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A mid-Cretaceous ambrosia fungus, *Paleoambrosia entomophila* gen. nov. et sp. nov. (*Ascomycota*: *Ophiostomatales*) in Burmese (Myanmar) amber, and evidence for a femoral mycangium

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A B S T R A C T

An ambrosia fungus is described from filamentous sporodochia adjacent to a wood–boring ambrosia beetle (*Coleoptera*: *Curculionidae*: *Platypodinae*) in mid-Cretaceous Burmese amber. Yeast-like propagules and hyphal fragments of *Paleoambrosia entomophila* gen. nov. et sp. nov. occur in glandular sac mycangia located inside the femur of the beetle. This is the first record of a fossil ambrosia fungus, showing that symbiotic associations between wood–boring insects and ectosymbiotic fungi date back some 100 million years ago. The present finding moves the origin of fungus-growing by insects from the Oligocene to the mid-Cretaceous and suggests a Gondwanan origin.

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1. Introduction

Ambrosia beetles (*Coleoptera*: *Curculionidae*: *Platypodinae*) are well known for co-habiting with symbiotic fungi in their galleries in dead and dying trees. These ambrosia fungi produce clusters of conidia and conidiophores (sporodochia) that nourish the beetle larvae and adults. Spores and hyphal fragments of these ectosymbiotic fungi are transported from tree to tree on the insect surface or in special pits, cavities, pouches (mycangia) of the adult beetles (Batra, 1967; Beaver, 1989; Harrington et al. 2010).

The present study describes a new genus of fossil ambrosia fungi based on conidiophores and conidia produced in superficial sporodochia adjacent to an ambrosia beetle in mid-Cretaceous Burmese (Myanmar) amber. Also characterized are glandular sac mycangia containing hyphal fragments and yeast-like propagules in the femur of the beetle. Based on the damaged elytra of the ambrosia beetle and associated wood fragments near the beetle (Fig. 1), we propose that a predator removed the beetle from its gallery near the surface of the host tree, along with some wood fibers. Associated with the ambrosia beetle was a symbiotic fungus and some of its sporodochia remained appressed to the insect. Thus, the fossil provides us with an ambrosia beetle, an associated ambrosia fungus with mycelium, conidiophores and conidia, mycangia with fungal stages, and wood fibers of the host tree.

This is the first fossil record of an ambrosia beetle associated with its symbiotic fungus (Taylor et al. 2015) as well as the first report of a mycangium in the femur of any ambrosia beetle.

2. Materials and methods

The specimen originated from the Noije Bum 2001 Summit Site mine excavated in the Hukawng Valley in 2001 and located southwest of Maingkhwan in Kachin State (26°20′N, 96°36′E) in Myanmar. Based on paleontological evidence this site was dated to the late Albian of the Early Cretaceous (Cruickshank and Ko, 2003), placing the age at 97–110 million years ago (Ma). A U–Pb zircon study determined the age to be 98.8 ± 0.6 Ma or at the Albian/Cenomanian boundary (Shi et al. 2012). Nuclear magnetic

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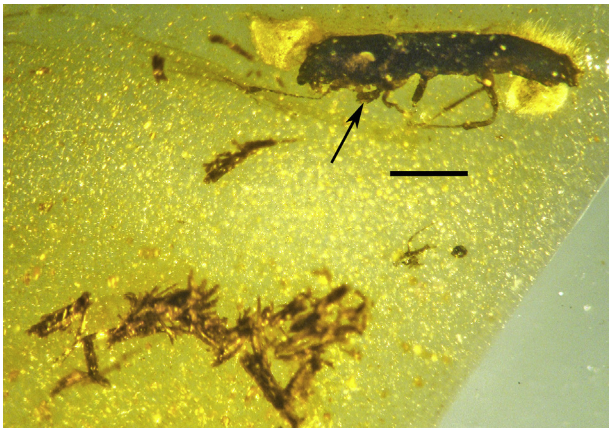

Fig. 1. Entire beetle specimen in Burmese amber. Note associated wood fragments in bottom left portion of figure. Ambrosia fungus can be seen adjacent to the anterior end of the abdomen. Arrow points at femur containing mycangium. Scale = 0.9 mm.

resonance (NMR) spectra and the presence of araucaroid wood fibers in amber samples from the Noije Bum 2001 Summit Site indicate an araucarian tree source for the amber (Poinar et al. 2007).

Observations and photographs were made with a Nikon SMZ-10 R stereoscopic microscope (Nikon Instruments, Tokyo, Japan) and Nikon Optiphot compound microscope (Nikon Instruments, Tokyo, Japan) with magnifications up to 800 X.

