Letters

Lichen-associated fungi from Paleogene amber

Lichens are highly specialized and obligate symbioses between fungi and green algae and/or cyanobacteria. In addition to the fungal partner (mycobiont) and algal or cyanobacterial symbionts (photobionts), lichen thalli often house diverse assemblages of associated microfungi and bacteria (Girlanda *et al.*, 1997; U'Ren *et al.*, 2012; Aschenbrenner *et al.*, 2014). More than 1750 species of obligately lichenicolous fungi have already been described and at least 5000 species are estimated to exist (Hawksworth, 1982a, 2003; Lawrey & Diederich, 2003, 2015; Werth *et al.*, 2013). The true lichenicolous fungi are obligate associates of lichen-symbiotic organisms and can thus only live on lichen thalli. The nature of their associations can vary from parasitism to commensalism and some lichen parasites appear to have evolved from saprotrophic ancestors (Rambold & Triebel, 1992).

Lichen-symbiotic fungi (mostly ascomycetes) produce many unique lichen compounds. Some of these secondary metabolites protect lichens from stress factors such as UV-radiation and herbivory (Asplund & Wardle, 2013; Nguyen *et al.*, 2013). However, many lichen-associated fungi are able to tolerate or even degrade bioactive lichen compounds and can thus effectively colonize lichen thalli (Hawksworth, 1982b; Lawrey *et al.*, 1999; Torzilli *et al.*, 1999). The high diversity of extant lichenicolous fungi and the concurrent diversity of bioactive lichen compounds suggest that some associations between lichen symbionts and associated saprotrophic and parasitic fungi have long coevolutionary histories (Lawrey *et al.*, 1999; Ranković *et al.*, 2007). However, direct evidence for this is difficult to find as lichen fossils are very rare and lichenicolous fungi have so far not had a fossil record. Despite their apparent low frequency in the fossil record the earliest lichen-like symbioses date back to the Lower Devonian (Taylor *et al.*, 1997, 2015; Karatygin *et al.*, 2009) and some of the early stratified fossils closely resemble modern lichens (Honegger *et al.*, 2013). It seems probable that lichens were a component of early terrestrial ecosystems, which bore a resemblance to modern cryptogam covers (Selosse *et al.*, 2015).

Cenozoic fossils of lichens clearly assignable to modern families and genera have been preserved as amber inclusions from different Paleogene deposits (Rikkinen & Poinar, 2002, 2008; Hartl *et al.*, 2015; Kaasalainen *et al.*, 2015). Amber, which is fossilized tree resin, has an ability to preserve tiny microorganisms in exquisite detail, and several fossils of filamentous microfungi have been discovered from different amber deposits around the world (Taylor *et al.*, 2015).

Here we report on the first fossils of lichen-associated filamentous fungi. Several distinct morphologies of darkly-pigmented fungi growing on crustose and foliose lichens are beautifully preserved, suggesting that hyphomycetous microfungi have colonized lichen surfaces at least since the Paleogene.

The amber fossils

Different darkly-pigmented hyphomycetes associated with corticolous lichen thalli (Fig. 1; Supporting Information Fig. S1 and Methods S1) were found enclosed in Bitterfeld amber from central Germany that has a minimum age of 24 million years (Dunlop, 2010). Two amber specimens contain darkly pigmented, torpedo-shaped conidia of fungi closely resembling the extant genus *Sporidesmium* growing on degraded crustose lichen thalli (Fig. 1a, c). Facultatively lichenicolous *Sporidesmium*-like fungi appear to be relatively common on extant lichens, but they have as yet hardly been studied. The fossils most resemble *S. lichenicola* described from decomposing *Leptogium* (a cyanobacterial lichen) in Venezuela (Iturriaga *et al.*, 2008), but have longer conidia with much longer apical extensions.

Another amber specimen contains a fungus closely resembling extant *Taeniolella* growing on what appears to be a decomposing crustose lichen (Fig. 1b). Extant species of the genus are principally saprophytes on decaying plant material such as bark or wood, but some species are also obligately lichenicolous (Hawksworth, 1979).

The associated fungi of the fossilized lichens also include robust, tapering hyphae with globular cells that are assignable to sooty moulds of the family Metacapnodiaceae (Capnodiales) which are common in Paleogene amber (Schmidt *et al.*, 2014), and some modern species of which are known to grow on extant lichens (Braun *et al.*, 2009). Sooty moulds usually grow on plant exudates and honeydew, and their occurrence on lichens is interpreted as incidental.

Palaeoecological implications

Possibly up to 95% of all presently known lichenicolous fungi are ascomycetes, but also some highly specialized basidiomycetes have adapted to grow on lichens (Lawrey & Diederich, 2003, 2015). Many of them cause visible symptoms on their hosts, such as discolorations or deformations on infected lichen thalli (Hawksworth, 1979).

