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A new record of fungus-beetle symbiosis in *Scolytodes* bark beetles (Scolytinae, Curculionidae, Coleoptera)

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Abstract
The most evolutionarily advanced form of symbiosis between wood-decaying fungi and wood-boring beetles (Coleoptera, Curculionidae: Scolytinae and Platypodinae) is the ambrosial habit, or fungus farming. Here we present a discovery of a new origin of the ambrosia symbiosis in *Scolytodes unipunctatus*. Feeding on symbiotic fungi and the spatial organization of the gallery system of *S. unipunctatus* is typical for ambrosia beetles, but not for phylogenetically related phloeophagous species. *S. unipunctatus* is associated with the fungal genera *Raffaelea*, *Graphium*, and *Gondwanamyces*; the association of the latter with scolytines is documented here for the first time. The fungi were identified using morphological characters and 18S, 28S and ITS regions of rDNA. We report four undescribed fungus species.

Keywords: *Scolytodes unipunctatus*, *Cecropia*, *Raffaelea*, *Gondwanamyces*, *Graphium*, ambrosia, xylomycetophagy, phloeophagy

1. Introduction

One of the most evolutionarily advanced forms of symbiosis between insects and wood-decaying fungi is "fungus farming", or obligate feeding of the insects on their saprophagous fungal symbionts, which are in turn dependent on the insect for dispersion. This symbiosis has evolved in only three separate groups of insects (Mueller et al., 2005): once each in termites (ca 330 farming species) and ants (ca 200 species), but repeatedly in wood-boring weevils within the subfamilies Scolytinae and Platypodinae (Coleoptera, Curculionidae; ca 3400 spp., 11 lineages; Farrell et al., 2001; Wood, 1986).

Morphological and molecular studies suggest that Scolytinae and Platypodinae represent one or possibly two clades within the family Curculionidae (Lyal, 1995; Marvaldi, 1997; Kuschel et al., 2000; Farrell et al., 2001; Marvaldi et al., 2002; Lyal et al., 2006). Scolytine and platypodine beetles display striking variation in reproductive strategies, including monogamy, harem polygamy, regular inbreeding, haplodiploidy, paternal genome loss, sperm-dependent and "normal" (thelytokous) parthenogenesis, and eusociality (Kirkendall, 1983, 1993; Kirkendall et al., 1997).

The two primary feeding strategies of scolytines and platypodines have been traditionally described as phloem-feeding, in true bark beetles, and fungus-feeding, in ambrosia beetles. However, we now realize that scolytine-fungus relationships represent a continuous and diverse gradient of dependency, including phloem-breeding beetles feeding preferentially on fungi (Six, 2003; Harrington, 2005), twig-borers which breed in wood with previously established ascomycete fungi (Deyrup, 1987), and fungus-farming ambrosia beetles that nevertheless consume host xylem tissue (Roeper, 1995). Many of these symbioses have evolved multiple times, and their evolution has been only partially correlated with phylogenetic grouping of either the fungi or the beetles (Cassar and Blackwell, 1996; Farrell et al., 2001).

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The majority of scolytine species breed primarily in dead phloem tissues (inner bark), a habit shared with their nearest weevil relatives (Sequeira and Farrell, 2001). Many such phloeophagous species benefit from an association with fungi, which either contribute nutrients to larval diets, protect the local inner bark from antagonistic fungi, or help the beetles overcome host tree defense mechanisms (Paine et al., 1997; Six, 2003; Klepzig and Six, 2004; Bentz and Six, 2006; Eckhardt et al., 2004).

However, more than a fourth of all scolytines, and all platypodines, feed exclusively on symbiotic fungi which they transport into and cultivate in dead woody tissues (ambrosia beetles, ca 3400 species, 11 independent lineages; Farrell et al., 2001; Mueller and Gerardo, 2002; Wood, 1986). Ambrosia beetles locate and penetrate the host tree tissues, usually sapwood, and allow the transported fungus to colonize the surrounding tissues. All stages feed either solely on their symbiont's mycelium (mycetophagy) or on a mixture of the mycelium and the sapwood tissue (xylomycetophagy, Roeper, 1995). The fungal symbiont extracts nutrients from a large volume of surrounding tissues, and consequently provides a richer diet for the beetles than would pure wood (French and Roeper, 1975; Beaver, 1989). Several physiological and developmental traits of the beetles depend on the fungal diet (Kok, 1979; Norris, 1979). On the other hand, ambrosia fungi are completely dependent on transmission provided by the beetles, as they have never been found in any environment except trees infested by their vectors (Beaver, 1989).

