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Paleomycology of the Princeton Chert II. Dark-septate fungi in the aquatic angiosperm *Eorhiza arnoldii* indicate a diverse assemblage of root-colonizing fungi during the Eocene

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Abstract: Tissues of the extinct aquatic or emergent angiosperm, *Eorhiza arnoldii* incertae sedis, were extensively colonized by microfungi, and in this study we report the presence of several types of sterile mycelia. In addition to inter- and intracellular proliferation of regular septate hyphae, the tissues contain monilioid hyphae with intercalary branching. These filamentous mycelia are spatially associated with two distinct morphotypes of intracellular microsclerotia. These quiescent structures are morphologically similar to loose and cerebriform microsclerotia found within the living tissues of some plants, which have been attributed to an informal assemblage of dematiaceous ascomycetes, the dark-septate endophytes. While there are significant challenges to interpreting the ecology of fossilized fungi, these specimens provide evidence for asymptomatic endophytic colonization of the rooting structures of a 48.7 million year old aquatic angiosperm.

Key words: cerebriform, dark-septate endophytes, *Leptodontidium*, microsclerotia, monilioid, paleomycology, *Phialocephala*, Princeton Chert

Introduction

Fungi are major ecological drivers in extant plant communities, where they play vital roles in decomposition and nutrient mobilization (Cromack and Caldwell 1992, Hoffland et al. 2004) and contribute to niche partitioning and plant species diversity (Gustafson and Casper 2006, Vogelsang et al. 2006). Mutualistic relationships with fungi are thought to have been integral to the colonization of land by plants (Pirozynski and Mallock 1975, Humphreys et al. 2010, Bidartondo et al. 2011); in the subsequent ~ 450 million y, intricate associations have evolved, ranging from obligate mutualism through commensalism, parasitism and pathogenicity. A substantial number of vascular plants also are host to internal fungal biota with which they form neither typical mycorrhizal associations nor produce responses associated with infection (Saikkonen et al. 1998, Jumpponen 2001). There is evidence that relationships between vascular plants and fungal endophytes occur within a continuum: endophytic fungi actively derive carbon from hosts (Barrow 2003) and their presence may inhibit herbivory (Saikkonen et al. 1998) and increase drought tolerance (Rodriguez et al. 2008), but there is also evidence for mutual antagonism between endophytes and hosts (Schulz et al. 1999), and these fungi are known to become weak pathogens or saptrophs with the decline of host plants (Schulz and Boyle 2005). Consequently the ecological functions of endophytic fungi are of interest, particularly in that they often are observed in plants growing in stressed or marginal habitats (Barrow 2003, Newsham 2011), where they may be more common than arbuscular mycorrhizal fungi (Read and Haselwandter 1981, Mandyam and Jumpponen 2005).

Interpreting the ecological role of fungi in the fossil record is a significant challenge. In some instances, there is anatomical or structural evidence that interactions between fossil fungi and host plants were mycorrhizal (Remy et al. 1994) or pathogenic (LePage et al. 1994). Given that a hallmark of an ascomycteous or basidiomycetous endophyte is asymptomatic persistence within a host, there is no proximal method by which to differentiate a fossil endophyte from a saprotroph, particularly because endophytic microfungi can persist as saprotrophs upon the death of their host (Menkis et al. 2005). Ecological interpretations of fossils therefore must take into account secondary lines of evidence, which include the taphonomic profile of host tissue, systematic affinities of fossils and associational data. This task is further complicated by the tendency of some fungi, particularly within Ascomycota, to exhibit multiple conidial and mycelial anamorphs (Seifert and Samuels 2000). In this study we describe several sterile structures systemically distributed within the

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rhizomes of an aquatic angiosperm, *Eorhiza arnoldii* Robison et Person. We interpret these fungal fossils to as monilioid and regular simple-septate sterile hyphae, which are in spatial association with two types of intracellular microsclerotia. These Eocene fungi are similar to the extant ascomycetes commonly referred to as dark-septate endophytes (DSE, Stoyke and Currah 1991), which inhabit the rhizosphere and living tissues of some vascular plants.

MATERIALS AND METHODS

Fungal body fossils described in this study occur within tissues of the extinct aquatic or emergent angiosperm *Eorhiza arnoldii*, which is known from anatomically preserved vegetative organs (Stockey and Pigg 1994) present in many of the individual bedding planes that constitute the Princeton Chert locality of southern British Columbia, Canada (UTM 10 U 678057 5472372; 49°22′40″N, 120°32′48″W). This well known paleobotanical locality comprises 49 layers of silicified peat interbedded with subbituminous coal; it has been K-Ar dated to ~ 48.7 Ma and thus is latest Ypresian to earliest Lutetian in age (Smith and Stockey 2007, Mustoe 2011, Klymiuk et al. 2013).

