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BRIEF COMMUNICATION

# FOSSIL ECTOMYCORRHIZAE FROM THE MIDDLE EOCENE[1]

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Fossil ectomycorrhizae were found recently among permineralized plant remains in the middle Eocene Princeton chert of British Columbia. The ectomycorrhizae are associated with roots of *Pinus* and have a Hartig net that extends to the endodermis, a pseudoparenchymatous mantle, and contiguous extramatrical hyphae that are simple-septate. The mycorrhizal rootlets lack root hairs and dichotomize repeatedly to form large, coralloid clusters. Reproductive structures are absent. Based on the morphological characteristics, and the identity of the host, the closely related basidiomycete genera *Rhizopogon* and *Suillus* are suggested as comparable extant mycorrhizal fungi. These exquisitely preserved specimens represent the first unequivocal occurrence of fossil ectomycorrhizae and demonstrate that such associations were well-established at least 50 million years ago.

**Key words:** Allenby Formation; ectomycorrhizae; Eocene; fossil fungi; *Pinus*; Princeton chert; *Rhizopogon; Suillus.*

Evolution of mycorrhizal associations is considered to be a key development in the world's land flora (Pirozynski and Malloch, 1975) and significant fossil evidence indicating that the endomycorrhizal symbiosis is an ancient one has been documented by Remy et al. (1994). Fossil evidence of ectomycorrhizae is nonexistent, although these would be expected in the counterparts of extant ectotrophic taxa. Ectotrophic mycorrhizae are morphologically distinctive and easily differentiated from other mycorrhizal types and nonmycorrhizal roots. In a cross section, ectomycorrhizae display an outer mantle or coating of densely packed fungal cells and the ''Hartig net'' mycelium that develops between the epidermal and cortical cells of the root.

Ectomycorrhizae represent <5% of the mycorrhizal associations known in vascular plants but are ubiquitous in the Pinaceae. The dominance of this family in a wide range of habitats where climate is strongly seasonal and soils are nutrient-poor has been credited to their ectomycorrhizal habit (Malloch, Pirozynski, and Raven, 1980). The subtropical, freshwater swamp or marsh flora of the middle Eocene Princeton chert (Cevallos-Ferriz, Stockey, and Pigg, 1991) of southern British Columbia, contains well-preserved material of the genus *Pinus* L. The widespread and distinctive ectotrophic habit of modern species of pine prompted us to search for ectomycorrhizae among the samples bearing roots of this taxon. Chert blocks containing the permineralized fossils were cut into slabs and studied using the cellulose acetate peel technique, modified for silicifications with hydrofluoric acid (Joy, Willis, and Lacey, 1956; Basinger and Rothwell, 1977; Basinger, 1981). Peels were mounted in coverbond and Eukitt for observation with the light microscope.

Permineralized ectomycorrhizae were found as coralloid clusters of small, dichotomously branched rootlets that are attached to larger roots (Fig. 1). The rootlets are 0.1–0.5 mm in diameter, dichotomize repeatedly (Fig. 2), and lack root hairs (Fig. 3). In cross section, the rootlets show a small stele surrounded by endodermis, several layers of parenchymatous cortex, and epidermis (Fig. 3). The cross sections also clearly show the presence of densely packed hyphae forming a labyrinthine system, one cell-layer thick, between and encapsulating the cortical cells (Figs. 3–5). This hyphal network represents Hartig net tissue and as such extends into the cortex as far as the endodermis. Traces of pseudoparenchymatous fungal mantle tissue also remain. Connected with the mantle are simple-septate, emanating hyphae, 1–2 μm in diameter. Thus far, ancillary structures, such as rhizomorphs, sclerotia, or fruiting bodies, have not been found.

Hormones produced by living ectomycorrhizal fungi suppress root-hair growth, cause cortical hypertrophy, and stimulate the fine rootlets to thicken and branch in a pattern characteristic of the plant and the fungus taxon involved (Moore-Landecker, 1990). In *Pinus*, mycorrhizal rootlets are usually dichotomously branched. Coralloid clusters consisting of second- and third-order dichotomous branching are found in only a few symbioses (Agerer, 1987–1993).