Except for two identifications of ambrosia fungi from basidiomycetes (Batra, 1972; Hsiau and Harrington, 2003), all other ambrosia fungi belong to Ascomycota. Some of the symbionts are yeasts (Batra, 1963; Francke-Grosmann, 1967), but most ambrosia fungi belong to the clade composed of the polyphyletic asexual ophiostomatoid genera *Ambrosiella* and *Raffaelea* and close relatives (Cassar and Blackwell, 1996; Jones and Blackwell, 1998; Gebhardt et al., 2005).

The specificity of the ambrosia associations is not well known. Batra (1966) showed that many ambrosia beetle species are able to develop on *in vitro* culture of fungal species with which they are not associated in nature, as well as on species not naturally involved in the ambrosia symbiosis. In most beetle species, a single fungus is the dominant symbiont, but the community usually includes less common species of both filamentous fungi, yeasts and bacteria (Batra, 1966; Kabir and Giese, 1966; Batra, 1985; Francke-Grosmann, 1967; Haanstadt and Norris, 1985; Kinuura, 1995).

One of the most apparent adaptations of the majority of ambrosia beetles are mycangia (sometimes called mycetangia) – organs specialized for transmission of spores and mycelial elements of the fungal symbiont. The structures range from simple pits on the surface of cuticle, to brushes of setae, to complex invaginations equipped with scraper setae and secretory glands (Francke-Grosmann, 1967; Six, 2003). Invaginated mycangia occur on various parts of the body, from the oral cavity to procoxae, prothorax, mesothorax, and elytral bases, among others (Beaver, 1989; Francke-Grosmann, 1967). The diversity, and thus presumably the number of independent origins of mycangia, suggest rather strong evolutionary pressure to develop such a structure.

Because of the variation in the ambrosia beetle feeding habit, feeding substrate, fungus transmission and specificity to particular ambrosia fungi, it is difficult to formulate an unambiguous definition of the ambrosia symbiosis. Three conditions can be proposed that define the relationship: 1) fungi represent the main source of the food consumed by ambrosia beetle larvae and adults, 2) the fungal symbiont or symbionts are non-random associates, transmitted by the beetles from one host to another and between generations, 3) the beetles are not able to survive and develop on a fungus-free diet composed only of plant tissue. A direct consequence of condition (1) is that larvae do not form their own feeding tunnels, although they may substantially participate in excavating a common breeding chamber or their own larval cradles. We do not consider the feeding substrate and the possession of mycangia to be defining characters of the symbiosis, since many exceptions exist.

This study presents a new record of fungus farming in Scolytinae, in *Scolytodes unipunctatus* (Blandford). This species was previously known only from a single collection from Guatemala, and its breeding behavior was unrecorded. *Scolytodes* is one of the most species-rich genera of Scolytinae, currently comprising 191 described species from subtropical and tropical America (Jordal, 1998a,b; Wood, 1982; Bright and Torres, 2006). As far as is known, species in Ctenophorini, the tribe which includes *Scolytodes,* are phloeophagous (breeding in dead inner bark) or myelophagous (breeding in the pith of twigs). However, nearly 30 species breed in unusual microhabitats afforded by large, woody, fallen *Cecropia* leaf petioles (Jordal, 1998a; Jordal and Kirkendall, 1998). Although one species, *S. multistriatus* (Wood) is reported to be a xylophage, and several other species to occasionally feed in pith or wood, ambrosia beetles are not known from the Ctenophorini. Here, we describe the habits of the insect, the associated mycoflora, and phylogenetic placement of the fungi.

2. Methods

We report our observations of active galleries of *Scolytodes unipunctatus* by LRK and JH at two sites in Costa Rica: at Zurquí de Moravia by LRK in July of 1997 (San José, Costa Rica, 1600 m.a.s.l., 10°03'N 84°01'W, montane cloud forest); and during collecting at the

Table 1. Feeding habits of *Scolytodes*.