Slabs of chert containing *E. arnoldii* rhizomes were selectively sectioned into 3–5 cm^2 samples and mounted on glass slides with Hillquist two-part mounting medium (Hillquist, USA). Serial thin sections, 50–200 μm thick, were cut with a Buehler Petrothin®. Serial photomicrographs, taken at different focal planes, were captured directly from the rock surface under oil immersion, with a Leica DC500 CCD attached to a Leica DM5000B transmitted-light compound microscope. Photomicrographs were compiled as composite focal-stacked images, optimizing visualization of specimens in z-space (after Bercovici et al. 2009); image processing was performed in Adobe Photoshop CS5 12.1. Specimens and slides are deposited in the Paleobotanical Collections, Natural History Museum and Biodiversity Institute, University of Kansas at Lawrence, under specimen accession numbers 17030 B_{bot} 001, 17030 C_{bot} 001, 17035 E_{top} 002, 17035 E_{bot} 002, 17035 F_{bot} 001, 17037 F_{bot} 001 and 17040 B_{bot} 001.

RESULTS

Monilioid hyphae.—Chains of dematiaceous monilioid cells, 12–14 μm long by 7–8 μm diam, are produced from acutely branched, melanized regularly septate hyphae, 2–5 μm diam, which exhibit septation ~ 10 μm below the branching point (FIG. 1A). Smaller monilioid cells occasionally occur at hyphal apices (FIG. 1B, arrow), which may indicate blastic yeast-like proliferation, but in many monilioid chains the individual hyphal elements do not show as much constriction at septa (FIG. 1C, lower arrow), which suggests formation by isodiametric enlargement of subdividing cells. A few monilioid cells also exhibit what appear to be inconspicuous lateral scars (FIG. 1C, upper arrow) but could be taphonomic artifacts. Intercalary branching within monilioid hyphae occurs frequently, and there is obvious septal constriction of cells at branching loci (FIG. 1D, E, H).

The regularly septate hyphae from which monilioid cells initially are produced may remain micronematous (FIG. 1A, D), or hyphal elements may be somewhat inflated, up to 7–8 μm diam (FIG. 1C, G). Within the pith of some *E. arnoldii* specimens, regular hyphae are absent or rare and proliferation of monilioid hyphae is extensive (FIG. 1E, F). This is in contrast to the cortex, where regular hyphae are frequently associated with monilioid growth (FIG. 1A–D, G–I), and also contrasts with the distribution of other fungal remains previously observed within these plants (Klymiuk et al. 2012), which likewise are restricted to cortical tissues.

Loose microsclerotia.—Monilioid hyphae are in close spatial association with clusters of monilioid cells that are constrained to host parenchyma cells (FIG. 1H, I). In this manner, aggregations of monilioid cells form loose microsclerotia, up to 65 μm long by 25 μm wide, that show no evidence of differentiation into rind or medullary zones (FIG. 1I). Microsclerotial initiation occurs via the production of monilioid cells constrained to the host parenchyma cell (FIG. 1H) and proceeds until the host cell is filled. Initiation occurs from normal hyphae (FIG. 1H, arrow), but closely associated inflated hyphal elements (FIG. 1I, arrow) suggest that microsclerotia also may develop concurrent with growth phases in which monilioid hyphae predominate.

Cerebriform microsclerotia.—Densely interwoven hyphal strands form cerebriform microsclerotia, 20–45 μm diam (FIG. 2). They are differentiated into medullary and rind zones; the rind typically is composed of a single layer of melanized hyphae, which are narrower in diameter than the medullary hyphae (FIG. 2A). External surfaces of these microsclerotia are undulating or ridged (FIG. 2B, C). Microsclerotia are associated with branching septate hyphae (FIG. 2A, C) and may be connected to one another by septate hyphal stolons (FIG. 2D, arrow).

