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ATRADIDYMELLA MUSCIVORA GEN. ET SP. NOV. (PLEOSPORALES) AND ITS ANAMORPH *PHOMA MUSCIVORA* SP. NOV.: A NEW PLEOMORPHIC PATHOGEN OF BOREAL BRYOPHYTES[1]

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During a survey of bryophilous fungi from boreal and montane habitats, 12 isolates of a hitherto unknown plant pathogenic member of the Pleosporales were recovered from *Aulacomnium palustre*, *Hylocomium splendens*, and *Polytrichum juniperinum*, and described as *Atradidymella muscivora* gen. et sp. nov. *Atradidymella* is characterized by minute, unilocular, setose pseudothecia having 2–3 wall layers; brown, fusiform, 1-septate ascospores; and a *Phoma* anamorph. The genus is distinguished from all other pleosporalean genera with brown, fusiform ascospores on the basis of ascospore and pseudothecium morphology and a highly reduced stroma that is localized within a single host cell. *Atradidymella muscivora* is distinguished by its minute pseudothecia (<115 μm) and ascospores that are slightly allantoid and constricted at the septum with the upper cell often wider than the lower. Its anamorph, *Phoma muscivora* sp. nov., is morphologically distinguishable from *P. herbarum* in having smaller conidia. Parsimony analysis of the ITS rDNA region indicates *A. muscivora* has affinities to the *Phoma-Ascochyta-Didymella* clade that is sister to the Phaeosphaeriaceae within the Pleosporales.

Key words: *Atradidymella muscivora*; bryophilous; pathogenesis; stroma development; *Phoma herbarum*, Pleosporales.

Bryophilous fungi are historically an understudied group (Davey and Currah, 2006), and the recent description of new species from bryophyte substrates (Döbbeler and Triebel, 2000; Tsuneda et al., 2000; Döbbeler, 2006; Rice and Currah, 2006; Davey and Currah, 2007; Döbbeler, 2007), as well as the taxonomically diverse suite of fungi reported from bryophyte sources (Racovitza, 1959; Felix, 1988; Kost, 1988; Döbbeler, 1997) suggest untold fungal diversity is associated with bryophytes. Members of the Pleosporales are well known for their predilection for vascular plant substrates and hosts (Schoch et al., 2006), and a variety of pleosporalean fungi have been reported from bryophytes, including representatives of the teleomorphic genera *Clathrospora* (Racovitza, 1959), *Coleroa* (Henderson, 1972; Fenton, 1983), *Didymosphaeria*, *Leptosphaeria* (Racovitza, 1959), *Massarina* (Döbbeler, 1978), *Phaeosphaeria* (Möller and Dreyfuss, 1996), *Pleospora*, *Protoventuria*, and *Pyrenophora* (Racovitza, 1959), and the anamorphic genera *Alternaria* (Racovitza, 1959; Thormann and Rice, 2007), *Ascochyta* (Thormann and Rice, 2007), *Phoma* (Kerry, 1990; Möller and Dreyfuss, 1996; Tosi et al., 2002), *Phyllosticta* (Racovitza, 1959), and *Stemphylium* (Prior, 1966). Given the taxonomic distribution of these genera through six different families in the order, it seems likely that the diversity of pleosporalean fungi with bryophyte hosts and substrates is similar to that found associated with vascular plants.

During a survey of fungi associated with common boreal bryophytes, 12 isolates of a pathogenic, pycnidium-forming fungus were obtained. When the model host *Funaria hygrometrica* was inoculated with the fungus, a teleomorph was produced with characters inconsistent with previously described pseudothecial genera. *Atradidymella muscivora* gen. et sp. nov. and *Phoma muscivora* sp. nov. are erected here on the basis of both molecular and morphological characters to accommodate the teleomorph and anamorph states of these isolates.

MATERIALS AND METHODS

Isolation and teleomorph induction—Individual gametophytes of *Aulacomnium palustre*, *Hylocomium splendens*, and *Polytrichum juniperinum* from various locales in Alberta, Canada (Table 1) were cleaned, surface sterilized, fragmented, and plated on Mycosel (MYC: 36 g Mycosel agar/L water; Becton, Dickinson & Co., Sparks, Maryland, USA) or oatmeal agar (OA: 20 g agar [Invitrogen, Carlsbad, California, USA], 20 g ground oatmeal, 1 L water) amended with 0.01% oxytetracycline (Sigma, St. Louis, Missouri, USA) as described by Davey and Currah (2007). Isolation plates were incubated in the dark at 20°C and examined weekly for 6–8 wk. Emerging hyphae were subcultured onto potato dextrose agar (PDA: 39 g potato dextrose agar/L water, Difco Laboratories, Detroit, Michigan USA), and OA. Isolates producing the *Phoma* anamorph of *A. muscivora* were obtained from all hosts after 3–6 wk of incubation.

The model host *Funaria hygrometrica* was cultured from spores in vitro on White's basal salt media in 100 mL glass tissue culture vessels as described in Davey and Currah (2007). Gametophytes having reached the five-leaf stage were inoculated with the ex-type strain of *A. muscivora* (UAMH 10909) by placing 5-mm-diameter plugs taken from near the margin of 15–30-d old colonies grown on OA among the moss gametophytes. Inoculated gametophytes were incubated for 16 wk at 15°C on a 12-h diurnal cycle with 75% relative humidity to induce the formation of the *Atradidymella* teleomorph.

