

## 11. Ecology and evolution of mycophagous bark beetles and their fungal partners

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### Introduction

Associations between bark beetles (Coleoptera: Curculionidae: Scolytinae, or family Scolytidae, depending on the classification used, Bright 1993; Marvaldi et al. 2002) and fungi are varied and well known, but mycophagy (fungal feeding) by bark beetles has received relatively little attention. This may be due to the rarity or relative unimportance of fungal feeding by bark beetles, which feed in a nutrient-rich substrate, the inner bark of trees, or it may be due to a bias in research towards the possible importance of plant pathogenic fungi carried by a few coniferous bark beetles. The best studied of the more than 3500 species of bark beetles (Wood 1982; Farrell et al. 2001) construct their egg galleries in the inner bark (secondary phloem) of living trees, especially conifers in the family Pinaceae (Raffa et al. 1993). These bark beetles kill their host and are among the most economically important of forest insects. Although much has been said about the role of fungi in aiding bark beetles in killing their tree hosts, there are inconsistencies in such associations (Harrington 1993b; Paine et al. 1997), and mycophagy may be the more important symbiosis between some of the most important tree-killing bark beetles and fungi. Other species of bark beetles feed in thin-barked branches or treetops and also exploit fungi to supplement their diet.

Probably all bark beetles feed at least briefly on plant tissue colonized by fungi and could thus be considered mycophagous. However, I am here restricting the term mycophagy to grazing by larvae or young adults on fungal spores, fruiting structures or hyphae (Lawrence 1989) on the surface of galleries or pupal chambers. Mycophagy does not appear to be obligatory for bark beetles, but I hypothesize that fungal feeding allows for more efficient use of the inner bark and gives these bark beetles a competitive edge over other species of bark beetles and phloeophagous (phloem feeding) wood borers. This scenario is consistent with the hypothesized evolution of xylomycetophagous (wood and fungal feeding) ambrosia beetles from phloeophagous bark beetles (Farrell et al. 2001). Mycophagy appears to have evolved many times in the bark beetles, as it has in the xylem-feeding ambrosia beetles (Farrell et al. 2001). There are also parallels and interesting contrasts between mycophagous bark beetles and ambrosia beetles in the way they carry their fungal symbionts, the range of fungi that have been exploited by the beetles, and the morphological adaptations that some of the fungi have made to maintain the symbioses.

### Ambrosia beetles

Approximately 3400 species of ambrosia beetles are found in 10 tribes of two subfamilies of the Curculionidae, the Platypodinae and the Scolytinae (Farrell et al. 2001). The adults of most ambrosia beetles lay their eggs and the larvae develop in wood. Lignified cellulose, the principal component of wood, is not readily digested by insects, and fungi serve as the ambrosia (the special food of the gods) of these beetles.

Many ambrosia beetles have specific symbiotic fungi that colonize the wood and produce special spores or modified hyphal endings for insect grazing (Hartig 1844; Hubbard 1897; Neger 1909; Baker 1963; Batra, 1963, 1967; Francke-Grosmann 1967; Beaver, 1989; Kok 1979; Norris 1979). The ambrosia fungi typically produce fruity volatiles in culture (Neger 1909; Francke-Grosmann 1967), and perhaps these odors direct adults and larvae to actively growing and sporulating areas in the dark galleries. The fungi are probably a richer source of protein than wood, and they may also supply sterols and B-group vitamins important to beetle development (Kok 1979; Beaver 1989). In many of the ambrosia beetles, special spore-carrying sacs, called mycangia (Batra 1963), are found in one or both sexes of the adults, and the specific fungal symbionts are transported from one tree to the next in these sacs (Nunberg 1951; Batra 1963; Francke-Grosmann 1966, 1967; Beaver 1989). Glandular secretions into the mycangium may facilitate growth of specific ambrosia fungi (Norris 1979).

Coniferous bark beetles are basal in the Scolytinae (Farrell et al. 2001; Sequeira and Farrell 2001), and the xylomycetophagous habit is thought to have evolved at least seven times in the subfamily, each origin following a shift to angiosperms (Farrell et al. 2001). The subfamily Platypodinae forms a monophyletic group of ambrosia feeders within the Scolytinae or is sister to it (Farrell et al. 2001; Marvaldi et al. 2002), and this clade and the tribes Corthylini and Xyleborini comprise 98% of the ambrosia beetles (Farrell et al. 2001). The Corthylini tribe contains seedeaters, pith borers, and cone borers,

as well as bark and ambrosia beetles (Wood and Bright 1992). The bark beetle genus *Dryocoetes*, species of which are found on both conifers and angiosperms, is basal to a monophyletic group of more than 1300 ambrosia beetle species in the tribe Xyleborini, which was thought to have arisen about 20 million years ago (Jordal et al. 2000; Farrell et al. 2001).

The fungal symbionts of only a small percentage of the ambrosia beetles have been identified (Baker 1963; Batra 1963, 1967; Francke-Grosmann 1967), and with many of these it is not clear if the identified fungus is the primary symbiont or a contaminating fungus in the system. An unidentified basidiomycete associated with *Xyleborus dispar* (Happ et al. 1976b) was found to be near *Antrodia* (Hsiau and Harrington 2003), a genus of brown rot fungi, but the basidiomycete associated with *X. dispar* may not be common or important to the beetle. Batra (1972) reported a *Tulasnella* sp. as an ambrosia fungus of *Trypodendron rufitarsus*. Aside from these two basidiomycetes, the identified ambrosia fungi are in the Ascomycota, though most are known only by their asexual states. Some of the symbionts are yeasts (Batra 1967; Francke-Grosmann 1967). Neger (1908, 1909) suggested and then questioned that the filamentous ambrosia fungi were derived forms of *Ophiostoma* species. Batra (1967) placed many of the ambrosia fungi in the anamorph (asexual) genera *Ambrosiella* or *Raffaelea*. Later phylogenetic studies (Cassar and Blackwell 1996; Jones and Blackwell 1998) have found that these species are closely related to the ascomycetous genera *Ceratocystis* and *Ophiostoma*, both of which have many sexual species associated with insects, especially bark beetles (Harrington 1993a, 1993b; Harrington and Wingfield 1998).

*Ceratocystis* and *Ophiostoma* are members of the pyrenomycetes, which probably arose more than 200 million years ago (Berbee and Taylor 2001), but the two genera are not closely related, and their ancestors may have diverged more than 170 million years ago (Farrell et al. 2001). The biology of *Ceratocystis* differs substantially from that of *Ophiostoma*, but they have converged on long-necked perithecia with sticky ascospore masses for insect dispersal (Harrington 1987). Most *Ophiostoma* species are saprophytes on wood and inner bark, often in association with coniferous bark beetles, while *Ceratocystis* species are principally plant pathogens on angiosperms.

Phylogenetic analyses (Cassar and Blackwell 1996; Jones and Blackwell 1998; Farrell et al. 2001; Rollins et al. 2001) place most of the ambrosia fungi within the large genus *Ophiostoma*, which may be more than 85 million years old (Farrell et al. 2001). *Ambrosiella* and *Raffaelea* species are each polyphyletic within *Ophiostoma* (Cassar and Blackwell 1996; Jones and Blackwell 1998; Farrell et al. 2001; Rollins et al. 2001). *Ceratocystis* may be younger than *Ophiostoma*, perhaps less than 40 million years (Farrell et al. 2001), and the three known *Ambrosiella* species within *Ceratocystis* appear to be closely related, but not necessarily monophyletic (Cassar and Blackwell 1996; Paulin-Mahady et al. 2002). Species of the relatively young tribe Xyleborini have ambrosia fungi from both the *Ophiostoma* and *Ceratocystis* groups (Farrell et al. 2001). It appears that various species of ambrosia beetles have independently acquired their symbionts from numerous species in these relatively old, insect-associated genera.

In contrast to the ambrosia beetles, most bark beetles lay their eggs in and their larvae feed on a relatively rich substrate, the inner bark of trees. The inner bark may be nutritionally complete for the developing brood, but there is a great deal of competition among bark beetles and wood borers (Coleoptera: Cerambycidae and Buprestidae) for this substrate, and some fungi quickly colonize the inner bark and may render it unsuitable for beetle brood development. Ambrosia beetles have avoided this competition in the inner bark by feeding in the xylem on symbiotic fungi (Farrell et al. 2001). Although little reported compared to mycophagy by ambrosia beetles, some bark beetle species are also known to supplement their diet on fungal hyphae and spores, some carry fungi in highly developed mycangia, and some may depend on fungi for optimal development.

### **Associations between fungi and bark beetles**

A wealth of fungi, principally ascomycetes, can be found in the egg and larval galleries and pupal chambers of coniferous bark beetles (Graham 1967; Francke-Grosmann, 1967; Dowding 1973, 1984; Whitney, 1982; Harrington, 1993a, b). Many of the fungi show adaptations for insect dispersal, that is, they produce asexual or sexual spores in sticky drops at the tip of fungal fruiting structures (Dowding 1984; Malloch and Blackwell 1993). Typically, the larvae construct a chamber for pupation in the inner bark at the terminus of the larval gallery, and sporulation in the pupal chamber is of particular importance for dissemination of the fungus to a new host tree. After pupation, the young (teneral or callow) adult may first go through a period of maturation feeding, perhaps feeding on fungi sporulating in the chamber as well as on bark tissue (Fig. 1). Then the adult bores through the outer bark and searches out new trees for breeding and egg laying, or the adults may first go through a period of hibernation or maturation feeding on trees. When the young adults emerge from their pupal chambers, they usually carry spores of many

different fungi on their exoskeleton or in their gut, and they introduce the fungi into new host trees during the construction of egg galleries.

The most conspicuous and first noted associations between bark beetles and fungi were between conifer bark beetles and ascomycetous bluestain fungi such as *Ophiostoma minus* (Fig. 2; Hartig 1878; Münch 1907). The bluestain fungi have melanized hyphae and get their name from the bluish-gray color of the sapwood they colonize. Species of *Ophiostoma* are the most common of the bluestain fungi on the Pinaceae, and both staining and non-staining *Ophiostoma* species and their anamorphs (asexual genera such as *Leptographium* and *Pesotum*) are conspicuous associates of conifer bark beetles (Harrington 1988, 1993a, 1993b; Harrington et al. 2001; Jacobs and Wingfield 2001). Seven species of the morphologically similar but unrelated genus *Ceratocystis* are also capable of causing bluestain in conifer sapwood, but only three of these species are known associates of conifer bark beetles (Harrington and Wingfield 1998). Yeasts are intimately associated with bark beetles, and various molds, mycoparasites, and some basidiomycetous wood decay fungi are also commonly found in bark beetle galleries (Whitney 1982; Harrington 1993a; Six 2003). While it is clear that the fungi benefit from these relationships by transportation to fresh phloem, the benefits to the vector are not always apparent.

