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A mid-Cretaceous trichomycete, *Priscadvena corymbosa* gen. et sp. nov., in Burmese amber

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ABSTRACT

Priscadvena corymbosa gen. et sp. nov., is described from thalli and sporangia emerging from the oral cavity of a click beetle (Coleoptera: Elateridae) in mid-Cretaceous Burmese amber. The fossil contains several features unknown in extant Trichomycetes including a click beetle (Coleoptera: Elateridae) host, spiny, aerial thalli with the entire thallus bearing numerous small uninucleate globular spores and stalks attached to the oral cavity of its host. Based on these features, *P. corymbosa* gen. et sp. nov. is placed in a new family, Priscadvenaceae fam. nov., and new order, Priscadvenales ord. nov. The new morphological and behavioral features of the fossil add to the diversity of the trichomycetes as currently defined.

1. Introduction

Fungi previously placed in four orders (Amoebidiales, Asellariales, Eccrinales, Harpellales) of the Trichomycetes (Zygomycota) constituted a group based on their ecology, their occurrence in the gut of arthropods, including aquatic insect larvae, mature insects, and marine water crustaceans. These organisms are mostly regarded as commensals, except for *Smittium morbosum*, which is pathogenic to mosquitoes (Sweeney, 1981).

Molecular analyses have shown that the Class Trichomycetes, previously placed in Phylum Zygomycota, is not monophyletic. Phylogenetic studies have resulted in the breakup not only of the Trichomycetes but also the Zygomycota (Spatafora et al., 2016, 2017). The Harpellales and Asellariales, which produce zygospores, are now in the fungal subdivision Kickxellomycotina (Zoopagomycota), while Amoebidiales and Eccrinales are excluded from Fungi and placed in the protist class Mesomycetozoea (Cafaro, 2005).

The group (trichomycetes) includes several lineages of microscopic fungi, most of which are associated with the digestive tract of various arthropods, including insects (Spatafora et al., 2017). They are attached by a holdfast to the gut lining and obtain nourishment from digested food in the alimentary track of the host. All stages normally occur in the gut lumen or rectum of the host (Lichtwardt, 1986; Lichtwardt et al., 2016). Only a few trichomycete fossils have been studied in detail and investigators have had difficulty understanding their structure and development. Arthropod hosts occur in both aquatic and terrestrial habitats and spores are released into the environment via infected individuals. Among those in the former habitat are aquatic larvae of flies (Diptera), mayflies (Ephemeroptera) and stoneflies (Plecoptera). Terrestrial insects are less common as hosts and include springtails (Collembola) and beetles (Coleoptera). Most trichomycetes appear to have a narrow host range and occur only on specific genera or species of arthropods (Lichtwardt, 1986; Benny, 2012; Lichtwardt et al., 2016).

Here we discuss the occurrence of what we interpret to be an unusual trichomycete that emerges from the oral cavity of a click beetle (Coleoptera: Elateridae). The specimen is from the Early Cretaceous, ca. 97–110 Mya, making it the oldest known trichomycete fossil.

2. Materials and methods

The holotype fossil specimen described below was obtained in 2018 from the Noije Bum Summit Site mine in the Hukawng Valley

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located southwest of Maingkhwan in Kachin State (26°20′N, 96°36′E) in Myanmar. In this and adjacent mines, the amber is dug out of rock and sediment layers by hand, washed and then examined for biological inclusions. Once a fossil in the amber has been selected for study, common lapidary techniques are used to prepare the amber for microscopic observation. These include reshaping the amber piece containing the fossil with a diamond trim saw followed by a belt sander. Final polishing for microscopic examination is carried out on a buffing wheel. The original environment in which the amber was deposited is believed to have been a lush, tropical to subtropical rain forest.

Paleontological evidence dated the amber site to the late Albian of the Early Cretaceous (Cruickshank and Ko, 2003), ca. 97–110 Mya. Shi et al. (2012) used U-Pb zircon dating and determined the age to be 98.79 ± 0.62 Ma or at the Albian/Cenomanian boundary. Nuclear magnetic resonance (NMR) spectra combined with the presence of araucaroid wood fibers in amber specimens from the Noije Bum Summit Site indicate an araucarian tree source for the amber (Poinar et al., 2007).

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

3. Results

The fossil is represented by a branched cluster of aerial thalli and sporangia arising from the oral cavity of an adult beetle (Coleoptera: Elateridae). The host beetle is complete except for the loss of the tip of the abdomen and the antennae. Placement of the beetle in the family Elateridae is based on the following characteristics: body elongate, narrow, nearly parallel-sided, labrum visible, antennal sockets located under the frontal margin near the eyes, thorax loosely connected to abdomen, hind angles of pronotum extending backward (White, 1983).

Taxonomy

Kindgom *Fungi*

Phylum *Zoopagomycota*

Subphylum *Kickxellomycotina*

Order *Priscadvenales* ord. nov.