Other sterile mycelia in host tissue.—In addition to catenulate monilioid hyphae and two microsclerotial morphologies, the cortical tissues of *Eorhiza arnoldii* exhibit extensive intracellular proliferation of assimilative mycelia (FIG. 3A). Hyphae, 2–5 μm diam, pass through cell walls as microhyphal strands, 0.25–0.5 μm wide (FIG. 3B, arrow), without eliciting any obvious host response. In addition to dense intracellular assimilative networks, in some specimens hyphal

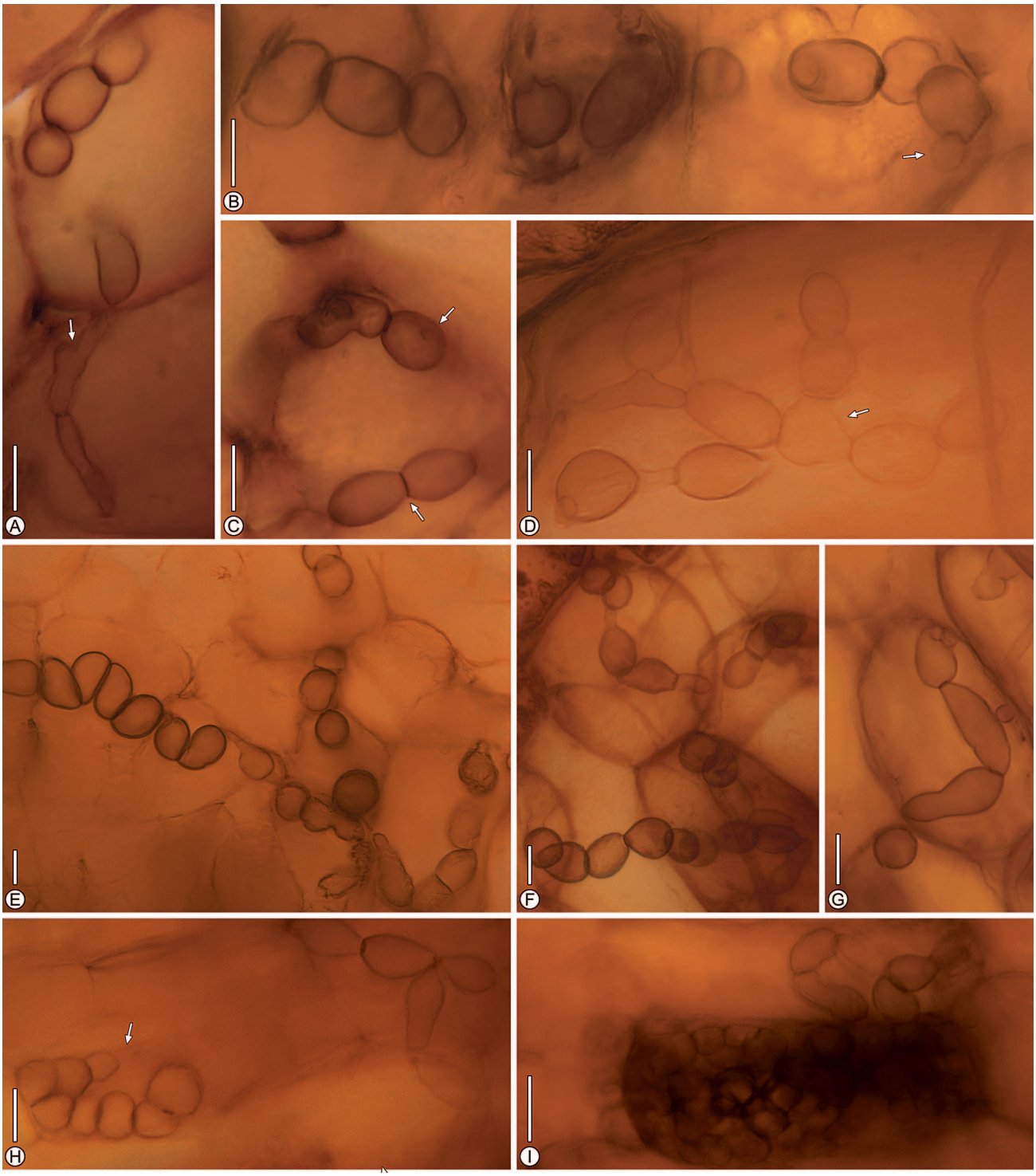

FIG. 1. Dematiaceous monilioid hyphae. A. Chains of monilioid cells in association with septate hyphae; note acute hyphal branching at arrow. B. Newly produced cells occur at the terminus of monilioid hypha (arrow). C. Branching hyphae may be inflated. Note relatively unconstricted septa between some cells (lower arrow) and presence of putative lateral bud scar (upper arrow). D. Intercalary branching (arrow). E–F. Extensive proliferation of monilioid hyphae through host tissue. G. Short, inflated hyphal segments frequently associated with isodiametric monilioid cells. H–I. Loose microsclerotia formed of monilioid hyphae that fill lumen of host cells. Monilioid hyphae are produced from regular simple-septate hyphae (H, arrow). Bars = 10 µm. A–F, H–I: 17037 F_{bot} 001; G: 17035 F_{bot} 001.