Given the niche available, that is, the highly nutritious, moist and uncolonized phloem, it is not surprising that many fungi have evolved stalked, sticky spore masses for acquisition by bark beetles. Based on observations of beetle galleries and isolations from adult beetles, the most successful genera of filamentous fungi associated with conifer bark beetles are *Ophiostoma* and its anamorphic genus *Leptographium* (Harrington 1988, 1993a). *Ophiostoma* may have a late Cretaceous origin (Farrell et al. 2001). The bark beetles are also believed to have their origins in the late Cretaceous (estimated at 67-93 million years before present), feeding on the coniferous genus *Araucaria*, and there may have been a diversification of bark beetles on the Pinaceae sometime near the Cretaceous/Paleocene border (Sequeira and Farrell 2001). It could be hypothesized that the diversification of *Ophiostoma* followed the advance of the coniferous bark beetles, whose tunnels provided an avenue to uncolonized phloem. The alternative hypothesis is that plant pathogenic *Ophiostoma* species allowed the bark beetles to colonize conifers by attacking the defensive resin canal system of the plant host (Farrell et al. 2001).

Some bark beetle associates have been shown to be capable of causing lesions in the inner bark and/or to invade the sapwood of living trees, and coalition of lesions or sapwood occlusion may contribute to the death of bark beetle-attacked trees (Paine et al. 1997). However, the fungal associates of most tree-killing bark beetles show little capability of colonizing a living host plant, and some aggressive tree-killing bark beetles are only inconsistently associated with plant pathogenic fungi (Harrington 1993b; Paine et al. 1997). The pattern of host colonization by fungi in artificial inoculations is not consistent with the natural colonization of living trees following bark beetle attack (Parmeter et al. 1992). The *Ophiostoma* species apparently invade the sapwood only after the tissue has ceased to conduct water, that is, after the host has died (Hobson et al. 1994). Antagonistic associations also have been noted between bark beetles and fungi that are capable of causing lesions in trees (Barras 1970; Yearian et al. 1972). Thus, a few species of fungi may in some cases benefit aggressive bark beetle species by attacking the host and providing a suitable substrate for brood development. However, most bark beetles attack dead or severely weakened trees, and it is unlikely that plant pathogenic *Ophiostoma* species have contributed greatly to the diversification of bark beetles.

Some fungi may be involved in the production of bark beetle pheromones, but this has only been investigated in the laboratory (Brand et al. 1976; Brand and Barras 1977; Hunt and Burden 1990). Other fungi are pathogens of bark beetles (Moore 1971; Whitney 1982; Whitney et al. 1984), or they may compete with the bark beetles for the phloem tissue and render the inner bark unsuitable for brood development (Barras 1970, 1973; Yearian 1972; Fox et al. 1992; Klepzig 2001a). The vast majority of bark beetle fungi, however, appear to be neither beneficial nor detrimental to the beetles (Harrington 1993a), and it would seem that the bark beetles are generally hapless taxis for the fungi.

Only a small fraction of the bark beetles have been seriously studied, and these investigations are heavily biased towards the bark beetles that attack living conifers, more specifically, towards the few bark beetles that attack living Pinaceae, especially species of *Pinus*, *Picea*, and *Abies*. Even with this small sampling, it is apparent that there are hundreds, if not thousands, of fungal species tightly associated with conifer bark beetles. With such a small number of studied beetles and such a large number of fungal associates, it would be difficult to make many generalizations about the associations between the beetles and their mycoflora. I feel safe in saying, however, that in most cases the beetles are indifferent to their fungal partners. Nonetheless, there are at least a few lineages of bark beetles that are known to utilize fungi as an important supplement to their diet, and most of these bark beetles have evolved special means of carrying fungi.

### **Bark beetle mycangia**

Adults of all bark beetles have pits and crevices in their exoskeleton that may contain fungal spores, but only nine bark beetle species are known to have well-developed mycangia (Table 1; Francke-Grosmann 1967; Beaver 1989; Bright 1993). As originally defined by Batra (1963), mycangia of ambrosia beetles are invaginations of the exoskeleton lined with secretory gland cells. The secretions into mycangia of ambrosia beetles are believed to favor the growth of specific symbiotic fungi (Norris 1979). However, the term *mycangium* has become much more broadly used with bark beetles and includes even mere pits in the exoskeleton that may accumulate fungal spores (Whitney 1982). Such simple pits and other crevices are found in virtually all bark beetles and may or may not be important in transporting fungal spores. Six (2003) recently suggested that these be considered nonglandular pit mycangia to distinguish them from the complex mycangia lined with secretory cells. However, even glandular pits may have a function other than fungal transport, and here I will discuss only setal brush mycangia and sac mycangia (Six 2003) because of their obvious role in fungal transport.

Female adults of *Pityoborus* spp. have a unique pubescent, prothoracic mycangium (Furniss et al. 1987), which could be considered a setal brush mycangium in the terminology of Six (2003). The pubescence acts as a comb to collect basidiospores from the galleries, and the fungi probably do not grow within the mycangium.

Relatively few bark beetles have sac mycangia, composed of pockets or tubes that may be glandular or nonglandular (Six 2003), and there is a remarkable coincidence of sac mycangia and mycophagy in the bark beetles. There is at least a suggestion of mycophagy in seven of the eight bark beetle species with sac mycangia (Table 1). It is not known if *Dryocoetes confusus* is mycophagous. Of the known mycophagous bark beetles, only *Ips avulsus* and *Tomicus minor* have not been shown to have either a sac or a setal brush mycangium (Table 1). However, fungal spores can be seen in the median suture and lateral folds of the elytra of *T. minor* (Francke-Grosmann 1963).

Two types of sac-like mycangia have been described for bark beetles: oral mycangia and prothoracic mycangia. Oral mycangia were first described for *Ips acuminatus*, in which there are small pouches behind the mandibles in both males and females (Francke-Grosmann 1967). *Dryocoetes confusus* also has oral, sac mycangia at the base of the mandibles of male and female adults (Farris 1969). The prothoracic mycangium of *Dendroctonus* species is glandular, and the secretions allow for growth of fungi within the mycangium (Fig. 3).

Most of the known mycangial bark beetles (Table 1) are near obligate parasites of pines and are within the genus *Dendroctonus*. This genus is in the tribe Tomicini, which is at the base of the bark beetle tree (Farrell et al. 2001), and the genus *Dendroctonus* is relatively old, estimated at 30 to 50 million years (Sequeira and Farrell 2001). Two types of mycangia, oral and prothoracic, have been described in *Dendroctonus* (Table 1), and the inferred phylogeny of *Dendroctonus* (Kelley and Farrell, 1998; Sequeira and Farrell 2001) shows that these respective mycangial types are synapomorphic (Fig. 4), suggesting their importance in the evolution of *Dendroctonus*.

Male and female adults of two sister species of *Dendroctonus* (*D. ponderosae* and *D. jeffreyi*, the Jeffrey pine beetle) have oral mycangia in the form of simple pouches in the maxillary cardines (Whitney and Farris 1970; Six and Paine 1997). Well-developed prothoracic mycangia were reported in female adults of *D. brevicomis*, *D. frontalis*, and *D. adjunctus* (Francke-Grosmann 1965, 1966, 1967), and *D. approximatus* also likely has a prothoracic mycangium (Hsiau and Harrington 2003). Similar prominent bulges in the prothorax of female *D. vitei* and *D. mexicanus* (Wood 1982; Lanier et al. 1988) suggest that these species have functional mycangia similar to those of their close relatives (Fig. 4). Secretory cells associated with the mycangium of *D. frontalis* (Happ et al. 1971), *D. brevicomis* (Francke-Grosmann 1967), and *D. adjunctus* (Barras and Perry 1971) suggest that material produced by these cells allows for the selective growth of special symbionts (Happ et al. 1976a; Paine and Birch 1983). As the fungi grow in the mycangium of the egg-laying females, the spores ooze out of the mycangium and inoculate the egg gallery.

Highly developed mycangia are not essential for the transport of fungi by bark beetles, and it is likely that all coniferous bark beetles at least occasionally carry *Ophiostoma* species. The non-mycophagous and non-mycangial *D. valens*, for instance, consistently carries *Leptographium terebrantis*, *L. procerum* and/or *O. ips* (Whitney 1982; Harrington 1988). Even bark beetles that carry specific fungi in a well-developed mycangium also carry fungi on their exoskeleton and in their gut. It would appear that mycangia have evolved to carry specific beneficial fungi. With the exception of *O. clavigerum*, the fungi listed in table 1 have not been shown to be pathogenic to trees in inoculation experiments. Rather, mycangial fungi appear to be sources of nutrition for the beetles.

### Fungal feeding by bark beetles

Mycophagous associations between phloem-feeding bark beetles and fungal symbionts appear to be relatively uncommon, presumably because the inner bark tissues of trees are relatively rich in nutrients. In some cases of mycophagy by bark beetle species, the larval galleries are relatively short and quickly widen to form a large pupal chamber, where the teneral adults can remain and feed on conspicuous fungal growth (Fig. 1) before emergence. In other cases, the larval galleries extend out into the xylem or the outer bark (Fig. 2), which would be dry and very low in nutrients, probably unsuitable for larval growth if not for the presence of fungi.

To date, mycophagy is known among only four genera of bark beetles, and the species are all conifer feeders, primarily on species of pine, and most of the mycophagous beetles are capable of attacking living trees (Table 1). Species of *Ips* and *Dendroctonus* are the best known mycophagous bark beetles, and a species of *Tomicus*, the lesser pine shoot borer, and another of *Pityoborus* also are thought to feed on fungi. The western balsam bark beetle, *Dryocoetes confusus*, has a well-developed mycangium (Farris 1969), but it is not known if it feeds on fungi.

The benefits of fungal feeding have been studied only in a few species, mostly in *Dendroctonus frontalis*, the southern pine beetle, which may carry one or both of two specific fungal symbionts in a well developed mycangium (Fig. 3; Table 1). Experimental manipulations with the mycoflora of bark beetles in a realistic environment have proven difficult but, as will be discussed later, such studies with *D. frontalis*, the mountain pine beetle (*D. ponderosae*), and the small southern pine engraver (*I. avulsus*) have shown that feeding on fungi or fungal colonized phloem can be beneficial to the bark beetle. The specific nutritional benefits conferred to the beetles are not clear, but it may be similar to the benefits suggested for ambrosia beetles (Kok 1979; Beaver 1989). Vitamins produced by the fungi may aid beetle development. Six (2003) speculated that the fungi produce essential sterols. Nutrients from the inner bark are likely concentrated by the fungi into larval galleries and pupal chambers, and the lowered C/N ratio is likely of benefit to the beetles (Barras and Hodges 1969; Ayers et al. 2000).

