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Surface wax in *Dinckleria, Lejeunea* and *Mytilopsis* (Jungermanniidae)

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Abstract – A scanning electron microscope study of leafy liverworts revealed the presence of surface wax in *Lejeunea flava* (Lejeuneaceae), *Mytilopsis albifrons* (Lepidoziaceae), *Dinckleria pleurata* and *D. fruticella* (Plagiochilaceae).

Jungermanniopsida / Lejeuneaceae / Lepidoziaceae / Plagiochilaceae / ultrastructure

INTRODUCTION

Molecular phylogenetic studies pointed to many unexpected relationships within liverworts such as the sister relationship of the leafy Haplomitriales and the thalloid Treubiales (Heinrichs *et al.*, 2005; Forrest *et al.*, 2006; He-Nygrén *et al.*, 2006), the presence of the thalloid liverwort *Mizutania* in the leafy Calypogeiaceae (Masuzaki *et al.*, 2010), or the position of the supposed Jungermanniaceae element *Rovainenia* in *Syzygiella* (Feldberg *et al.*, 2010). The gross morphology of the liverwort gametophyte is often not suited to support such relationships whereas ultrastructural and anatomical features may lend support to newly detected molecular affinities (Renzaglia *et al.*, 2007).

Liverwort surfaces are usually described as being "smooth" or "papillose", however, there is growing evidence that the "papillose" structures visible in the light microscope have different origins and do not always belong to the cell wall. Already Suessenguth (1951) reported wax threads and needles from cushions of the leafy liverwort *Anthelia* (Antheliaceae). These structures (Figs 11-12) are visible with the naked eye, and had earlier been interpreted as fungal hyphae. Other local wax projections look similar to papillae but can be removed with chloroform (Heinrichs *et al.*, 2000). To date, such surface waxes have been reported for at least single representatives of the leafy liverwort families Calypogeiaceae (*Calypogeia*), Plagiochilaceae (*Plagiochila*, *Plagiochilion*), and Scapaniaceae (*Douinia*) (Heinrichs *et al.*, 2002; Pócs, 2005; Pressel *et al.*, 2011). In *Plagiochila*, local wax projections seem to occur only in a single clade, *P.* sect. *Fuscoluteae*, and allow to separate this clade from its sister lineage (Heinrichs, 2002). Statements on the systematic relevance of wax structures in other groups await more comprehensive investigations.

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During revisionary studies in several groups of leafy liverworts we conducted SEM investigations of the gametophyte surface, and detected wax in three further genera. Here we present SEM micrographs of these structures.

MATERIALS AND METHODS

Gametophyte fragments of air-dried herbarium specimens were examined by scanning electron microscopy (SEM) using a ZEISS DMS 960 after fixing on double adhesive tape and sputter coating with gold. In order to determine whether or not surface waxes were present, gametophyte fragments from the same plants were examined by SEM following washing of the shoots in chloroform 1-3 minutes prior to coating. Classification and terminology of surface waxes follows Barthlott *et al.* (1998).

