Mycol. Res. **109** (8): 956–960 (August 2005). © The British Mycological Society
doi:10.1017/S0953756205003497 *Printed in the United Kingdom.*

A fossil *Aspergillus* from Baltic amber

Heinrich DÖRFELT[1] and Alexander R. SCHMIDT[2]*

[1] *Martin-Luther-Universität Halle, Institut für Geobotanik und Botanischer Garten, Neuwerk 21, D-06108 Halle/Saale, Germany.*
[2] *Museum für Naturkunde, Humboldt-Universität zu Berlin, Invalidenstr. 43, D-10115 Berlin, Germany.*
E-mail: *alexander.schmidt@museum.hu-berlin.de*

Received 17 May 2004; accepted 23 May 2005.

A piece of Baltic amber (Tertiary, Eocene) contains an inclusion of a springtail (*Collembola*) which is overgrown by an *Aspergillus* species. The fossil fungus is described as *A. collembolorum* sp. nov. The excellent mode of preservation of the numerous conidiophores is remarkable and can be explained by sporulation in liquid resin. This is the second report of a fossil *Aspergillus*, the first being from Dominican amber.

INTRODUCTION

A springtail (*Collembola*), densely overgrown by conidiophores of an *Aspergillus* species, was found in a piece of Baltic amber. The Baltic amber forests grew during the Eocene epoch (50–35 Myr) in a temperate to subtropical climate and consisted, apart from conifers, mainly of species in the *Arecaceae* and *Fagaceae* (Ganzelewski 1997). The fossil is early Tertiary in age and, together with a new world find from Dominican amber, the only fossil specimen of a representative of the genus *Aspergillus* to be found.

MATERIAL AND METHODS

The piece of Baltic amber investigated originated from the Kaliningrad area on the eastern coast of the Baltic Sea (western Russia). Syninclusions with the overgrown springtail are a caddis fly (*Trichoptera*) and several hairs from macrophytes. The springtail is 2 mm in length and lies near the *Trichoptera* (Figs 1–3). The protruding mesonotum, the body shape and the relative length of the extremities (third leg) of the springtail allow a safe assignment to the suborder *Entomobryomorpha*. It is probably a representative of the genus *Entomobrya s. lat.* or *Protoentomobrya*. Similar springtails have been found repeatedly in fossil resins (Wolfram Dunger, pers. comm.).

The springtail was slightly damaged laterally as the amber was ground and polished (Figs 1, 3) because the insect was located close to the weathered surface. This allowed for microscopic investigation from both the outer surface as well as in longitudinal-section.

In order to protect the amber from oxidation and breakage the polished amber piece of $20 \times 15 \times 2$ mm was embedded using polyester resin (GTS cured with addition of MEKP hardener Vosschemie, Uetersen) as described by Hoffeins (2001). The highly transparent artificial resin has almost the same refractive index as the amber and therefore the fungus was easy to investigate at high magnifications. The amber piece was investigated under a transmitted light, differential interference contrast microscope (Axioplan, Carl Zeiss, Jena) with long distance objectives ($10\times$, $20\times$ and $40\times$) and alternative incident light.