Fungal feeding does not appear to be obligatory for the completion of the life cycle of any of these bark beetles. Rather, the concentration and production of nutrients, sterols and vitamins in fungal hyphae and spores may supplement the diet and allow for shorter larval galleries in the inner bark, which may reduce intraspecific competition and the intense interspecific competition that exists with other bark beetles and wood borers. Such interspecific competition is particularly acute with subcortical insects (Denno et al. 1995), especially in *Pinus* spp. Five of the six mycophagous *Dendroctonus* species attack *P. ponderosa* (Table 1), which hosts more than 75 species of Scolytidae, including many species of *Ips*, and at least 40 species of wood borers (Farrell et al. 2001). In such a competitive environment, effective transport and introduction of particularly nutritious fungi could give a distinct advantage.

#### *Mycophagy by Ips spp.*

The genus *Ips* is economically important and well known, and there are a number of reports of fungal feeding by *Ips* species, or at least of the nutritional superiority of phloem colonized by fungi. For instance, Fox et al. (1992) reported that larvae of *I. paraconfusus* could develop successfully to adults without fungi, but the presence of associated fungi in the phloem led to shorter galleries for first and second instar larvae, and larvae were larger when compared to fungus-free larvae. In contrast, Colineau and Lieutier (1994) found little difference between brood of *I. sexdentatus* raised from axenic adults and those from naturally contaminated adults.

Leach et al. (1934) observed young adults of both *Ips pini* and *I. grandicollis* feeding on conidia and hyphae of *Ambrosiella ips*, which was sporulating heavily in some pupal chambers. When *A. ips* was present, the young adults fed upon the growth and consumed it before exiting the tree. No special transport vesicles have been characterized in adults of these two species of *Ips*, and because *A. ips* was not frequently seen in the galleries, Leach et al. (1934) did not consider fungal feeding important to these *Ips* species.

Later work with three species of *Ips* that commonly compete in freshly-killed southern pine trees tends to support the claim that fungal feeding is not important for *I. grandicollis*. Wild adults of *I. grandicollis*, *I. calligraphus*, and *I. avulsus* were introduced into pine bolts, and the developing broods were compared to those from fungus-free adults and adults with only bluestain fungi (*O. ips*) added (Yearian et al. 1972). In general, there was little difference among the treatments for *I. grandicollis* and *I. calligraphus*. However, wild *I. avulsus* adults constructed longer egg galleries, laid more eggs, and the broods matured faster and weighed more than broods from fungus-free adults or from adults with only *O. ips* added.

*Ips avulsus*, unlike the other *Ips* and *Dendroctonus* species attacking southern yellow pines, is relatively small, can have a life cycle as short as 18 to 25 days, and can breed in small branches (Berisford 1980; Drooz 1885), perhaps because of its short larval galleries and mycophagy. The larval galleries of *I. avulsus* soon open out into a feeding/pupal chamber (Drooz 1985) that contains luxurious fungal growth (Fig. 1; Yearian et al. 1972). Yearian (1966) reported that the fungal growth in the pupal chambers was that of a *Tuberculariella* species, and Gouger (1971) referred to the fungus as an *Ambrosiella* sp. Klepzig et al. (2001b) concluded that these observations were instead of the basidiomycete observed by Brian Sullivan (unpublished), which is now known as an *Entomocorticium* (Hsiau and Harrington 2003). *Ophiostoma ips* also sporulates in these pupal chambers (Yearian et al. 1972). Teneral adults of various *Ips* species feed on perithecia of *O. ips*, and ascospores of *O. ips* are commonly present in the gut and feces of adult *I. avulsus* and other *Ips* species (Leach et al. 1934; Yearian et al. 1972; Gouger et al. 1975). No mycangium was found in *I. avulsus* (Gouger et al. 1975).

Francke-Grosmann (1952) found an intimate association between *I. acuminatus* and its fungal associates. Mycangia stuffed with spores were identified behind the mandibles of the adults. Mathiesen-Käärik (1951) and Francke-Grosmann (1952) found *O. clavatum* to be tightly associated with *I. acuminatus*. Francke-Grosmann (1952) also noted yeasts and a second symbiont unique to *I. acuminatus*, *Trichosporium tingens* var. *macrospora*, later placed in *Ambrosiella* by Batra (1967). *Ambrosiella macrospora* may be the most important fungal source of nutrition for *I. acuminatus* (Francke-Grosmann 1952), but more work is needed on this beetle and its fungal symbionts.

#### *Mycophagy by Tomicus minor*

Mathiesen-Käärik (1950) found that *O. canum* and *Trichosporium tingens* (syn. *Ambrosiella tingens*) were tightly associated with this beetle and not found with other bark beetles in Sweden. Francke-Grosmann (1952) observed luxuriant fungal growth of *O. canum* and *A. tingens* in the galleries of *T. minor* and speculated that feeding on fungi allowed the beetle to develop normally in small branches and under very thin bark. The larvae leave their relatively short larval galleries after the second molt and enter the nutritionally poor xylem, where they become strictly fungal feeders. Thus, this beetle species is both a bark beetle and a xylomycetophagous ambrosia beetle. *Ophiostoma canum*, *A. tingens*, and some yeasts are found in the median suture and the lateral folds of the elytra of *T. minor* (Francke-Grosmann 1963).

#### *Mycophagy by Pityoborus comatus*

*Pityoborus* is within the omnivorous tribe Corthylini, which includes species of pith borers, cone borers, seed borers, and ambrosia beetles (Wood and Bright 1992). Although not economically important and thus little studied, *Pityoborus* species are interesting in that they breed in small branches (1-8 cm diameter) of *Pinus* spp. (Furniss et al. 1987). *Pityoborus comatus* adults are less than 2 mm long and breed in the underside of living pine branches, deeply engraving the xylem (Drooz 1985).

Mycophagy may allow *Pityoborus* species to feed in the thin bark of these branches (Furniss et al. 1987). Adult females of *Pityoborus* species have a unique pubescent mycangium in the prothorax (Furniss et al. 1987). Spores are apparently combed into the mycangium of the adults and transported to new branches. Furniss et al. (1987) isolated a fungus from the basidiospore-filled mycangium of *P. comatus*, and Hsiau and Harrington (2003) placed this fungus in the genus *Entomocorticium*.

#### *Mycophagy by Dendroctonus spp.*

This genus of bark beetles is arguably the most economically important of forest insects in North America and also has the greatest number of mycophagous species. *Dendroctonus*, meaning "tree killer," is a relatively old genus of largely North American bark beetles that is restricted to the Pinaceae. The tribe Tomicingini is basal to the Scolytinae, and xylomycetophagy has not been reported in the tribe (Farrell et al. 2001; Sequeira and Farrell 2001), except for that by the later instar larvae of *Tomiscus minor*. As discussed above, well-developed mycangia are not known for most species of *Dendroctonus*, but six species have been shown to carry fungi consistently in their mycangia (Table 1), and two others (*D. mexicanus* and *D. vitei*) likely have mycangia (Fig. 4). *Dendroctonus frontalis* has a rich mycoflora and well developed prothoracic mycangium (Fig. 3). The related *D. brevicomis* is also a major economic pest and has a biology and fungal relationships very near those of *D. frontalis*. In addition to similar mycangial fungi, *D. brevicomis* and *D. frontalis* are associated with *O. minus* and *O. nigrocarpum* (Table 1; Whitney and Cobb 1972; Paine and Birch 1983; Harrington and Zambino 1990; Hsiau and Harrington 1997, 2003; de Beer et al. 2003). *Dendroctonus adjunctus* and *D. approximatus* also have prothoracic mycangia, and fungal feeding is likely important for these beetles, though it appears that the fungal symbionts of the latter two *Dendroctonus* species differ from those of the better-studied *D. frontalis* and *D. brevicomis* (Table 1).

Two other mycophagous *Dendroctonus* species, *D. ponderosae* and *D. jeffreyi*, form a sister clade to the *Dendroctonus* species with prothoracic mycangia (Fig. 4).

#### *The mycoflora of Dendroctonus ponderosae and D. jeffreyi*

The maxillary mycangia, mycoflora, and biology of the sister species *D. ponderosae* and *D. jeffreyi* are very similar, except that *D. jeffreyi* is restricted to *Pinus jeffreyi* and *D. ponderosae* attacks most *Pinus* species in its range, except *P. jeffreyi* (Wood and Bright 1992). There is less genetic variation in *D. jeffreyi* than in *D. ponderosae* (Kelley et al. 1999), suggesting that the geographically- and host-limited *D. jeffreyi* population has gone through a genetic bottleneck. It could be speculated that the specialist *D. jeffreyi* diverged from the more generalist *D. ponderosae* (Kelley et al. 1999).

The larvae of *D. ponderosae* primarily feed on phloem devoid of fungal growth, as the fungi lag behind the larvae in emanating from the egg gallery (Whitney 1971). During pupation, the fungi continue to advance and colonize the pupal chamber, where they form copious amounts of spores in an ambrosial growth. Teneral adults of *D. ponderosae* consume the fungal growth and enlarge the pupal chamber by eating phloem and phellem before emergence (Whitney 1971; Whitney et al. 1987). Fungal spores enter the oral mycangium during this grazing on fungi, and in this manner the fungi are transported to the new host trees (Whitney 1971).

Many fungi have been found sporulating in the pupal chambers of *D. ponderosae* (Table 1; Whitney 1971; Whitney et al. 1987; Hsiao and Harrington 2003). Special isolation techniques and media would be needed to isolate basidiomycetes from mycangia of *D. ponderosae*, but spores of the basidiomycetes listed in table 1 are copious in pupal chambers and likely enter the maxillary mycangia during grazing. Yeasts, molds, *Ophiostoma montium*, and *O. clavigerum* were isolated from the mycangia of *D. ponderosae* (Whitney and Farris 1970), suggesting that the mycangia do not select for the growth of specific fungi. Only *O. clavigerum* was isolated from the mycangia of *D. jeffreyi* (Six and Paine 1997). More work needs to be done to identify the mycoflora of *D. jeffreyi*, but if this bark beetle is recently derived from a *D. ponderosae* population, then it might be expected that the diversity of its mycoflora also has gone through a bottleneck.