3. Results

The Burmese amber fossil is represented by mycelium, conidiophores and conidia in sporodochia adjacent to the beetle (Figs. 1–5), and yeast-like propagules and hyphal fragments in the femoral mycangium of the beetle (Figs. 6 and 7).

Taxonomy

Phylum *Ascomycota* (Berk.) Cavalier-Smith (1998)
Class *Sordariomycetes*
Order *Ophiostomatales* Benny & Kimbrough (1980)
Family *Ophiostomataceae* Nannfeldt (1932)
Paleoambrosia Poinar and Vega **gen. nov.**
Diagnosis: as for type species (monotypic)

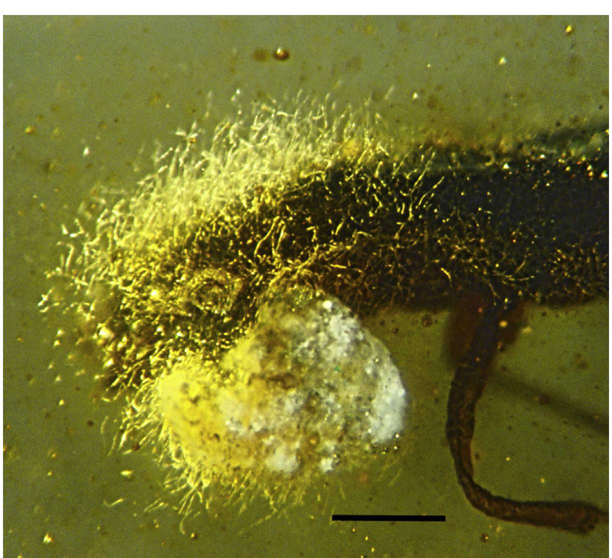

Fig. 2. Anterior end of adult platypodine beetle in Burmese amber with sporodochia of *Paleoambrosia entomophila* gen. nov. et sp. nov. Bar = 380 μm.

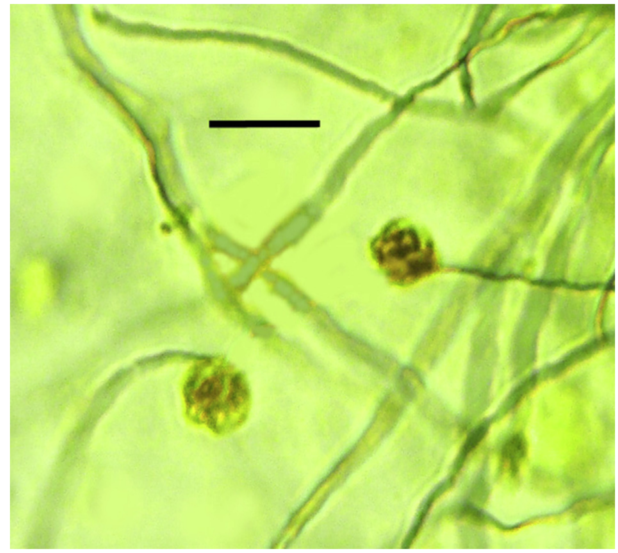

Fig. 3. Conidiophores with apically borne conidia of *Paleoambrosia entomophila* gen. nov. et sp. nov. in Burmese amber. Bar = 12 μm.

Fig. 4. Terminal cluster of four remaining conidia at the tip of a conidiophore of *Paleoambrosia entomophila* gen. nov. et sp. nov. in Burmese amber. Bar = 9 μm. Insert shows conidia in a mucilage head. Bar = 11 μm.