Phloem	*amoenus, cecropicolens, cedrelae, clusiacolens, clusiae, clusiavorus, erineophilus, exiguus, facetus, ficivorus, genialis, impressus, ingavorus, irazuensis, lepidus, marginatus, micidus, nanellus, obscurus, ochromae, perditus, phoebeae, piceus, plumeriae, plumeriae, proximus, pseudpiceus, pubescens, pumilus, punctiferus, reticulatus, rugicollis, schwarzi, venustulus, venustus, ovalis, pilifrons, volcanus*
Leaf petioles	*acares, atratus, atratus, blanfordi, cecropiavorus, cecropii, glabrescens, levis, maurus, parvulus, acuminatus, caudatus, festus, imitans, jucundus, nitidissimus, suturalis, hondurensis, ovalis, acuminatus, chapuisi, punctifer, anceps, borealis, suspectus, pacificus*
Live trees	*alni, guyanensis*
Twigs	*immamis, punctiferus, tenuis*
Xylophagous	*multistriatus*
Vines	*hirsutus*
Xylomycetophagous	*unipunctatus**
Unknown	*amabilis, canalis, costabilis, crassus, culcitatus, melanocephalus, minutissimus, obesus, pannuceus, pelicerinus, pelicipennis, radiatus, setosus, spadix, striatus, swieteniae, trispinosus*

(Wood, 1982; Jordal, 1998a,b; Jordal and Kirkendall, 1998; *this paper).

Table 2. The incidence of fungal taxa (%) isolated from A) adults or from the gallery's segments directly plated on the isolation medium, B) processed with fractional-sterilization method of Francke-Grossmann (1956), and C) processed with fractional sterilization method with subsequent surface sterilization (1.5 min in 1% $HgCl_2$).

	A		B	C
	Adults	Gallery	Adults	Adults
C. destructans	86.7	100	0	0
Yeast 1	100	100	0	0
Yeast 2	0	0	100	20
Graphium sp.	93.3	0	95.4	0
Gondwanamyces sp.1	46.7	80	76.9	70
Gondwanamyces sp.2	46.7	10	15.4	10
Raffaelea sp.	0	100	4.6	20
No. of samples examined	15	10	65	10

Arthropods of La Selva project's elevational transect (300–2000 m a.s.l., http://viceroy.eeb.uconn.edu/ALAS/ALAS.html4). Active galleries were collected for studying the fungal symbionts at Finca Morillo, Heredia, Costa Rica, 1500 m, 10°14'N 84°06'W, montane cloud forest.

Two 10 cm pieces of an infested *Cecropia* trunk were collected and shipped to the laboratory (Prague) for examination of presence of symbiotic fungi. The cultivation media used were PDA (potato dextrose agar) and YEME (yeast-extract-malt extract agar) according to Batra (1967). Teneral adult males and females were used for fungal isolation. Fifteen adults from the total number of 90 extracted from the active galleries were crushed and directly plated on glass slides covered with fine layer of YEME medium. Another 75 adults were first processed with the fractional sterilization method of Francke-Grossmann (1956) (adults were left 12 h on moist filter paper and 20 h on dry filter paper). These beetles were crushed and plated on glass slides with YEME medium immediately or after the additional surface sterilization procedure with the modified White's solution (1 g of $HgCl_2$ in 1 l H_2O, exposure time 1.5 min). The slides were observed daily until mycelium of growing colonies was transferred to agar plates to obtain pure cultures. Additional cultures were obtained from fragments of the galleries held in moisture chambers and by lifting ascospore masses from the apex of perithecia necks.

The isolated cultures were incubated in the dark or in incidental light at 25°C for 14 days on 4% MEA and YEME. Fungal structures were mounted in lactophenol with cotton blue and examined with phase or differential interference contrast (Olympus BX-51). Cycloheximide tolerance was determined on MEA plates amended with 100 μg l^{-1} cycloheximide at 25°C. Growth rates were measured by placing 5 mm disks from one week old cultures on the surface of three Petri dishes of the tested media. All examined cultures were sorted to morphotypes – groups of highly similar phenotypes, based on micro- and macromorphology, growth rates and cycloheximide tolerances. Selected isolates of each morphotype were used in further molecular analyses and were lyophilized in skimmed milk and deposited to the Czech Collection of Fungi in Prague (Table 3).