*Ophiostoma clavigerum* appears to be more beneficial in the *D. ponderosae* system compared to *O. montium*. Adults of *D. ponderosae* with *O. clavigerum* produced more progeny, and emergence of the brood occurred sooner than with *O. montium* (Six and Paine 1998). No progeny were produced by fungus-free *D. ponderosae*. However, larvae of *D. ponderosae* developed into apparently normal adults in axenic pine phloem (Whitney 1971), so fungal feeding is not obligatory. *Entomocorticium dendroctoni* is also a likely nutritional symbiont of *D. ponderosae*, apparently superior to the bluestain fungi *O. clavigerum* and *O. montium* (Whitney et al. 1987), but the numerous basidiomycetous fungi found sporulating in pupal chambers of *D. ponderosae* and *D. jeffreyi* (Table 1) have not been well studied.

#### *The mycoflora of Dendroctonus frontalis*

Most of the investigations and observations of the mycoflora of the southern pine beetle have focused on three primary associates of the beetle, the bluestain fungus *O. minus* and two mycangial fungi (Barras and Perry 1972), now known as *Ceratocystiopsis ranaculosus* and *Entomocorticium* sp. A, respectively (Klepzig et al. 2001a, 2001b). However, *O. nigrocarpum* is also a common associate of *D. frontalis* (Harrington and Zambino 1990). Further, there has been considerable confusion over the identity of the ascomycete mycangial fungus *C. ranaculosus*, which has been known as SJB 133, *Sporothrix* sp., *Raffaelea* sp., and *Ceratocystis minor* var. *barrasii* (Harrington and Zambino 1990).

Because of the wealth of information and confusion concerning the fungi associated with *D. frontalis*, its mycoflora needs to be characterized more clearly. In an unpublished study, we (I, Bob Bridges, and the late Thelma Perry of the U.S. Forest Service) collected bark infested by *D. frontalis* in eight outbreak regions from east Texas to North Carolina. At each national forest site sampled, bark from two heights of 3 to 12 trees was collected, and adults were reared from the samples in the laboratory. From each bark sample, 10 males and 10 females were ground in sterile water, and dilution-plating techniques on cycloheximide-amended medium were used to quantify the presence of fungi (Harrington 1992; Hsiao and Harrington 1997). Aside from some yeasts, especially *Candida* spp., which were present in relatively small numbers, three filamentous fungi were recovered. *Ophiostoma minus* and *O. nigrocarpum* were recovered in equal frequency from males and females and in similar frequency to each other, although their relative incidence varied greatly among the eight national forests (Fig. 5). *Ceratocystiopsis ranaculosus* was rarely isolated from the male beetles, but it was very numerous in the females, often in the tens of thousands of colony forming units (CFUs) per beetle, in some cases in concentrations hundreds of times higher than the concentrations of *O. nigrocarpum* and *O. minus*.

The numerous small conidia of *C. ranaculosus* found in female mycangia (Fig. 3C) are the result of conidiogenesis within the mycangium. The basidiomycete *Entomocorticium* sp. A. also grows in the mycangium (Happ et al. 1976a), but mostly as hyphae with swollen cells (Fig. 3D), and dilution plating failed to recover the fungus, even with media lacking cycloheximide. To quantify the presence of the basidiomycete, the pronota of 21 to 70 females per national forest site were excised, surface sterilized, and plated on medium amended with benomyl (Harrington et al. 1981). The surface sterilization procedure killed much of the fungal material in the mycangium. In spite of the severe surface sterilization, however, *Entomocorticium* sp. A was isolated from nearly 75% of the pronota at Crockett National Forest, but it was not isolated from the 21 pronota from Cherokee National Forest (Fig. 5).

It is clear that the relative abundance of the four main associates of *D. frontalis* can vary greatly, with little correlation between the relative frequencies of the various fungi. Among the populations from 13 sites, only two significant ( $P < 0.05$ ) correlations were found among the frequencies of the four fungal species. The strongest was a negative correlation ( $r = -0.379$ ) between the incidence of *C. ranaculosus* and *Entomocorticium* sp. A. The mycangial fungi probably compete with each other within the pupal chambers, where the propagules enter the mycangia of exiting adults, and a negative correlation of the two fungi might be expected. Bridges (1985) found that most individual mycangia had either *C. ranaculosus* or *Entomocorticium* sp. A, but rarely were both species present.

Although it has been speculated that competition between *O. minus* and the mycangial fungi is important to the biology of the beetle (Bridges and Perry 1985; Harrington 1993a; Klepzig 2001a, 2001b), there was a positive correlation ( $r = 0.360$ ) between the incidence of *Entomocorticium* sp. A and *O. minus*. However, this significant correlation was primarily due to the population at Crockett National Forest, which had the highest incidence of *O. minus*, *O. nigrocarpum*, and *Entomocorticium* sp. A (Fig. 5). *Ophiostoma minus* primarily contaminates the beetles via the sporothecae of fungal-feeding mites that are phoretic on the adult beetles (Bridges and Moser 1983; Moser and Bridges 1986), and *O. nigrocarpum* may be acquired by adult beetles in a similar manner. Thus, the frequencies of *O. minus* and *O. nigrocarpum* may not be directly correlated with the frequencies of the mycangial fungi as there may not be much direct competition between mycangial and non-mycangial fungi in the pupal chambers.

*Ophiostoma minus* is the most conspicuous associate of *D. frontalis* because it causes an intense bluing of the sapwood of the beetle-attacked trees. The role of *O. minus* in the biology of *D. frontalis* has received much speculation (Harrington 1993b; Paine et al. 1997; Klepzig et al. 2001a, 2001b), and it was once thought that the fungus aided the beetle in killing the host tree. I (unpublished) inoculated loblolly pine seedlings and large trees with *O. minus*. A few seedlings died within 3 months of inoculation, and small lesions developed in the larger trees. The lesions were much smaller than those caused by the more aggressive fungus *L. terebrantis*, which was used as a positive check and killed one of the trees and more seedlings than did *O. minus*. No seedling mortality or lesions greater than controls were found in inoculations with *O. nigrocarpum*, *C. ranaculosus*, or *Entomocorticium* sp. A. These results are very similar to those using closely related fungi from *D. brevicornis* (Owen et al. 1987; Parmeter et al. 1989) and suggest that *O. minus* is not an aggressive plant pathogen.

Often *O. minus* colonizes only a small portion of the inner bark and sapwood (Fig. 2A), usually around the entrance holes of the egg galleries. When the developing larvae encounter *O. minus*, it is clear that it is detrimental to the development of the beetle brood (Bridges 1983; Bridges and Perry 1985; Goldhammer et al. 1990; Klepzig 2001a). It has been speculated that the habit of migration of late instar larvae to the dry outer bark evolved as a way to avoid phloem colonized by *O. minus* (Harrington 1993b). Because of the low moisture content and nutritional value of the outer bark, the larvae might only survive there through mycophagy, and the mycangium of *D. frontalis* would assure that only beneficial fungi would dominate in the galleries and pupal chambers.

Broods developing with either *Entomocorticium* sp. A or *Ceratocystiopsis ranaculosus* are larger, have higher lipid contents and are more fecund than beetles developing without these fungi, and *Entomocorticium* sp. A gives a greater benefit than *C. ranaculosus* (Barras 1973; Bridges 1983; Goldhammer et al. 1990; Coppedge et al. 1995). Because *Entomocorticium* sp. A is more beneficial to the beetle brood than is *C. ranaculosus*, and *C. ranaculosus* is well adapted to dispersal by mites phoretic on bark beetles (Moser et al. 1995), the evolution of mycangia in *D. frontalis* may have initially been to carry *Entomocorticium* sp. A. However, *C. ranaculosus* competes effectively with the basidiomycete in entering and sporulating in the mycangium of *D. frontalis*.

### Fungi fed upon by bark beetles

Ascomycetes and basidiomycetes are both fed upon by bark beetles and carried in mycangia (Table 1). All of the known examples of mycophagy by bark beetles are by conifer bark beetles, which have well-known

associations with members of *Ophiostoma* and their *Leptographium* and *Pesotum* anamorphs. So, it is not surprising that the ascomycetes fed upon by coniferous bark beetles or carried in their mycangia are all members of the genus *Ophiostoma* or their close relatives (Table 1). As discussed earlier, *Ophiostoma* apparently predates *Dendroctonus* and other mycophagous bark beetle groups. If a bark beetle species were to begin to feed on fungi and in this manner shorten the feeding galleries and lessen competition with other beetles, then the conspicuous, large masses of conidia of numerous *Ophiostoma* species would be readily available. Minor morphological adaptations, such as spreading conidiogenous apparatus and larger conidia, could greatly facilitate feeding on *Ophiostoma* species by beetles. Such minor adaptations can be seen in many specialized *Ophiostoma* species associated with mycophagous bark beetles. However, *Ophiostoma* species may not be the most nutritious fungi.

The known basidiomycete associates of mycophagous bark beetles are *Phlebiopsis gigantea* and species in the genus *Entomocorticium* (Hsiau and Harrington 2003). The relatives of these bark beetle associates are wood decaying basidiomycetes that degrade complex, lignified carbohydrates in the secondary xylem and produce macroscopic, sexual fruiting bodies on the outside of trees (Hibbett and Thorn 2001). The basidiospores are borne on basidia formed in a dense palisade and are forcibly discharged from distinctive sterigmata on the basidia, and the basidiospores fall free from the hymenium to be carried passively in wind currents. Spores of *Peniophora aurantiaca* have been trapped more than 1000 km away from the nearest fruit body (Hallenberg and Kuffer 2001). Some of the wood decay fungi also produce conidial states that might form an ambrosial layer in beetle galleries. One such basidiomycete is *Phlebiopsis gigantea*, which forms arthroconidia as well as airborne basidiospores (Hsiau and Harrington 2003). In contrast, species of *Entomocorticium* have apparently lost the capacity to produce airborne basidiospores and have more dramatic, irreversible adaptations that provide for symbioses with bark beetles (Hsiau and Harrington 2003).

#### *Ophiostoma* and *Leptographium* as food for beetles

All of the ascomycete species listed in Table 1 are species of *Ophiostoma*, their anamorphs, or other closely related genera. *Ophiostoma* is a relatively old group of more than 85 species (Seifert et al. 1993) that have slimy ascospore masses at the tip of perithecial necks. *Leptographium* and *Pesotum* have conidia produced in a slimy mass at the tip of stalks, and the mass of sticky spores readily adheres to the exoskeleton of insects. *Pesotum* species have synnemata, which are stalks comprised of many parallel strands of conidiophores, and most *Pesotum* species have known *Ophiostoma* sexual states (Harrington et al. 2001). *Leptographium* species have mononematous (individual) conidiophores but are similarly associated with insects (Harrington 1988; Jacobs and Wingfield 2001). In addition to named *Ophiostoma* species with *Leptographium* anamorphs, there are 28 species of *Leptographium* (Jacobs and Wingfield 2001), most of which are likely anamorphs of *Ophiostoma*.

