

One hundred million year old ergot: psychotropic compounds in the Cretaceous?

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Abstract

A fungal sclerotium similar to those of the genus *Claviceps*, commonly known as ergot, was discovered infecting a grass floret in Early–mid Cretaceous Myanmar amber. The fungus, described as *Palaeoclaviceps parasiticus* gen. n., sp. n. is characterized by an erect black sclerotium with flattened hyphal outgrowths and adjacent conidia consistent in morphology with those of extant species of *Claviceps*. The lobed hyphal outgrowths on the distal end of the sclerotium separate the fossil from all extant species of *Claviceps* as well as other sclerotium-producing fungi in the Clavicipitaceae. The fossil shows that the original hosts of the Clavicipitaceae could have been grasses and establishes the presence of the Clavicipitaceae and Poaceae in the Early–mid Cretaceous.

Key words: *Claviceps*, Early–mid Cretaceous grass, Ergot fossil, sclerotium, *Palaeoclaviceps parasiticus*, Myanmar amber.

1. Introduction

Few fungi have had a greater historical impact on society than ergot, the common name given to the sclerotia of fungi belonging to the genus *Claviceps* (BOVE 1970). The most common and well known of the *Claviceps* species is *C. purpurea*, which parasitizes the unfertilized ovaries of rye, wheat and many other grasses, producing a dense tissue mass, the sclerotium, with a black to purple-black rind that often protrudes beyond the tip of the infected spikelet (BOVE 1970; LEWIS & ELVIN-LEWIS 1977). This parasitic association was described in detail by LUTTRELL (1980).

Ergot sclerotia contain hundreds of different compounds, some of which have been used to treat various human conditions, especially in relation to childbirth, migraines and psychiatric disturbances. Some ergot alkaloids however are quite poisonous and during periods of famine in the middle ages when the only bread available was made with ergot-infected grain (black grain bread), the heat-stable alkaloids caused a disease known as ergotism, resulting in delirium, convulsions, severe pain, gangrenous limbs and death (BOVE 1970). Some 40,000 people died during an ergot epidemic in France in 994 AD and thousands died throughout the Middle Ages (LEWIS & ELVIN-LEWIS 1977). Human poisonings from ergot still occur (KING 1979), but the primary victims today are farm animals such as cattle, horses, pigs and poultry that eat the infested grains (KADA 2005). The most notorious compound isolated from ergot is lysergic acid diethylamide (LSD), which is used as a recreational hallucinatory drug as well as a medicinal psychiatric drug (BOVE 1970; TAYLOR 1965). Ergot remains problematic in cereal and grass seed production as well as in pastures and grazing lands.

The present study describes the first fossil ergot fungus infecting an Early–mid Cretaceous grass in amber from Myanmar (Burma) and discusses its significance in deciphering the evolution of the Clavicipitaceae.

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2. Material and methods

The ergot infected grass floret is embedded in a rectangular piece of amber 12 mm long, 5 mm wide and 4 mm deep. The specimen originated from the Noiye Bum 2001 Summit Site in the Hukawng Valley, southwest of Maingkhwan in the state of Kachin, Myanmar (26°20'N, 96°36'E). Amber-bearing strata in this mine were assigned to the Upper Albian (97–110 Ma) of the Early Cretaceous on the basis of paleontological (ammonite) evidence (CRUICKSHANK & KO 2003). Nuclear magnetic resonance (NMR) spectra and the presence of araucaroid wood fibers in amber samples from the Noiye Bum 2001 Summit site indicate an araucarian tree source for the amber (POINAR et al. 2007). The Myanmar amber mines are on the Burma Plate (MITCHELL 1993), which is part of Laurasia, thus all inclusions are of Old World origin. Based on lithologic indicators, the habitat of the amber bearing region when the resin-producing trees were living some 100 million years ago was a tropical-subtropical forested area (BOUCOT et al. 2013).

