

# The sporophyte of the Paleogene liverwort *Frullania varians* Caspary

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## Abstract

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## Key Words

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We document the sporophyte of the extinct *Frullania varians* based on an inclusion in Late Oligocene Bitterfeld amber from Germany. The sporophyte consists of a short, ca. 45 µm thick seta that exceeds the perianth only slightly; the elongate-ovate, acute valves of the opened capsule are about 225 µm long, curved backwards and consist of an epidermal and an internal layer. Cell walls of both layers possess nodulose trigones. Several trumpet-shaped, unispiral elaters are fixed to the upper third of the internal valve layer. They have a length of ca. 150 µm and a diameter of 15–18 µm. A subglobose structure of 19 µm diameter is interpreted as a degraded spore. Fossil elaters and spores as well as capsule wall details of Frullaniaceae are described for the first time.

## Introduction

Numerous inclusions of leafy liverworts (Jungermanniiidae) have been recognized in Eocene Baltic and Late Oligocene Bitterfeld amber but well preserved sporophytes are exceedingly rare (Grolle & Meister 2004; see Standke 1998; Blumenstengel 2004; and Standke 2008 for stratigraphic assignments of the amber deposits). Liverwort sporophytes are delicate structures with fragile setae, and many extant species produce them only infrequently. Their infrequent occurrence and short lifetime may explain their rarity in the liverwort fossil record.

Here we describe the sporophyte of *Frullania* (subg. *Microfrullania*) *variens* Caspary, 1887, based on a liverwort inclusion in a piece of Bitterfeld amber. With more than 300 extant species (von Konrat et al. 2012), *Frullania* Raddi, 1818, is the largest genus of the Porellales, the epiphyte clade of the leafy liverworts (Heinrichs et al. 2005). *Frullania* is also the most diverse genus preserved in Baltic and Bitterfeld amber. Some

25 species have been described from these ambers, of which nine are currently accepted (Grolle 1985, 2003; Grolle & Meister 2004; Heinrichs et al. 2012). *Frullania* is characterized by its often reddish pigmentation, incubous leaves divided in a dorsal lobe, a ventral laminar stylus and a ventral lobule often forming a water-sac, rhizoids originating at the base of usually bilobed underleaves, male inflorescences located on short, almost leafless spicate branches, and beaked perianths often provided with ridges and tubercles (Schuster 1992; Hentschel et al. 2009). The supraspecific classification of *Frullania* is largely based on gametophytic characters (von Konrat & Braggins 2001a); however, Schuster (1992) and von Konrat et al. (1999) emphasized the importance of sporophytic characters for the classification of *Frullania*. Fossil sporophytes have so far been recognized only in the Eocene species *Frullania baltica* Grolle, 1985 (Grolle 1998) and *F. schumannii* (Casp.) Grolle, 1981 (Grolle & Meister 2004). However, their state of preservation only allowed for appraisals of their dimensions; details of the wall struc-

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tures and the content of the capsules remain unknown. In contrast, the newly described *Frullania varians* sporophyte allows for a description of elaters, spores, and some details of the capsule wall.

## Material and methods

Bitterfeld amber pieces (Grabenhorst collection) with inclusions of *Frullania* were ground and 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 for the investigation. All inclusions were studied using an incident-light microscope (Carl Zeiss Stemi 2000) and a transmitted-light microscope (Carl Zeiss AxioScope A1), each equipped with a Canon 450D digital camera. In some instances, incident and transmitted light were used simultaneously. The images of Figures 1A–G were obtained from several optical sections using the software package HeliconFocus 5.0 for a better illustration of the three-dimensional inclusions. Identification of the fossil plant material was carried out with the keys and descriptions of Grolle (1985) and Grolle & Meister (2004); in some cases the inclusions were already identified by the late Riclef Grolle. Specimens of extant *Frullania* species from the following herbaria were examined using light microscopy and scanning electron microscopy as described by von Konrat et al. (1999) and von Konrat & Braggins (2001a, b): Auckland War Memorial Museum, New Zealand (AK), University of Auckland, New Zealand (AKU), Natural History Museum London, UK (BM), Australian National Herbarium Canberra, Australia (CANB), Bairro Universitário, Criciúma, Brazil (CRI), Field Museum of Natural History, Chicago, USA (F), Conservatoire et Jardin botaniques de la Ville de Genève (G), University of Göttingen, Germany (GOET), Royal Botanic Gardens Melbourne, Australia (MEL), University of Melbourne, Australia (MELU), Massey University, Palmerston North, New Zealand (MPN), Hattori Botanical Laboratory, Nichinan, Japan (NICH), New York Botanical Garden, New York, USA (NY), Muséum National d'Histoire Naturelle, Paris, France (P), Swedish Museum of Natural History, Stockholm, Sweden (S), Museum of New Zealand, Wellington, New Zealand (WELT) and University of Washington, Seattle, Washington (WTU).