As mentioned above, it had been speculated that some *Ophiostoma* species aid beetles in killing host trees, but these fungi are not particularly strong plant pathogens, with the exception of the species that cause Dutch elm disease and black stain root disease (Harrington 1993b; Harrington et al. 2001). The most aggressive of the bark beetles are not necessarily associated with aggressive *Ophiostoma* species (Paine et al. 1997). Harrington (1993b) suggested that some *Ophiostoma* species associated with tree-killing bark beetles are pathogenic to their conifer host because of the selective advantage over other *Ophiostoma* species that cannot colonize living phloem and sapwood.

Many of the *Ophiostoma* species listed in Table 1 show interesting characteristics that may indicate evolutionary adaptations to insect feeding. *Ophiostoma clavigerum*, among the most important food sources for young adults of *D. ponderosae* and its sibling species, *D. jeffreyi*, has a *Leptographium* state that is frequently found in the pupal chambers of these beetles. The conidia are extremely long and often septate (Fig. 6), unusual characters for *Leptographium* species. Also, the conidiophores tend to branch at the base and form a shaving-brush cluster of conidiogenous cells at the apex, producing columns of waxy spore masses. The waxy columns of elongated conidia appear well suited for consumption and for entering the opening of the maxillary mycangia. Surprisingly, genetic analyses (Six et al. in press) showed that *O. clavigerum* is very closely related to *L. terebrantis*, which is loosely associated with a number of bark beetles and weevils (Harrington 1988; Jacobs and Wingfield 2001) and does not appear to serve as food for bark beetles. In contrast to the ambrosia-like *O. clavigerum*, *L. terebrantis* produces only short, single-celled conidia and does not produce the shaving-brush type of conidial apparatus (Fig. 6A, 6B). In addition to the typical ambrosial-type conidiophores and clavate conidia, degenerative cultures of *O. clavigerum* also produce simpler conidiophores and small, single-celled conidia (Tsuneda and Hiratsuka 1984), thus reverting to a *L. terebrantis* morphology. Six et al. (in press) speculated that *O. clavigerum* evolved from a *L. terebrantis*-like ancestor and filled a new niche created when the *D. ponderosae/D.*

*jeffreyi* lineage of mycophagous bark beetles evolved. *Ophiostoma clavigerum* is capable of causing lesions in inoculated ponderosa pine seedlings and large trees (Owen et al. 1987; Parmeter et al. 1989; Yamaoka et al. 1990; Yamaoka et al. 1995), as does *L. terebrantis*, though the unique conidia and conidiophores of *O. clavigerum* suggest its role as food rather than as plant pathogen.

*Leptographium pyrinum*, a mycangial fungus from *D. adjunctus* (Six and Paine 1996), is also related to *L. terebrantis* and *O. clavigerum* (Six et al. in press). Mycophagy by *D. adjunctus* has not been well studied, but the well-developed mycangium of *D. adjunctus* would suggest a biologically important role for fungi. The conidia and conidiophores *L. pyrinum* do not differ greatly from those of the non-ambrosial *L. terebrantis* (Six et al. in press).

In addition to *O. clavigerum*, *O. montium* is a common associate of *D. ponderosae*. Based on morphology and DNA sequence comparisons, *O. montium* is very closely related to *O. ips* (Kim et al. 2003), a widespread species found on many species of *Pinus* and associated with many species of bark beetles (Francke-Grosmann 1963; Whitney 1982). Again, it could be speculated that the specialized mycangial fungus *O. montium* evolved from a generalist ancestor like *O. ips*.

Another intriguing evolutionary relationship between a generalist *Ophiostoma* and its specialized sister species is that of *O. piceae* and *O. canum*. *Ophiostoma canum* is believed to be a food source for *Tomicus minor* (Francke-Grosmann 1952) and is only found with this beetle (Mathiesen-Käärik 1950). In rDNA sequence and morphology of the teleomorph, *O. canum* is indistinguishable from *O. piceae* (Harrington et al. 2001), a widespread species loosely associated with many conifer insects and a common colonizer of sapwood in the apparent absence of bark beetles. The distinguishing feature of *O. canum* is the masses of large, round conidia, which resemble the ambrosia growth of many *Ambrosiella* species, except the conidia are found at the tip of synnemata (Fig. 7). The large conidia could be a morphological adaptation for serving as food for *T. minor*. *Ophiostoma canum* and *O. piceae* also differ in the nutrients they utilize (Mathiesen-Käärik 1960).

Francke-Grosmann (1952) reported that larvae of *Ips acuminatus* fed upon *O. clavatum*, although there also is a species of *Ambrosiella* found in association with this mycophagous bark beetle. It is not clear which of these fungi is most common in the oral mycangium of *I. acuminatus*. The conidia of *O. clavatum* are relatively small compared to other ascomycetes that serve as food for bark beetles, but the ramifying synnemata of this species (Mathiesen-Käärik 1951) may compensate for small spore size by producing large, copious conidial masses for grazing. The nearest relatives of *O. clavatum* are not known, but morphologically it is quite similar to *O. brunneo-ciliatum* (Mathiesen-Käärik 1953), a common associate of *Ips sexdentatus*. *Ophiostoma brunneo-ciliatum* was reported to be associated with *I. acuminatus* in France (Lieutier et al. 1991). Guerard et al. (2000) inoculated Scots pine with *O. brunneo-ciliatum* and concluded that it was not very pathogenic to the tree host.

#### *Ceratocystiopsis as food for beetles*

Although considered synonymous with *Ophiostoma* by some (Hausner et al. 1993, 2003), *Ceratocystiopsis* species may prove to be a monophyletic group with distinctive, long, thin ascospores and relatively small perithecia and necks, perhaps adaptations for dispersal by mites phoretic on bark beetles. However, the phylogenetic picture of *Ceratocystiopsis*, especially of the variable *C. minuta*, is cloudy (Hausner et al. 1993, 2003). Ascospores of the best known of these species, *C. minuta*, *C. ranaculosus*, and *C. brevicomis* have been found in the sporothecae of *Tarsonemus* spp. and on other genera of mites (Moser et al. 1989, 1995; Moser and Macias-Samano 2000). *Ceratocystiopsis collifera*, associated with *D. valens*, may be a synonym of *C. ranaculosus*, or it is a very closely related species (Hsiau and Harrington 1997).

*Ceratocystiopsis ranaculosus* and *C. brevicomi* produce large conidia in the pupal chambers of their associated beetles, *D. frontalis* and *D. brevicomis*, respectively. The large conidia are believed to be the ambrosial growth on which the beetles feed and are the likely propagules for entering the mycangium (Harrington and Zambino 1990; Hsiau and Harrington 1997). It is clear that *C. ranaculosus* can proliferate within the mycangium of *D. frontalis* as small conidia (Fig. 3C) and occur in thousands of CFUs per mycangium (Fig. 5). Small conidia of *C. ranaculosus* and *C. brevicomi* are also present in phloem tissue and may serve as spermatia in these heterothallic ascomycetes (Harrington and Zambino 1990; Moser et al. 1995; Hsiau and Harrington 1997). However, ascospores of these species seem particularly effective for dispersal by mites. For example, ascospores of *C. ranaculosus* were abundant on *Tarsonemus* mites phoretic on *D. frontalis* in Chiapas, Mexico (Moser and Macias-Samano 2000).

It is possible that *C. ranaculosus* and *C. brevicomis* share a most recent common ancestor, perhaps a mite-dispersed generalist species such as *C. minuta*, which is frequently found in old bark beetle galleries (Mathiesen-Käärik 1960). *Ceratocystis ranaculosus* and *C. brevicomis* appear to be mycangial

interlopers, adapted to produce large conidia for beetle grazing and entering mycangia, proliferate in prothoracic mycangia, and compete with *Entomocorticium* sp. A. and sp. B, respectively. It is not clear how nutritionally beneficial *C. ranaculosus* and *C. brevicomi* are to their beetles. As discussed above in the section on *D. frontalis*, *C. ranaculosus* is less beneficial to its beetle than is *Entomocorticium* sp. A, with which it competes (Fig. 5), though *C. ranaculosus* may be important in competition with the detrimental bluestain fungus *O. minus*.

#### *Ambrosiella* as food for beetles

Batra (1967) erected this genus to accommodate the asexual states of a number of fungi associated with ambrosia beetles. *Ambrosiella* species produce sporodochial-like masses of undifferentiated conidiophores and large masses of relatively large conidia in moniloid chains, presumably adaptations that allow for easy grazing by beetles. No teleomorphs of these fungi have been discovered, but rDNA analysis (Cassar and Blackwell 1996) separated *Ambrosiella* species associated with ambrosia beetles into two groups, one clade closely related to *Ceratocystis* teleomorphs and the other related to *Ophiostoma* teleomorphs. Not surprisingly, the *Ambrosiella* species associated with bark beetles are all within the *Ophiostoma* group (Rollins et al. 2001).

There are three described species of *Ambrosiella* that are associated with coniferous bark beetles: *A. ips*, *A. macrospora*, and *A. tingens* (Batra 1967). Isolates of two undescribed *Ambrosiella* species from two other conifer bark beetles were recently studied (Rollins et al. 2001) and found to be related to *A. macrospora*, but these undescribed *Ambrosiella* species may not be important sources of nutrition for their bark beetles, *Polygraphus polygraphus* and *Hylurgops palliatus*. Phylogenetic relationships among the *Ophiostoma* species are not clear, but the *Ambrosiella* species associated with bark beetles are related to each other, though they are not necessarily monophyletic, and the bark beetle symbionts are distinct from the ambrosia beetle symbionts (Farrell et al. 2001; Rollins et al. 2001).

Leach et al. (1934) observed grazing of teneral adults of *Ips pini* and *I. grandicollis* on *A. ips*, but he did not consider fungal feeding important to these beetles. Francke-Grosman (1967) concluded that *A. tingens* and *A. macrospora* are important food sources for *Ips acuminatus* and *Tomicus minor*, respectively, although mycophagy by these bark beetles has received little attention since her work. As discussed earlier, *O. clavatum* and *O. canum*, respectively, also are specifically associated with these mycophagous bark beetles (Table 1).