Characterization—Single-point inoculations of the ex-type strain (UAMH 10909) made on PDA, OA, and malt extract agar (MEA: 20 g malt extract [Becton, Dickinson & Co.], 15 g agar, 1 L water) were incubated in the dark at 20°C and observed every 5 d for 1 month. Colony measurements were

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TABLE 1. Isolates of *Atradidymella muscivora* from the boreal mosses *Aulacomnium palustre*, *Hylocomium splendens*, and *Polytrichum juniperinum* in Alberta, Canada.

Site no.	Location	Site description	Isolate	Host	GenBank no.	UAMH no.
1	Jackfish Lake Bog	Mixed *Sphagnum* bog with an overstory of	Ap1-Q	*A. palustre*	EU817826	—
	Along HWY 2, 7 km E of Jackfish	*Picea mariana*	Ap1-R	*A. palustre*	—	—
	Lake		Ap1-S	*A. palustre*	EU817827	—
					EU817830	
2	Redwater Natural Area	Mixed *Sphagnum* wetlands with overstory of	Hs7-C	*H. splendens*	EU817828	UAMH 10910
	NE 20–57–20 W4	*Picea mariana* and *Larix laricina* and uplands transitioning	Hs7-D	*H. splendens*	—	—
		to *Pinus banksiana*-dominated dunes	Hs7-J	*H. splendens*	—	—
			Pj9-A	*P. juniperinum*	—	—
			Pj9-C	*P. juniperinum*	EU817829	UAMH 10911
3	Wolf Lake	Wet lakeshore with an overstory of *Picea glauca, Betula*	Pj8-C	*P. juniperinum*	—	—
	NW 35–65–7 W4	*papyrifera*, and *Salix* sp.	Pj8-D^{T}	*P. juniperinum*	EU817825	UAMH 10909
	54°40′N 110°57′E		Pj8-G	*P. juniperinum*	—	—

Note: Superscript T indicates the isolate is ex-type.

calculated by averaging two perpendicular measurements of the diameter and are given as an average of three colonies. The anamorph state produced on PDA and infected host moss material were mounted in water or polyvinyl alcohol with acid fuchsin (0.05 g acid fuchsin in 10 mL lactic acid and 1 mL glycerine mixed with 1.66 g polyvinyl alcohol dissolved in 10 mL water). Infected host mosses bearing both pycnidia of *P. muscivora* and pseudothecia of *A. muscivora* were fixed in FAA (10 mL 40% formaldehyde, 50 mL ethanol, 5 mL acetic acid, 35 mL water) for a minimum of 24 h, dehydrated in an ethanol series, and embedded in paraffin wax. Sections were stained using safranin O (Sigma), counterstained with fast green FCF (Sigma), and mounted using DPX mountant (Sigma). Light micrographs of all preparations were taken using an Olympus BX50 microscope with a DP-12 digital camera (Olympus, Tokyo, Japan). Line drawings are freehand representations of the material observed. Measurements of structures are expressed as range (mean ± SD), $N = 20$.

For SEM, infected gametophytes were fixed in 2% glutaraldehyde (Sigma) overnight. Fixed gametophytes were rinsed in distilled water and placed in 2% tannic acid-2% guanidine hydrochloride (Sigma) solution for 4–5 hours and then postfixed overnight in 2% OsO_4 (Sigma) at 5°C. Fixed material was dehydrated in ethanol series, taken to amyl acetate, and critical-point dried in a CPD 030 dryer (BAL-TEC, Balzers, Liechtenstein) using carbon dioxide. Dried samples were coated with gold and examined with a Hitachi (Tokyo, Japan) S-510 scanning electron microscope at 10 or 15 kV.

Phylogenetic analyses—Isolates were grown on PDA or OA overlaid with a Cellophane membrane (UCB Films, Somerset, UK) for 15–30 d at ambient light and temperature. Genomic DNA was extracted as described by Davey and Currah (2007) and the internal transcribed spacer (ITS) region was amplified as described by Gibas et al. (2002) using the forward and reverse primer set ITS 4 (White et al., 1990) and BMB-CR (Lane et al., 1985). The 18S SSU region of the ex-type strain was amplified using the forward and reverse primer set NS1 and NS8 (White et al., 1990). PCR amplicons were purified and sequenced as described by Davey and Currah (2007) using forward and reverse primers ITS1, ITS2, ITS3, ITS4, (White et al., 1990) and BMB-CR (Lane et al., 1985) for the ITS region, and NS1, NS2, NS3, NS4, NS6, NS8 (White et al., 1990) NS13, and NS151 for the 18S SSU region. Consensus sequences were determined from overlapping sequence data using the software Sequencher (Gene Codes Corp., Ann Arbor, Michigan, USA). Sequence identity was compared between strains across the ITS region using bl2seq (Tatusova and Madden, 1999). A data matrix of ITS sequences including *A. muscivora* and members of the *Phoma-Ascochyta-Didymella* complex and representative pleosporalean families was created from sequences retrieved from GenBank. Two representatives of the Dothideales were used as outgroup taxa. ITS sequences were aligned using the program MAFFT version 5.8 (Katoh et al., 2005) and the subsequent alignment manually verified. This matrix was subjected to parsimony analysis using the program PAUP* version 4.0b10 (Swofford, 2003) with simple stepwise addition of taxa, tree bisection-reconnection (TBR) branch swapping, and gaps treated as missing data. Support for branching topologies was evaluated using 1000 resamplings of the data by bootstrapping analysis (Felsenstein, 1985). All trees were scored for length in steps, consistency index (CI), retention index (RI), and homoplasy index (HI). The data matrix and resulting trees were deposited in TreeBASE (accession S2351, website http://www.treebase.org). Bayesian analysis was also conducted on the matrix using the program Mr.Bayes version 3.1 (Ronquist and Huelsenbeck, 2003) with a general time reversible model of DNA substitution (GTR) with gamma distributed rate variation across sites (invariance, partitioning across genes) for two independent Markov chain Monte Carlo runs with 1.5×10^6 generations each, sampling trees every 100th cycle. A final standard deviation of <0.01 for the split frequency was taken as an indication that convergence had been achieved. The first 25% of sampled trees were discarded as burn-in and posterior probabilities for each node of the 50% majority rule consensus tree were recorded. Sequence identity was also compared between *A. muscivora* and *Phaeodothis winteri* (GenBank accessions EU817830, DQ678021) across the 18S small subunit (SSU) region because of the lack of available ITS data for *P. winteri*.