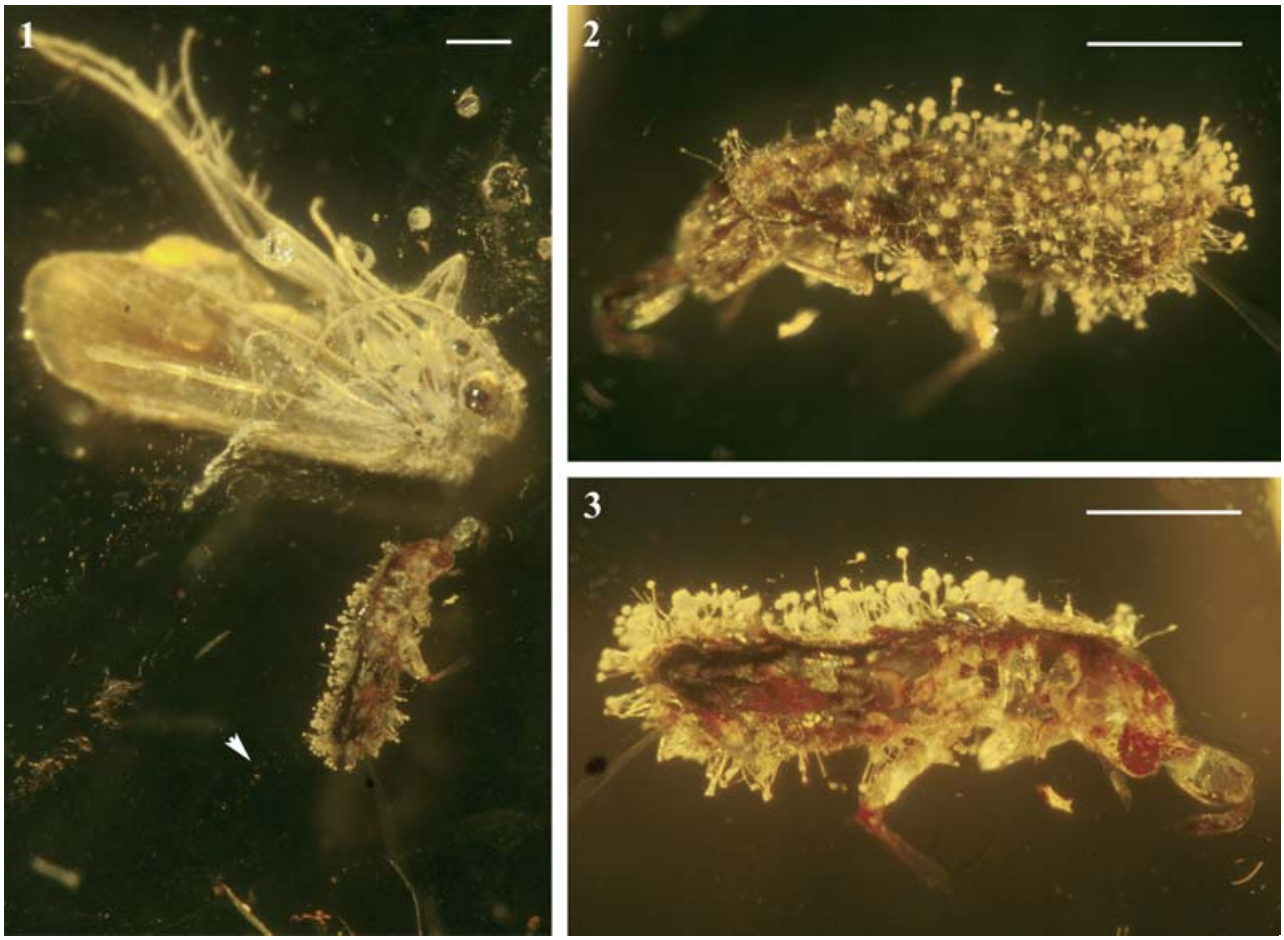
TAXONOMY

The surface of the springtail is loosely (to densely) covered by hyphae and conidiophores (Figs 2–3). Most conidiophores grew in clusters of 2–6 directly out of the surface of the insect but some originated from superficial hyphae at the cuticle (Fig. 10). Inside, the springtail is loosely penetrated by branched substrate hyphae.

Aspergillus collembolorum Dörfelt & A. R. Schmidt, **sp. nov.**

Conidiophora (sine vesiculis, phialidibus et sporis) circiter 125–225 μm longa et 5–8 μm diam, hyalina et glabra usque aspera; vesiculae globosae usque subglobosae, circiter 12–22 μm diam; phialides semper sine metulis, 8–10 μm

* Corresponding author.



Figs 1–3. Overview of the inclusion of the springtail (*Collembola*, *Entomobryomorpha*), overgrown by the fossil *Aspergillus collembolorum*. **Fig. 1.** Springtail with the caddis fly syninclusion. Note the streak of abscised ripe conidia (arrow). **Fig. 2.** Intact side. **Fig. 3.** Polished side that allows for viewing of the springtail in longitudinal-section. Bars = 0.5 mm.