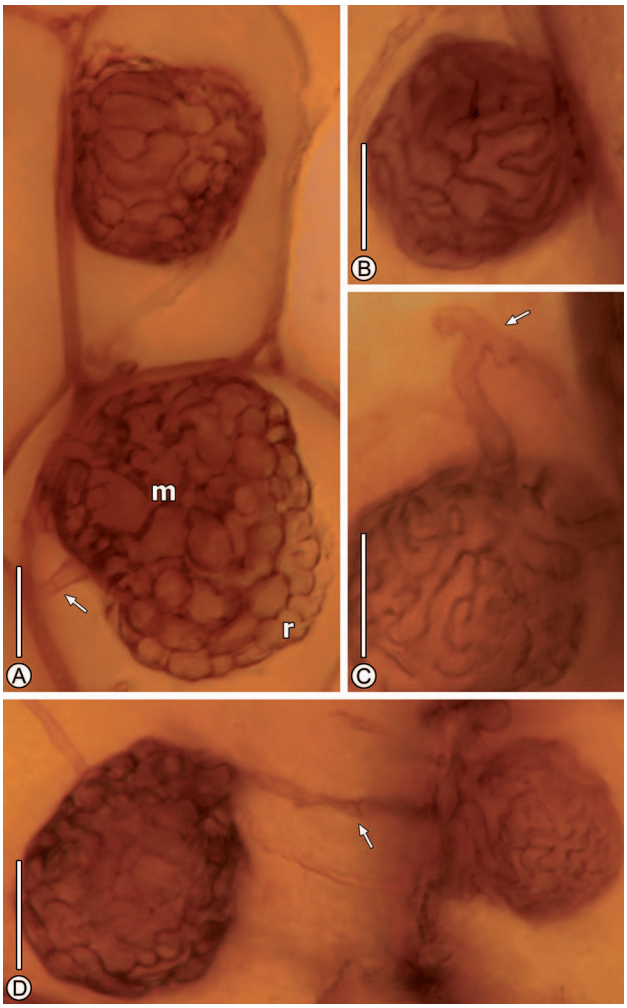

FIG. 2. Cerebriform microsclerotia. A. Microsclerotia in transverse section, exhibiting differentiation into rind (r) and medulla (m), and attachment to septate hyphae (arrow). B, C. Microsclerotia in plan or surficial view; hyphae that form the rind are tightly adpressed. Note attachment to branching hyphae (C, at arrow). D. Multiple microsclerotia may be attached by hyphal stolons (arrow). Bars = 10 μm. A, B, D: 17030 B_{bot} 001; C: 17037 F_{bot} 001.

growth appears to respond to the architecture of host tissue, in that hyaline to slightly pigmented hyphae are entirely constrained to intercellular spaces (FIG. 3C). Finally, larger, 8–10 μm wide, septate hyphae may form loose coils that fill the lumen of host cells (FIG. 3D, E). Short, 6 μm by 8 μm, distinctively lobed or invaginated fungal propagules (FIG. 3F) also occur within several outer cortex cells of a single *E. arnoldii* specimen, and are not close to hyphae. In addition to knobby lobes, these cells also are characterized by the presence of a medial, light, peg or dot-like structure (FIG. 3F, arrow), interpreted here as penetration pegs.

DISCUSSION

Monilioid hyphae.—In an early review of known Princeton Chert fungi, LePage et al. (1994) observed several monilioid cells within cortical tissues of *Eorhiza arnoldii* and suggested that they strongly resembled monilioid hyphae of *Rhizoctonia* DC. To date, relatively few examples of monilioid hyphae have been recognized as such within the fossil record, with the notable exception of the Permian palynomorphs *Reduviasporonites*, which have been interpreted as *Rhizoctonia*-like sclerotia (Visscher et al. 2011). It is probable that this paucity within the fossil record results from conflation of monilioid hyphae with conidiogenesis.