RESULTS

Isolation and teleomorph induction—Pycnidia of *Phoma muscivora* formed within 10–20 d of plating gametophyte fragments of all three hosts on either MYC or OA. The *Atradidymella*

teleomorph was not observed in culture, but formed on *Funaria hygrometrica* 30–60 d after inoculation with the ex-type strain of *A. muscivora* (UAMH 10909).

Phylogenetic analysis—Pairwise identity analysis of the ITS region of isolates of *A. muscivora* revealed 100% sequence identity between isolates, and 100% similarity to two strains deposited in GenBank as *Phoma herbarum* (GenBank accessions AY337712, DQ912692). *Atradidymella muscivora* shared 97% sequence identity across the 18S SSU region with *Phaeodothis winteri*, another genus with 1-septate, darkly pigmented ascospores. The aligned ITS data matrix consisted of a representative sequence of *Atradidymella muscivora*, sequences of *Phoma herbarum*, members of the *Phoma-Ascochyta-Didymella* clade, and representative members of the Pleosporales, was composed of 46 taxa, and included 816 characters, of which 357 were parsimony informative. Parsimony analysis generated 21 equally parsimonious trees of 1914 steps [consistency index (CI) = 0.511, retention index (RI) = 0.623, homoplasy index (HI) = 0.489) whose topologies were consistent in the phylogenetic placement of *Atradidymella* relative to other species. Results of both the bootstrap analysis and Bayesian inference are shown on a single most parsimonious tree (Fig. 1). *Atradidymella muscivora* and two GenBank isolates identified as *Phoma herbarum* (AY293803, DQ132841) form a strongly supported group (95% bootstrap proportion [BP]/100 Bayesian posterior probability [BPP]) nested within clade A of the *Phoma-Ascochyta-Didymella* clade that is weakly supported (59% BP/64 BPP) as sister to the Phaeosphariaceae. Other pleosporalean genera with 1-septate, darkly pigmented ascospores (e.g., *Montagnula*, *Munkovalsaria*, *Roussoëlla*, *Venturia*) are allied to taxa outside the *Phoma-Ascochyta-Didymella* complex (Fig. 1).

Taxonomy: Atradidymella Davey & Currah, gen. nov.—*Mycobank accession*—
MB 511986

Etymology—"Atra" refers to the darkly pigmented ascospores that differentiate this genus from the phylogenetically close and morphologically similar genus *Didymella*

Typus generis—*Atradidymella muscivora* Davey & Currah.

Teleomorphosis—Pseudothecia minuta (<200 μm), fusco-brunnea, uniloculata, subglobosa ad pyriformia, setosa circum ostiolum, cum tunicis pseudoparenchymatosis. Hamathecium pseudoparenchymatosum in ascomatibus novis, quod restat in ascomatibus maturis in forma septata et filamentosa. Asci bitunicati, octo-spori, cylindrici ad clavati. Ascosporae brunneae, fusiformes, mono-septatae, tenuiter constrictae prope septum. Anamorphosis *Phoma*

Pseudothecia minute (<200 μm), dark brown, uniloculate, subglobose to pyriform, setose around ostiole, with pseudoparenchymatous walls. Hamathecium pseudoparenchymatous in young ascomata, persisting as septate filamentous remnants in mature ascomata. Asci bitunicate, IKI-negative, 8-spored, cylindrical to clavate. Ascospores brown, fusiform, 1-septate, slightly constricted at septum. Anamorph *Phoma*.

Taxonomy: Atradidymella muscivora Davey & Currah, sp. nov.—*Mycobank accession*—
MB 511987

Etymology—"Muscivora" refers to the pathogenic nature of the fungus on bryophyte hosts.