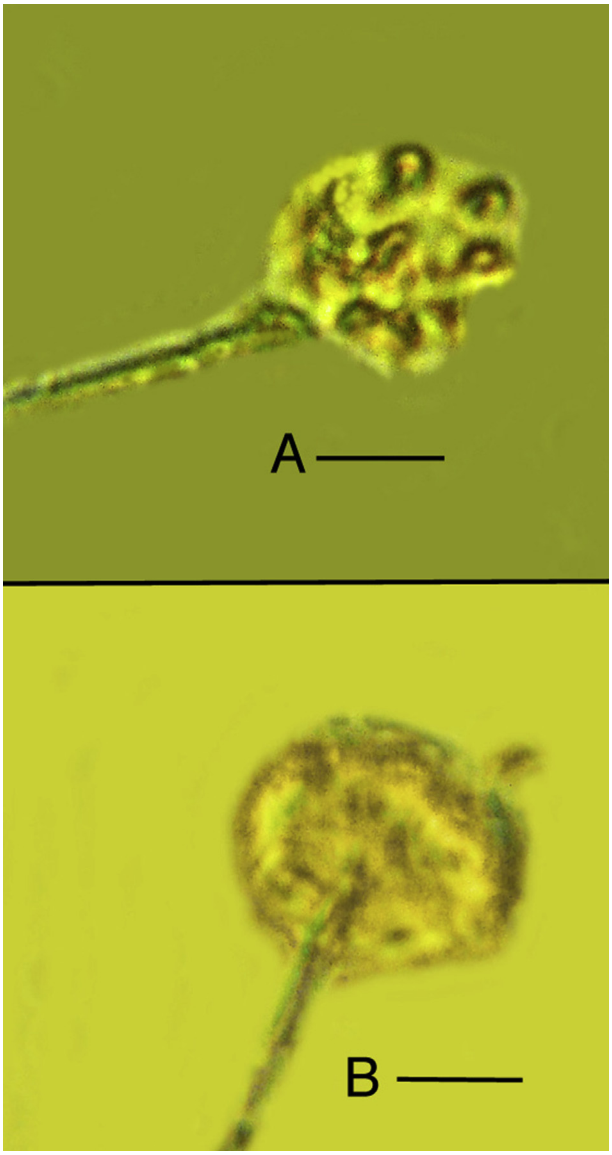

Fig. 5. Spore clusters of *Paleoambrosia entomophila* gen. nov. et sp. nov. in Burmese amber. (A) The mucilage deposit is obvious in this spore head. Bar = 5 μm. (B) A conidium in the process of leaving the spore head. Bar = 5 μm.

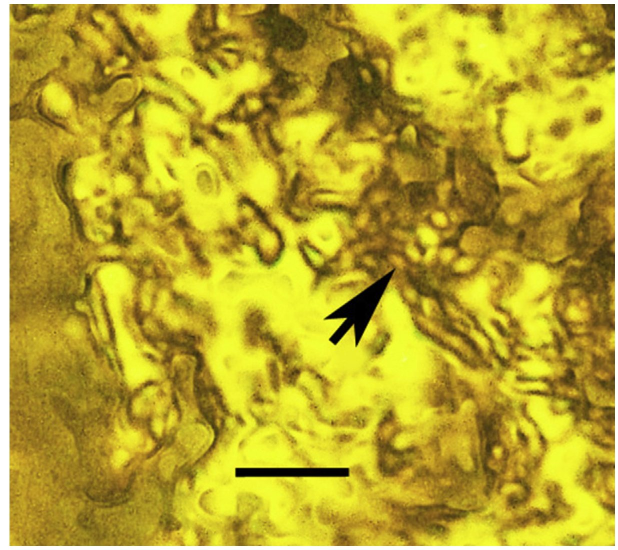

Fig. 6. Detail of a portion of the femoral mycangium showing hyphal fragments and yeast-like propagules (arrow) of *Paleoambrosia entomophila* gen. nov. et sp. nov. in Burmese amber. Bar = 7 μm.

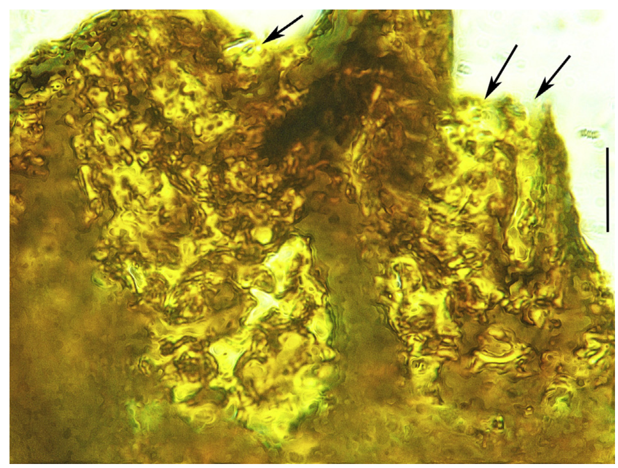

Fig. 7. Femoral mycangium of *Paleoambrosia entomophila* gen. nov. et sp. nov. in Burmese amber. Note two large cavities separated by a central column of glandular tissue. Arrows show openings to the exterior. Bar = 18 μm.

Type species: ***Paleoambrosia entomophila*** Poinar and Vega **sp. nov.** (Figs. 1–7).

Etymology: Generic name from the Greek "palaios" = ancient and the Greek "ambrosia" = immortal. Specific epithet from the Greek "entomo" = insect and the Greek "philia" = love.

Description: Mycelium septate, hyaline; conidiophores unbranched, variable in length, one-celled, fuscous, 2.1–2.4 μm in width, bearing terminal spore clusters; conidia non-septate, straight, smooth, fuscous, globose to obovoid, length 2.7–3.5 μm, width 2.1–2.7 μm, borne in apical yellowish slime droplets. Yeast-like propagules, length 2.5–4.2 μm, and hyphal fragments present in glandular sac mycangia extending 118 μm in length and located in the femur.