## Results

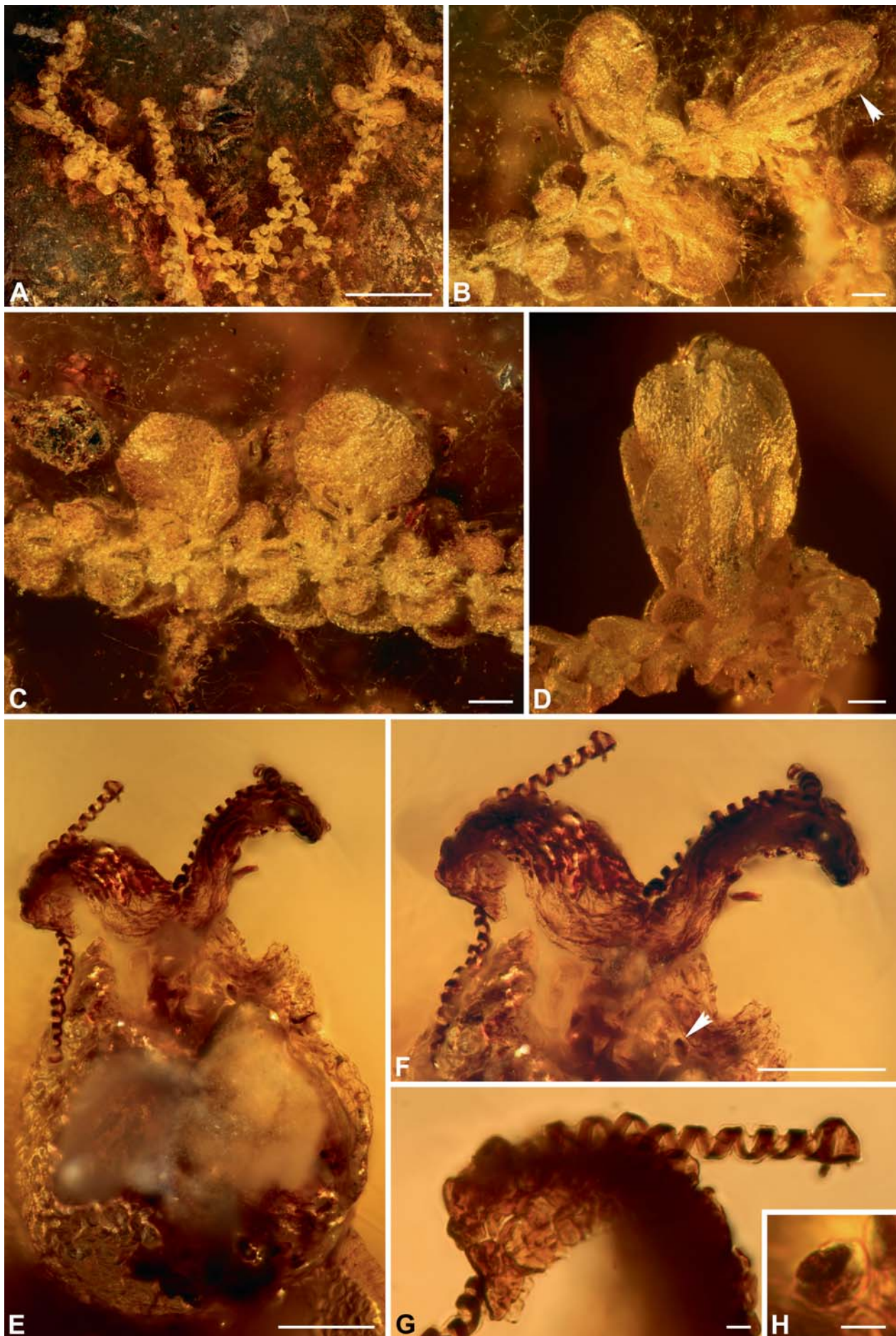
The inspection of some 30 amber inclusions of liverworts revealed several new fossils assignable to *Frullania varians*. Besides several sterile gametophytes we recognized autoecious gametophytes with short, bud-like androecia and at best indistinctly beaked perianths with entire-margined gynoeical leaves and underleaves (Figs 1A–D). Bilobed, laterally ciliate underleaves were seen on several shoots (Fig. 1C). Amber piece Le 69 of