Typus—Canada, Alberta, Wolf Lake, 78 km north of Bonnyville, Alberta, 54°40′N 110° 57′E, dried *Funaria hygrometrica* gametophytes bearing pseudothecia (holotype) (UAMH 10909: ex-type culture, isolated from gametophytes of *Polytrichum juniperinum*)

Teleomorphosis—Pseudothecia solitaria, a substrato erumpentia, fusco-brunnea, uniloculata, subglobosa ad elliptica vel pyriformia (75–115 × 58–95 μm), setae breves et apicales, tunicae pseudoparenchymatosae. Hamathecium pseudoparenchymatosum in ascomatibus novis, quod restat in ascomatibus maturis in forma septata et filamentosa (1–3 μm diam). Asci cylindrici at clavati, bitunicati, octo-spori, 6–13 μm diam. Ascosporae aureo-brunneae ad fusco-brunneae, late fusiformes (14–20 × 4–5.5 μm), rectae ad alantoidae, mono-septatae, tenuiter constrictae prope septum, cellula superior aliquando brevior et latior quam cellula inferior.

Anamorphosis—*Phoma muscivora* M.L. Davey & R.S. Currah, MB 511988

Etymology—"Muscivora" refers to the pathogenic nature of the fungus on bryophyte hosts.

Typus—Canada, Alberta, Wolf Lake, 78 km north of Bonnyville, Alberta, 54°40′N 110°57′E, dried colony on potato dextrose agar (PDA), isolated from gametophytes of *Polytrichum juniperinum* (UAMH 10909: ex-type; UAMH 10910, 10911: paratypes)

Pycnidia solitaria vel in glomeribus, a substrato erumpentia, globosa ad subglobosa, collum breve, (70-212.5 x 85-150 um), alba cum collo fusco, pycnidium totum fuscum quando maturant. Conidia elliptica, unicellularia (2.5–5 × 1–2 μm).

On leaves, stems, and rhizoids of mosses, causing chlorosis and often death (Figs. 2, 8, 9). On *Funaria hygrometrica*, hyphae hyaline and frequently penetrating host tissues, forming intracellular clumps that become dematiaceous and mature to form pycnidia or pseudothecia that are erumpent 10–60 d post inoculation (Figs. 2, 8, 9). On PDA, young (7 d) colonies dense, floccose with funiculate projections, often forming sectors of variously pigmented sterile and fertile mycelia. Young colonies white, cream, pale orange, or pale pink, producing pink or red pigments visible in reverse that turn blue with the addition of 1 M NaOH, mature (>10 d) colonies often darkening to gray or gray-brown. Colonies attaining 33 mm diameter after 7 d at 20°C and producing pycnidia after 7–20 d, predominantly on funiculate projections (Fig. 7). On MEA, young colonies white, cream, or pale orange, with sparse white aerial hyphae, and a pale margin of submersed hyphae, reverse white. Colony darkening with age to grey-brown with dark brown reverse, becoming floccose with funiculate projections, and attaining 35 mm after 7 d at 20°C (Fig. 7). Pycnidia sparse, not immersed in media, forming after 15–30 d. On OA, young colonies dense, floccose to velvety with funiculate projections, white, cream, pale orange or pale pink, and producing pink-orange or red pigments visible in reverse. Maturing to off-white or pale grey-brown with dark brown reverse, attaining 37 mm after 7 d at 20°C, producing pycnidia after 10–20 d (Fig. 7).

Teleomorph—Pseudothecia solitary, erumpent from underlying host cell (Figs. 3, 12), dark brown, uniloculate, subglobose