The coralloid conformation of these mycorrhizal clusters, the pseudoparenchymatous mantle, and the apparent

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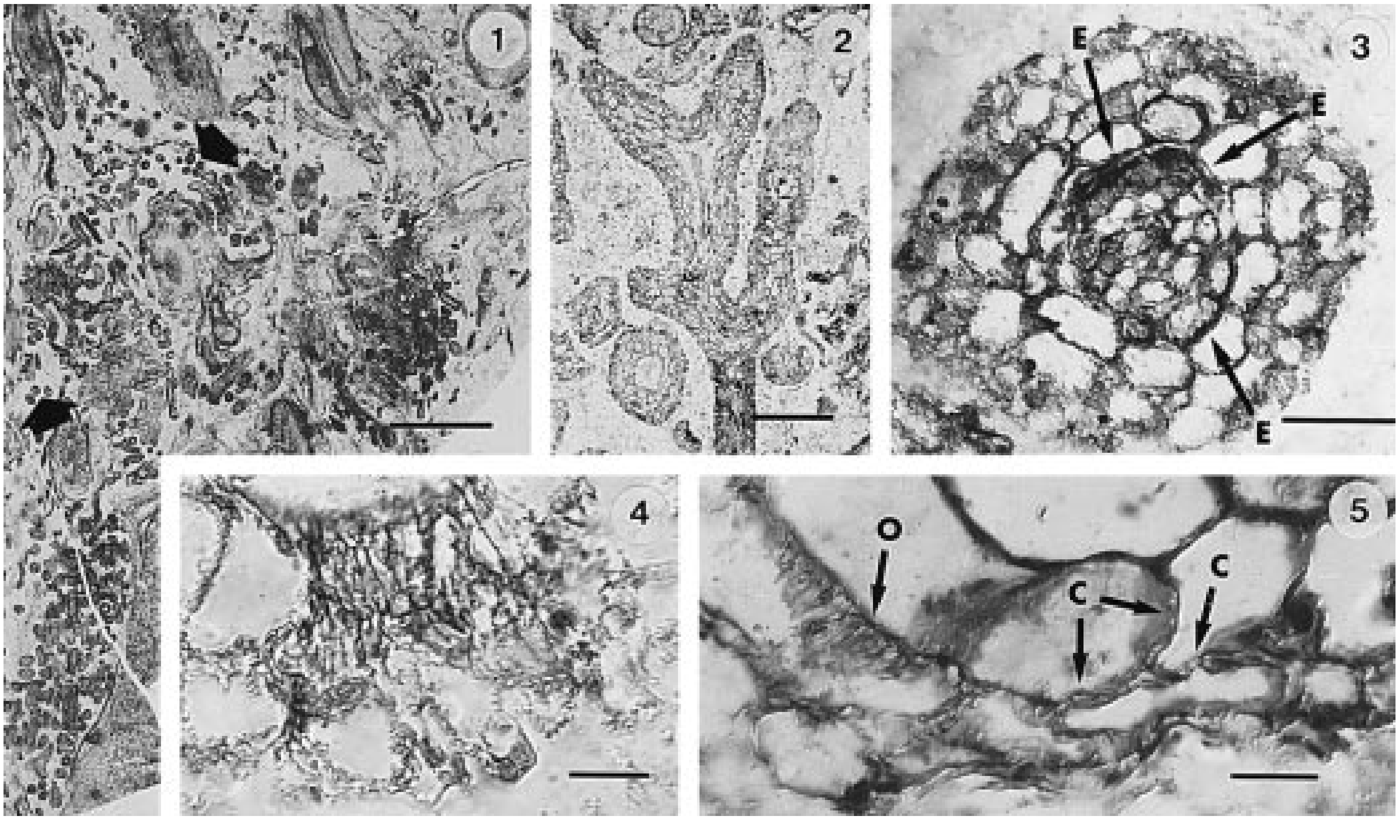