Observations and photographs were made with a Nikon SMZ-10 R stereoscopic microscope and Nikon

Optiphot compound microscope with magnifications up to 600X. Helicon Focus Pro X64 was used to stack photos for better clarity and depth of field.

3. Description

Order Hypocreales

Family Clavicipitaceae

Genus *Palaeoclaviceps* nov.

Type species: *Palaeoclaviceps parasiticus* gen. n., sp. n. POINAR, ALDERMAN & WUNDERLICH.

Diagnosis: As for the type species (monotypic).

Palaeoclaviceps parasiticus g. n., sp. nov.

Figs. 1–6

Mycobank no.: MB810772.

Etymology: The generic name is taken from the Greek “paleo” = ancient and *Claviceps*, an extant genus of ergot fungi.



Fig. 1. Grass spikelet with attached sclerotium (arrow) of *Palaeoclaviceps parasiticus* gen. n., sp. n., holotype, F26481BUIC (ex coll. WUNDERLICH) in Early–mid Cretaceous amber from Myanmar. Bar = 1.6 mm.

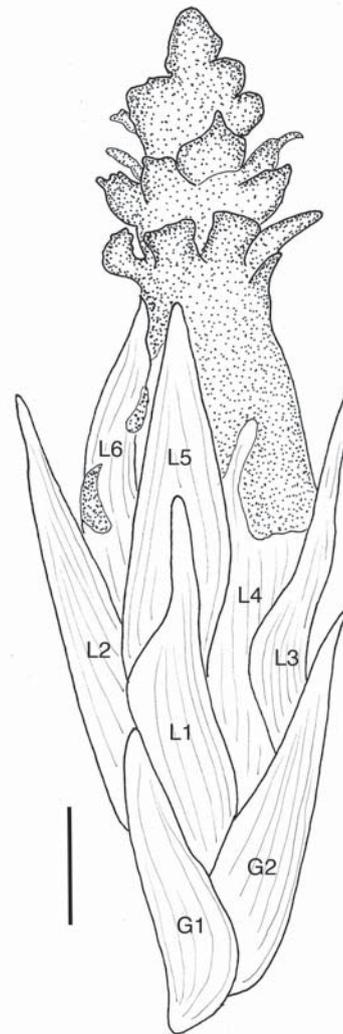


Fig. 2. Drawing of grass spikelet with attached sclerotium (stippled region) of *Palaeoclaviceps parasiticus* gen. n., sp. n., holotype, F26481BUIC (ex coll. WUNDERLICH) in Early–mid Cretaceous amber from Myanmar. G1= first glume, G2 = second glume, L1= first lemma, L2 = second lemma, L3 = third lemma, L4 = partial fourth lemma, L5 = fifth lemma, L6 = partial sixth lemma. Bar = 1.6 mm.