Palynological maceration techniques may disarticulate chains of cells, and unicellular fungal propagules are regarded typically as amerospores by palynologists (Kalgutkar and Jansonius 2000). For example, the palynological form genus *Haplographites* Felix is used for moniliform chains of ellipsoidal unicells, and despite the absence of diagnostic features of conidiogenesis these cells are considered amerospores (Kalgutkar and Jansonius 2000, O'Keefe et al. 2011). Similarly, Krings et al. (2009) interpret short chains of spherical to ovoid cells as amerosporic conidia, but supposed conidiogenous loci are undifferentiated (micronematous), hyphae are not uniformly present near clusters of cells, neither ramoconidia or connectives are present and the cells are irregularly arranged in three dimensions within plant cells.

An interpretation of the monilioid cells preserved within *Eorhiza arnoldii* as hyphomycetous conidia can be confidently dismissed. Among extant fungi, amerosporic microconidia of aspergilloid or penicilloid fungi are typically produced from fixed, phialidic conidiogenous loci, whereas the fossil cells do not arise from obvious conidiogenous cells or conidiophores. While catenulate macroconidia are produced from micronematous conidiogenous loci by some species of *Monilia* Bonord., *Phaeomonilia* R.F. Castañeda, Heredia & R.M. Arias, *Seifertia* Partr. & Morgan-Jones and *Sorocybe* Fr., these genera tend to have conidiomata that are sporodochial, or formed of distinctive, macronematous hyphae (Seifert et al. 2011). Although catenulate conidia of *Cladosporium* Link and *Toxicocladosporium* Crous & U. Braun do resemble the monilioid cells observed in this study, members of these genera also produce numerous septate ramoconidia (Sivanesan 1984, Crous et al. 2007, Seifert et al. 2011). In the fossils, intercalary branching does not result in the production of septate ramoconidia.

We thus concur with LePage et al. (1994), in that these chains of fungal cells are monilioid hyphae.

