceae* species, such as those discussed in the introduction. Species in the genera *Tympanis* and *Durandiella* with solitary rather than clustered ascomata have a similar macro-morphology. Some of these species produce apothecia that are approximately the same size as *Micraspis*, which are also erumpent from the host tissue and have similar stromatic tissues (Quijada 2015). *Grovesiella* is the only *Tympanidaceae* genus with a covering layer over the hymenium that tears apart and forms a lacerate margin like *Micraspis*; however, the excipulum is composed of *textura angularis* to *prismatica* (*op. cit.*), whereas in *Micraspis* it is *textura epidermoidea* (Fig. 3). Also, the spores of *Grovesiella* are acicular to cylindrical-fusoid and larger than in any species of *Micraspis* (*op. cit.*). *Pragmopora* is the *Tympanidaceae* genus most similar to *Micraspis*, but the hymenium of *Pragmopora* is embedded in a brownish to olivaceous gelatinous matrix, with abundant hyaline or yellowish, amorphous, intercellular resinous drops. Besides, the ectal excipulum is composed of *textura oblita* (*op. cit.*). Neither of these features are present in any *Micraspis* species. Several species in *Tympanidaceae* produce conidia or ascoconidia from the ascospores (Baral 1999, Quijada 2015, Quijada *et al.* 2019). Some species in *Tympanidaceae* produce conidia budding directly from the ascospore wall, inside the dead asci or after the ascospores are ejected, for example *Holwaya mucida*, *Pragmopora* spp., *Claussenomyces prasinulus* and *C. kirschsteinianus* (Quijada 2015, Quijada *et al.* 2019). Whereas, *Tympanis* spp., *Myriodiscus sparassoides* and *Claussenomyces atrovirens* produce ascoconidia from germ tubes or directly from the spore walls, and they are always packed in balls inside the living asci and ejected as one unit (Quijada *et al*. 2019). Darker (1963) illustrated germinating ascospores of *M. acicola*, but he did not provide any information about this feature in his description. No *Micraspis* species were described as producing conidia from ascospores (Darker 1963, Dennis 1971, Graddon 1984). Darker's drawing of the ascospores show structures similar to conidia that are formed in the median cells, and the long germ tubes extending from the basal and apical cells, but he simply wrote "ascospores at various stages of development and germination". Darker (1963) probably misinterpreted the conidia formed on the spore walls as germ tubes, but as we show here, the germ tubes in *Micraspis* are only produced from the poles, and conidia can be formed directly in any part of the spore walls or germ tubes (Fig. 3E5, E6, G1–G3, M1–M3, S). This combination of features observed in *Micraspis*, conidia formed directly from ascospore walls and germ tubes, is never present in any member of the *Tympanidaceae*. These differences are reflected in our phylogenetic analysis, which shows *Tympanidaceae* to be distantly related to *Micraspis* (Fig. 2).

Phacidiaceae contains species reported as endophytes, saprotrophs and pathogens from a wide range of plant hosts, especially *Ericaceae*, *Pinaceae* and *Rosaceae* species (Tanney

& Seifert 2018). Darker (1963) described the symptoms caused by *M. acicola* on *Picea mariana* as suggestive of Phacidium snow blight (*Phacidium infestans,* now *Gremmenia infestans*; *Phacidiaceae*, *Phacidiales*), but distinct from *Lophophacidium hyperboreum* (*Phacidiaceae*), which also causes a snow blight of *Picea*. The reasons for Darker's placement of *Micraspis acicola* within *Phacidiaceae* were largely superficial, such as its dark excipulum that is more-or-less attached to the host cuticle and the lacerate tearing of the covering layer. Other similarities between *Micraspis* and *Phacidiaceae* include apothecia and conidiomata being immersed and becoming erumpent from the host tissue, some species having 4–8-spored asci (*e.g. M. tetraspora* and *Darkera parca*), and ascospores lacking mucilaginous sheaths or appendages. However, there are salient differences that morphologically distinguish *Micraspis* from *Phacidiaceae*. The covering layer of *Micraspis* apothecia and conidiomata are distinctly *textura epidermoidea*, whereas *Phacidiaceae* species have a covering layer consisting of vertically-oriented cells that form a *textura globulosa* with the innermost layer composed of hyaline periphysoids invested in mucilage (DiCosmo *et al.* 1983, 1984). *Phacidiaceae* ascal apices are primarily amyloid (except *Pseudophacidium*) while those of *Micraspis* are inamyloid. *Micraspis* ascospores are 1–3-septate and produce blastic-phialidic conidia directly from the ascospore cell wall or germ tubes, which are features that are atypical of *Phacidiaceae*. Finally, the asexual morphs of *M. acicola* and *M. strobilina* are morphologically dissimilar to those from *Phacidiaceae*.

In summary, the morphology of *Micraspidaceae* (*Micraspidales*) could be confused with *Tympanidaceae* (*Leotiales*) and *Phacidiaceae* (*Phacidiales*), but the combination of the following features distinguish *Micraspidaceae* (*Micraspidales*): (1) apothecial covering layer formed of *textura epidermoidea* and lacking periphysoids immersed in mucilage; (2) ectal excipulum also of *textura epidermoidea* but covered outside in the margin and flanks by a shiny yellowish gel; (3) ascospores germinating only at poles and producing conidia on the germ tubes and also budding from ascospores walls; and (4) conidiomata macroscopically indistinguishable from ascomata with covering layer formed of *textura epidermoidea*.