#### *Phlebiopsis gigantea* as food for beetles

This fungus is a well-known saprophytic wood decay fungus of conifers that produces wind-disseminated basidiospores from basidiocarps produced on the outside of logs or stumps. It is found in the polyphyletic family Corticiaceae (Hibbett and Thorn 2001). Like a minority of basidiomycetous wood decay fungi, it also produces a conidial state (Hibbett and Thorn 2001), consisting of hyphal tips that disarticulate into arthrospores. This conidial state is used commercially for the treatment of fresh stump tops as a biological control agent against the root rot fungus *Heterobasidion annosum* (Vainio and Hantula 2000).

It was shown (Hsiau and Harrington 2003) that the arthrospore-producing basidiomycete noted in galleries of *D. ponderosae* (Tsuneda et al. 1993) is *P. gigantea*, and it was speculated that its conidial state forms a suitable ambrosial growth on which the young adults can feed. Isolations and PCR amplifications from DNA extracted from the pronota of *D. approximatus* showed that *P. gigantea* is a mycangial fungus of *D. approximatus* (Hsiau and Harrington 2003). Isolates of *P. gigantea* from basidiomes (basidiocarps) and isolates from bark beetles have identical sequences of the internal transcribed spacer regions of the nuclear rDNA, but the isolates from bark beetles have wider hyphae and conidia and grow slower in culture than do those from basidiomes (Hsiau and Harrington 2003). Thus, it is a possibility that the beetle isolates represent a newly-derived taxon. The wider conidiogenous cells and conidia would seem to be particularly advantageous to mycophagous bark beetles. However, the number of isolates examined was low. It may be that the evolution and persistence of the conidial state by *P. gigantea* (and perhaps other wood decaying basidiomycetes with conidial states) is an adaptation for insect dissemination that supplements the wind dissemination of basidiospores. A similar dual life history exists for some species of *Amylostereum*, which produce arthroconidia or fragments of hyphae in the mycangia of horntails (*Sirex* spp.) but also are free-living and produce windborne basidiospores (Tabata et al. 2000).

#### *Entomocorticium* as food for beetles

Most basidiomycetous species associated with bark beetles are of the genus *Entomocorticium*, a recently evolved, monophyletic genus within the paraphyletic genus *Peniophora* (Fig. 8). *Peniophora* is a large and complex genus of wood decay fungi with rather simple basidiocarps, classified in the Corticiaceae

(Eriksson et al. 1978; Hallenberg et al. 1996), but phylogenetic analyses place *Peniophora* in the "Russuloid clade" of the "Euagarics" (Hibbett and Thorn 2001). Hallenberg and Parmasto (1998) suggested that *Peniophora* is near *Amylostereum*, the genus of horntail symbionts mentioned above.

*Amylostereum* and *Peniophora* species produce their basidiocarps on exposed bark or wood, the basidia forcibly discharge their basidiospores from the basidia by way of specialized sterigmata, and the spores are then dispersed by wind (Hallenberg and Kuffer 2001). In *Entomocorticium*, however, the sterigmata are merely pegs and do not discharge the basidiospores, and the sticky basidiospores accumulate on the surface of the hymenium for grazing by the beetles and adherence to the exoskeleton of the adult beetles (Whitney et al. 1987; Hsiao and Harrington 2003). The loss of forcible discharge of basidiospores would appear to be an irreversible but successful adaptation. The phylogenetic analyses (Fig. 8, Hsiao and Harrington 2003) strongly support the monophyly of *Entomocorticium* and suggest that this is a young clade with a rapid radiation of species, particularly in association with *D. ponderosae*.

Only one species of *Entomocorticium* has been formally described, and only a few others have been studied morphologically. Cultures of *Entomocorticium* species G, H, and I (Fig. 8) form basidia with short sterigmata identical to those described for *E. dendroctoni* (Whitney et al. 1987), and some of these species produce cystidia similar to those found in some of the *Peniophora* species. Most of the *Entomocorticium* species were discovered through examinations of pupal chambers of *D. ponderosae* (Table 1). The spores of these *Entomocorticium* species likely collect in the maxillary mycangia of *D. ponderosae* while the young adults are grazing, but no basidiomycetes have been isolated from the mycangia.

An undescribed basidiomycete was seen in pupal chambers of *Ips avulsus* (Brian Sullivan, unpublished; Klepzig et al. 1991b). Small stems of loblolly pine were sent to me by Brian Sullivan, and many of the pupal chambers of *I. avulsus* had luxurious, white fungal growth, which proved to be the hymenium and basidiospores of an *Entomocorticium*. When the bark was peeled away from the wood, teneral adults were seen grazing on this fungal growth (Fig. 1).

Basidiospores have not been observed in two of the mycangial *Entomocorticium* species (A and B), and it is not clear if the cells forming in the pupal chambers of *D. frontalis* were asexual spores or basidiospores. However, Klepzig et al. (2001a) illustrated a basidiomycete from a pupal chamber of *D. frontalis* that had the characteristic basidiospores and cystidia of an *Entomocorticium* species, and that fungus may have been *Entomocorticium* sp. A. The mycangial species of *D. frontalis* and *D. brevicomis* (*Entomocorticium* sp. A and B, respectively) grow more slowly than the other *Entomocorticium* species, perhaps indicative of their more specialized habitat. The ambrosial-like growth in the pupal chambers of *D. frontalis* and *D. brevicomis*, whether due to the accumulation of basidiospores or conidia, likely serves as the propagules for entrance into the mycangium of the young adults. In the mycangium *Entomocorticium* sp. A grows in a yeast-like phase (Fig. 3D; Happ et al. 1976a).

#### *Other basidiomycetes associated with bark beetles*

*Gloeocystidium ipidophilum* was described by Siemaszko (1939) to be an associate of *Ips typographus*, though it is not apparently a food source for this beetle. *Gloeocystidium ipidophilum* was thought to be related to *E. dendroctoni* (Whitney et al. 1987), but this speculation was not supported by mitochondrial small subunit rDNA sequence data (Hsiao and Harrington 2003). *Heterobasidion annosum* was reported to be loosely associated with Curculionidae (Bakshi 1952; Himes and Skelly 1972; Hunt and Cobb 1982; Kadlec et al. 1992), although it would appear to be more detrimental than mutualistic with the beetles. The proposition (Castello et al. 1976) that exiting bark beetles can acquire fragments of mycelium of wood decaying basidiomycetes was not supported by later studies (Harrington et al. 1981). *Cryptoporus volvatus* is commonly found producing fruiting bodies near the entrance and exit holes of bark beetles, but bark beetles do not acquire basidiospores or mycelial fragments of the fungus from beetle galleries (Harrington 1980; Harrington et al. 1981). A number of wind-disseminated basidiomycetes have been isolated from bark beetles that have been trapped in-flight, but these appear to be casual contaminants of the bark beetles and are probably unimportant to the biology of the beetles (Harrington et al. 1981). A *Sebacina*-like species was reported from galleries of *Ips avulsus* (Moser and Roton 1971). Basidiomycetous yeasts and mycoparasites also have been found in galleries of bark beetles (Kirschner et al. 1999; Kirschner and Chen 2003).

#### **Evolution of mycophagy in bark beetles**

Mycophagy appears to be important in many of the well-studied species of bark beetles, but the biology of few bark beetles is well known. Strict dependence on fungal feeding to complete the bark beetle life cycle has not been demonstrated, but the nutritional benefit of mycophagy has been suggested. The fungi could

provide a source of sterols, B vitamins, and perhaps a concentration of nitrogen and readily digestible carbohydrates. This extra nutritional boost could result in adults more robust and fit for emergence, flight, mating, gallery construction, and egg-laying. It also could lessen the amount of phloem tissue needed to complete development and shorten the life cycle, which could prove advantageous in a highly competitive environment.

As discussed by Denno et al. (1995), interspecific competition tends to be most intense among mandibulate herbivores feeding in concealed niches, and such competition may be a major driving force in the evolution of mycophagy by bark beetles. The known mycophagous bark beetles all have pine hosts, which have an exceptionally large number of phloeophagous insects, especially bark beetles on some of the North American *Pinus* species like *P. ponderosa* (Farrell et al. 2001), a host of most of the mycophagous bark beetles. Two of the five highly competitive species of bark beetles on southern yellow pines, *D. frontalis* and *Ips avulsus*, also are known to be mycophagous. A common characteristic of mycophagous bark beetles is their relatively short larval galleries, which likely results in more efficient use of the tree for brood development and less competition with other species of bark beetles and wood borers.

Ambrosia beetles apparently escaped the intense interspecific competition in phloem by feeding on fungal symbionts in the xylem (Farrell et al. 2001). Mycophagous bark beetles feed in thin-barked branches or tops of pines, often deeply grazing the xylem, their later instars are found often in the xylem or the dry outer bark, or their teneral adults feed on fungi in pupal chambers before exiting. Fungal feeding probably allows for shorter larval galleries and feeding in nutritionally poor plant tissues.

It is likely that many bark beetle species feed frequently on fungal spores and fruiting structures, and at times the fungal growth forms a thick, sporulating mass of tissue, well suited for grazing by the beetles. The most luxuriant fungal growth is often seen in the pupal chambers, and those adults that feed upon fungi would most likely carry spores of fungi from this ambrosial growth, especially if the adult beetles have specialized mycangia near their mouthparts or head. Like many ambrosia beetles, some mycophagous bark beetles have mycangia into which excretions apparently provide nutrition for specific fungi to grow and sporulate, and bark beetles with such mycangia carry only one or two species of uniquely adapted fungi. Well-developed mycangia are not essential for transport of fungi, but almost all known mycophagous bark beetles have sac or setal brush mycangia. Various types of mycangia are found in such phylogenetically diverse genera as *Dendroctonus*, *Dryocoetes*, *Ips*, and *Pityoborus* (Farrell et al. 2001), suggesting that mycophagy has evolved many times among the phloeophagous bark beetles. Two related clades of *Dendroctonus* appear to have adapted to mycophagy, and the respective clades have their respective mycangial types. Dependence on fungi for nutrition is apparently a derived character in the bark beetles, and in *Dendroctonus* mycophagy and mycangia are found in what might be considered the two most advanced clades within the genus.