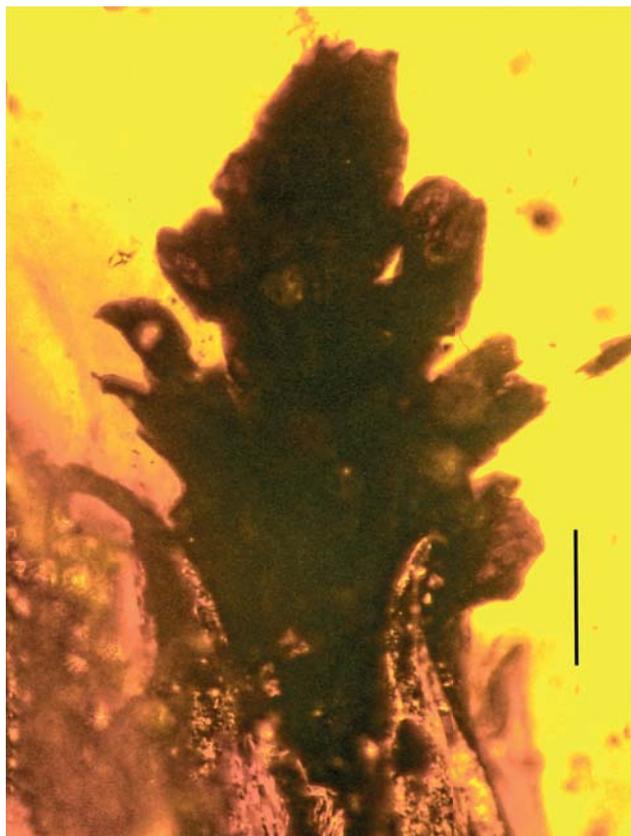


Fig. 3. Detail of lobed sclerotium of *Palaeoclaviceps parasiticus* gen. n., sp. n., holotype, F26481BUIC (ex coll. WUNDERLICH) in Early–mid Cretaceous amber from Myanmar. Bar = 0.5 mm.

Type: Holotype presently deposited in the JOERG WUNDERLICH collection (F26481BUIC). At a later date, it will be deposited in the Senckenberg Museum and Research Institute, Frankfurt-am-Main, Germany.

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.

Diagnosis: The erect, black sclerotium with flattened outgrowths of hyphal tissue and its presence in an Early–mid Cretaceous grass floret separate *Palaeoclaviceps parasiticus* gen. n., sp. n. from all extant species of *Claviceps* as well as from other fungi in the Clavicipitaceae (PAZOUTOVA 2002; ALDERMAN et al. 1999).

Description: Length entire grass spikelet with sclerotium, 9.5 mm; sclerotium black, elongate, thick, protruding from infected floret, 5.6 mm in length; greatest width across sclerotium, 2.2 mm; sclerotium bearing flattened hyphal outgrowths (lobes) ranging from 0.3 mm to 1.0 mm in length and 0.2 mm to 0.6 mm in width; outgrowths composed of thick, branching, anastomosed hyphae; conidiophores hyaline, simple, septate,

11µm–14µm in length, bearing conidia terminally; conidia small, simple, hyaline, ovoid, 1-celled, 5µm – 8µm in length, distributed in a dense mass adjacent to the sclerotium.

Remarks: The thick, branching, anastomosed hyphae in the sclerotial outgrowths of *Paleoclaviceps parasiticus* are similar to the arrangement of mycelium in developing sclerotia of extant *Claviceps purpurea* (FR.) TUL. (TAVEL 1892). The conidial mass adjacent to the fossil sclerotium is assumed to represent the “honey dew” droplets that in *Claviceps* spp. are comprised of conidia in a high sugar syrup produced during the early stages of ovary infection (Fig. 6) (MOWER & HANCOCK 1975). These