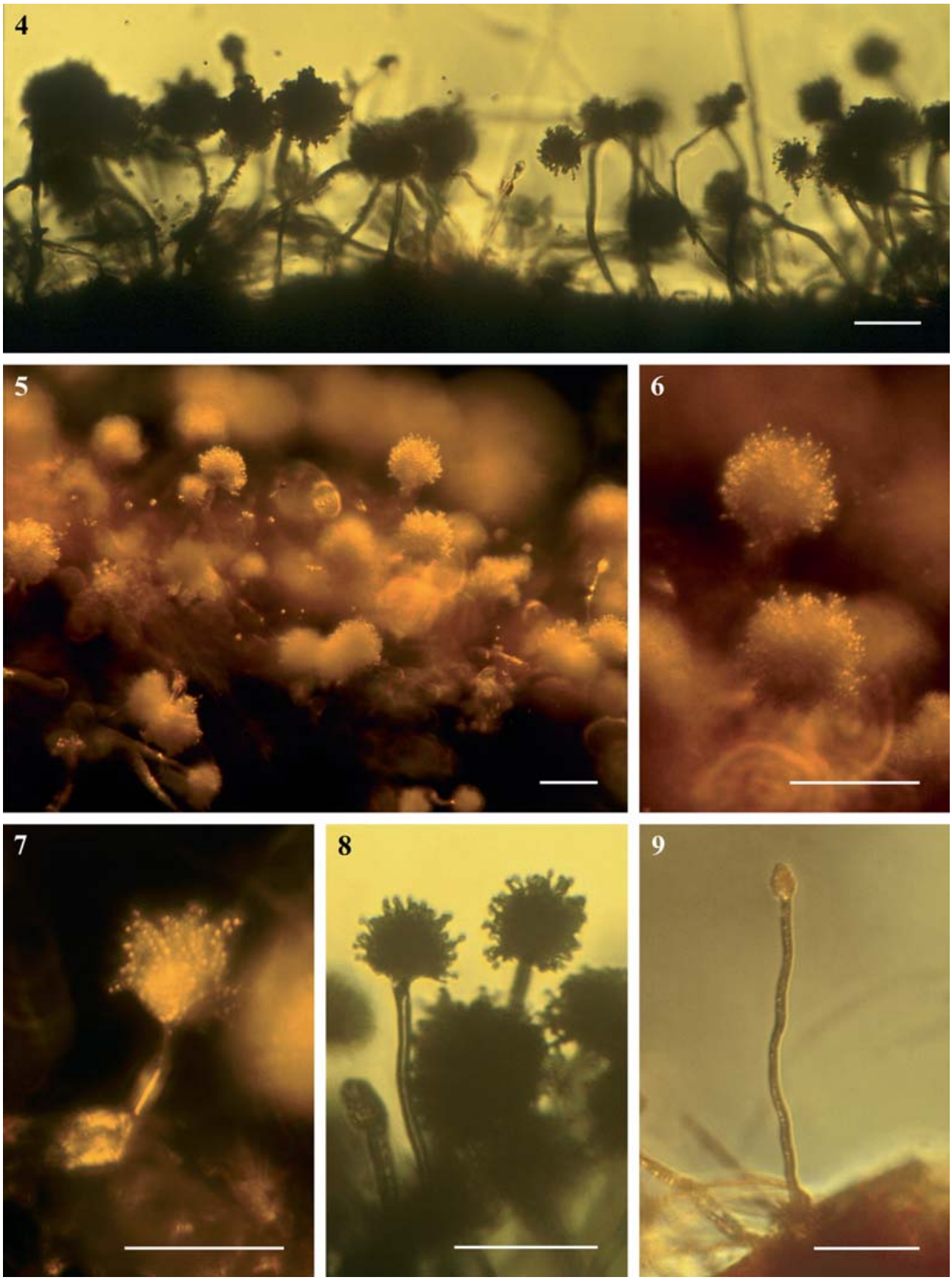
longae et 4–5 μm latae; sporae flavescentes globosae usque subglobosae, circiter 3–4 μm diam.

Typus: **Russia:** Kaliningrad (Koenigsberg), in succinum Balticum, in exemplare subordines *Entomobryomorpha* (*Collembola*), *C. & H. W. Hoffeins* (coll. Hoffeins, Hamburg, no. 805, holotypus).