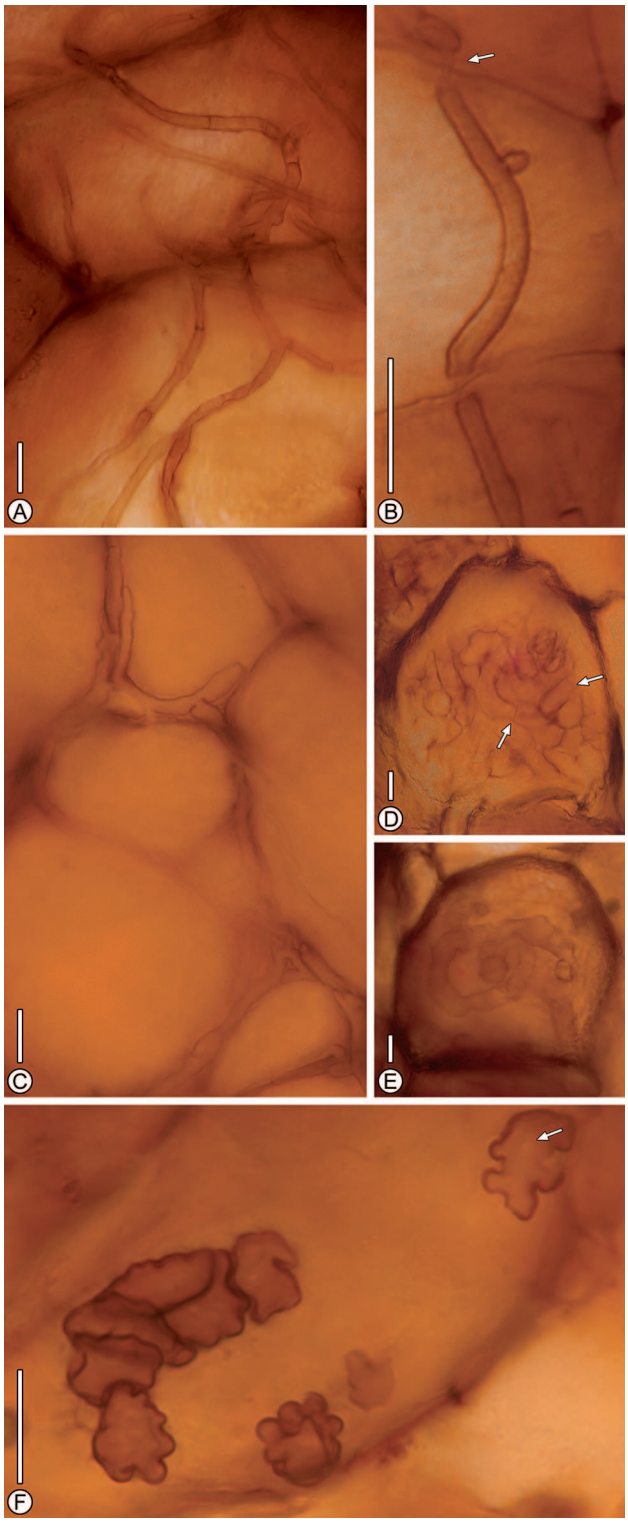

FIG. 3. Variation in mycelial growth through tissue of host plant. A, B. Extensive intracellular hyphal proliferation with microhyphal cell wall penetration (B, arrow). C. Restriction of hyphae to intercellular spaces. D, E. Loose coils of large diameter (10 µm) hyphae within host cell lumens; septa visible at arrows. F. Vegetative hyphal elements with irregularly lobed or invaginated morphology; note medial cellular structures interpreted as penetration pegs, all cells and indicated at arrow. Bars = 10 µm. A, C: 17035 E_{bot} 002; B: 17030 C_{bot} 001; D, E: 17035 E_{top} 002; F: 17040 B_{bot} 001.

However, in that *Rhizoctonia* originally was erected as a form genus for soil-borne pathogens and endophytes, and is now understood as a polyphyletic assemblage (Moore 1987, Anderson and Stalpers 1994, García et al. 2006) the taxonomic affinities of these fossil fungi bear reassessment. By examining *E. arnoldii* tissue in palaeontological thin section, we have been able to observe numerous examples of these monilioid hyphae, and it is now apparent that their proliferation through host tissue frequently is extensive. Furthermore, we now know that the monilioid hyphae are produced from simple-septate, acutely branching regular hyphae, and also occur as loose microsclerotia. These new data suggest that these fossils are unlikely to represent *Rhizoctonia* s.s. or other basidiomycetous species previously classified within the morphotaxon: the regular hyphae associated with the monilioid hyphae tend to be smaller in diameter (< 5 µm) than those of *Rhizoctonia* s.l.; they lack clamp connections and dolipore septa; there is no evidence for orthogonal branching in any of the assimilative mycelia observed; and the infection process in *Rhizoctonia* s.l. involves the production of profusely branching masses of hyphae (Parmeter and Whitney 1970, García et al. 2006), whereas only hyphopodium-like cells (FIG. 3F) have been observed in association with the fossils. On the basis of these morphological characters, it is unlikely that the fossils share an affinity with the basidiomycetous *Rhizoctonia*-like soil pathogens, but hyphal features alone do not permit us to more precisely identify them, especially because monilioid growth is common to many fungi that colonize vascular plants, including both plant pathogens and endophytes (Melin 1923, Parmeter and Whitney 1970, Currah et al. 1988). Unambiguous identification of sterile mycelia in living fungi depends upon observing their association with conidial or sexual phases, characterizing substrate utilization, or molecular taxonomy (Addy et al. 2005, García et al. 2006).

Microsclerotia.—Survival anamorphs, which include aleuriospores, chlamydospores and sclerotia, represent dormant or quiescent stages of fungal life cycles; they are produced by microfungi in response to changing environmental conditions and function in long-term survival and dispersal of microfungi (Chet and Hennis 1975, Willets and Bullock 1992, Willets

1996, Siefert and Samuels 2000). Sclerotia in particular have been extensively studied and are known to be produced in response to accumulation of metabolic staling products, changing temperature and light regimes and mechanical trauma to the vegetative mycelium (Chet and Hennis 1975). The two types of microsclerotia found within *Eorhiza arnoldii* are consistent in size and morphology with true, anatomically differentiated sclerotia (FIG. 2) and with the undifferentiated monilioid sclerotia (FIG. 1F–G) produced by non-clavicipitaceous endophytic fungi (Chet and Hennis 1975, Willets 1997, Rodriguez et al. 2009).

The development of true sclerotia is typified by three stages: the formation of sclerotial initials from interwoven hyphae is followed by an increase in size and septation of hyphal initials to form the medulla; as the developing sclerotium matures, the pseudoparenchymatous exterior surface, or rind, thickens and becomes melanized (Chet and Hennis 1975, Willets and Bullock 1992, Erental et al. 2008). The cerebriform microsclerotia present within some rhizomes of *Eorhiza* are fully mature, as the tightly adpressed hyphae forming the rind are deeply pigmented in comparison to vegetative hyphae with which the microsclerotia are associated (FIG. 2). Coiled and interwoven strands of hyphae that occur within some host cells (FIG. 3D, E) may represent initial stages in sclerotial development, but they do not occur near mature sclerotia, and intermediate forms have not been observed.