Diagnosis: as for type species (monotypic)

MycoBank: MB829255

Family *Priscadvenaceae* fam. nov.

Diagnosis: as for type species (monotypic)

MycoBank: MB829256

Priscadvena Poinar and Vega gen. nov.

Diagnosis: as for type species (monotypic)

MycoBank: MB829257

Type species: ***Priscadvena corymbosa*** Poinar and Vega **sp. nov.** (Figs. 1–6).

Etymology: Generic name from the Latin “priscus” = ancient and the Latin “advena” = stranger. Specific epithet from the Latin “*corymbosa*” = clustered in relation to the bouquet of thalli and sporangia.

MycoBank: MB829258

Description: Thalli and sporangia long, slender, branched (Figs. 1 and 3), acute-tipped, septate (Fig. 4), bearing spines on outer surface. Stalks attached to the oral cavity of click beetle vary in width from 7 to 18 μm (Fig. 2). Thalli and sporangia 280–570 μm in length. Sporangiospores (Figs. 3,5 and 6), more or less circular in outline, 9–11 μm in diameter.

Diagnosis: Branched, aerial, septate, spiny, thalli with the entire thallus functioning as a sporangium producing small uninucleate spores; symbiont of click beetles (Coleoptera: Elateridae).

Fig. 1. Dorsal view showing branched thalli and sporangia of *Priscadvena corymbosa* gen. et sp. nov. attached to the oral cavity of an elaterid beetle in Burmese amber. Bar = 1 mm.

Holotype: Holotype No. B-F-9 deposited in the Poinar amber collection maintained at Oregon State University.

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

Associated beetle: Coleoptera: Elateridae

Comments: P. corymbosa gen. et sp. nov. is characterized by branched, aerial, septate, spiny thalli with the entire thallus functioning as a sporangium that produces numerous small uninucleate spores. The stalks are attached to the oral cavity of a click beetle (Elateridae). This unique combination of characters does not occur with any currently known genus, family or order of extant Trichomycetes (Lichtwardt, 1986; Misra and Lichtwardt, 2001; Lichtwardt et al., 2016), which justifies placing the fossil in a new family, the Priscadvenaceae fam. nov. and new order, Priscadvenales ord. nov.

4. Discussion

The trichomycetes evolved a very successful life style that has enabled them to survive for millions of years (Lichtwardt, 2012; Lichtwardt et al., 2016). By restricting their development to the alimentary tract of the arthropod host, they cause little or no physical damage, which is beneficial for both commensal and host.

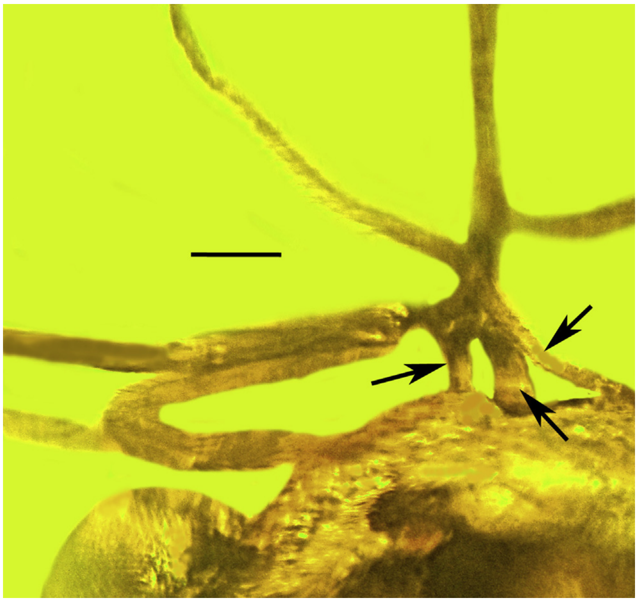

Fig. 2. Ventral view showing stalks (arrows) of *Priscadvena corymbosa* gen. et sp. nov. attached to the oral cavity of an elaterid beetle host in Burmese amber. Bar = 50 μm.