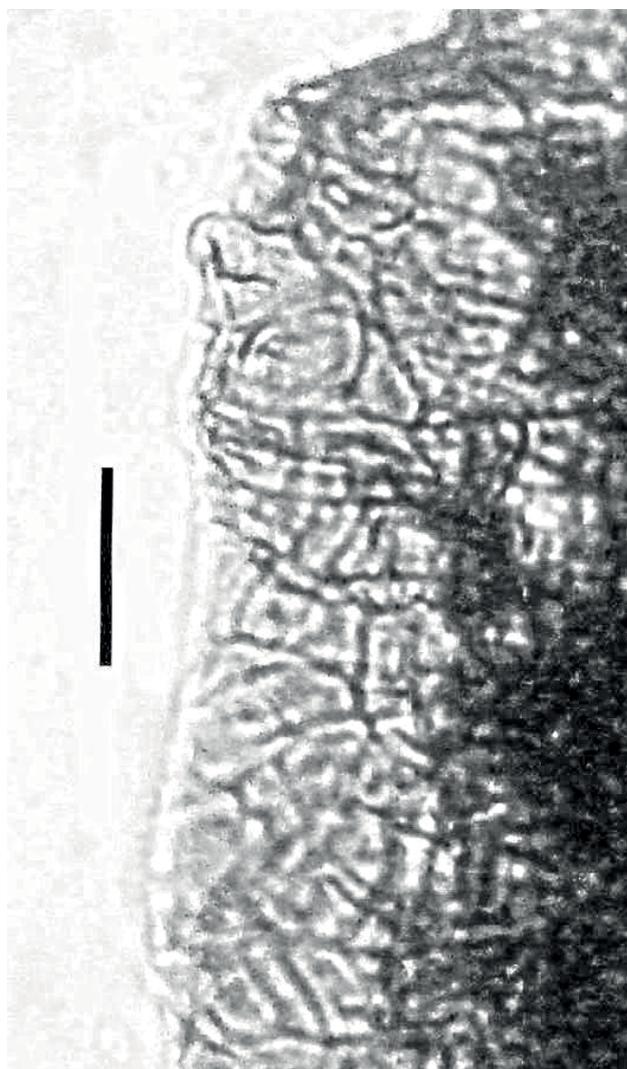


Fig. 4. A sclerotial lobe of *Palaeoclaviceps parasiticus* gen. n., sp. n., holotype, F26481BUIC (ex coll. Wunderlich) in Early–mid Cretaceous amber from Myanmar. Note anastomosed arrangement of mycelium. Bar = 0.9 mm.

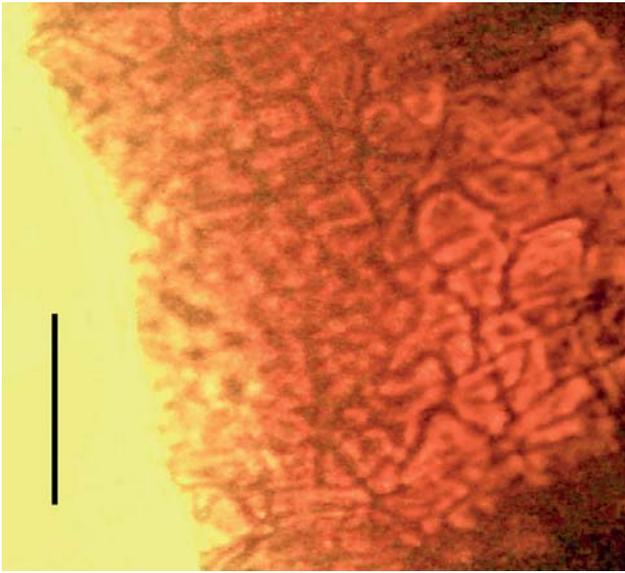


Fig. 5. Detail of arrangement of anastomosed hyphae in a second lobe of the sclerotium of *Palaeoclaviceps parasiticus* gen. n., sp. n., holotype, F26481BUIC (ex coll. WUNDERLICH); in Early–mid Cretaceous amber from Myanmar. Bar = 0.133 mm.

sweet droplets attract insects, especially flies, which can carry the conidia to healthy florets, facilitating new infections. While no extant *Claviceps* sclerotia are known to