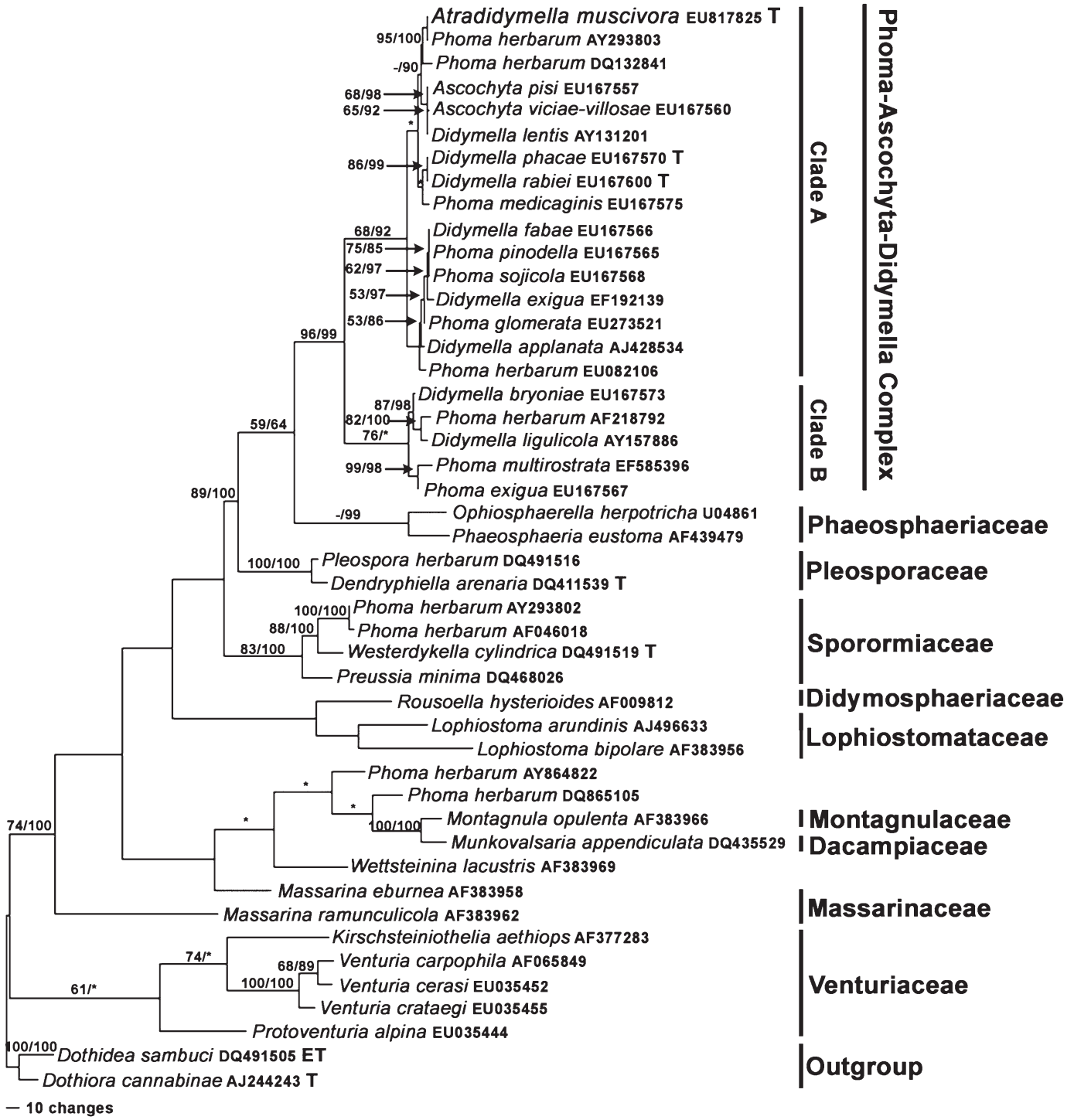
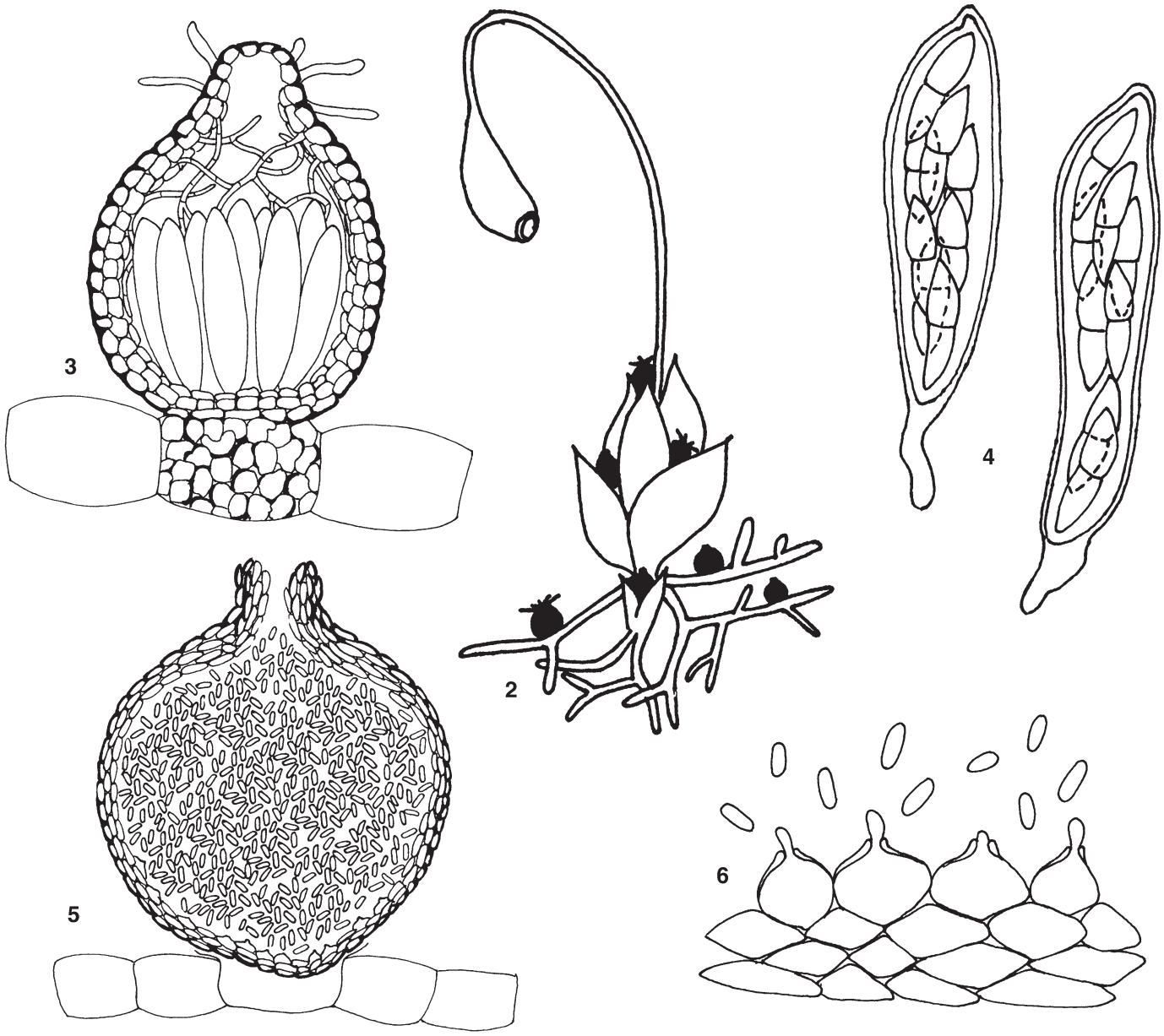