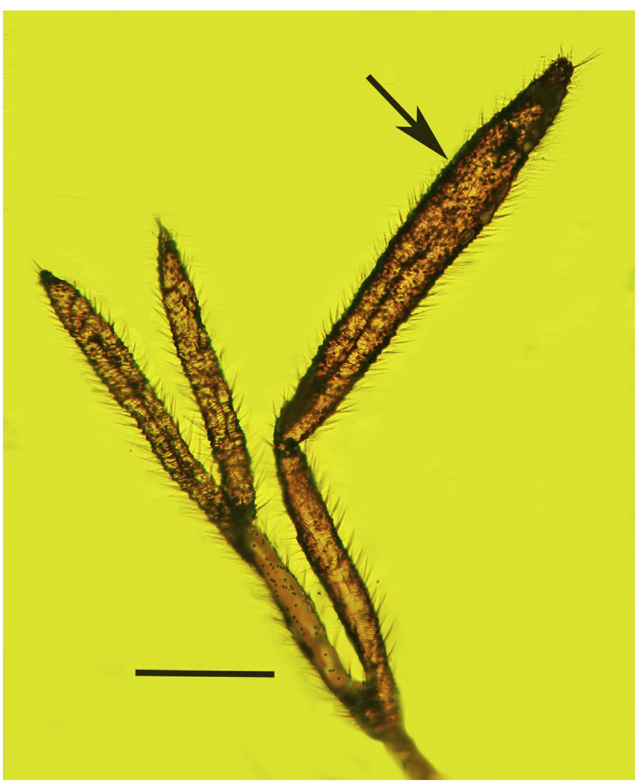

Fig. 3. Terminal branched thalli and sporangium (arrow) of *Priscadvena corymbosa* gen. et sp. nov. in Burmese amber. Bar = 160 μm.

Fig. 4. Developing segmented thallus of *Priscadvena corymbosa* gen. et sp. nov. in Burmese amber. Bar = 113 μm.

They also have a constant supply of nutrients and do not encounter host defense systems, such as encapsulation or melanization, since they normally do not enter the host's hemocoel. Dissemination of propagules is via the alimentary tract except in special cases when the vegetative stages become aerial as with the present fossil (Lichtwardt, 2012; Lichtwardt et al., 2016).

Fossil trichomycetes are very rare (Taylor et al., 2015). One possible specimen was described from the Triassic of Antarctica (White and Taylor, 1989); however, it was contested since it contained certain features absent in extant lineages and there was no definite association with an arthropod (Cafaro, 2005).

It is not known if *P. corymbosa* gen. et sp. nov. established itself in the hindgut, which is typical of trichomycetes, or in the foregut, since it was attached to the oral cavity. Beetles are rare hosts of trichomycetes and there is no previous report of an Elateridae serving as a host to these fungi (Lichtwardt, 1986; Misra and Lichtwardt, 2001; Lichtwardt et al., 2016).

The spines on the sporangia, which is an autapomorphy of *P. corymbosa* gen. et sp. nov., are curious. However, if this lineage consistently produced aerial sporangia, then the spines could be associated with dispersal. They could attach the sporangia to the feathers or pelage of small avian or terrestrial vertebrates or even to the bodies of invertebrates that were attracted to the host bodies. A variety of spore dispersal methods have been reported for zygomycetes, including sterile spines on the sporulating heads of members of the Mucorales, which Benny (2012) suggests could be adaptations for animal dispersal. To our knowledge, no extant trichomycete possesses such spines.

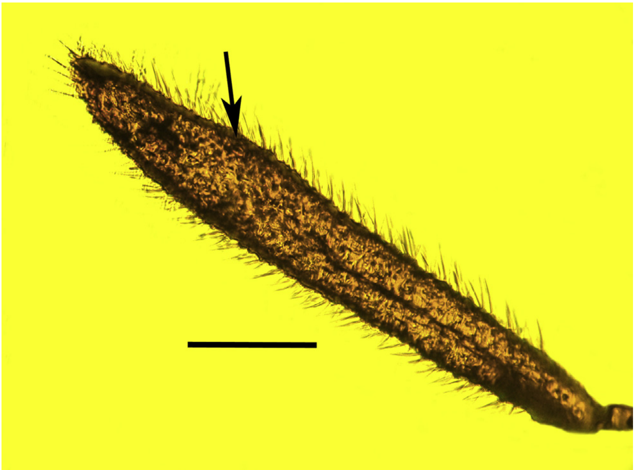

Fig. 5. Sporangium of *Priscadvena corymbosa* gen. et sp. nov. in Burmese amber filled with developing spores, especially noticeable along upper margin (arrow). Bar = 110 μm.

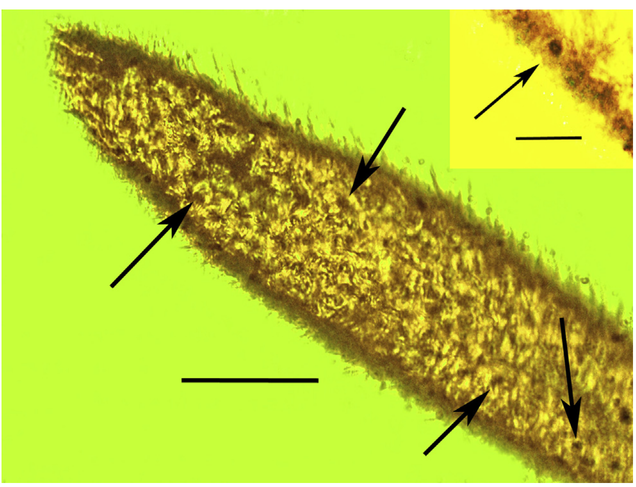

Fig. 6. Terminal portion of a sporangium of *Priscadvena corymbosa* gen. et sp. nov. in Burmese amber filled with developing spores (arrows show spore nuclei). Bar = 40 μm. Insert shows edge of sporangium with a developing spore (arrow). Bar = 20 μm.