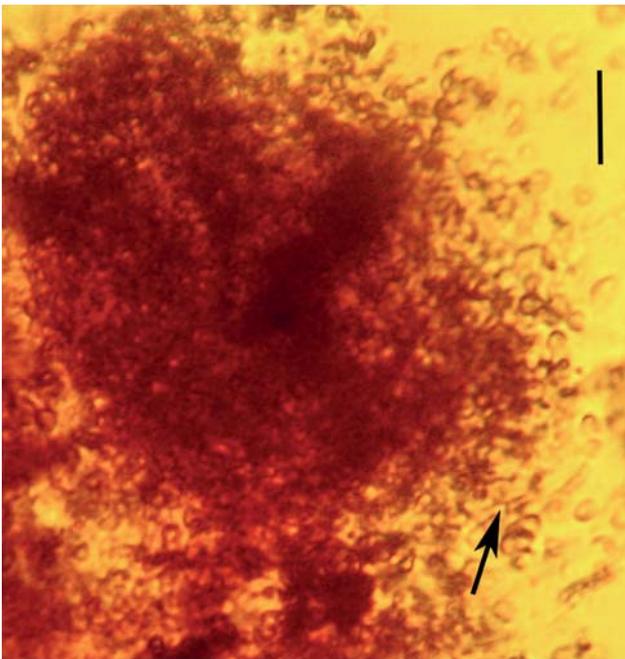


Fig. 6. Group of *Sphacelia* conidia and conidiophores (arrow) adjacent to the developing sclerotium of *Palaeoclaviceps parasiticus* gen. n., sp. n., holotype, F26481BUIC (ex coll. WUNDERLICH) in Early–mid Cretaceous amber from Myanmar. Bar = 0.13 mm.

have flattened lobes as occurs in *Palaeoclaviceps parasiticus* gen. n., sp. n., some sclerotia of *C. purpurea* infecting rye do occasionally have a rugose surface with some short thick lobes (Fig. 7).

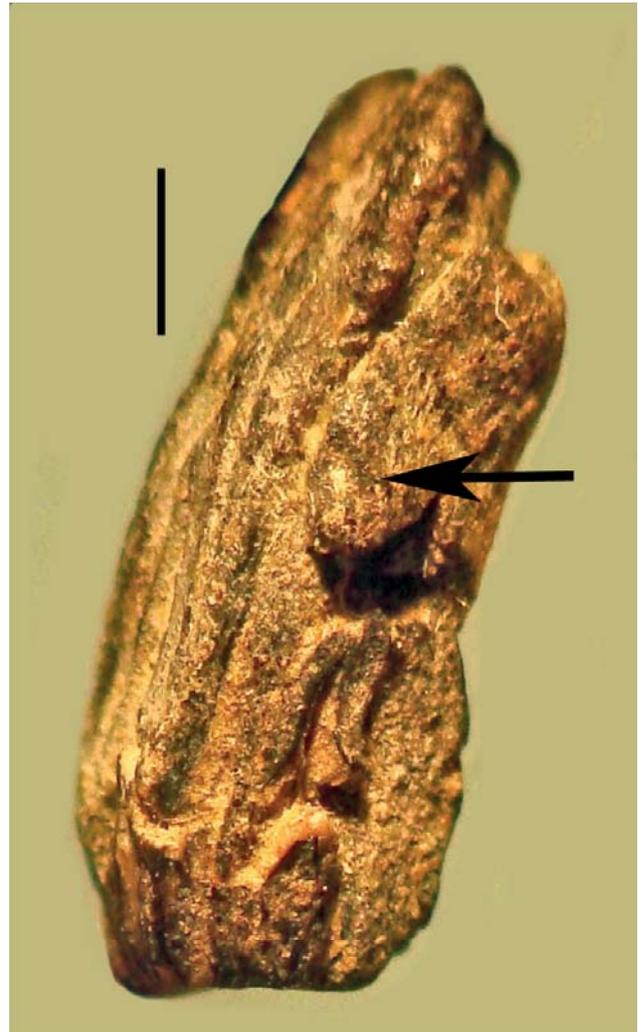


Fig. 7. An extant sclerotium of *Claviceps purpurea* showing irregular surface with a folded lobe (arrow). Bar = 1.5 mm.

Type host: While grasses are the most common hosts of ergot fungi today, some members of the Cyperaceae and Juncaceae are also attacked. However, grasses are the only members of this group that lack bracts subtending the inflorescence branches and the only group with spikelets possessing a pair of sterile glumes subtending the florets (LINDER 1986; DICKISON 2000; METCALFE 1960). Members of the Cyperaceae only have a single bract (prophyll) subtending each flower and the Juncaceae have trimerous flowers with two or more prophylls situated dorsally and ventrally in the medial plane, with an arrangement of 3+3 tepals (DAHLGREN et al. 1985).