Conidiophores (without vesicles, phialides and chains of conidia) mostly 125–175 μm, rarely to 225 μm, in length and (3–) 5–7.5 μm diam, mostly of equal diameter throughout and sometimes slightly thickened at the base (Figs 4, 8–9); vesicles spherical to ovoid or subglobose and *ca* 18.5–20 μm diam or, when subglobose to ovoid, around 17–22 × 11–15 μm (Fig. 9). *Conidiogenous cells* close, around 8–10 μm in length and 4–5 mm broad, uniseriate and always extending directly from the vesicles. *Supporting cells* (metulae) absent. *Conidia* spherical and 3–4 μm diam, probably almost smooth or very finely punctate at the surface; conidial heads including the radial chains of conidia 35–70 μm diam (Figs 4–8); hyphae within the springtail *ca* 2–4 μm diam and hyaline. *Sclerotia and cleistothecia* not found.

Compared to the surface of the insect, the fungal structures appear lighter in colour. Under incident light the conidial heads are coloured yellowish to light brown or whitish. The colours of fossils do not correspond exactly to that of the living organism; however, it can be assumed that the living hyphae were hyaline and the conidia were light in colour, or at least they were not darkly pigmented.

In general, it is not possible to determine *Aspergillus* species without examination of features from pure culture. The phenotypic plasticity of a genotype may be very high and mainly depends on the substrate. The excellent preservation of the fossil, however, allows for observation of the most important features. We can estimate the colour of hyphae, conidiophores and conidia and the mode of life, but an assignment to the *Aspergillus flavus* group (subgen. *Circumdati* sect. *Flavi*) is not sure. Rough stipe walls are characteristic of most members of sect. *Flavi*. The morphological features, especially the small phialides extending directly from the vesicles, show similarity to *A. parasiticus*. However, the conidiophores of that species are 0.2–1 mm in length and the conidial heads are much larger being over 100 μm diam. The morphological differences from other species of the *A. flavus* group, the probable parasitic mode of life (see below) as well as the occurrence in the Early Tertiary leads to the



Figs 4–9. Transmitted light differential interference contrast (Figs 4, 8) and incident light (Figs 5–7, 9) photomicrographs of the fossil *Aspergillus collembolorum*. **Figs 4–5.** View of the surface of the springtail with numerous sporulating conidiophores. **Figs 6–8.** Conidial heads with radial chains of conidia. **Fig. 9.** Single conidiophore with subglobose vesicle. Bars = 50 μm.

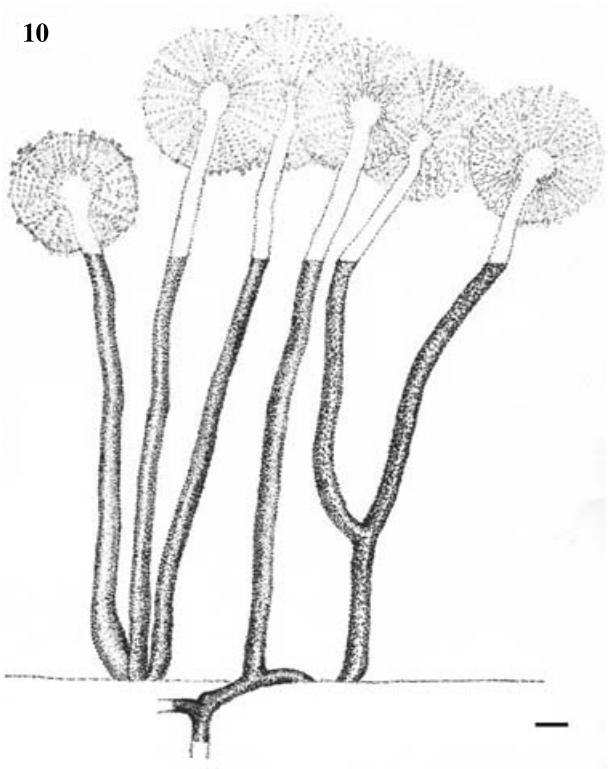


Fig. 10. Basal and median parts of the conidiophores of *Aspergillus collembolorum*. The septa are not perceptible because of the light scattering in the fossil resin. The horizontal line indicates the surface of the fossil springtail. Bar = 10 μm.

conclusion that the fossil cannot be identical to any modern species or subgenus. Furthermore, we assume that the species have changed genetically over time because of parasexuality and mutations, and thus the morphological differences have a genotypic background as well. Therefore, we decided to describe the fossil as a new species.