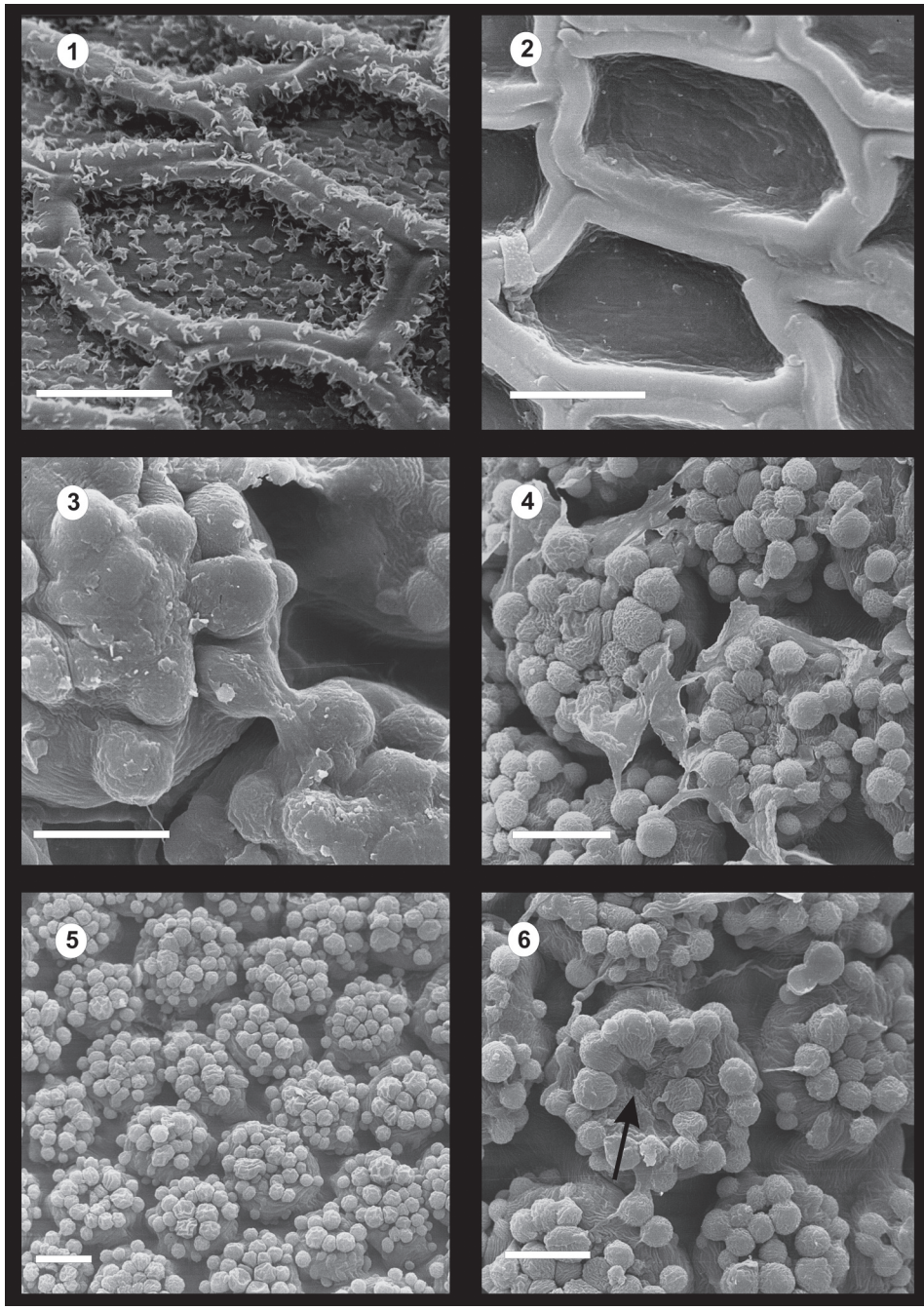
RESULTS AND DISCUSSION

Lejeuneaceae

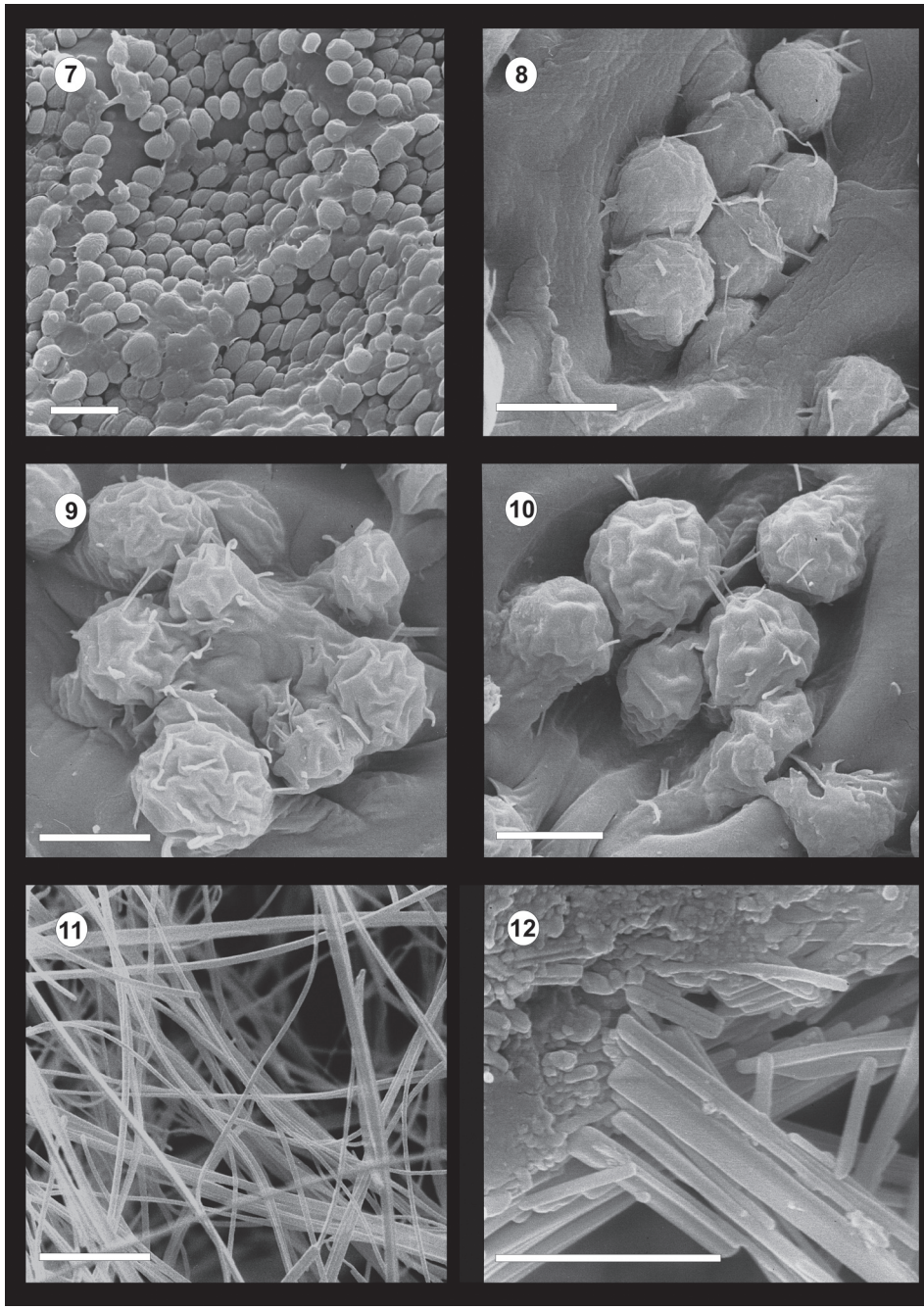
Many representatives of *Lejeunea* have papillose leaf surfaces, e.g. *L. controversa* Gottsche (Reiner-Drehwald & Goda, 2000, Fig. 20c), *L. magohukui* Mizut. (Zhu & So, 2001, Fig. 63l), *L. perpapillosa* M.E.Reiner & Pôrto (Reiner-Drehwald & Pôrto, 2007, Fig. 3), and *L. pulverulenta* (Gottsche *ex* Steph.) M.E.Reiner (Reiner-Drehwald, 2005, Figs. 3-4). The pantropical *Lejeunea flava* (Sw.) Nees is described as having a smooth (Schuster, 1980) or finely papillose cuticle (Paton, 1999). A slightly rough surface is indeed visible in the light microscope; however, our SEM study demonstrates that the papillose structures are wax projections (Fig. 1) that can be removed with chloroform (Fig. 2). Our SEM study indicates that wax is present in the form of membraneous platelets. This is the first record of surface wax in Lejeuneaceae, with an estimated number of 1000 species the largest family of liverworts (Wilson *et al.*, 2007). It remains to be seen if wax is more widely distributed in this family.

