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Lejeuneaceae (Marchantiophyta) from a species-rich taphocoenosis in Miocene Mexican amber, with a review of liverworts fossilised in amber

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ABSTRACT

We describe a diverse taphocoenosis in a piece of Mexican amber that includes two morphotypes assignable to the leafy liverwort family Lejeuneaceae, an angiosperm seed, a putative bud scale, dematiaceous hyphomycetes as well as dipteran and hymenopteran insects belonging to Phoridae (genera *Megaselia*, *Puliciphora*, and *Apocephalus*), Cecidomyiidae, Psychodidae and Mymaridae (genus *Alaptus*). Liverworts are known from eight amber deposits but have only rarely been observed in Mexican amber. A perianth-bearing liverwort gametophyte in the piece of amber is classified as *Mastigolejeunea extincta* sp. nov., and several sterile gametophytes are described as *Ceratolejeunea sublaetefusca* sp. nov. With these new species, approximately 60 liverworts have been described from amber to date. Remarkable syninclusions include a hyphomycete which is the first darkly-pigmented filamentous microfungus with clear reproductive structures reported from Mexican amber. Fourier Transform Infrared Spectroscopy of a fragment of the investigated amber piece revealed a "Type I" Mexican amber. A whole solubility test suggested a mature resin, although the resin was not much oxidized during the process of amberization. Available evidence suggests that the amber was produced by a *Hymenaea* tree and that the resin was exposed on its bark, behaving as a sticky trap for insects and vascular plant fragments, and also embedding some epiphytic liverworts and fungi.

1. Introduction

Liverworts, mosses, and hornworts are non-vascular land plants collectively known as bryophytes. Liverworts or Marchantiophyta play a crucial role in land plant evolution, having diverged from other lineages during the earliest diversification of plants on land (Kenrick and Crane, 1997; Wellman et al., 2003; Groth-Malonek et al., 2005; Qiu et al., 2006; Fiz-Palacios et al., 2011; Kenrick et al., 2012; Cox et al., 2014; Wickett et al., 2014). Extant liverworts are distributed worldwide and currently comprise around 7,500 species (von Konrat et al., 2010). Liverworts occur in a wide range of habitats and are especially common in humid tropical forests, which are considered of prime importance for carbon dioxide fixation and climate stability (Goodman and Herold, 2014). Combined with mosses, liverworts can make up more of the photosynthetically active (i.e., green) epiphytic biomass in these forests than all the other plant groups put together (Hofstede et al., 1993). Moreover, liverworts are sensitive to ecological change and have been employed as indicators of air pollution (Wielgolaski, 1975), water pollution (Whitton et al., 1982) and of climate change (Gignac, 2001). Finally, liverworts contain many novel organic compounds with antitumour, antiviral and antimicrobial properties as well as insecticidal, piscicidal and cytotoxic activity (Asakawa, 2001; Asakawa et al., 2013).

Despite their ecological, phylogenetic and chemical importance, many open questions remain in the study of liverworts. Little is known about their morphological evolution through time and only a few well preserved fossils are known from the Palaeozoic and the Mesozoic (Oostendorp, 1987). The situation turns for the better in the Cenozoic

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but most geographical regions are essentially poor in liverwort fossils. The Neotropics are a hotspot of extant liverwort diversity (Gradstein et al., 2001); however, the past diversity of liverworts in the region is still incompletely known, owing to their sparse fossil record. The majority of tropical American liverwort fossils is preserved in Miocene Dominican amber (15–20 Ma, Iturralde-Vinent and MacPhee, 1996). This fossilized resin originated from trees of the Fabaceae genus *Hymenaea* L. (Poinar, 1991; Langenheim, 1995) and has so far yielded fossils assigned to 16 extant genera of the largely epiphytic leafy liverwort family Lejeuneaceae as well as to *Frullania* Raddi (Frullaniaceae), *Radula* Dumortier (Radulaceae) and *Bazzania* Gray (Lepidoziaceae) (Grolle, 1984a, 1984b, 1987, 1990, 1993a, 1993b; Grolle and Braune, 1988; Gradstein, 1993; Rikkinen and Poinar, 2008; Heinrichs and Schmidt, 2010; Reiner-Drehwald et al., 2012). The Dominican liverwort fossils thus point to generic stasis of the regional epiphytic diversity since the Miocene. Study of epiphytic mosses (Frahm and Newton, 2005) and ferns (Gomez, 1982; Lóriga et al., 2014; Schneider et al., 2015) provided comparable results, strongly suggesting stability in the gross morphology of local cryptogams. However, it is unclear whether this situation in epiphytic communities in the Caribbean is an exception for the Neotropics. Thus, it is desirable to reconstruct the species composition of other neotropical epiphytic communities of the Miocene.