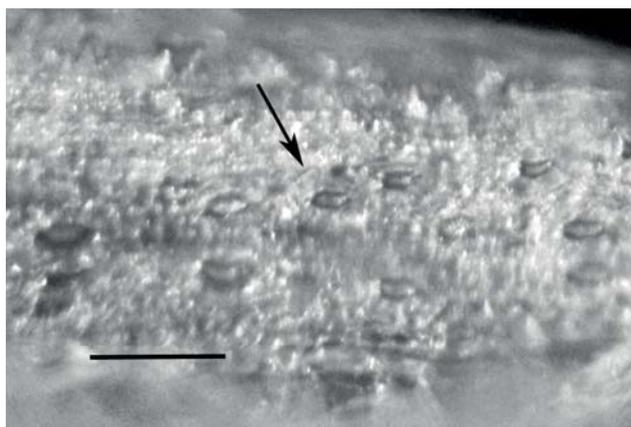


Fig. 8. View of exposed surface of lemma showing rows of stomata in the grass host of *Palaeoclaviceps parasiticus* gen. n., sp. n., holotype, F26481BUIC (ex coll. WUNDERLICH) in Early–mid Cretaceous amber from Myanmar. Arrow shows border separating rows of epidermal cells with and without stomata. Bar = 0.14 mm.

The infected spikelet is laterally-compressed, 9.5 mm in length, with a rachilla bearing two complete basal sterile glumes and a series of 5 lemmas: bracts glabrous, awnless: lengths, first glume, 2.7 mm in length; second glume, 3.4 mm in length; first lemma, 3.3 mm in length, second lemma, 3.9 mm in length; third lemma, 3.3 mm in length; fourth lemma, 3.4 mm in length; fifth lemma (partial), 3.3 mm in length: stamens not observed; short rows of stomata occur on the exposed surfaces of some lemmas (Fig. 8); stomata 75–99 μm in length and 48–59 μm in width;

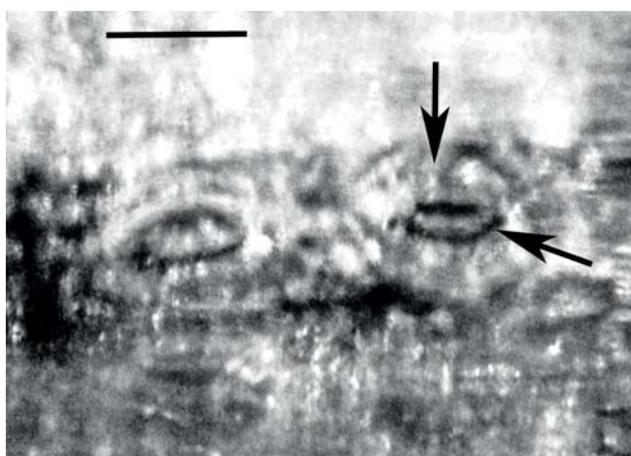


Fig. 9. Detail of two stomata in the lemma of the grass host of *Palaeoclaviceps parasiticus* gen. n., sp. n., holotype, F26481BUIC (ex coll. WUNDERLICH) in Early–mid Cretaceous amber from Myanmar. Top arrow shows subsidiary cell. Lower right arrow shows slightly expanded terminus of guard cell nucleus. Bar = 0.06 mm.

guard cells reniform, with thickened inner wall; guard cell nuclei prominent and elongated with slightly bulbous ends, 32–40 μm in length, guard cells flanked by low-dome-shaped subsidiary cell (paracytic) (Fig. 9). The above characters place the infected spikelet in the family Poaceae.

