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## **Fossil evidence of lichen grazing from Paleogene amber**

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Amber is fossilized tree resin and famous for its lifelike preservation of arthropods, plant remains, and microorganisms. A piece of Paleogene Bitterfeld amber (23-24 million years minimum age) includes a fossilized lichen with marks of grazing and faecal pellets. Light microscopy and scanning electron microscopy (SEM) were used to study the specimen. Additionally, in order to identify the source(s) of the feeding marks and faecal pellets, an actuo-paleontological experiment was designed using extant lichens and known lichen grazers including mites, collembolans, psocoptera, coleoptera, and gastropods. The morphological characters of the fossil lichen, especially the structure of the lower side pseudocortex, suggest an affiliation to the extant genus *Phyllopsora*. Based on the results of our grazing experiment and published data we identified gastropods and/or mites as the most likely grazers of the fossil lichen.

# Fossil evidence of lichen grazing from Paleogene amber

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**A piece of Paleogene Bitterfeld amber includes a fossilized lichen with marks of grazing and fecal pellets. The morphological characters of the fossil lichen suggest an affiliation to the extant genus *Phyllopsora*. Comparison of the grazing marks and fecal pellets of the fossil lichen with extant material suggests gastropods and/or mites as the most likely grazers of the fossil lichen.**

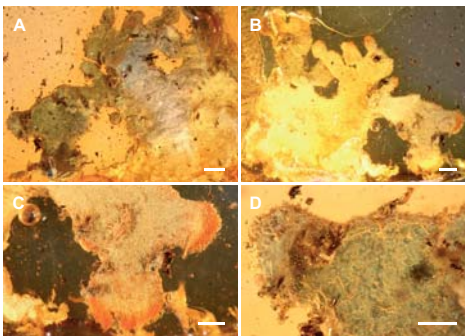


Figure 1. **Lichen fossil.** General appearance of the A) upper and B) lower sides (scale bars 0.5 mm). The most distinctive features include C) the lack of lower cortex and the presence of pseudocortex-like structure, the orange hue of the lobe tips (scale bar 0.3 mm), and D) the robust hyphae evolving around the thallus margins (scale bar 0.2 mm).

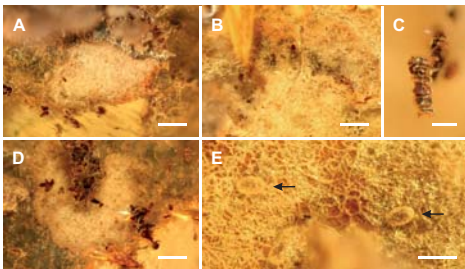


Figure 2. **Fossil feeding marks and fecal pellets.** A, B & D) Feeding marks on the upper side of the fossil lichen (scale bars 0.1 mm in A & B, and 0.2 mm in D). C) Spindle-shaped and E) oval (marked with black arrows) fossil putative fecal pellets (scale bars 0.1 mm).

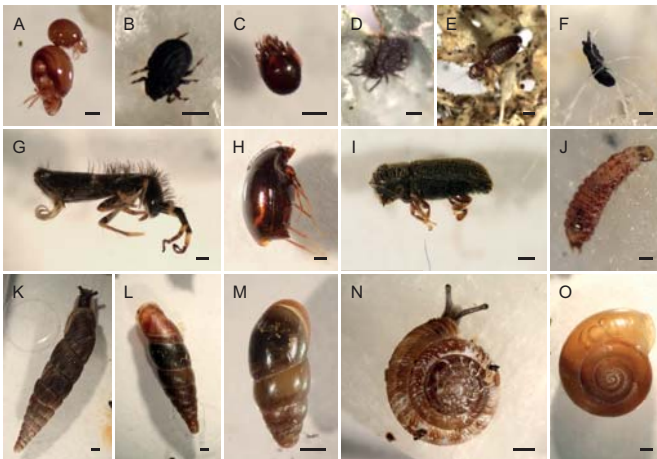


Figure 3. Arthropods in the experiment included mites A) Phthiracaridae (Oribatidae), B) Carabodoidae (Oribatidae), C) Euzetidae (Oribatidae), and D) an unidentified Acaridae; E) booklice Liposcelidae (Psocoptera); springtails (Collembola) of the F) Poduridae and G) Entomobryidae; beetles (Coleoptera) H) Coccinellidae, I) Scolytidae, and J) coleopteran larvae (scale bars 200  $\mu$ m). Gastropods in the experiment included snails from several families K) *Laciniaria* cf. *biplicata* (Clausiliidae), L) *Cochlodina* cf. *laminata* (Clausiliidae), M) cf. *Cochlicopa lubrica* (Cochlicopidae), N) *Discus rotundatus* (Discidae), and O) cf. *Helicigona lapicida* (Helicidae; scale bars 1 mm).