External surface of collected male and female adults was separated to abdomen, thorax, head, mandibles and maxillae and examined for presence of structures facilitating transport of fungi (mycangia). Quanta 200 Scanning Electron Microscope in low vacuum mode (130 Pa) was used.

DNA was extracted from active fungal colonies using UltraClean Microbial DNA kit (MoBio Laboratories, California, USA). Amplifications of 18S rDNA were made using the PCR protocol of White et al. (1990) using the primers NS1, NS3, NS5, NS24. Amplification of the ITS region of rDNA cluster followed procedures described in Kolarik et al. (2005). Amplification of the 5′ region of the 28S rDNA gene was conducted using primers NL1 and NL4 as described by Begerow et al. (1997). The same primers were used for sequencing. The templates were purified with the aid of the Qiagen PCR Purification Kit.

BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) was used in combination with an ABI 3100 Genetic Analyser to determine the sequences. Sequences were deposited to NCBI – GenBank (accession numbers AM267260–AM267272, Table 3).

A data set containing 71 nucSSU rDNA sequences of ophiostomatoid fungi and other Ascomycota was created. Sequences were aligned using ClustalX 1.81 with the default settings (gap opening 10.00, gap extension 0.20) (Thompson et al., 1997). Phylogenetic trees were constructed using the maximum parsimony method implemented in PAUP* 4.0b10 (Swofford, 1998) and by the Bayesian method in MrBayes 3.0 (Huelsenbeck and Ronquist, 2001). A heuristic search in PAUP* included ten replicates of random taxon addition followed by TBR branch swapping was used. Bootstrapping was performed with 1000 replicates. In MrBayes, base frequencies, rates for six different types of substitutions, number of invariant sites, and shape parameter of the gamma correction for rate heterogeneity with four discrete categories were allowed to vary. One million generations of the Markov Chain Monte Carlo were run with four simultaneous chains and heating temperature 0.2. The first 40% of trees were discarded as the burn in.

3. Results

Natural history

From the elevational transect, most *S. unipunctatus* were from intermediate elevations, between 1100 and 1500 m a.s.l.; single specimens were collected at 500 m and 2000 m. Active galleries were found in 4–10 cm diameter branches or trunks of dead *Cecropia insignis* and *Cecropia angustifolia* (=*C. polyphlebia*). The conditions of the woody material varied from very moist in a shaded forest, to moderately moist with patches of dry wood at the edge of a clearing. In one instance the trunk was colonized by multiple species of ambrosia beetles.

The gallery systems of *S. unipunctatus* consist of circumferentially oriented tunnels both in the phloem layer at the surface of the sapwood, and deeper in the sapwood (Fig. 1). The tunnels in phloem engrave the wood lightly. The deeper tunnels are connected to the surface by a single vertical tunnel, and are between 3 and 20 mm deep in the wood. Both sexes of the parental generation participate in building the gallery. Surface tunnels are constructed first, and up to 3 eggs are laid in them before the deeper tunnels are excavated. Eggs are placed in distinct niches and packed in with a thin covering of fine macerated wood (as is typical of phloeophagous *Scolytodes*). We did not have the opportunity to observe larval behavior in the cambial part of the gallery. In the deeper tunnels, each larva occupied its own chamber (Fig. 1). The larval chambers are arrayed perpendicularly to the main gallery tunnel. Chambers of the first instar larvae are smaller than chambers of later instars. There are no larval tunnels in the sapwood, only the individual chambers. The wood surrounding *S. unipunctatus* tunnel systems was stained by the associated fungi (Fig. 1), as is typical of ambrosia beetle tunnels in wood.

Fungal associates

Isolations from the untreated adults and the galleries yielded similar spectra of fungi (Table 2). The dominant filamentous fungus was *Cylindrocarpon destructans*, a common plant-parasitic and saprophytic fungus, most likely not an ambrosia associate. It was identified according to Booth (1966) and according to the ITS-rDNA sequence similarity with published sequences (98%, GenBank AF220968). It has not been isolated from the surface-sterilized adults, suggesting that it is not specifically associated with *S. unipunctatus*. Two other fungi that occurred on untreated adults or in galleries were yeasts of unclear taxonomic affiliation and biological role.

The remaining four fungal morphotypes were repeatedly cultivated from most substrates, including surface-sterilized adult beetles. We assume that one or more of these fungi

Table 3. Fungal strains isolated from *S. unipunctatus* with GenBank accessions numbers of their rDNA sequences.