Cerebriform microsclerotia have not been reported extensively in the literature. This sclerotial morphology is best known in association with slow-growing colonial ascomycetes called ''meristematic fungi'', which are found predominantly on rock, including marble buildings and monuments. They usually proliferate by short hyphal stolons, although yeast-like phases have been observed (Sterflinger et al. 1999, Sterflinger 2006). Phylogenetically, these fungi are members of orders that contain saprotrophic and plant pathogenic black yeasts (Ruibal et al. 2009). Cerebriform microsclerotia, however, are reported only rarely within plants but are probably common in that they have been observed within a broad taxonomic range of hosts (Hambleton et al. 2003, Ahlic and Sieber 2006, Fernandez et al. 2008).

Unlike the fossil cerebriform microsclerotia, which invariably are born from regular hyphae, the dense aggregations of monilioid cells that fill the lumens of host plant parenchyma are associated with both regular and monilioid hyphae. Although some true (differentiated) sclerotia may initiate in this fashion (Townsend and Willets 1954), there is no evidence that these fossil microsclerotia ever became further differentiated into rind or cortex, which is a development that is normally attendant with maturation of a true sclerotium (Willets and Bullock 1992). Willets (1997) considers these structures ''multihyphal reproductive anamorphs'', but in most literature they are regarded simply as microsclerotia and thought to function in the same capacity as other sclerotia (Currah et al. 1988, Anderson 1996, Jumpponen and Trappe 1998). Loose, monilioid microsclerotia similar to the fossils are produced by a number of root-colonizing fungi (Currah et al. 1988, Ahlic and Sieber 2006), and the affinities of these fossil fungi likely lie within the informal assemblage commonly referred to as dark-septate endophytes (DSE).

Similarities to extant dark-septate endophytes.—DSE, which have also been termed DS fungi (DSF) and *Mycelium radicis atrovirens* (MRA), comprise a heterogenous assemblage of predominantly ascomycetous fungi that have been isolated from more than 600 species of vascular plants and can grow asymptomatically within the living tissue of their hosts (Jumpponen and Trappe 1998, Jumpponen 2001, Rodriguez et al. 2009). In contrast to plant shoots, endophytic colonization of roots often is extensive, with both inter- and intracellular proliferation (Schulz and Boyle 2005) of dematiaceous septate hyphae, monilioid hyphae and yeast-like arthroconidia (Melin 1923, Currah et al. 1988, Dalpé et al. 1989). Unlike arbuscular mycorrhizal fungi, DSE do not form obvious assimilative structures at their interface with host tissues. Instead, there is evidence that DSE are intimately associated with host sieve elements via mucilaginous hyphae that form integrated networks between the host's vascular system and the hyphae present within the cortical tissue (Barrow 2003). Intracellular microsclerotia occur in the outer cortex, developing in response to stress or host senescence (Fernando and Currah 1996, Jumpponen and Trappe 1998, Barrow 2003).

Distinctive microsclerotia that co-occur with monilioid and regular hypha growth in the cortical tissues of *Eorhiza arnoldii* are morphologically similar to known DSE (Currah et al. 1988, Ahlich and Sieber 2006, Fernandez et al. 2008). Because the host-fungus interface of DSE involves a network of non-chitinous mucilaginous hyphae (Barrow 2003), direct evidence by which to discriminate an asymptomatic endophyte from a saprotrophic root colonizer is unlikely to be observed in the fossil record, although additional investigations may yield associated conidia. Conidiogenesis can be diagnostic for a number of root endophytes (Fernando and Currah 1995, Addy et al. 2005) but is often rare, frequently occurring only after a period of vernalization (Wilson et al. 2004,

Addy et al. 2005). Because the sterile mycelia of most DSE are morphologically similar, we currently are unable to more precisely delimit the systematic affinities of these fossils, although the occurrence of two types of survival anamorphs indicates that more than one species of root colonizing fungi might have been present.

As previously mentioned, several lobed or invaginated cells (FIG. 3F) also occur within cortical tissue that hosts monilioid hyphae. Similar cells, co-called germlings, have been observed in association with microthyriaceous epiphyllous fungi (Dilcher 1965), but this morphology, particularly with respect to the presence of penetration pegs, is consistent with hyphopodia of the cereal pathogen *Gaeumannomyces graminis* (Sacc.) Arx & D.L. Olivier (van Geel et al. 2011). A hyphopodial growth phase also has been observed in the DSE *Phialocephala fortinii* Wang & Wilcox (Ahlich and Sieber 2006) and may represent the mode of primary infection for other endophytic fungi. The fossil hyphopodia may represent infection propagules of either of the two DSE-type anamorphs or a hitherto unknown pathogen of the aquatic host plant, *Eorhiza arnoldii*.