4. Discussion

The preservation of *Paleoclaviceps parasiticus* gen. n., sp. n. was excellent and the grass florets and sclerotium appeared unaltered. Such preservation of amber fossils is due to rapid dehydration and tissue fixation by chemicals in the original resin. While there is often some collapsing due to dehydration in embedded arthropods, it is rare to find distorted fungal tissues in amber. Even the fragile pileus of the Early–mid Cretaceous Myanmar amber agaric, *Palaeoagaracites antiquus* POINAR & BUCKLEY 2000 that was partly decomposed by a mycoparasite, showed no signs of distortion, nor was there any evidence of damage to the phialides of *Paleoophiocordyceps coccophagus* SUNG, POINAR & SPATAFORA in Myanmar amber (SUNG et al. 2008).

Based on molecular evidence, SPATAFORA et al. (2005) and SUNG et al. (2008) hypothesized that the archetype of the Clavicipitaceae was an invertebrate pathogen with an age estimate of its crown node being at least 117 Mya. The present discovery provides evidence that the archetype could just as well have been a plant pathogen such as the present fossil or another possible grass endophyte (e.g., *Balansia* and *Epichloe*) and that invertebrates were infected later through interkingdom host shifts. SUNG et al. (2008) estimated the crown node of grass symbionts (including *Claviceps* spp.) to be at least 81 Mya, suggesting that fungal–grass symbioses originated in the Late Cretaceous. This date is congruent with finding a *Claviceps*-like fossil that is roughly 100 million years old.

PAZOUTOVA (2002) suggested that the ancestors of *Claviceps* evolved on panicoid grasses in the American tropics sometime in the Upper Cretaceous. This was based on the interpretation of ancestral and advanced characters and finding *Claviceps* species with primitive undifferentiated sclerotia in that region. These primitive strains of *Claviceps* then migrated from South America to North America and then to Europe and Africa. With the discovery of a *Claviceps*-like fossil infecting a floret of an Early–mid Cretaceous Asian grass, we propose that the progenitor of *Claviceps* evolved in Asia among early grasses sometime in the mid- to Late Jurassic.

Fossil evidence has established the presence of grasses not only in the Late Cretaceous (CREPET et al. 2004; PRASAD et al. 2005) but also in the Early–mid Cretaceous (POINAR 2004, 2011). The Myanmar amber *Programinis burmitis* POINAR was described from a spikelet possessing characters similar to the present infected fossil spike-

let (POINAR 2004). Without additional characters, it is not possible to assign the infected spikelet to *P. burmitis* at this time. However, the present infected grass fossil adds indisputable evidence that the Poaceae was established by the Early–mid Cretaceous.

Ergot is a veritable pharmacopeia and over a thousand compounds have been extracted or derived from *Claviceps* spp. (BOVE 1955). At least some of these probably served to repel herbivores since raw ergots are reported to be bitter and cause nausea (ATANASOFF 1920). Early researchers who fed ergot-infected seeds to farm animals also noted that after a few feedings, the livestock refused to eat the diseased grain (BOVE 1955). If serving as a deterrent against herbivores was the primary purpose of the chemical compounds in ergot, then they were likely already present in *P. parasiticus*. It is also possible that psychotropic compounds in *Palaeoclaviceps* also served as repellants and were present at this stage in the evolution of *Claviceps* (BOVE 1955).

Cretaceous herbivores that would have consumed grasses were mammals, birds and dinosaurs. One study has in fact identified grass remains in coprolites of a Cretaceous sauropod (PRASAD et al. 2005). An analysis of the feeding strata of sauropod dinosaurs (from hatchlings to adults) at the Myanmar amber site indicated that they fed in the herb and shrub zones (POINAR & POINAR 2008), where the grass host of *Palaeoclaviceps parasiticus* would have grown.

The present discovery establishes: 1) the first fossil record of a progenitor of *Claviceps*. 2), a minimum date for the establishment of plant parasitism in the Clavicipitaceae. 3), supportive evidence for the earliest known fossil Poaceae in the Early–mid Cretaceous, and 4), a minimum time for the conceivable presence of psychotropic compounds. This fossil also provides new insights into the origin and evolution of plant parasitism by the Clavicipitaceae.

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