Figs. 1–5. Fossil ectomycorrhizae associated with *Pinus* roots. **1.** Coralloid cluster of ectomycorrhizal rootlets among larger roots. Arrows indicate some of the mycorrhizal rootlets. P2480 C top no. 9a. Bar = 3 mm. **2.** Longitudinal section through an attenuated, thickened, ectomycorrhizal rootlet with multiple dichotomies. P2566 B bottom no. 10. Bar = 500 μm. **3.** Cross section of an ectomycorrhizal rootlet with Hartig net between the cortical cells and penetrating as far as the endodermis (E). P1126 B bottom no. 16a. Bar = 100 μm. **4.** Densely packed and labyrinthine hyphae of the Hartig net in face view. P1126 F bottom no. 1a. Bar = 20 μm. **5.** Oblique (O) and cross sections (C) of a single layer of cells of the Hartig net among the cortical cells. P1126 B bottom no. 8a. Bar = 20 μm.

absence of clamp connections on the emanating hyphae, are characteristics associated with *Pinus* mycorrhizae involving the closely related basidiomycete genera *Rhizopogon* and *Suillus* (Bruns et al., 1989; Molina and Trappe, 1994). These associations are most common throughout the Northern Hemisphere in regions with dry, sandy, noncalcareous soils, but they also occur in lowlands and swamps (Agerer, 1987–1993). *Rhizopogon luteolus* Fr. & Nordh. is known to occur with *Pinus sylvestris* L., one of the most widely distributed species of *Pinus* and one that is adapted to a diverse range of habitats including marshlands.

It is not known when ectomycorrhizal associations first developed, but the taxonomic distribution of fungi with the ability to form the symbiosis indicates that the habit evolved independently a number of times among the fungi. Progenitors from both ascomycetes (e.g., Pezizales) and basidiomycetes (e.g., Agaricales) were probably derived from saprotrophs (Malloch, 1987). Based on their molecular clock model, Berbee and Taylor (1993) suggest that ectomycorrhizal fungi first appeared during the Early Cretaceous, ≈130 million years ago.

The earliest fossil evidence for *Pinus* is also Early Cretaceous (Alvin, 1960). Throughout their evolutionary history, representatives of the genus *Pinus* frequently dominated new and often stressful environments (Axelrod, 1986). If the earliest representatives of *Pinus* had established ectomycorrhizal associations, these would have provided an adaptive advantage over other pioneer species and may have contributed to the evolutionary radiation and ultimate success of the group.

While undisputed endomycorrhizae are known in the fossil record from a number of geologic time periods (Wagner and Taylor, 1982; Stubblefield and Banks, 1983; Stubblefield, Taylor, and Trappe, 1987; Cantrill and Douglas, 1988; Remy et al., 1994; Phipps and Taylor, 1996), ectomycorrhizae have not been demonstrated previously. Because of their microscopic size, soft tissues, and ephemeral nature, ectomycorrhizae are not good candidates for preservation as fossils. However, the permineralized material in the Princeton chert is proving to be a rich source of beautifully preserved fossil Mycota in which cellular morphology is intact as well as in situ with respect to host tissues (Currah and Stockey, 1991; LePage, Currah, and Stockey, 1994; Hill-Rackette, Currah, and Stockey, 1995). The Princeton chert ectomycorrhizal fossils display Hartig net, pseudoparenchymatous mantle, and extramatrical hyphae. These features combined with *Pinus*-like root morphology including the coralloid root clusters, dichotomized roots, and lack of root hairs, clearly demonstrate that ectomycorrhizal associations evolved at least 50 million years ago.

## LITERATURE CITED

Agerer, R. 1987–1993. Colour atlas of ectomycorrhizae. Einhorn Verlag Eduard Dietenberger, Schwäbisch Gmünd.

