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AMBRODISCUS, A NEW GENUS OF INOPERCULATE DISCOMYCETES FROM AMBROSIA BEETLE GALLERIES

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ABSTRACT

The discomycete genus *Ambrodiscus* is described from Ambrosia beetle galleries in the sapwood of a recently fallen 130-year-old tree of *Pseudotsuga menziesii*. The features of this fungus are extremely reduced, apparently from adaptations to insect dispersal. The placement of *Ambrodiscus* in the Leotiales is discussed.

Key Words: *Ambrodiscus*, Ambrosia fungi, fungus-insect interactions, Leotiales, wood decomposition.

Ambrodiscus Carpenter, *gen. nov.*

Apothecia sessilia, pulvinata, ad 0.5 mm diam, in labyrinthus insecti crescens. Texturae steriles deminutae, friablissimae. Asci octospori, clavati, pariete iodo tincto haud caerulescente, poro apicali nullo. Ascosporae cylindracae, hyalinae, laeves. Vagina gelatinosa ascosporas cingens. Species typica: *Ambrodiscus pseudotsugae.*

Apothecia sessile, pulvinate, less than 1 mm diam; asci 8-spored, clavate, lacking an apical pore, J-; ascospores cylindrical, smooth, hyaline, surrounded by a hyaline, gelatinous sheath; growing in scolytid beetle galleries.

Etymology: From a combination of the shortened words ambrosia (ambro) and discomycete (discus).

Ambrodiscus pseudotsugae Carpenter, *sp. nov.* FIGS. 1, 2

Apothecia sessilia, pulvinata, superficialia, circa 350–400 μm diam, translucida, viridi-flava pallida. Texturae steriles deminutae, friablissimae. Excipulum ectalum cellulis globosis, hyalinis, parietibus tenuibus. Asci octospori, clavati, 38–41 × 5–6 μm, pariete iodo tincto haud caerulescente, poro apicali nullo, apice late rotundatis, basin versus gradatim. Ascosporae cylindricae, hyalinae, laeves, aseptatae, 8–9 × 1.5–2.0 μm, extremis rotundatis. Vagina gelatinosa ascosporas cingens. Holotypus: OSC #46720.

Apothecia pulvinate, superficial, *ca* 350–400 μm diam, translucent pale greenish-yellow. Sterile tissues reduced and extremely fragile. Outer ectal excipulum of the apothecium composed of cells which are globose, thin-walled and hyaline. Asci 8-spored, arising from croziers, J-, clavate, 38–41 × 10–11 μm, the ascus apex broadly rounded and lacking a pore, gradually tapered to a truncate base.

Ascospores irregularly triseriate and biseriate in the ascus, cylindrical with the ends rounded, 8–9 × 1.5–2.0 μm, aseptate, smooth, hyaline, with a single minute guttule at each end of the spore, the entire spore surrounded by a hyaline gelatinous sheath, the sheath extending to *ca* 2.0 μm from the spore wall.

Paraphyses filamentous with rounded apices, smooth, hyaline, branched from the base, 1–2-septate, *ca* 1.0 μm wide, equal to or slightly shorter than the asci.

Habitat: Growing in the gallery of an unidentified ambrosia beetle, either *Trypodendron lineatum* (Ol.), *Gnathotrichus retusus* (LeC.), or *G. sulcatus* (LeC.), located in sapwood of a 1-year fallen log of *Pseudotsuga menziesii.*

HOLOTYPE: NORTH AMERICA. UNITED STATES. OREGON, Linn Co., Andrews Experimental Forest, *ca* 80 km east of Eugene, 19.63 km NE of Blue River, R5E, T15S, S14 NE, LTER Site #1, from an unidentified ambrosia beetle gallery in a Douglas-fir log, September 10, 1986, *S. E. Carpenter s.n.*, OSC #46720.

Etymology of the specific epithet: From *Pseudotsuga menziesii*, the species of tree in which the fungus was discovered.

DISCUSSION

Ambrodiscus was discovered in the examination of a control treatment of a long term study of conifer log decomposition. In September, 1985.

FIG. 1. Asci with ascospores. A. Asci showing internal arrangement of ascospores. B. Whole asci, mature (left) and immature (right). Holotype, crush-mount in cotton-blue lactic acid, ×2000.

over one hundred 130-year-old trees of *Pseudotsuga menziesii* were harvested and placed in selected sites in a 450-year-old *Tsuga mertensiana-Pseudotsuga menziesii* forest in the Cascade mountain range of Oregon. Temperatures at the study site range from a January mean of 1 C to a July mean of 20 C. Annual precipitation averages *ca* 250 cm. The logs were *ca* 50 cm in diameter and at least 6 m long, with a sapwood layer *ca* 3–4 cm thick.

By April, 1986, the sapwood of these logs was colonized by the ambrosia beetles *Trypodendron lineatum, Gnathotrichus sulcatus* and *G. retusus.* These insects burrow through the relatively thick bark layer and into the sapwood of fallen trees, tunneling up to the heartwood. The galleries dug by these insects subsequently fill with a specialized microbial community upon which the beetles graze and complete their life-cycle (Haanstad and Norris, 1985; Batra, 1963). The gallery which contained *Ambrodiscus* was vacant of ambrosia beetles. Unfortunately, I do not know exactly which species excavated the gallery, but the tunnel in which I found *Ambrodiscus* was made by either *Trypodendron lineatum, Gnathotrichus sulcatus* or *G. retusus,* the only three local species of ambrosia beetle.