All the newly found fossil fungi appear to be saprotrophic or at most weakly parasitic fungi growing on dying or dead and decomposing lichen thalli. However, we suppose that some of them were able to also infect weakened lichens and then persist on the substrate after the host's death (Hawksworth, 1982a,b). The intact superficial reproductive structures (conidiophores) and the generally excellent condition of the fossil fungi demonstrate that they had already developed on the lichens before the thalli were

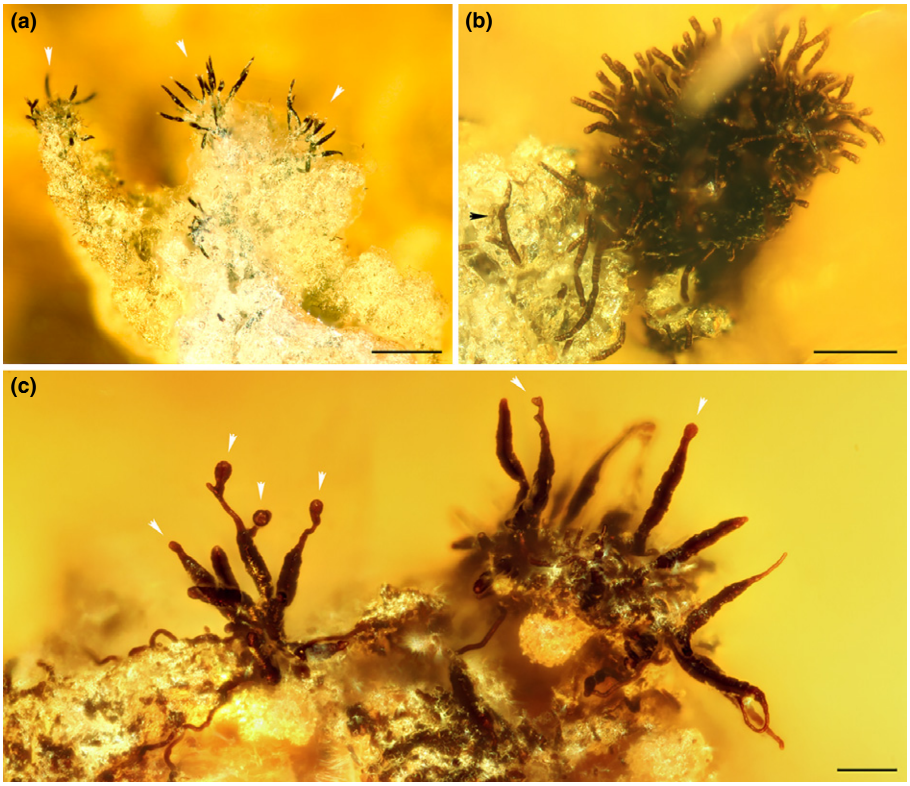

Fig. 1 Lichen-associated microfungi from Bitterfeld amber. (a) *Sporidesmium*-like fungus on crustose lichen thallus (Geoscientific Collections of the University of Göttingen GZG.BST.27298). The filamentous fungus grew mostly inside the lichen thallus but also produced superficial clusters of dark, upright hyphae (conidiophores) from which asexual spores (conidia) were produced (arrowheads). The structural details of two such colonies are illustrated in (c), and the general placement of the colonies on the fossil lichen is shown in the Supporting Information Fig S1(a). (b) *Taeniolella*-like fungus on crustose lichen thallus (GZG.BST.27299). Also this fungus mainly grew immersed in the lichen thallus and produced dark superficial colonies of multicellular conidia in simple or sparingly branched chains. Some detached conidial chains can be seen on the lichen surface just left of the main colony (arrowhead). (c) Close up of *Sporidesmium*-like fungal colonies (GZG.BST.27294). The long, multicellular conidia were produced solitarily and have apical cells tapering gradually towards the tip. Some of the tips have produced globular extensions likely representing initial stages of new conidia (arrowheads). Bars: (a) 200 μm; (b) 100 μm; (c) 50 μm.

engulfed by fresh resin. While none of the fossil species are here interpreted as being truly lichenicolous (i.e. obligate parasites or commensals of lichen symbionts), they most probably were not growing on lichens by accident. This interpretation is supported by the growth patterns and non-random placement of the fungi on their substrates. For example, the *Sporidesmium*-like fungi distinctly favoured elevated spots on the lichen surface and produced most conidia on thallus ridges (Fig. 1a,c). This may have increased the likelihood of wind dispersal and also ensured rapid drying of conidia after rain.

Iturriaga *et al.* (2008) noted that not only *Sporidesmium* but also several other genera of tropical lichenicolous hyphomycetes include species with 'torpedo-shaped' conidia and suggested that such a conidial shape would have some adaptive value. Possibly the relatively robust but very streamlined conidia can effectively penetrate the boundary layer and become attached to lichen surfaces in often windy epiphytic habitats. In any case, the fossils show that torpedo-shaped conidia were produced by lichen-associated hyphomycetes already in the Paleogene.

Conclusions

The new fossils demonstrate that Paleogene lichens already supported lichen-associated, presumably saprotrophic fungi. As the fossils are essentially identical to their modern analogues, the evolutionary associations between lichen-associated microfungi and their substrate must extend back much further, most probably to the Mesozoic.

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Author contributions

E.K., A.R.S. and J.R. planned the research and H.G. provided fossil specimens. E.K., A.R.S., P.D. and J.R. analysed the fossils and wrote the manuscript.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Location of the lichen-associated fungi on the surface of the lichen thalli in three Bitterfeld amber pieces.

Methods S1 Geology, preparation and imaging.

Key words: Ascomycota, coevolution, dematiaceous hyphomycetes, lichen compounds, sooty moulds.