A promising contemporaneously formed amber comes from Mexico (Solórzano Kraemer, 2010). It derives from the same angiospermous genus as Dominican amber but so far has yielded only very few botanical inclusions. The only bryophytes described from Mexican amber are the leafy liverworts *Lejeunea palaeomexicana* Grolle (Grolle, 1984c), *Ceratolejeunea antiqua* Heinrichs et Schäfer-Verwimp (Heinrichs et al., 2014a), and two further poorly preserved inclusions tentatively assigned to the genus *Mastigolejeunea* (Spruce) Stephani (Scheben et al., 2014).

Here we present an exceptionally species-rich piece of Miocene Mexican amber in which a large number of botanical and zoological inclusions are preserved, including two species of Lejeuneaceae, an angiosperm seed, a fragment of a putative bud scale, fungi as well as various hymenopteran and dipteran insects. Our study leads to the description of two new liverwort species and provides further evidence for dominance of the Lejeuneaceae in both Miocene and extant Caribbean epiphytic liverwort floras. In addition, we discuss the entire amber fossil record of liverworts as well as problems in the classification of incompletely preserved Neogene fossils resembling extant species.

2. Material and methods

2.1. Initial preparation and light microscopy

A 3.3 × 1.7 × 0.6 cm sized drop-shaped piece of Miocene Mexican amber with several biological inclusions of the Matthias Svojtka collection (Plate I, 1) was investigated. The piece is now part of the Mexican amber collection of the Natural History Museum Stuttgart (SMNS Mx-443). The resin surface was polished manually with a series of wet silicon carbide abrasive papers [grit from FEPA P 600–4000 (25.8 μm to 5 μm particle size), firm Struers] to minimize light scattering during the investigation. The prepared specimen was placed on a glass microscope slide with a drop of water applied to the upper surface of the amber, and covered with a glass coverslip. Inclusions were analyzed with a Carl Zeiss Stemi 2000 dissection microscope and a Carl Zeiss AxioScope A1 compound microscope, each equipped with a Canon 60D digital camera. In most instances, incident and transmitted light were used simultaneously. Oblique incident light was obtained using a goose-neck light guide 200 of a Carl Zeiss CL 1500 Eco cold light source. All presented illustrations are digitally stacked photomicrographic composites of up to 70 individual focal planes obtained by using the software package HeliconFocus 5.0.

2.2. Resin analyses

A 5 mm long fragment from the narrow end of the amber piece was separated for Fourier Transform Infrared Spectroscopy (FTIR) analyses. FTIR analyses were performed to elucidate whether it represents Miocene amber produced by angiosperm trees related to the genus *Hymenaea* or rather a piece of subfossil resin (copal). FTIR is a standard procedure for a general characterization of extant and fossil resins (Beck et al., 1964; Langenheim and Beck, 1965). FTIR analysis was performed on the freshly powdered specimen, dispersed in a potassium bromide pellet using a Perkin Elmer 1600 Series FTIR Spectrophotometer (Perkin Elmer, Monza, Italy) with a wavelength range of 2–15 μm (5000–670 cm^{-1}) (transmittance mode). Solubility of the resin in organic solvents was determined by exposing the specimen to a small drop of solvent (acetone) for 30 s, and looking for any dissolution of the surface (Roghi et al., 2006). A whole solubility test was performed with a minute part of the fragments obtained as abrasion. These were kept in acetone for 12 hours.

3. Results

3.1. Resin analyses

The main features of the FTIR spectrum obtained from the separated resin piece (Fig. 1) are summarized in Table 1. They correspond to reference spectra of *Hymenaea* and Mexican amber. The acetone test led to a slight surface dissolution of the resin, but in the whole solubility test, the sample still remained as compact mass and did not completely dissolve.

3.2. Systematic palaeontology

3.2.1. Liverworts

All liverwort inclusions possess underleaves as well as incubously inserted, entire, complicate bilobed leaves being divided into a lobe and a lobule, with the ventral lobule folded against the dorsal lobe. Thus, they clearly belong to Lejeuneaceae. Two different morphotypes are present: a single perianth-bearing shoot with undivided ("holostipous") underleaves and a ventral merophyte 4–6 cells wide

Plate I. Mexican amber specimen SMNS Mx-443. Scale bars: 1 mm (1), and 200 μm (2–12).

1 Overview with holotypes of the liverworts *Mastigolejeunea extincta* (with perianth) and *Ceratolejeunea sublaetefusca* visible in the upper left corner of the image.
2 Liverworts *Mastigolejeunea extincta* (right) and *Ceratolejeunea sublaetefusca* (left).
3 Dematiaceous hyphomycete on a leaf lobe of *Ceratolejeunea sublaetefusca*.
4 Winged angiosperm seed.
5 Putative bud scale of seed plant.
6 Female individual of *Megaselia* sp. (Phoridae).
7 and 8 Male individuals of *Megaselia* sp. (Phoridae).
9 Male individual of *Apocephalus* sp. (Phoridae).
10 Male individual of *Puliciphora* sp. (Phoridae).
11 Female individual of Psychodidae, probably *Psychoda* sp.
12 Male individual of *Alaptus* (Mymaridae).