Diagnosis: General features of *P. entomophila* gen. nov. et sp. nov., such as the shape and character of the conidiophores, conidia and spore heads, align the fossil with members of the family *Ophiostomataceae*. Within this family are several genera of ambrosia fungi, with *Raffaelea* spp. having many features (conidia shape, size, mucilage heads) of the fossil fungus. However, the small size of the conidia of the fossil differentiate it from extant members of the genus (Batra, 1967; Kubono and Ito, 2002; Harrington et al. 2010; Simmons et al. 2016). The fuscous hue of the conidiophores and conidia and yellowish mucilage surrounding the conidial heads (Figs. 2–5), as well as the age of the fossil and its association with an extinct genus of platypodine beetles, distinguishes it from all extant members of the *Ophiostomataceae*.

Holotype: Holotype No. B–F-7 deposited in the Poinar Amber Collection maintained at Oregon State University, Corvallis, OR, USA.

Type locality: Myanmar (Burma), state of Kachin, Noije bum 2001 Summit Site amber mine in the Hukawng Valley, SW of Maingkhwan (26°20′N, 96°36′E).

Associated beetle: Coleoptera: *Curculionidae*: *Platypodinae*.

4. Discussion

Fungus-growing has been reported in three insect orders: *Coleoptera* (*Scolytinae* and *Platypodinae*), Hymenoptera (*Attini*, or leaf-cutter ants), and *Blattodea* (*Macrotermitinae* termites).

Platypodinae originated in the mid-Cretaceous (119–88 Ma) and are the oldest group of fungus-growing insects (Jordal, 2015). The first platypodine fungus-growing tribe, the *Tesserocerini*, arose 88.5–103.4 Ma (Jordal, 2015). *Ophiostomatales* originated ca. 101 Ma, with fungus-growing in the *Tesserocerini* originating ca. 86 Ma (Vanderpool et al. 2017). Fungus-growing in *Attini* occurred 61–57 Ma (Branstetter et al. 2017), followed by termites (39.5–25.8 Ma; Roberts et al. 2016).

Until now, the oldest evidence of fungus-growing behavior by an insect was based on ca. 25 Ma fossilized termite nests in Tanzania (Roberts et al. 2016). Referring to ambrosia beetles, Roberts et al. (2016) stated: “Unfortunately, no fossil evidence, either in the form of fungal gardens or unequivocal ambrosia beetle borings, exists to validate molecular age estimates for ambrosia beetles or to provide direct geographic evidence on where this symbiosis originated.” The present finding moves the origin of fungus-growing by insects from the Oligocene (Roberts et al. 2016) to the mid-Cretaceous based on the age of Burmese amber specimens (110–97 Ma; see above) and suggests a Gondwanan origin (Poinar, 2018).

Glandular sac mycangia (pocket like expansions in various body parts) are often found in extant ambrosia beetles that bear symbiotic fungi (Harrington, 2005). Such mycangia play an important role not only in fungal transmission but also in fungal survival since even if the conidia are covered with mucilage, they can still be vulnerable to desiccation. The mucilage holds the spore masses together, protects the conidia from desiccation, aids adhesion of conidia to the exoskeleton of the beetle and keeps the spores from being digested in the beetle's gut (Beaver, 1989). These mycangia can occur on various body parts, including the head, thorax and legs. They can be tube-like or shaped like pits and cavities. They often contain a lining of glandular cells that provide nourishment for the enclosed fungal stages. Femoral mycangia with *P. entomophila* gen. nov. et sp. nov. are lined with glandular cells (Fig. 7) that probably provided nourishment to the developing yeast-like propagules and hyphal fragments (Fig. 6). It is assumed that the contents of the mycangia are stages of *P. entomophila* gen. nov. et sp. nov., however, it is known that more than one ambrosia species can be transported in the mycangia of ambrosia beetles (Hulcr and Stelinski, 2017). All mycangia found in the legs of extant beetles are restricted to the coxae (Beaver, 1989). Our findings are not only the first evidence of a mycangium in an amber-preserved insect, but also the first evidence for a mycangium in the femur of an insect.

5. Conclusions

The Myanmar amber *P. entomophila* gen. nov. et sp. nov. represents the only known fossil ambrosia fungus, showing that these ectosymbiotic fungi, together with their Platypodinae associates, were well established some 100 Ma. The present fossil broadens our knowledge of the diversity of fossil fungi and establishes a date for the evolution for the *Ophiostomataceae*.

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