Fig. 1. One of 21 equally parsimonious trees (1914 steps, CI = 0.511, RI = 0.623, HI = 0.489) inferred from a maximum parsimony analysis of ITS sequences showing the placement of *Atradidymella muscivora* (including its anamorph, *P. muscivora*) among members of the Pleosporales. *Dothidea sambuci* and *Dothiora cannabinae* (Dothideales) serve as outgroup taxa. Bootstrap values greater than 50% calculated from 1000 replicates and Bayesian posterior probabilities greater than 50 are given above the branches as bootstrap proportion/Bayesian posterior probability. Gaps (-) indicate a collapsed node and asterisks indicate the presence of a differently resolved node in the Bayesian analysis. GenBank accession numbers are given following species names. Ex-type sequences are indicated with a "T" following the accession number. "ET" indicates ex-epitype.

to elliptic or pyriform (75–115 × 58–95 μm) with short concolorous, occasionally septate setae around ostiole (Figs. 3, 11, 12). Peridium approximately 10 μm wide with three layers of pseudoparenchymatous cells, the outermost with darkly pigmented walls (Figs. 3, 11). Preascogenous centrum of hyaline pseudoparenchymatous elements and darkly pigmented, branched,

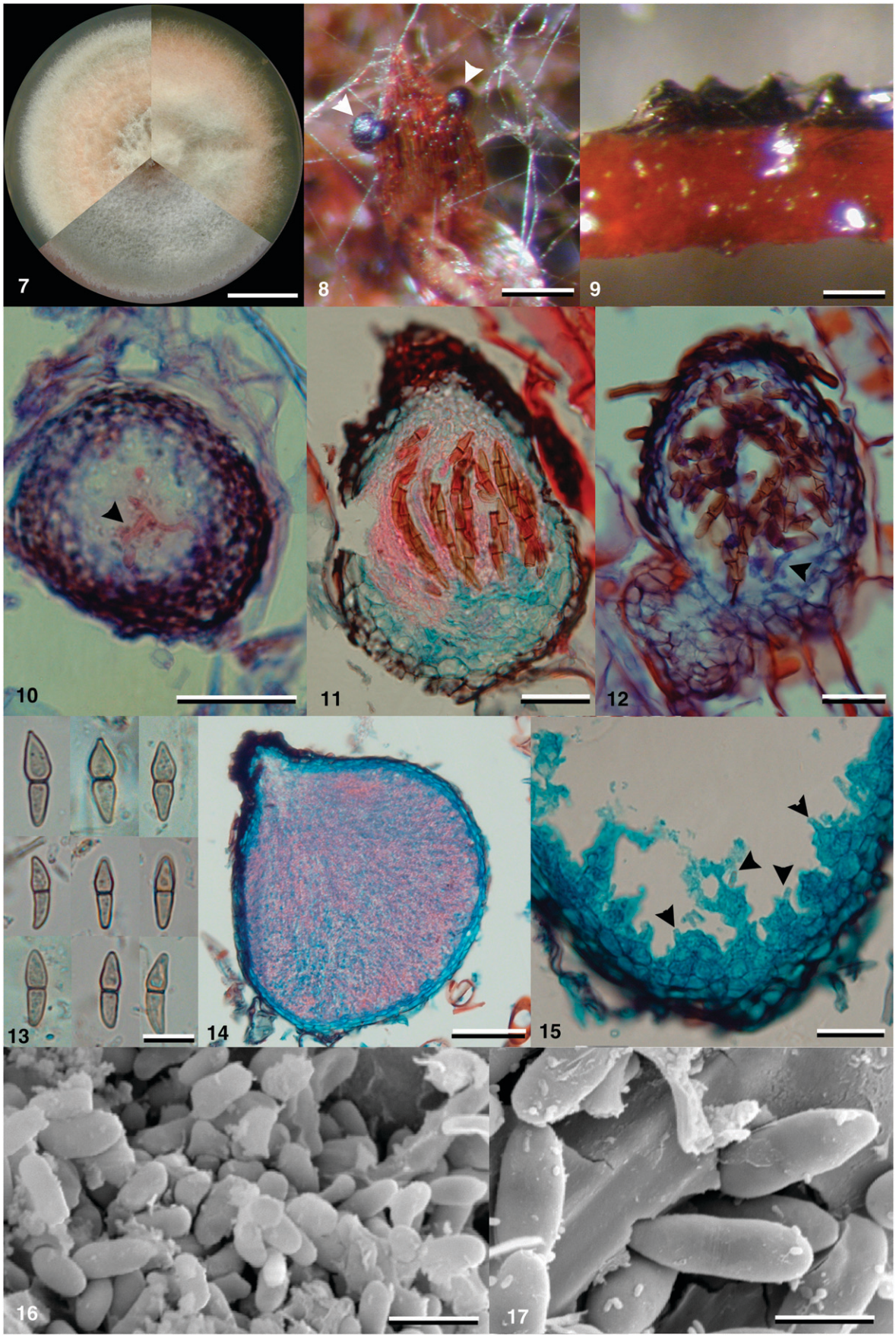


Figs. 2–6. Line drawings of *Atradidymella muscivora* and its anamorph, *Phoma muscivora*. **2.** *Funaria hygrometrica* plant bearing both pseudothecia and pycnidia on leaves and rhizoids of gametophyte. **3.** Pseudothecium and stroma of *A. muscivora* on a moss leaf. **4.** Asci and ascospores of *A. muscivora*. **5.** Pycnidium of the *P. muscivora* on a moss leaf. **6.** Phialides and conidia of *P. muscivora*.