Ecological interpretations.—In the absence of defining features of conidiogenesis that would permit clear attribution to extant DSE lineages, it is impossible to conclusively identify the ecological role of these fossil fungi. Hyphae associated with the putative DSE, however, do appear to have interacted with the cell wall structure of the host plant: penetration across cell walls is via microhyphal strands (a feature that to our knowledge has not been demonstrated previously for fossil fungi), and in some specimens hyphal growth has been constrained to intercellular spaces of host tissue. Finally, the *Eorhiza* tissue contains several loose intracellular coils of hyphae, which are similar to ericoid mycorrhizae and to ''peloton-like'' DSE structures observed in some boreal orchids that have been interpreted as functioning as ectendomycorrhizae (Currah et al. 1988, Petersen et al. 2004). We favor the latter interpretation because the structures are isolated and rare and the host plant is thought to represent an extinct family of basal angiosperms perhaps most closely affiliated with Nympheales (Stockey and Pigg 1991, 1994). Currah et al. (1993) noted, however, that the peloton-like, coiled, branching hyphae associated with some DSE also can occur in moribund tissues and therefore are not necessarily indicative of a biotrophic relationship.

Previous assessments of fungal diversity within *E. arnoldii* have revealed the presence of several microfungi, some of which are known to be saprotrophic (LePage et al. 1994, Klymiuk et al. 2012), providing indication that the host tissue was moribund at the time of fossilization. In that chitin is a highly resistant biopolymer (Briggs 1999), it is likely that the fungi preserved within the plants of the Princeton Chert are a palimpsest of fungal succession: endophytes colonized living tissue, which senesced, died and was incorporated into the organic substrate of a peat-forming mire, where it was subject to biodegradation by saprotrophs. We hypothesize that *E. arnoldii* was colonized by dark-septate endophytes that persisted commensally within the cortex during the plant's life; during this period, regular hyphal growth was likely restricted to intercellular spaces, with monilioid growth occurring predominantly in association with sclerotial development, which occurred within the confines of host cells. Subsequent to the death of the host and upon incorporation into the inundated substrate the fungi persisted as saprotrophs, with assimilative mycelia proliferating through the degrading host tissue.

Our suggestion that the relationship between the *E. arnoldii* plant and the dark-septate endophytes was one of commensalism should be understood only as a parsimonious hypothesis, especially because the ecology of living endophytic microfungi remains largely unknown. It has been demonstrated experimentally that DSE can function as both pathogens and saprotrophs (Wilcox and Wang 1987; Menkis et al. 2004, 2005). In addition, they display little host specificity (Ahlich and Sieber 2006, Walker et al. 2011) and are known to colonize species that simultaneously host AMF or ectomycorrhizal fungi (Wagg et al. 2008, Ghanta et al. 2012). Nevertheless, they are ubiquitous in alpine, boreal, arctic and arid environments (Gardes and Dahlberg 1996, Barrow 2003, Schmidt et al. 2008), and there is some indication that DSE can form mutualistic mycorrhizal-like associations with plants that lack typical mycorrhizae (Petersen et al. 2008). It has been hypothesized that they can positively contribute to plant growth through nutrient solubilization or by water retention (Mandyam and Jumpponen 2005). The fact that some heliotealean DSE also have been shown to enhance nitrogen uptake in graminoids and eriocoids (Zijlstra et al. 2005, Newsham 2011) is of particular interest when considering plants growing in inundated peat-forming mires, in that these environments generally are nitrogen poor.

Many of the plants preserved in the Princeton Chert, including *E. arnoldii*, have structural adaptations to an aquatic habitat and obviously grew within or near the periphery of the Eocene mire that has been preserved as a succession of silicified peats (Cevallos-Ferriz et al. 1991). Exquisite preservation of botanical remains has allowed insight into the

microbial constituents of this environment, which in turn provide new information about the ecology of this renowned paleobotanical locality. The sterile mycelia described here provide an important new fossil record for plant-fungal interactions and simultaneously expand our understanding of the diversity of root-colonizing fungi within the chert. In addition to arbuscular mycorrhizae and ectomycorrhizae (LePage et al. 1997, Stockey et al. 2001), there is now evidence for the presence of dark-septate endophytes. We anticipate that continued research into the distribution and prevalence of these enigmatic fungi will better enable us to draw ecological parallels between modern temperate mires and the fossil biota of these Eocene peats.

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