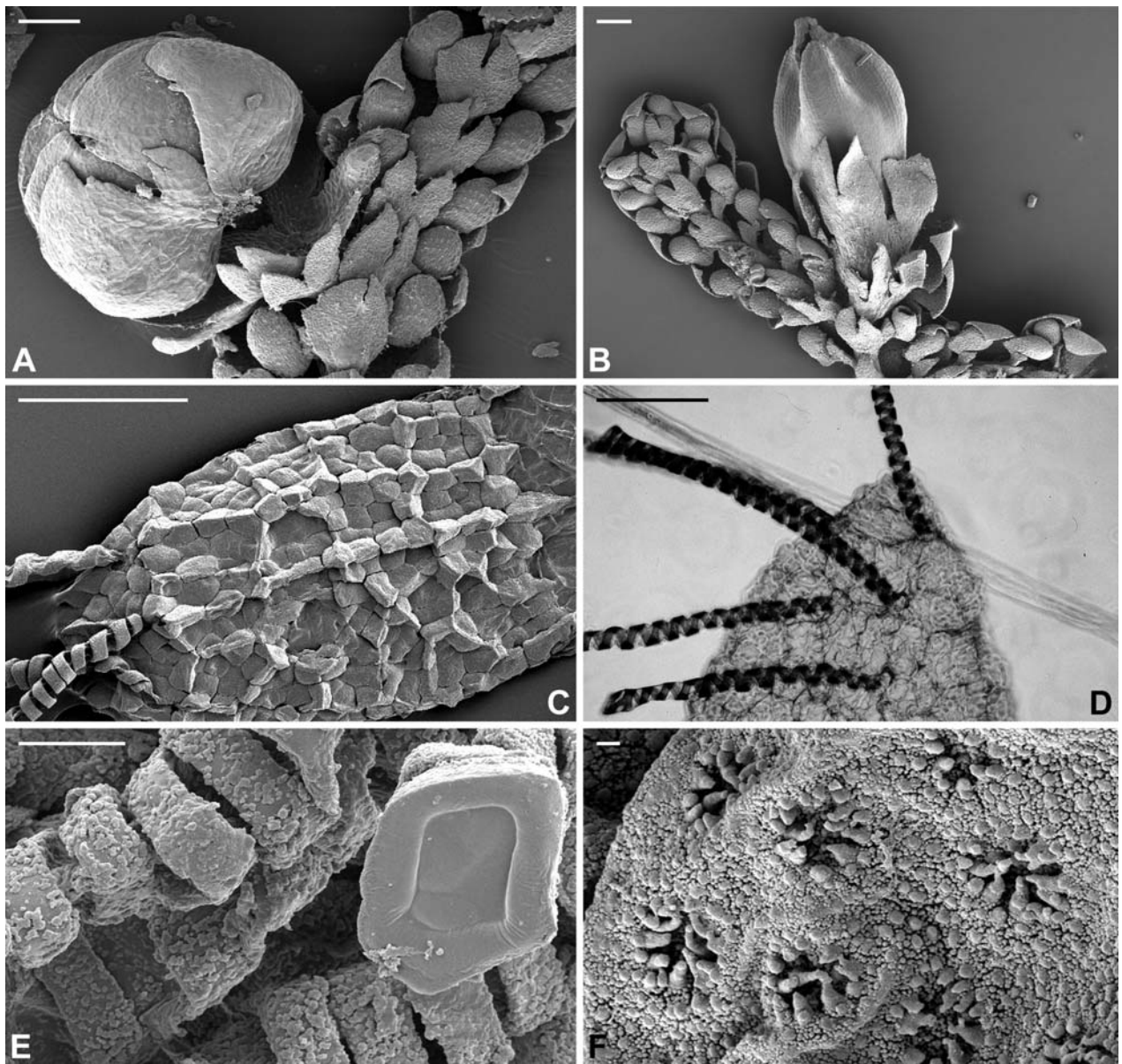
the Grabenhorst collection comprises a few shoots of *F. varians* with a single sporophyte that exceeds the perianth only slightly. Both the sporophyte and the perianth under it were slightly damaged by former grinding and polishing; thus parts of the perianth wall and the sporophyte capsule were removed. However, the ca. 45 µm thick seta and two valves of the opened capsule are still visible (Figs 1E, F). The elongate-ovate, acute valves have a length of 225 µm, and are curved backwards; their maximum diameter cannot be determined because of their embedding situation, and may be larger than 80 µm. Their epidermal layer has a brownish colour; cellular details are visible in the apical part of one valve (Fig. 1G). The apical epidermal cell has a triangular shape, the subtending epidermal cells are more or less rectangular with indistinctly visible corner thickenings in the form of short, rounded trigones extending towards the centre of the cell, and more elongate intermediate thickenings, possibly extending over the outer cell wall. The internal layer of the capsule valves is visible for the lower 2/3 of one valve, and consists of +/- rectangular to elongate rectangular cells with irregularly shaped coarse wall thickenings; the basalmost cells without wall thickenings. Cells of the internal layers seem to form low ridges, however, it is possible that these structures are artifacts from the embedding process within resin. Seven unbranched, trumpet-shaped, unispiral, smooth elaters are visible with their apical ends fixed to the upper third of the internal layer of the valves (one valve with two, one with five elaters; Figs 1E–G). They have a length of ca. 150 µm and a diameter of 15–18 µm (central part). A subglobose structure with a diameter of 19 µm at the perianth mouth likely represents a spore (Fig. 1H); details of the spore surface are not visible.