Micraspis acicola is known from *Picea mariana*, rarely *P. glauca* and *P. rubens*, across eastern Canada, from Northwestern Ontario to Newfoundland. *Micraspis acicola* is not considered a significant pathogen and reports of its occurrence are rare (*e.g.*, Davis & Myren 1990). Here we report the isolation of *M. acicola* from surface-sterilized needles from a culture-dependent study of *P. rubens* foliar endophytes (Tanney 2017, Tanney *et al.* 2018a). *Micraspis acicola* endophyte strains were initially unidentifiable because of the absence of pertinent reference sequences and *in vitro* morphological characters; however, collection and subsequent culturing and sequencing of *M. acicola* conidiomata from dead, attached needles of *P. rubens* permitted their identification. This is yet another example demonstrating the identification of unknown endophytes by means of connecting them with identifiable field (or herbarium) specimens (*e.g.*, Tanney *et al.* 2016, McMullin *et al.* 2019, Tanney & Seifert 2019), and underlines the importance of taxonomic work to support biodiversity surveys (Truong *et al.* 2017). In this study, we provide cultural and genetic evidence establishing the connection between *Micraspis acicola* and its purported asexual morph (= *Periperidium acicola*) on symptomatic needles and also report its occurrence as an endophyte on asymptomatic needles. Our phylogenetic analyses also support the relationship between *M. acicola* and *M. strobilina* (Fig. 2). But also, our study of type specimens verified the similarities among the three species in the genus (Fig. 3). In our type study we found the asexual morph of *M. strobilina*, which was not described by Dennis (1971). No asexual morph is reported for *M. tetraspora* but it presumably co-occurs with the ascomata on decorticated wood of *Picea sitchensis* and possibly other conifer hosts.

Although the biology and ecology of *Micraspis* are poorly understood, we use the type species here to understand more about their association with their hosts. Our results identify the occurrence of *M. acicola* as a pathogen and endophyte and provide some insight into the ecology of this species in the genus. We hypothesize that *M. acicola* causes prolonged asymptomatic foliar infections (*i.e.*, endophytic) in *Picea* spp. in eastern North America and switches to an opportunistic pathogenic or saprotrophic phase following host needle stress or senescence, where it then produces conidiomata and apothecia. The infection pathway is unknown, although collection data show that ascospore dispersal occurs in late summer (July and August) and is presumably facilitated by air current, rain splash, and canopy throughfall. Conidia, which readily germinate on MEA, are aggregated in a more-or-less slimy mass on the surface of the conidioma and are possibly dispersed by insects along with rain splash and canopy throughfall. Microcyclic conidiation is observed in both ejected ascospores and ascospores still bound within asci (DiCosmo *et al.* 1984). The function of the resulting microconidia is unknown, *e.g.*, as spermatia, increasing inoculum load, or a survival mechanism for ascospores that encounter unfavorable conditions (Hanlin 1994). Microcyclic conidiation of ascospores is a diagnostic character of *Micraspis* and is a relatively uncommon phenomenon in *Leotiomycetes*, most notably occurring in *Tympanidaceae*.

As with *Micraspis acicola*, little is known about the ecology of *M. strobilina* and *M. tetraspora* because of limited collections and studies. *Micraspis strobilina* was described from fallen cones of *Pinus sylvestris* in Scotland (Dennis 1971) and later collected from *P. sylvestris* cones in other localities in the UK (Minter 1980) and the Leningrad region of Russia (Popov 2007). Minter (1980) described *Sporonema diamandidis* as the purported asexual morph of *M. strobilina*, noting that *M. strobilina* ascomata later occurred on the same cone scales; however, he did not establish this connection with cultural studies. *Micraspis strobilina* is not uncommon in the UK and therefore material should be available for future studies including establishing the asexual morph connection and elucidating its ecology. Dennis (1971) did not give an account of the asexual morph in his type description; therefore, we provided a morphological description of the co-occurring asexual morph discovered while examining Dennis' *M. strobilina* holotype (Fig. 3E–G).

Micraspis tetraspora was described from decorticated wood of *Picea sitchensis* in Great Britain and is apparently only known from its initial collection by Graddon (1984); recent attempts to re-collect *M. tetraspora* from the type locale were unsuccessful as appropriate tree hosts appear to be lost (B. Douglas, pers. comm.). *Picea sitchensis* is native to the western coast of N. America and was introduced to the UK in 1831 by David Douglas, where it is now an important commercial timber species and is naturalized in some localities. Future work should explore the biogeography of *M. tetraspora* by means of field collecting within the natural range of *P. sitchensis* and from introduced and naturalized conifers within the UK. For example, does the presence of *M. tetraspora* in the UK represent a co-introduction event (*i.e.*, *M.*

tetraspora is endemic to the western coast of North America) or is *M. tetraspora* endemic to the UK and capable of host-jumping from native or naturalized conifers (*e.g. Picea abies*)? More collections are required to establish the overall host and substrate preferences of *Micraspis* species, especially for *M. strobilina* and *M. tetraspora*.

This study is part of a global effort to improve the current systematics of *Leotiomycetes*, a large class of ascomycetes including many important plant pathogens, saprotrophs and mutualists. We provide reference sequences linked to morphological data, accurate descriptions and a key to species. This information facilitates the identification of *Micraspis* species from both field specimens and environmental sequences, which will incrementally provide more insight into the frequency of occurrence, biogeography and species diversity of this previously obscure genus.

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