Alvin, K. L. 1960. Further conifers of the Pinaceae from the Wealden Formation of Belgium. *Institut Royal des Sciences Naturelles de Belgique, Memoirs* 146: 1–39.

Axelrod, D. I. 1986. Cenozoic history of some western American pines. *Annals of the Missouri Botanical Garden* 73: 565–641.

Basinger, J. F. 1981. The vegetative body of *Metasequoia milleri* from the Middle Eocene (Allenby Formation) of British Columbia. *Canadian Journal of Botany* 54: 2379–2410.

———, and G. W. Rothwell. 1977. Anatomically preserved plants from the Middle Eocene (Allenby Formation) of British Columbia. *Canadian Journal of Botany* 55: 1984–1990.

Berbee, M. L., and J. W. Taylor. 1993. Dating the evolutionary radiations of the true fungi. *Canadian Journal of Botany* 71: 1114–1127.

Bruns, T. D., R. Fogel, T. J. White, and J. D. Palmer. 1989. Accelerated evolution of a false-truffle from a mushroom ancestor. *Nature* 339: 140–142.

Cantrill, D. J., and J. G. Douglas. 1988. Mycorrhizal conifer roots from the Lower Cretaceous of the Otway Basin, Victoria. *Australian Journal of Botany* 36: 257–272.

Cevallos-Ferriz, S. R. S., R. A. Stockey, and K. B. Pigg. 1991. The Princeton chert: evidence for *in situ* aquatic plants. *Review of Paleobotany and Palynology* 70: 173–185.

Currah, R. S., and R. A. Stockey. 1991. A fossil smut fungus from the anthers of an Eocene angiosperm. *Nature* (*London*) 350: 698–699.

Hill-Rackette, G. L., R. S. Currah, and R. A. Stockey. 1995. A fungal blight on the fruits of an Eocene aquatic dicot. *American Journal of Botany* 82: 86 (Abstract).

Joy, K. W., A. J. Willis, and W. S. Lacey. 1956. A rapid cellulose peel technique in paleobotany. *Annals of Botany* (*London*), *NS,* 20: 635–637.

LePage, B. A., R. S. Currah, and R. A. Stockey. 1994. The fossil fungi of the Princeton chert. *International Journal of Plant Sciences* 155: 828–836.

Malloch, D. W. 1987. The evolution of mycorrhizae. *Canadian Journal of Plant Pathology* 9: 398–402.

———, K. A. Pirozynski, and P. H. Raven. 1980. Ecological and evolutionary significance of mycorrhizal symbioses in vascular plants (a review). *Proceedings of the National Academy of Sciences, USA* 77: 2113–2118.

Molina, R., and J. M. Trappe. 1994. Biology of the ectomycorrhizal genus, *Rhizopogon. New Phytologist* 126: 653–675.

Moore-Landecker, E. 1990. Fundamentals of the fungi, 3rd ed. Prentice Hall, Englewood Cliffs, NJ.

Phipps, C. J., and T. N. Taylor. 1996. Mixed arbuscular mycorrhizae from the Triassic of Antarctica. *Mycologia* 88: 707–714.

Pirozynski, K. A., and D. W. Malloch. 1975. The origin of land plants: a matter of mycotrophism. *BioSystems* 6: 153–164.

Remy, W., T. N. Taylor, H. Hass, and H. Kerp. 1994. Four hundred-million-year-old vesicular arbuscular mycorrhizae. *Proceedings of the National Academy of Sciences, USA* 91: 11841–11843.

Stubblefield, S. P., and H. P. Banks. 1983. Fungal remains in the Devonian trimerophyte *Psilophyton dawsonii. American Journal of Botany* 70: 1258–1261.

———, T. N. Taylor, and J. M. Trappe. 1987. Vesicular-arbuscular mycorrhizae from the Triassic of Antarctica. *American Journal of Botany* 74: 1904–1911.

Wagner, C. W., and T. N. Taylor. 1982. Fungal chlamydospores from the Pennsylvanian of North America. *Review of Palaeobotany and Palynology* 37: 317–328.