The apothecia of *Ambrodiscus* were found growing on a dark brown microbial mat, which was colonized by mites, nematodes, bacteria and fungi. Isolations made from galleries and the surrounding sapwood revealed that the fungal community in which *Ambrodiscus* was found is composed mostly of species of *Ophiostoma, Trichoderma* anamorphs of *Hypocrea* spp., the

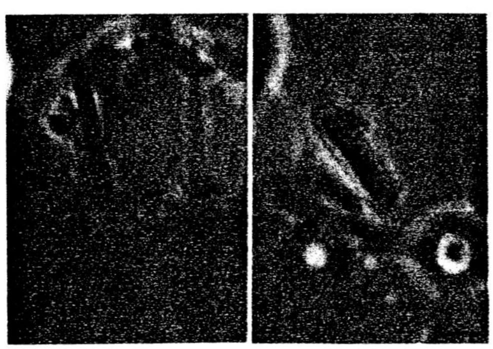

FIG. 2. Ascospores. A. Clump of ascospores adhering by gelatinous sheathes. B. Single ascospore demonstrating polar guttule and gelatinous sheath. Holotype, crush-mount in cotton-blue lactic acid, phase contrast, ×2000.

anamorphic form-genus *Penicillium*, and ascomycetous yeasts.

I found apothecia of *Ambrodiscus* in only one insect gallery, although galleries from ten other trees from the same experimental treatment were examined. Whether *Ambrodiscus* itself is rare remains to be seen, for its frequency can only be determined from its successful culture followed by an extensive survey for it in nature.

The apothecia of *Ambrodiscus* are so small that they are only observable with a high-powered dissecting microscope, and I also found them to be extremely fragile. Using a fine insect-mounting pin, I transferred apothecia to a drop of water on a microscope slide and found that the weight of a #1 glass coverslip placed on the drop completely flattened the apothecia. I was thus unable to section it to determine the orientation of its excipulum.

I transferred whole apothecia of *Ambrodiscus* to Petri plates of malt extract agar (Anonymous, **1968**) amended with 100 ppm streptomycin-sulfate and incubated them at 15 C with fluorescent lights set at a 12 hr diurnal cycle. The plates were examined daily for two weeks, and yielded only a contaminant species of *Ophiostoma*. I was unable to perform micromanipulation of ascospores for subsequent culture attempts.

The ambrosia beetle galleries of the type in which *Ambrodiscus* is found are convoluted and narrow, with a diameter ranging from 1.68 mm (*Trypodendron lineatum*) to 1.30 mm (*Gnathotrichus sulcatus*) (Kinghorn, **1957**). Because the galleries lie deep in the sapwood, dispersal of fungi specialized to this habitat is problematic. As a consequence, the majority of fungi found in those galleries, such as the anamorph and teleomorph states of *Ophiostoma* spp., have passive dispersal mechanisms specifically adapted to vectoring by invertebrates. For example, almost all of the fungi we have found in insect galleries produce sexual and asexual fruiting structures which are are long and slender, and hold at their apex an orb of a mucoid, spore-filled liquid which easily adheres to the bodies of passing insects. The spores are subsequently carried by those insects to new logs (Batra, **1966**, **1985**).

The morphology of *Ambrodiscus* follows the adaptive pattern of other insect-vectored fungi of ambrosia beetle galleries. I have observed its asci with phase-contrast microscopy and can detect no specialized apical dehiscence apparatus. I therefore hypothesize that mature asci deliquesce or break open when an insect brushes over the apothecium. The gelatinous sheath around the ascospores may act in two or more functions: 1) it may enable ascospores to adhere to scolytids crawling through the gallery, eventuating in their transport to a new scolytid gallery; or 2) if the fungus is selectively grazed by animals inhabiting the galleries, then the sheath may serve as a protective layer, enabling the spores to survive ingestion and subsequent dispersal in frass. The very small size and lack of complex sterile tissue structures of the apothecia indicate extensive adaptation to the insect gallery. The environment of the gallery is very stable and wet, with moisture content of surrounding sapwood reaching 100–150% of dry weight and sapwood temperatures reflecting only 50% of the air temperature fluctuation (Mark Harmon, pers. comm.). In the laboratory, I observed an insulating effect of sapwood: when it was chiseled open to reveal the galleries, the exposed fungi and gallery surfaces dried in just a few minutes under fiber-optic illumination. The apothecia of *Ambrodiscus* have more hymenium volume than that of sterile tissue: in the very moist environment of the gallery, elaboration of sterile tissues to protect the hymenium from desiccation tissues is probably unnecessary, and may have been lost from ancestral morphology. This extreme adaptation to insect galleries indicates that the teleomorphic state of *Ambrodiscus* probably could not survive outside of a gallery or another equally moist and stable environment.

The morphology of *Ambrodiscus* is that of the order Leotiales *sensu* Carpenter (**1988**). *Ambrodiscus* may have evolved from a lignicolous saprophyte with functionally dispersive asci, altering its apothecial form and ascus dehiscence mechanism as it adapted to insect dispersal: alternatively, *Ambrodiscus* may have evolved from some other group of ascomycetes, such as the morphologically simpler Hemiascomycetes or the closely related Pyrenomycetes. At present I believe that its affinities are closest to the Leotiales. I contend that *Ambrodiscus* has undergone such extensive adaptations to its specialized environment that these adaptations have obscured its relationship with other genera in the Leotiales. The globose excipular cells suggest affinities with the Dermateaceae, but similar excipular cells may also be found in genera of the Leotiaceae. I will defer placement of this genus in any family until more material can be found and for an analysis of its life-history.

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