## Discussion

The extinct species *Frullania varians* is the most abundant liverwort of the Paleogene Baltic and Bitterfeld amber forests (Grolle & Meister 2004). It has been assigned to the extant Southern hemispheric *Frullania* subg. *Microfrullania* (R. M. Schust.) R. M. Schust., 1982 (Figs 2A–D, F) on account of its minute size, *Lejeunea*-type branches, equally thickened, subquadrate leaf lobe cells, and entire-margined gynoeical leaves and underleaves (Grolle 1985). On the other hand, *Fru-*

**Figure 1.** Inclusions of the leafy liverwort *Frullania varians* Caspary in Bitterfeld amber from latest Oligocene strata of central Germany. **A.** Overview showing several gametophytes in ventral view; **B.** Autoecious gametophyte with a perianth (arrowhead) and two male inflorescences on short, lateral branches (ventral view); **C.** Portion of gametophyte with two lateral, bud-like androecia (ventral view); **D.** Portion of shoot (ventral view) with a perianth and entire-margined gynoeical leaves and underleaf; **E.** Perianth and sporophyte; two valves are visible, each with several trumpet shaped, unispiral elaters with their apical ends fixed to the inner layer of the valves; **F.** Ibid, close up with arrowhead pointing to a degraded spore; **G.** Recurved valve with two elaters; the epidermal cells with indistinctly visible corner thickenings in the form of short, rounded trigones extending towards the center of the cell, and more elongate intermediate thickenings, possibly extending over the outer wall of the cell; **H.** Degraded spore. A–C: Grabenhorst collection Le 41; D–H: Grabenhorst collection Le 69. Scale bars 1 mm in A, 100 µm in B–F, and 10 µm in G and H.





**Figure 2.** Morphology of extant *Frullania*. **A.** Part of shoot with androecium, ventral view; **B.** Part of shoot with gynoecium, ventral view; **C.** Internal layer of capsule valve with ridges and three elaters attached to upper third of valve; **D.** Upper third of valve with five elaters; **E.** Parts of unispiral elaters; end of one elater visible in the upper right part; **F.** Detail of spore with rosettes. **A, C:** *Frullania* (subg. *Microfrullania*) *lobulata* (Hook.) Dumort., 1835; **B, D and F:** *Frullania* (subg. *Microfrullania*) *rostrata* (Hook.f. & Taylor) Hook.f. & Taylor, 1845, and **E:** *Frullania* (subg. *Frullania*) *incumbens* Mitt., 1855. Scale bars 100  $\mu\text{m}$  in **A–D**, 10  $\mu\text{m}$  in **E**, and 1  $\mu\text{m}$  in **F**.

*lania varians* has narrow styli and helmet-shaped water-sacs that are oriented parallel to the stem, with low distance, resembling those of the extant *Frullania* (subg. *Frullania*) *fugax* (Hook. F. & Taylor) Gottsche, 1845 (Hentschel et al. 2009) rather than extant representatives of subg. *Microfrullania*. Classification systems for extant *Frullania* species can be tested using molecular phylogenies (Hentschel et al. 2009). These phylogenies allow to identify characters that support supraspecific entities as well as homoplasious characters that cannot be used for classification. However, such kinds of data are usually not available for fossil taxa, hence; a certain probability of error must be accepted in taxonomical assessments of fossils. The more complete a morpholo-

gical description of a fossil is, the more reliable its taxonomic treatment.