	Strain No.	SSU rRNA	ITS region of rRNA	D1/D2 domain of LSU rRNA
Cylindrocarpon destructans (anamorph of *Neonectria radicicola* var. *radicicola*)	CCF 3571		AM267272	
Graphium sp.	CCF 3570		AM267265	AM267263
	CCF 3566	AM267260	AM267264	
Gondwanamyces sp.1	CCF 3569	AM267268	AM267268	AM267262
Gondwanamyces sp.2	CCF 3565		AM267266	
	CCF 3568	AM267267	AM267267	AM267267
Raffaelea sp.	CCF 3572	AM267261	AM267270	AM267270
	S28		AM267271	

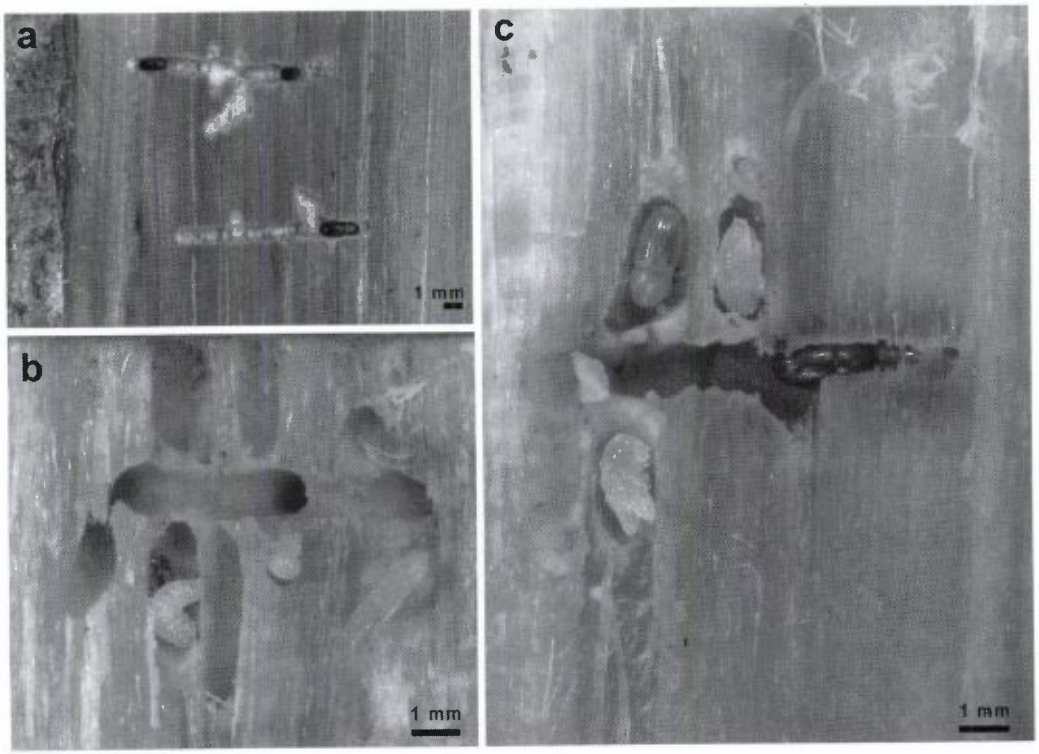

Figure 1. Structure and development of *Scolytodes unipunctatus* gallery in the trunk of *Cecropia angustifolia*: a) The initial stage of gallery building – tunnels in the inner bark engraved into the sapwood. A parental adult is seen in each gallery (one of a pair), and a vertical tunnel leading into deeper levels of the upper gallery. b) Larval cells and an egg cell adjacent to the maternal tunnel in sapwood. Note the size variation of the larval cells and its correlation with the size of the larva. c) Pupal and teneral adult cells, and two nearly-mature beetles in the maternal tunnel. Note the extensive fungal staining surrounding the galleries in each picture.