DISCUSSION

Taphonomy and palaeoecology

The conidiophores are better preserved in the resin than would be possible using any artificial preparation method. The excellent preservation and, especially, the natural orientation of the conidiophores and chains of conidia show that the fungus sporulated after being covered by liquid resin. A streak of abscised mature conidia (Fig. 1) originating from a few conidial heads indicates that the resin was still viscous when some conidia matured and that the inclusion moved slightly at that stage. In contrast to the numerous abscised hairs from the caddis fly, the streak of conidia is not attached to the contact zone of two successive resin outflows in the amber. This confirms the assumption of sporulation after embedding. Processes of growth in liquid resins have been described for bacteria embedded in fresh resins for actualistic studies (see Schmidt 2003) and from resinicolous fungi (Rikkinen & Poinar 2000).

The resin was usually a trap for living and not for dead animals. Possibly the fungus already penetrated and parasitized the living springtail and, continued to grow rapidly within the embedded dead body. Furthermore, the dominance of only one insecticolous organism and the excellent preservation of the springtail indicate the probability of a parasitic mode of life of the fungus.

Numerous parasitic fungi are known to infest insects, some of which have increasing importance as biological controls and medicines (Dörfelt 2001). Fungal taxa such as the *Harpellales* (*Trichomycetes*), *Entomophthorales* (*Zygomycetes*) and *Laboulbeniales* (*Ascomycota*) contain highly specialized entomopathogenetic species. Many of the widespread anamorphs of the order *Eurotiales*, in which this fossil belongs, however, are not specialized and are saprotrophic or facultatively parasitic.

Parasitic fungi specialized on *Collembolae* are not known. Dead arthropods overgrown by fungi occur frequently and in these cases it is assumed that dead and not living animals were invaded (Wolfram Dunger, pers. comm.). However, there are indications that some *Aspergillus* species may live as facultative parasites on insects, for example on species of the *A. ochraceus* and *A. flavus* groups; *A. flavus* is known to be a facultative parasite of some pyralid moths (*Pyralidae*, *Lepidoptera*), and *A. flavus* and *A. parasiticus* can invade different *Orthoptera via* tracheal openings and may become established in the haemolymph or in the tissue of the insects (Raper & Fennell 1965). Some *Aspergillus* species which produce pathogenic mycotoxins may live as facultative parasites in endothermic animals. *Aspergillus* infections are well known in birds, and mammals, including humans (Weber 1993).

Entomogenous fungi of the genera *Gibellula* and *Pseudogibellula* also have conidiophores with vesiculae, but differ from the fossil fungus in some morphological features, such as the presence of synnemata, ellipsoid to fusoid spores, and by large intervening supporting cells between the vesicles and phialides (Samson & Evans 1973).

Palaeomycological importance

Aspergillus collembolorum is the second fossil *Aspergillus* record. Thomas & Poinar (1988) previously described a sporulating *Aspergillus* from Dominican amber, but there is uncertainty regarding the age of the amber, which originated from the Palo Alto amber mine. It has been reported as ranging from Lower Miocene-Oligocene (*ca* 23–30 Myr. Poinar 1992) to Eocene (*ca* 40 Myr; Thomas & Poinar 1988). The identification of that *Aspergillus* specimen was difficult because the conidiogenous cells were not visible in the amber. It was assigned to the *A. versicolor* group, and

those authors found similarities with the white-spored phase of *A. janus*, characterized by long conidiophores 2–2.5 mm in length, large conidial heads of up to more than 200 μm diam with clavate vesicles, conidiogenous cells born on supporting cells (metulae), and smooth small conidia up to 3 μm diam. Most features of the Dominican fossil *Aspergillus* correspond to extant *A. janus*, except that the features of the phialides were not visible and larger spores of 6–9 μm were observed.