It is not surprising that the ascomycetous fungi fed upon by bark beetles have proven to be related to *Ophiostoma* species, which may have diversified with the diversification of the earliest conifer bark beetles. Thus, *Ophiostoma* predates the mycophagous bark beetles. *Ophiostoma* and *Ceratocystiopsis* species and their related anamorphic genera are generally the fungi most abundant and frequently found in galleries of coniferous bark beetles. Many of the species fed upon by bark beetles are associated with specific beetle species, and the fungal symbionts appear to have morphological adaptations that make them more suitable as food, more readily adherent to bark beetles, and, for some, able to grow in beetle mycangia. In most cases, these specialized fungi are close relatives of more generalist fungi, fungi associated with many different species of coniferous bark beetles. *Ophiostoma montium* is very closely related to the generalist *O. ips*, for instance. *Ophiostoma clavigerum* is genetically almost indistinguishable from its generalist sister species *L. terebrantis*, *O. canum* has an identical rDNA sequence with its generalist sister species *O. piceae*, and the mycangial *Ceratocystiopsis ranaculosus* and *C. brevicomis* are closely related to the mite-associated *C. minuta*. In most of these cases, relatively large asexual spores are produced in an aggregated, ambrosial-like layer, while their generalist counterparts produce spores that are much smaller and accumulate on more discrete conidiophores.

Aside from associations with mycophagous bark beetles, basidiomycetes are not common associates of Scolytinae. However, basidiomycetes may be the preferred food source of some of the best-studied mycophagous bark beetles. It appears that these basidiomycetes evolved at least twice from wind-disseminated wood decay fungi. An asexual basidiomycete associated with some *Dendroctonus* species is either the common wood decay fungus *Phlebiopsis gigantea*, which may have a dual life history, or the bark beetle symbiont is recently derived from *P. gigantea*. The other known associated basidiomycetes appear to reside in a young, recently derived, monophyletic lineage nested within the paraphyletic genus *Peniophora*. Loss of forcible discharge appears to be a recently derived, irreversible character in

*Entomocorticium*, and DNA sequence analyses suggest that this adaptation has led to a relatively rapid radiation of species. Most of the known *Entomocorticium* species are associated with *D. ponderosae*.

It has been speculated that the phloeophagous, saprophytic habit is a primitive character in the Scolytinae and that xylomycetophagy is more advanced (Wood 1982; Kirkendall 1983; Beaver 1989; Berryman 1989; Farrell et al. 2001). The habit of feeding in the nutritionally poor xylem by ambrosia beetles likely evolved several times with the feeding on fungal fruiting structures and spores, mostly with shifts to angiosperm hosts (Farrell et al. 2001), and many of these ambrosia beetles have well-developed mycangia for transporting their symbionts. Similarly, but independently, mycophagous bark beetles appear to have evolved from non-mycophagous bark beetles, probably many times, and mycangia are a common characteristic of mycophagous bark beetles. Like the evolution of mycophagy within the ambrosia beetles, a number of independent evolutionary events are needed to explain the diversity of fungi that are adapted to being fed upon and transported by mycophagous bark beetles. Although the adaptations were often dramatic and perhaps irreversible in both the beetles and the fungi, there does not appear to be a clear case for the co-evolution of the mycophagous bark beetles and the fungi upon which they feed. Rather, mycophagous bark beetles have exploited a number of different fungi for their fungal partners, and based on the morphological adaptations evident in these fungi, they appear to be willing and successful symbionts.

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Table 1. Species of bark beetles thought to be mycophagous, their hosts, mycangial types, and the principal ascomycetous and basidiomycetous fungi upon which they feed or carry.

Bark Beetle	Tribe	Principal Plant Hosts	Mycangial Type	Ascomycete Associates	Basidiomycete Associates
<i>Dendroctonus frontalis</i>	Tomicini	<i>Pinus</i> spp.	Prothoracic, glandular	<i>Ceratocystiopsis ranaculosus</i>	<i>Entomocorticium</i> sp. A
<i>D. brevicomis</i>	Tomicini	<i>P. ponderosae</i> , <i>P. coulteri</i>	Prothoracic, glandular	<i>C. brevicomis</i>	<i>Entomocorticium</i> sp. B
<i>D. approximatus</i>	Tomicini	<i>Pinus</i> spp.	Prothoracic, glandular	Unknown	<i>Phlebiopsis gigantea</i>
<i>D. adjunctus</i>	Tomicini	<i>Pinus</i> spp.	Prothoracic, glandular	<i>Leptographium pyrinum</i>	Unknown
<i>D. ponderosae</i>	Tomicini	<i>Pinus</i> spp.	Maxillary	<i>Ophiostoma clavigerum</i> , <i>O. montium</i>	<i>Entomocorticium dendroctoni</i> , <i>E. sp. D, E, F, G, H</i> , <i>P. gigantea</i>
<i>D. jeffreyi</i>	Tomicini	<i>P. jeffreyi</i>	Maxillary	<i>O. clavigerum</i>	<i>Entomocorticium</i> sp. E
<i>Tomicus minor</i>	Tomicini	<i>Pinus</i> spp.	Unknown	<i>O. canum</i> , <i>Ambrosiella tingens</i>	Unknown
<i>Ips acuminatus</i>	Ipini	<i>Pinus</i> spp.	Mandibular	<i>O. clavatum</i> , <i>A. macrospora</i>	Unknown
<i>I. avulsus</i>	Ipini	<i>Pinus</i> spp.	Unknown	<i>O. ips</i>	<i>Entomocorticium</i> sp. I
<i>Pityoborus comatus</i>	Corthylini	<i>Pinus</i> spp.	Prothoracic, pubescent	Unknown	<i>Entomocorticium</i> sp. C

Fig. 1. *Ips avulsus* and *Entomocorticium* sp. I. A, B. Hymenium, basidia and basidiospores of *Entomocorticium* sp. I. C. Egg gallery (top), short larval galleries (descending), and pupal chambers of *I. avulsus* with white hymenia and basidiospores of *Entomocorticium* sp. I. D. Teneral adult of *I. avulsus* feeding on the basidiospores and hymenium of *Entomocorticium* sp. I.

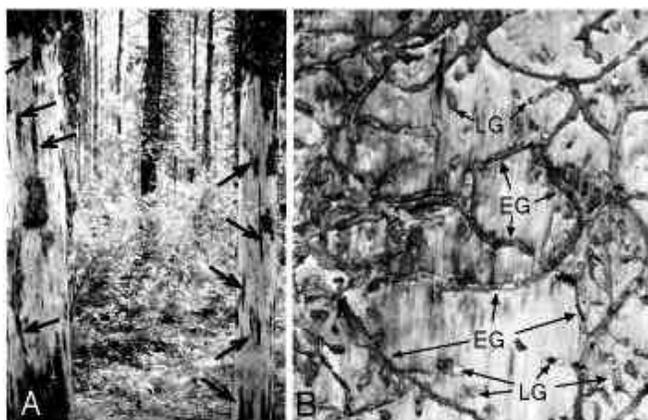
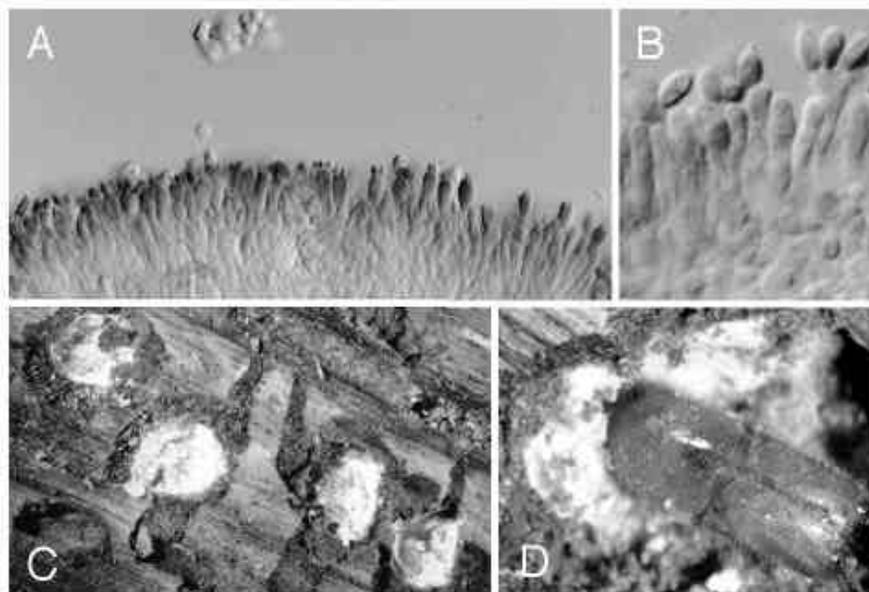


Fig. 2. Loblolly pine trees attacked and killed by *Dendroctonus frontalis*. A. Two trees in foreground with bark stripped to show the small, dark patches with black microsclerotia and perithecia of *Ophiostoma minus*, a bluestain fungus commonly associated with *D. frontalis*. The sapwood below these dark patches is stained blue-gray. B. Winding egg galleries (EG) and short larval galleries (LG) in the inner bark of loblolly pine.

Fig. 3. Mycangia of *Dendrotonus frontalis*. A. Adult, female *D. frontalis* with a bulge (arrow) in the prothorax where the mycangium resides. B. Prothorax removed from a decapitated, female adult to show the mycangium, a pale tube-like structure indicated by white arrows. C. Squashed mycangium with numerous, small conidia and conidiogenous cells of *Ceratocystiopsis ranaculosus*. D. Prothorax of *D. frontalis* torn apart to show the budding growth of *Entomocorticium* sp. A within the mycangium.