New, unique fungi from Burmese amber insects may be more common than presumed. In addition to the present fossil, a new ectoparasitic fungus (*Speciophila adercia*) in a new family (Spheciophilidae) but unknown order and class, was described from the body of a wingless adult ceraphronid wasp (Hymenoptera: Ceraphronoidea) that itself was placed in an extinct family (Poinar, 2016).

5. Conclusions

The Burmese amber fossil, *P. corymbosa* gen. et sp. nov., represents an extinct lineage and provides new characters (spiny, aerial thalli and sporangia; sporangia containing numerous uninucleate, globular spores; click beetle host) not previously known in extant members of this group. The fossil shows that such features, which add to the diversity of the Trichomycetes, were well established by the mid-Cretaceous.

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References

Benny, G.L., 2012. Current systematics of Zygomycota with a brief review of their biology. In: Misra, J.K., Tiwari, J.P., Deshmukh, S.K. (Eds.), Systematics and Evolution of Fungi. Science Publishers, Enfield, pp. 55–105.

Cafaro, M.J., 2005. Eccrinales (Trichomycetes) are not fungi but a clade of protists at early divergence of animals and fungi. Mol. Phylogenet. Evol. 35, 21–34.

Cruickshank, D., Ko, K., 2003. Geology of an amber locality in the Hukawng Valley, northern Myanmar. J. Asian Earth Sci. 21, 441–455.

Lichtwardt, R.W., 1986. The Trichomycetes: Fungal Associates of Arthropods. Springer-Verlag, New York, p. 343.

Lichtwardt, R.W., 2012. Evolution of trichomycetes. In: Misra, J.K., Tewari, J.P., Deshmukh, S.K. (Eds.), Systematics and Evolution of Fungi. Science Publishers, Enfield, pp. 107–114.

Lichtwardt, R.W., Cafaro, M.J., White, M.M., 2016. The Trichomycetes. Fungal Associates of Arthropods. Revised edition of Lichtwardt (1986). Available online: https://keyserver.lucidcentral.org/key-server/data/0b08020c-0f0c-4908-8807-030c020a0002/media/Html/monograph/text/mono.htm.

Misra, J.K., Lichtwardt, R.W., 2001. Illustrated Genera of Trichomycetes: Fungal Symbionts of Insects and Other Arthropods. Science Publishers, Inc., Enfield, p. 155.

Poinar Jr., G., 2016. A mid-Cretaceous ectoparasitic fungus, *Spheciophila adercia* gen. et sp nov. attached to a wasp in Myanmar amber. Fungal Genom. Biol. 6, 145.

Poinar Jr., G.O., Lambert, G.J.B., Wu, Y., 2007. Araucarian source of fossiliferous Burmese amber: spectroscopic and anatomical evidence. J. Bot. Res. Inst. Tex. 1, 449–455.

Shi, G., Grimaldi, D.A., Harlow, G.E., Wang, J., Wang, J., Yang, M., Lei, W., Li, Q., Li, X., 2012. Age constraint on Burmese amber based on U-Pb dating of zircons. Cretac. Res. 37, 155–163.

Spatafora, J.W., Chang, Y., Benny, G.L., Lazarus, K., Smith, M.E., Berbee, M.L., Bonito, G., Corradi, N., Grigoriev, I., Gryganskyi, A., James, T.Y., O'Donnell, K., Robertson, R.W., Taylor, T.N., Uehling, J., Vilgalys, R., White, M.W., Stajich, J.E., 2016. Phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. Mycologia 108, 1029–1046.

Spatafora, J.W., Aime, M.C., Grigoriev, I.V., Martin, F., Stajich, J.E., Blackwell, M., 2017. The fungal tree of life: from molecular systematics to genome-scale phylogenies. Microbiol. Spectr. 5 (5). FUNK-0053-2016.

Sweeney, A.W., 1981. An undescribed species of *Smittium* (Trichomycetes) pathogenic to mosquito larvae in Australia. Trans. Br. Mycol. Soc. 77, 55–60.

Taylor, T.N., Krings, M., Taylor, E.L., 2015. Fossil Fungi. Elsevier, Amsterdam, p. 382.

White Jr., J.F., Taylor, T.N., 1989. A trichomycete-like fossil from the Triassic of Antarctica. Mycologia 81, 643–646.

White, R.E., 1983. A Field Guide to the Beetles of North America. Houghton Mifflin Company, Boston, p. 368.