Berkeley (1848) mentioned *A. penicillatus* from Baltic amber, however, it clearly are aerial hyphae forming arthroconidia as in the genera *Geotrichium*, *Monilia* or *Chrysonilia*. The description of *Aspergillites torulosus* by Trivedi & Verma (1969) from a Tertiary coalbed from Malaysia is based on chains of five to many sphaerical spores. The chains of dark sphaerical spores to 17 μm diam, however, suggest the rust genus *Xenodochus* and not *Aspergillus*. Neither in the compilations and overviews of Pia (1927), Tiffney & Barghoorn (1974), or Pirozynski (1976), is a fossil *Aspergillus* mentioned. The lack of further fossil records can easily be explained by the low fossilisation rate of such soft-bodied microorganisms.

ACKNOWLEDGEMENTS

In particular, we would like to express our gratitude to the amber collectors Christel and Hans Werner Hoffeins (Hamburg) for the kind permission to investigate the fossil and for their information regarding the origin and preparation of the amber piece. We are indebted to Wolfram Dunger (Görlitz) for his remarks regarding the systematics of the springtail. We thank Heike Heklau (Halle), Kerstin Schultz (Jena) and Jeffrey K. Stone (Corvallis) for helpful information and advice. The work on amber is supported by the German Research Foundation, project Graduate Research Programme no. 503.

REFERENCES

Berkeley, M. J. (1848) On three species of mould detected by Dr. Thomas in the amber of East Prussia. *Annals and Magazine of Natural History, series 2* **2**: 381–383.

Dörfelt, H. (2001) *Eumycota* (*Fungi*). In *Mansfeld's Encyclopedia of Agricultural and Horticultural Crops* (P. Hanelt, ed.) **1**: 14–49. Springer Verlag, Berlin.

Ganzelewski, M. (1997) Entstehung und Lagerstätten des Baltischen Bernsteins. In *Bernstein – Tränen der Götter* (M. Ganzelewski & R. Slotta, eds): 11–18. Glückauf, Bochum.

Hoffeins, H. W. (2001) On the preparation and conservation of amber inclusions in artificial resin. *Polish Journal of Entomology* **70**: 215–219.

Pia, J. (1927) Fungi. In *Handbuch der Paläobotanik* (M. Hirmer, ed.) **1**: 112–130. R. Oldenbourg, Munich.

Pirozynski, K. A. (1976) Fossil fungi. *Annual Review of Phytopathology* **14**: 237–246.

Poinar, G. O. jr. (1992) *Life in Amber*. Stanford University Press, Stanford.

Raper, K. B. & Fennell, D. I. (1965) *The Genus Aspergillus*. Williams & Wilkins, Baltimore.

Rikkinen, J. & Poinar, G. O. jr. (2000) A new species of resinicolous *Chaenothecopsis* (*Mycocaliciaceae*, *Ascomycota*) from 20 million year old Bitterfeld amber, with remarks on the biology of resinicolous fungi. *Mycological Research* **104**: 7–15.

Samson, R. A. & Evans, H. C. (1973) Notes on entomogenous fungi from Ghana. I. The genera *Gibellula* and *Pseudogibellula*. *Acta Botanica Neerlandica* **22**: 522–528.

Schmidt, A. R. (2003) *Das fossile Harz von Schliersee* (*Bayerische Alpen*) *und seine Mikroinklusen*. Doctoral thesis, Friedrich-Schiller-Universität, Jena.

Thomas, G. M. & Poinar, G. O. jr. (1988) A fossil *Aspergillus* from Eocene Dominican Amber. *Journal of Paleontology* **62**: 141–143.

Tiffney, B. H. & Barghoorn, E. S. (1974) The fossil record of the fungi. *Occasional Papers of the Farlow Herbarium* **7**: 1–42.

Trivedi, B. S. & Verma, G. L. (1969) Fungal remains from Tertiary coal bed of Malaya. *Journal of Palynology* **5**: 68–73.

Weber, H. (1993) *Allgemeine Mykologie*. Gustav Fischer Verlag, Jena.

Corresponding Editor: J. K. Stone