serve as the ambrosia associates. Phylogenetic analysis of 18S ribosomal RNA suggested the affinity of the four morphotypes to the ophiostomatoid genera *Gondwanamyces* (2 spp.), *Graphium*, and *Raffaelea* (Fig. 2). The phylogenetic placement is in agreement with the morphological characteristics of each genus. The cultures derived from ascospores lifted from perithecia inside the galleries were identical with one of the *Godwanamyces* morphotypes. The morphotype CCF 3572 exhibited some phenotypic features of *Raffaelea arxii* (Scott and du Toit, 1970), including the presence of pleomorphy typical for ambrosia fungi. However, none of the rDNA sequences of our strains were similar to any published sequences of ophiostomatoid fungi, in both the conservative SSU region or the more divergent ITS and LSU regions (similarity not exceeding 96% in the latter two, Table 3). The phenotypic characters of the other strains do not fit any known species of ophiostomatoid fungi. This suggests the discovery of four new distinct species. Taxonomic treatment of these fungi will be published elsewhere.

Mycangia

The beetles were examined for presence of mycangia. The only structures observed to contain fungal spores were dense shallow punctures with setae on the surface of head and prothorax (Fig. 3). No glands associated with the punctures were seen.

4. Discussion

The majority of *Scolytodes* species live in dead tissues of their host plants: phloem (inner bark), pith of twigs, or leaf petioles (Wood, 1982; Jordal, 1998a,b; Jordal and Kirkendall, 1998; Table 1). Feeding behavior in most *Scolytodes* is typical for phloeophagous or pith-feeding species – parental adults excavate linear tunnels within the host tissue, and larvae extend the gallery by individual mines. The breeding behavior of *S. unipunctatus* is radically different from other *Scolytodes*, but exhibits attributes typical for ambrosia beetles. Of the three characteristics of ambrosia symbiosis, *S. unipunctatus* and its fungal associates were observed to exhibit two; we did not test the condition no. 3, i.e., whether beetles are able to develop on fungus-free plant tissues. The other criteria fit our observations:

1) *S. unipunctatus* larvae feed almost exclusively on fungus. Were this not so, some form of larval mining would have been observed. The larval chambers are apparently being expanded as the larvae mature, since there is a correlation between the size of a larva and the volume of its chamber. However, the amount of xylem tissue removed from each chamber was negligible.

2) Four out of seven fungal morphospecies found in the galleries *S. unipunctatus* are probably non-random associates (Table 2).