**Amber** is fossilized resin and famous for its lifelike preservation of arthropods, plant remains, and microorganisms. The fossil record of many lineages of fungi, plants, and arthropods is actually restricted to amber inclusions and even the preservation of soft-bodied microorganisms may have cellular and ultrastructural fidelity. Bitterfeld amber has a minimum age of 23–24 million years and it is excavated from the Bitterfeld brown coal mines in eastern Germany.

## Actuopaleontological experiment

1. Fresh lichens were prepared by freezing to kill any existing animals. After freezing the lichens were studied under a microscope. Pieces with undamaged surface were selected for the experiment and documented and imaged from both sides.
2. Small arthropods and gastropods (Fig. 3) were collected and placed in experiment boxes with moistened pieces of lichen. The experiment was continued for several weeks.
3. After the experiment the grazers were conserved in 70% ethanol and lichens examined under a dissection microscope. The general appearance, size, shape, margins, and the appearance of medulla were examined, imaged (Figs 4 and 5), and compared with the fossilized grazing marks (Fig. 2A, B and D).
4. The size, shape, characteristics, and content of fresh fecal pellets (Fig. 6) produced during the grazing experiment were imaged and compared with the potential fossilized fecal pellets (Fig. 2C and E).

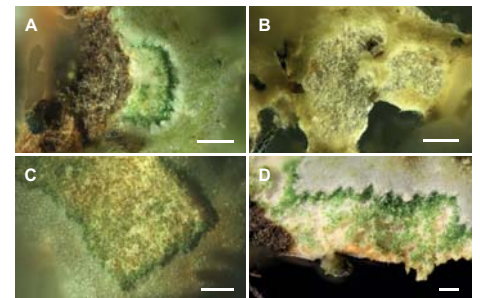


Figure 4. **Feeding marks of extant arthropods.** A) Mite grazing damage on the upper side of *Parmelia* sp. with regular incisions are visible. B) Poduridae grazing damage on the upper side of *Physcia* sp. D) Psocopteran grazing damage on the upper side of *Parmelia* sp. E) Entomobryidae grazing damage on the upper side of *Parmelia* sp. with regular incisions. Scale bars 0.1 mm.

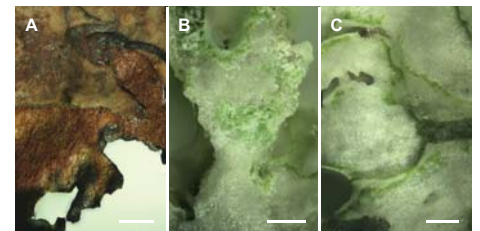


Figure 5. **Feeding marks of extant gastropods.** A) Grazing damage of *Laciniaria* cf. *biplicata* on the upper side of *Parmelia* sp. B) Grazing damage of *Discus rotundatus* on the upper side of *Physcia* sp. C) Grazing damage of *Cochlodina* cf. *laminata* on the upper side of *Physcia* sp. Scale bars 0.2 mm.

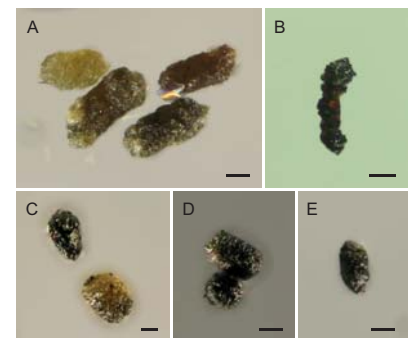


Figure 6. **Fecal pellets of extant arthropods** harvested during the actuopaleontological experiment. A) Barrel-shaped fecal pellets of the springtail Entomobryidae, B) spindle-like fecal pellet of the coleopteran larvae, C) oval fecal pellets of the booklice (Psocoptera), D) barrel-shaped and globular pellets of the springtail Poduridae, and E) oval fecal pellet of a mite. Scale bars 50  $\mu$ m in A and B, and 30  $\mu$ m in C–E.