Lepidoziaceae

Mytilopsis albifrons Spruce is a small Neotropical species with a strongly papillose outer leaf surface (Figs. 3-6). The strongly protruding papillae are well visible in the light microscope and occasionally peel off from herbarium specimens when studied in water. SEM examination demonstrates that scars remain after detachment (Fig. 6, arrow). Currently it is unclear why *Mytilopsis* discards some of its papillae. Ultrastructural studies of living plants are needed to gain deeper insights into the observed processes. Our study also demonstrates the presence of smooth wax layers (Fig. 4) that come off the papillose outer leaf surface, possibly in consequence of shrinking-stretching processes of the gametophytes through dehydration-moisturization. Occasionally, papillae from different cells are connected by wax coatings that can be removed by chloroform treatment (Fig. 5).



Figs 1-6. **1-2.** Ventral leaf surface of *Lejeunea flava* (*Schäfer-Verwimp 6764*, GOET) showing membraneous wax platelets. **2.** Ibid., washed in chloroform. **3-6.** Outer leaf surface of *Mytilopsis albifrons* (*Gradstein 5375*, GOET). **3.** Wax overlay connecting papillae from two different cells. **4.** Wax overlay peeling off from papillae. **5.** Cell surface washed in chloroform. 6. Cell surface with cavity (arrow), likely as consequence of papilla separation. Scale bars Figs 1-6: 10 µm.



Figs 7-12. **7.** Papillose ventral leaf surface of *Dinckleria fruticella* (*Mues s.n.*, GOET) with wax overlay. **8-10.** Papillose cell surface of *Dinckleria pleurata* (*Schäfer-Verwimp & Verwimp 13777*, GOET) with membraneous wax platelets and polygonal rodlets. **11-12.** Wax rodlets on the gametophytes of *Anthelia julacea* (*Heinrichs s.n.*, GOET). Scale bars Figs 7-12: 5 µm.

Plagiochilaceae

Surface waxes are known from two genera of Plagiochilaceae, *Plagiochila* and *Plagiochilion* (Heinrichs *et al.*, 2000). Our study demonstrates the presence of surface wax in a third genus, *Dinckleria*. Both *Dinckleria* species have a strongly papillose cuticle that is at least partly covered by wax. Smooth wax layers could be demonstrated for *Dinckleria fruticella* (Hook. f. *et* Taylor) J.J. Engel *et* J. Heinrichs (Fig. 7); the leaves of *D. pleurata* (Hook.f. *et* Taylor) Trevis. are partly covered by membraneous wax platelets and rodlets (Figs. 8-10).

Concluding remarks

Our study demonstrates the presence of surface wax in three further genera of leafy liverworts. Wax has now been proven for representatives of five families of Jungermanniales, Antheliaceae, Calypogeiaceae, Lepidoziaceae, Plagiochilaceae, Scapaniaceae and one family of Porellales, Lejeuneaceae (Heinrichs, 2002; Pócs, 2005; Pressel *et al.*, 2011; this study). SEM micrographs in Engel & Schuster (1982, Figs. 36-37) provide evidence for the presence of surface wax in *Brevianthus flavus* (Grolle) J.J. Engel *et* R.M. Schust. of the monospecific family Brevianthaceae (Crandall-Stotler *et al.*, 2009). All these data indicate that surface waxes are much more widespread in leafy liverworts than currently thought. The presence of thin, smooth wax layers is possibly only detectable by SEM studies when papillose species are investigated from which the wax layers peel off. Thin wax films on species with a smooth cuticle are likely not visible by SEM investigation but can possibly be detected by chemical analyses of chloroform extracts (Heinrichs *et al.*, 2002). It remains to be seen if waxes are diffusely distributed in the above families, or if they are restricted to certain lineages.

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