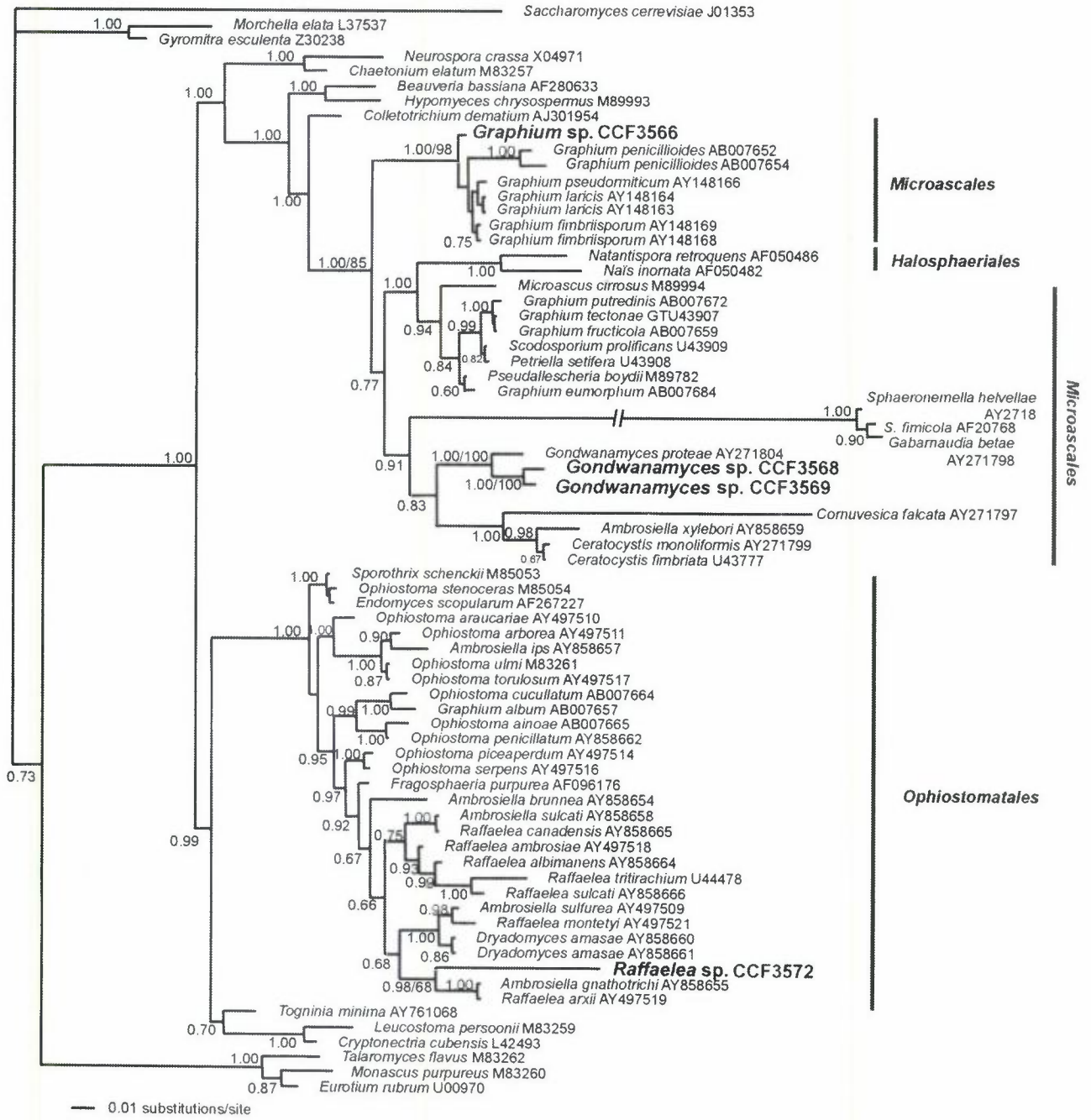

Figure 2. Phylogenetic placement of fungi associated with *S. unipunctatus*. The alignment of nucSSU-rDNA sequences contained 1692 nucleotides of which 969 were constant. The tree was constructed by Bayesian analysis with Markov chain Monte Carlo (MCMC) in MrBayes. Bayesian posterior probabilities ≥0.6 are shown at the nodes. The Maximum Parsimony analysis generated nearly the same topology. Bootstrap values are given only for the clades containing the strains from *S. unipunctatus*. The topology was rooted with *Saccharomyces cerevisiae*.

S. unipunctatus displays several other behavioral patterns characteristic for ambrosia beetles in other scolytine groups, but absent in other *Scolytodes* and most other non-ambrosia species. The tunnel system of *S. unipunctatus* resembles most closely those of some *Camptocerus*, with their combination of parallel tunnel systems in the inner bark and wood (Beaver, 1972; Wood, 1982; Kirkendall, pers. obs.). In the deeper tunnels, larvae

occupy individual cradles arrayed perpendicularly along the maternal tunnel. This arrangement is common in galleries of Platypodinae, *Camptocerus*, *Scolytoplatypus*, and the ambrosia-feeding genera in Corthylini (Beeson, 1941; Browne, 1961; Wood, 1982).

Ambrosia fungi in the galleries of many ambrosia beetle species may form a layer of mycelium lining the gallery walls, although such a layer is frequently absent in active ambrosia beetle tunnels due to the constant grazing by parents and their offspring. Such a layer was not observed in galleries of *S. unipunctatus*; however, some of the perithecia of the ophiostomatoid fungi in the galleries lacked their necks, suggesting the beetles' feeding.

No specialized mycangia were found on the exoskeleton of the beetles, but fungal spores were seen in shallow punctures scattered over the body surface (Fig. 3). Nonspecialized punctures serving as means for fungal transfer are classified as nonglandular pit mycangia (Six, 2003). Some species of bark beetles are known to efficiently transmit fungal associates without possessing specialized organs (Beaver, 1986) but few true ambrosia beetles lack mycangia.

Of the fungal genera associated with *S. unipunctatus*, only *Raffaelea* is commonly found in an ambrosia symbiosis. *Graphium* has been recorded as a rare associate of two scolytine species (Baker and Noris, 1968; Funk, 1970), and the association of the genus *Gondwanamyces* with ambrosia beetles is reported here for the first time. It is not yet clear what role these fungi play in nutrition of *S. unipunctatus*. The genus *Gondwanamyces* currently comprises two known species, both isolated only from decaying infructescences of *Protea* spp. in South Africa (Wingfield et al., 1988; Wingfield and van Wyk, 1993). Our finding suggests that this genus has a much broader ecological spectrum and geographical distribution than previously known.