septate filamentous elements (Fig. 10). Hamathecium pseudoparenchymatous in young ascomata (Fig. 11), persisting as septate filamentous remnants (1–3 μm in diameter) in mature ascomata (Fig. 12). Crozier formation was not observed. Asci cylindrical to clavate, bitunicate, 8-spored, IKI negative, 6–13 μm in diameter, grouped in a small fascicle of 10–20 at base of pseudothecium (Figs. 3, 4). Ascospores arranged bi- or triseriately in ascus (Figs. 4, 11), golden brown to dark brown, broadly fusiform, 14–20 (16.8 ± 1.5) × 4–5.5 (4.5 ± 0.6) μm, smooth, straight to allantoid, 1-septate, slightly constricted at septum, the upper cell sometimes shorter and broader than the lower (Figs. 4, 13, 17).

Anamorph—Pycnidia solitary or in clusters, erumpent from substrate (Figs. 8, 9), globose to subglobose with short neck (Fig. 5), 85–250 (140.6 ± 39.5) × 70–212.5 (121.1 ± 34.4) μm. Young pycnidia white with dark neck, entire pycnidium becoming dark with age. Pycnidium wall 3–9 μm thick, consisting of 3–4 layers of flattened pseudoparenchymatous cells, the outermost becoming darkened and compressed with age (Fig. 14). Phialides hyaline, broad ampulliform, mostly wider than tall (Figs. 6, 15), 3–7 (4.5 ± 1.1) × 3–5 (3.9 ± 0.7) μm. Conidia hyaline, elliptic, smooth, unicellular (Fig. 16), 2.5–5 (3.3 ± 0.5) × 1–2 (1.2 ± 0.3) μm, exuded as a slimy cream, pale yellow, or pale pink droplet.

Additional specimens examined—Canada: Alberta, Jackfish Lake Bog, 7 km east of Jackfish Lake along HWY 2, isolated from gametophytes of *Aulacomnium palustre*, M. L. Davey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(Ap1-R); Alberta, Redwater Recreation Area, 12 km east of Redwater, isolated from gametophytes of *Hylocomium splendens*, M. L. Davey, (UAMH 10910, Hs7-D, Hs7-J); Alberta, Redwater Recreation Area, 12 km east of Redwater, isolated from gametophytes of *Polytrichum juniperinum,* M. L. Davey, (UAMH 10911, Pj9-A); Alberta, Wolf Lake, 78 km north of Bonnyville, isolated from gametophytes of *Polytrichum juniperinum,* M. L. Davey, (UAMH 10909, Pj8-C, Pj8-G).

DISCUSSION

Among previously established pseudothecial genera with reduced stromata, darkly pigmented pseudothecia, and fusiform, 1-septate ascospores, *Atradidymella* is morphologically similar to *Coleroa*, *Venturia*, *Didymella*, *Didymosphaeria*, *Phaeodothis*, and *Roussoëlla*, of which only *Coleroa* (Henderson, 1972; Fenton, 1983) and *Didymosphaeria* (Racovitza, 1959) have previously been reported from mosses. However, *Atradidymella* can be distinguished from these genera on the basis of ascospore and pseudothecium morphology, and stroma ontogeny. *Atradidymella* has smooth, darkly pigmented ascospores that lack a gelatinous sheath, which distinguish it from the pigmented, sheath-bearing ascospores of *Phaeodothis* (Aptroot, 1995b), the hyaline ascospores of *Didymella* (Corbaz, 1957) and the reticulately or striately ornamented ascospores of *Roussoëlla* (Aptroot, 1995b). *Atradidymella* produces an extremely reduced stroma within a single host cell, from which the pseudothecium ultimately emerges. This limited stroma formation distinguishes *Atradidymella* from *Coleroa* and *Venturia*, both of which form more extensive stromata that originate both inter- and intracellularly in the epidermal and subepidermal regions of vascular plant leaves and stems (Barr, 1968). Its minute pseudothecia and pseudoparenchymatous peridium distinguish it from *Didymosphaeria*, which has larger pseudothecia and a hyphal peridium of textura intricata (Aptroot, 1995a). Although the small pseudothecia and reduced stromata of *Atradidymella* are superficially similar to those of *Phaeodothis,* they can be distinguished by the number of peridial layers (2–3 in *Atradidymella,* 1–2 in *Phaeodothis*).