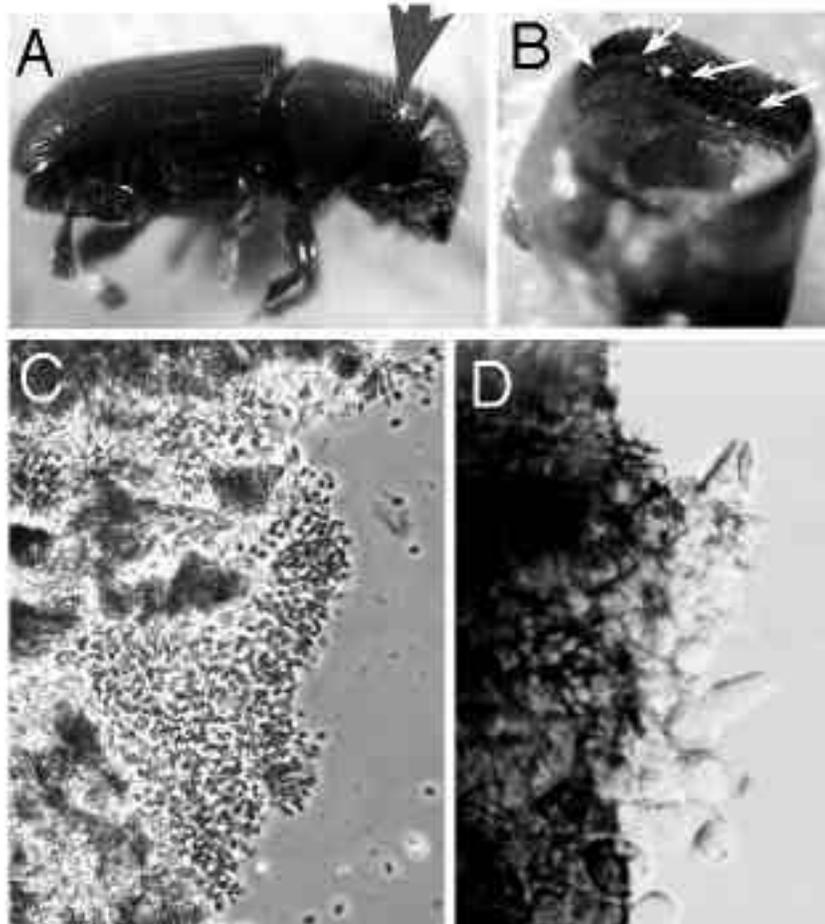


Fig. 4. Mycangial types in a cladogram of the inferred phylogeny of *Dendroctonus* species based on cytochrome oxidase I mitochondrial gene sequences (Kelley and Farrell 1998). The tree includes all recognized *Dendroctonus* species except the non-mycangial *D. parallelcollis*, which is near *D. terebrans*.

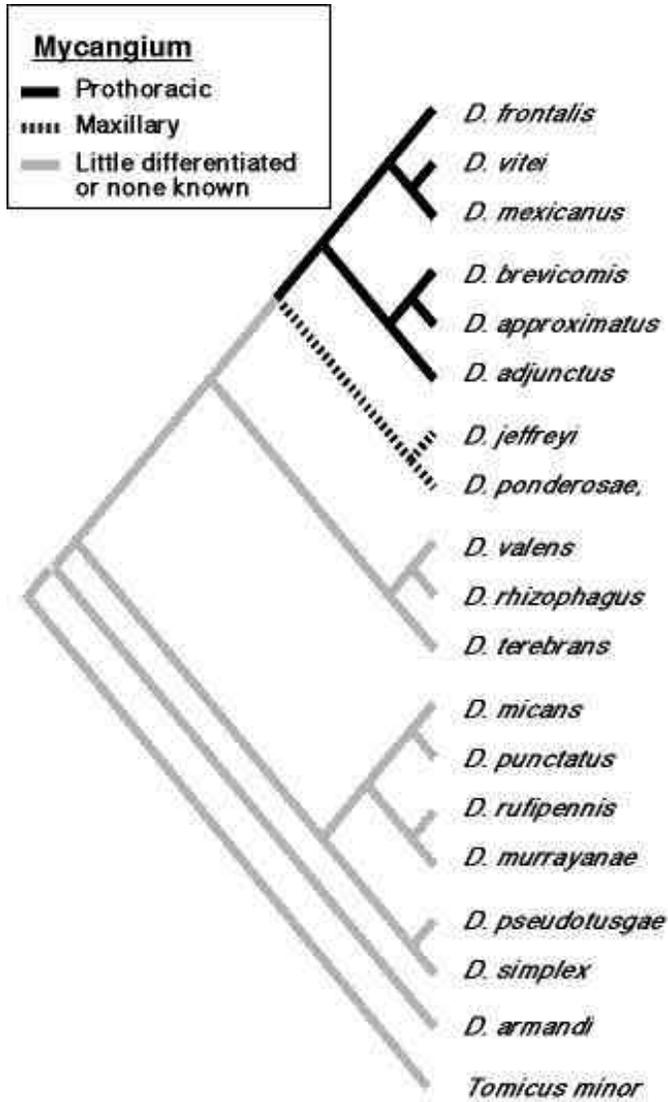


Fig. 5. Incidence of *Ophiostoma minus*, *O. nigrocarpum*, *Ceratocystiopsis ranaculosus* (colony forming units, CFUs), and *Entomocorticium* sp. A. (percent female pronota colonized) on populations of *Dendroctonus frontalis* from eight national forests in the southeastern USA.

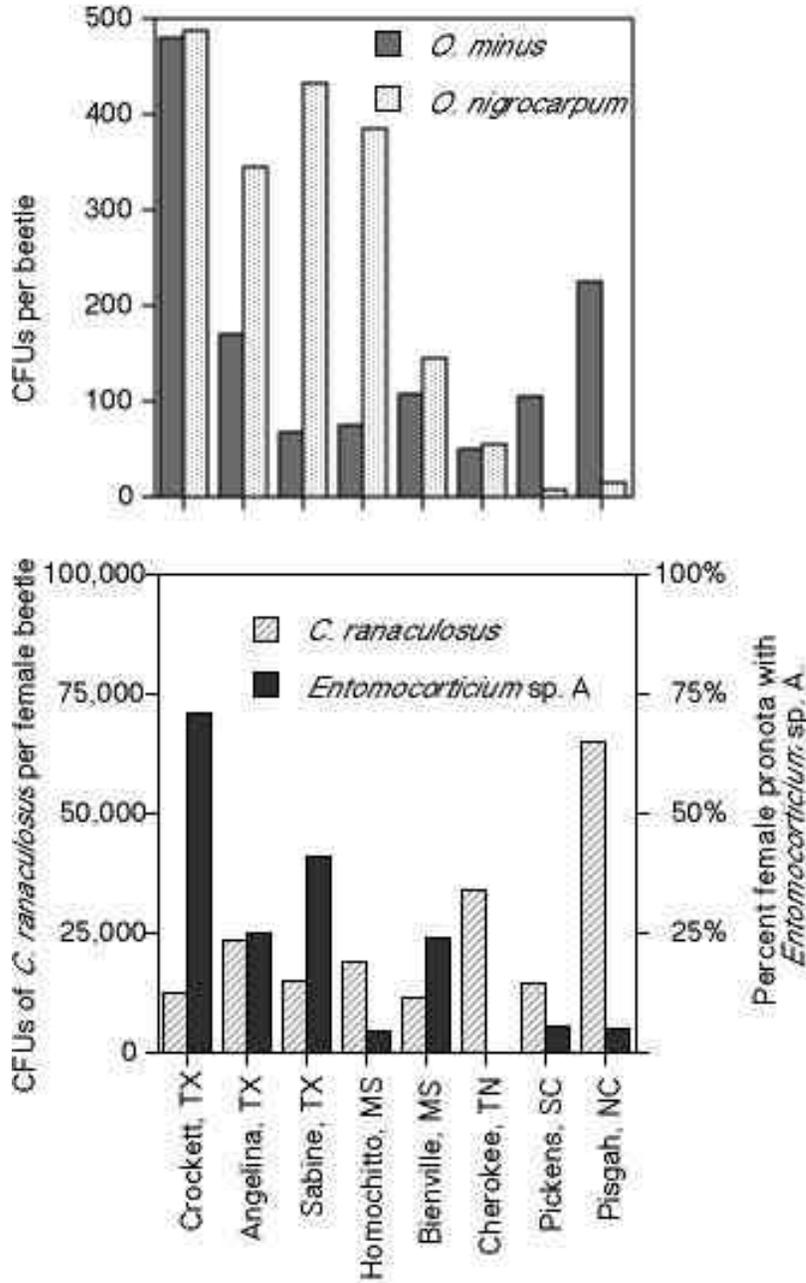


Fig. 6. Conidiophores and conidia of the mycangial fungus *Ophiostoma clavigerum* (A and B) and its non-mycangial, generalist sister species *Leptographium terebrantis*. A and C are to the same scale, as are B and D.

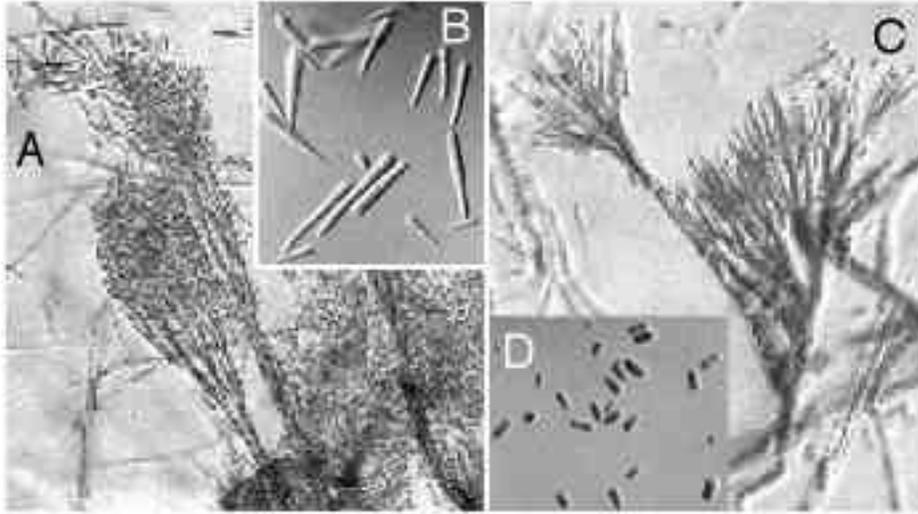


Fig. 7. Synnemata and conidia of *Ophiostoma canum* (A and B), a species associated solely with the mycophagous *Tomicus minor*, and conidia of its generalist sister species *Ophiostoma piceae* (C). B and C are to the same scale.

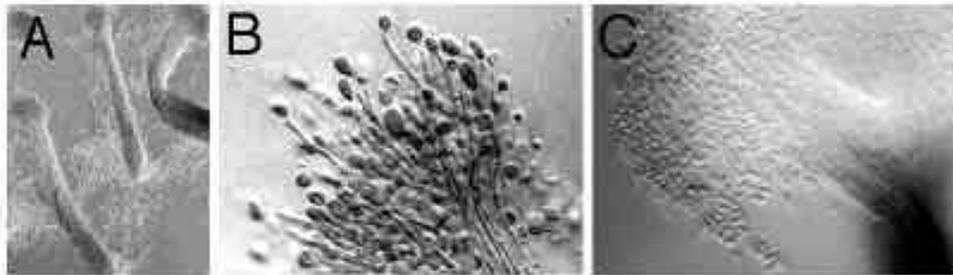


Fig. 8. One of eight most parsimonious trees of 254 steps from the internal transcribed spacers and 5.8S rDNA sequences of select *Peniophora* species and the known *Entomocorticium* species (CI = 0.6850, RI = 0.8072). The tree is rooted to *Dendrophora albobadia*. Bootstrap values greater than 50% are shown above branches.