A remarkable number of *Scolytodes* species are associated with *Cecropia* trees (Jordal, 1998b). The majority of these species feed on phloem of twigs and branches, but numerous species utilize the large woody petioles of fallen leaves (Table 1). According to Jordal (1998b), colonization of *Cecropia* by *Scolytodes* has evolved repeatedly, and the association is stable within several monophyletic groups. Despite numerous collections of ambrosia beetles from both Zurquí de Moravia and Finca Murillo, *S. unipunctatus* has only been found in trunks and larger branches of *Cecropia*. This is surprising, since most ambrosia beetles are host generalists (Atkinson and Equihua, 1986; Beaver, 1979; Browne, 1958, 1961; Hulcr, 2007; Kirkendall, 2006; Noguera-Martinez and Atkinson, 1990). While the restricted host range could reflects physiological constraints on the beetles or fungi, it is more likely due to retention of ancestral host recognition mechanisms.

Only a few groups of Scolytinae are capable of true xylophagy (Browne, 1961; Wood, 1982, 1986; Kirkendall, 1983). The ability to extract sufficient amounts of nutrients (in particular, nitrogen) from dead xylem by scolytid beetles unaided by symbionts is probably difficult, as sapwood is one of the most nutrient-poor tissues in trees. Several scolytine groups have evolved the ability to utilize xylem, such as the tribe Micracini (Kirkendall, 1984) or a congener of *S. unipunctatus, Scolytodes multistriatus* (Wood, 1982; the possibility of a presence of internal symbionts have not been studied in these species). However, most scolytines utilize either relatively nitrogen-rich tissues such as phloem (inner bark), or symbiotic fungi (Ayres et al., 2000). Since woody tissue with ambrosia fungus is much richer in nitrogen and other nutrients than is the surrounding wood (French and Roeper, 1975), evolving the ability to utilize it is presumably extremely beneficial for a scolytine species.

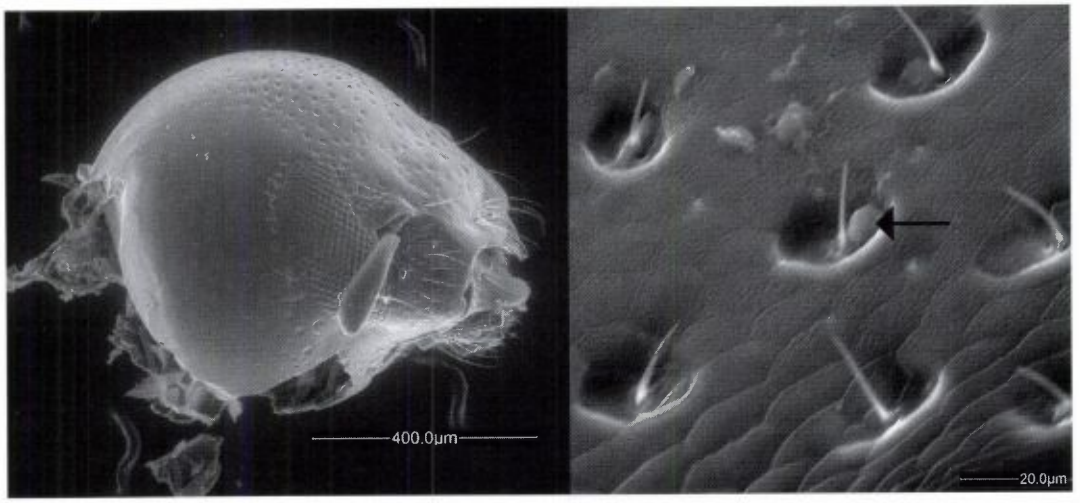

Figure 3. Punctures with setae on the head of *Scolytodes unipunctatus* classified as nonglandular pit mycangia. Arrow points to a fungal spore carried in the puncture.

In *S. unipunctatus*, the apparent lack of specialized means of transporting fungal spores, the host specificity, retention of cambial tunnels, and the lack of related species with similar breeding behavior, all argue for a very recent origin of this ambrosial symbiosis in *Scolytodes*.

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