Parsimony analysis of the ITS region supports morphological data suggesting *Atradidymella muscivora* represents both a new species and genus. The only teleomorphic genera with fusiform, pigmented, 1-septate ascospores that have been reported with *Phoma* or *Ascochyta* anamorphs are *Didymosphaeria* (Farr et al., 1989), *Massarina* (de Hoog, 1979), and *Otthia* (Grove, 1935), all of which belong to families that are phylogenetically distant from *Atradidymella*. Although nested within the *Phoma-Ascochyta-Didymella* clade and resembling *Didymella* in hamathecium development, and ascospore and pseudothecium shape and size, *Atradidymella* cannot be accommodated within *Didymella* on account of its pigmented ascospores and intracellular stroma formation (Corbaz, 1957; Corlett, 1981; Skarshaug, 1981; De Neergaard, 1989). Pairwise sequence identity comparison between *A. muscivora* and the morphologically similar *Phaeodothis winteri* shows the two are not congeneric, as the level of divergence observed (97% similarity) is consistent with levels of intergeneric divergence among the Pleosporales (Rossman et al., 2002; Pinnoi et al., 2007).

Isolates of *Atradidymella muscivora* have a high degree of ITS sequence similarity (99–100%) to four sequences deposited in GenBank as *Phoma herbarum* (GenBank accessions AY337712, AY293803, DQ132841, DQ912692) that were isolated independently from a variety of synthetic and organic substrates. However, sequence data are not available for the type specimen of *P. herbarum*, and phylogenetic analysis of available sequences for the species (Fig. 1) and analysis of its physiological and morphological characters (Montel et al., 1991) suggest that the current species concept is polyphyletic. The *Phoma* anamorph of *Atradidymella muscivora* is also morphologically most similar to *P. herbarum* with its pink to yellow or white spore masses; unicellular, ellipsoid to cylindric conidia; ampulliform phialides; and pycnidia that are initially pale brown with a dark ostiolar neck, and later darken with age. However, *P. muscivora* is morphologically differentiated from *P. herbarum* by its slightly smaller conidia (1–2 × 2.5–5 μm vs. 1.5–3 × 3.5–8 μm) (Boerema, 1964, 2004).

Despite the potential for taxonomic confusion between *P. herbarum* and *P. muscivora*, the *Phoma* anamorph of *Atradidymella* was formally named because it is the predominantly visible state in the disease cycle of *A. muscivora*, is the morph most easily induced to sporulate in vitro, and can be morphologically distinguished from *P. herbarum* based on conidium size. Given that *P. muscivora* is morphologically very similar to *P. herbarum*, it is possible that those isolates with high ITS similarity to *P. muscivora* could also be accommodated within this species upon verification of conidium size. *Phoma herbarum* has previously been reported from mosses (Kerry, 1990; Tosi et al., 2002), and some of these may also represent further records of *P. muscivora* from bryophyte hosts.

Atradidymella muscivora represents new diversity within the bryophilous Pleosporales. The isolates described herein are the first report of a teleomorph allied to the *Phoma-Ascochyta-Didymella* clade from a bryophyte host and only the second

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Figs. 7–17. *Atradidymella muscivora* and its anamorph, *Phoma muscivora*. (Figs. 7, 10–13: UAMH 10909; Figs. 8, 14–17: UAMH 10911; Fig. 9: Ap1-S; Figs. 10–12, 14, 15: paraffin sections stained with safranin O-fast green FCF; Fig. 13: wet mount; Figs. 16, 17: SEM) **7.** Composite figure of colonies of the ex-type strain of *Atradidymella muscivora* (UAMH 10909) after 10 d incubation at 20°C. Clockwise from top left: PDA, OA, MEA. Scale bar = 15 mm. **8.** *Funaria hygrometrica* gametophyte infected with *P. muscivora* (UAMH 10911). Two pycnidia (arrowheads) are erumpent from the leaf surface. Scale bar = 1 mm. **9.** Stem of *Funaria hygrometrica* inoculated with *P. muscivora* (Ap1-S) showing emerging pycnidia. Scale bar = 150 μm. **10.** Cross section of immature pseudothecium containing both hyaline and dematiaceous (arrowhead) stromatal elements. Scale bar = 25 μm. **11.** Longitudinal section of young pseudothecium with intact asci and pseudoparenchymatous hamathecial elements. Scale bar = 25 μm. **12.** Longitudinal section of mature pseudothecium of *A. muscivora*. Asci have deliquesced, leaving a jumbled mass of ascospores within the pseudothecium, and hamathecium consists of septate, filamentous remnants (arrowhead). The stroma of the pseudothecium is restricted to a single gametophyte cell (bottom left). Scale bar = 20 μm. **13.** Composite figure showing variation in ascospore morphology between pseudothecia of the ex-type strain. Scale bar = 12 μm. **14.** Longitudinal section of a pycnidium of *P. muscivora*. Note the neck has darkened, while the remainder of the pycnidium walls are compressed, but have yet to darken. Scale bar = 40 μm. **15.** Longitudinal section of a pycnidium showing conidia produced by stout, ampulliform phialides (arrowheads). Scale bar = 15 μm. **16.** Scanning electron micrograph of the smooth, ellipsoid to short cylindric conidia of *P. muscivora*. Scale bar = 5 μm. **17.** Smooth, broad fusiform, 1-septate ascospores of *A. muscivora* with a slight constriction at the septum. Scale bar = 10 μm.

description of a Pleosporalean pathogen actively causing chlorosis in its host, after *Coleroa turfosorum* (Fenton, 1983). Finally, the extreme stroma reduction and localization to a single host cell observed in *A. muscivora* has not been previously reported among pleosporalean bryophilous fungi and may represent a unique specialization to a bryophyte habit.

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