The newly described sporophyte of *F. varians* allows to further complete the detailed species description of Grolle (1985) and Grolle & Meister (2004), and lends further support to the position of the Paleogene *F. varians* in *F.* subg. *Microfrullania*. The capsule of extant *Frullania* consists of four valves, each possessing an epidermal and an internal layer (Kamimura 1961; Yuza-wa 1991; Schuster 1992; von Konrat et al. 1999). The capsule wall is often heavily pigmented and in smaller species, especially those of *F.* subg. *Microfrullania*, the valves may only range from 250–700  $\mu\text{m}$  long and 180–450  $\mu\text{m}$  wide. Both capsule wall layers consist of

cells with variously shaped cell wall thickenings. These characters are present in *F. varians*, which possesses very small valves with a length of only ca. 225 µm. We do not know if the dimensions of the valves would be similar when studied in water or whether the embedding process in amber is connected with some shrinking processes. However, we do not expect extreme size deviations of valves embedded in resin or water respectively; hence the small size of the observed valves further supports an assignment of *F. varians* to *F.* subg. *Microfrullania*. Ridges on the inner capsule wall layer have also been seen in extant representatives of *F.* subg. *Microfrullania* (Fig. 2C).

Like most other liverworts, the capsules of extant *Frullania* species include both spores and elaters. In general, spores of *Frullania* are subglobose with diameters from (20)35–55(70) µm and possess a surface that is covered with rosettes; each rosette is formed of several variously shaped stelae (Schuster 1992; Weis 2001; von Konrat et al. 2011). Stelae of *F.* subg. *Microfrullania* representatives lack secondary branches and deposits (Hentschel et al. 2009). The observed single *F. varians* spore is at the lower end of size variation observed for spores of extant *Frullania*; details of the spore wall are unfortunately not visible. *Frullania* elaters have trumpet-shaped ends and remain connected to the inner layer at the distal end of the valves after spore release. They are nearly all consistently unispiral (Figs 2C–E), however, partly bispiral elaters have been observed in the extant species *F. ericoides* (Nees) Mont., 1839 (Schuster 1992), *F. grandistipula* Lindenb., 1845 (Nath 1984) and *F. incumbens* Mitt., 1855 (Weis 2001 [as *F. "procumbens"*]). The dimensions of the elaters vary from (110–)150–450 µm long and 15–35 µm in diameter (Schuster 1992; von Konrat & Braggins 2001b; Weis 2001). A range in the number of elaters per capsule occurs from 10–20 for *F.* (subg. *Microfrullania*) *microcaulis* Gola, 1923, to 110–120 for *F.* (subg. *Chonanthelia*) *riojaneirensis* (Raddi) Spruce, 1884 (Yuzawa 1991; Schuster 1992; von Konrat et al. 2011); however, the vast majority of *Frullania* species has more than 5 elaters per valve, with low interspecific variation (Schuster 1992). Although it is possible that a few elaters of the studied *F. varians* sporophyte were removed during the embedding process, their low number remains indicative of *F.* subg. *Microfrullania*.

The fragile seta of extant *Frullania* sporophytes exceeds the perianths only slightly and varies in size from the base to the apex. In cross section it is made up of 16–40 rows of epidermal cells and 4–55 rows of internal cells (Hattori & Mizutani 1982; Schuster 1992); representatives of subg. *Microfrullania* possess only some 16–26 rows of epidermal cells and 4–22 rows of internal cells. Although we were unable to count the epidermal cells of the *F. varians* seta, we expect a low number of cells on account of its small diameter.

In summary, the *Frullania varians* sporophyte fits the morphology of *F.* subg. *Microfrullania*, corroborating the importance of sporophytic characters for the su-

praspecific classification of *Frullania* and liverworts in general. *Mastigolejeunea contorta* (Göpp. & Berendt) Gradst. & Grolle, 2004, is the only other liverwort from Baltic or Bitterfeld amber of which some details of capsule walls and elaters have been described (Weis 2001 [as *Trocholejeunea sandvicensis* (Gottsche) Mizutani, 1962]; Grolle et al. 2004). Weis (2001: 112–13) recognized funnel shaped elaters with 1–2 spiral bands and an internal capsule wall layer with plurifenestrate incassations, characters that are also seen in extant *Mastigolejeunea* (Spruce) Schiffner, 1893, species. Additional liverwort sporophytes have been recognized in Paleogene amber inclusions of *Radula oblongifolia* Caspary, 1887 (Grolle 1989) and *Scapania hoffeinsiana* Grolle, 2001 (Grolle & Schmidt 2001), however, preservation of the latter allowed only for descriptions of the capsule shape or valves respectively. It remains to be seen if the increased interest in plant inclusions in amber (Grolle & Meister 2004; Frahm 2010) will lead to the discovery of further fossil liverwort sporophytes, and whether these sporophytes will also match the morphology of extant taxa.

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