

Development and regeneration ability of the wax coverage in *Nepenthes alata* pitchers: a cryo-SEM approach

Elena V. Gorb*, Martina J. Baum and Stanislav N. Gorb

Supplementary Information

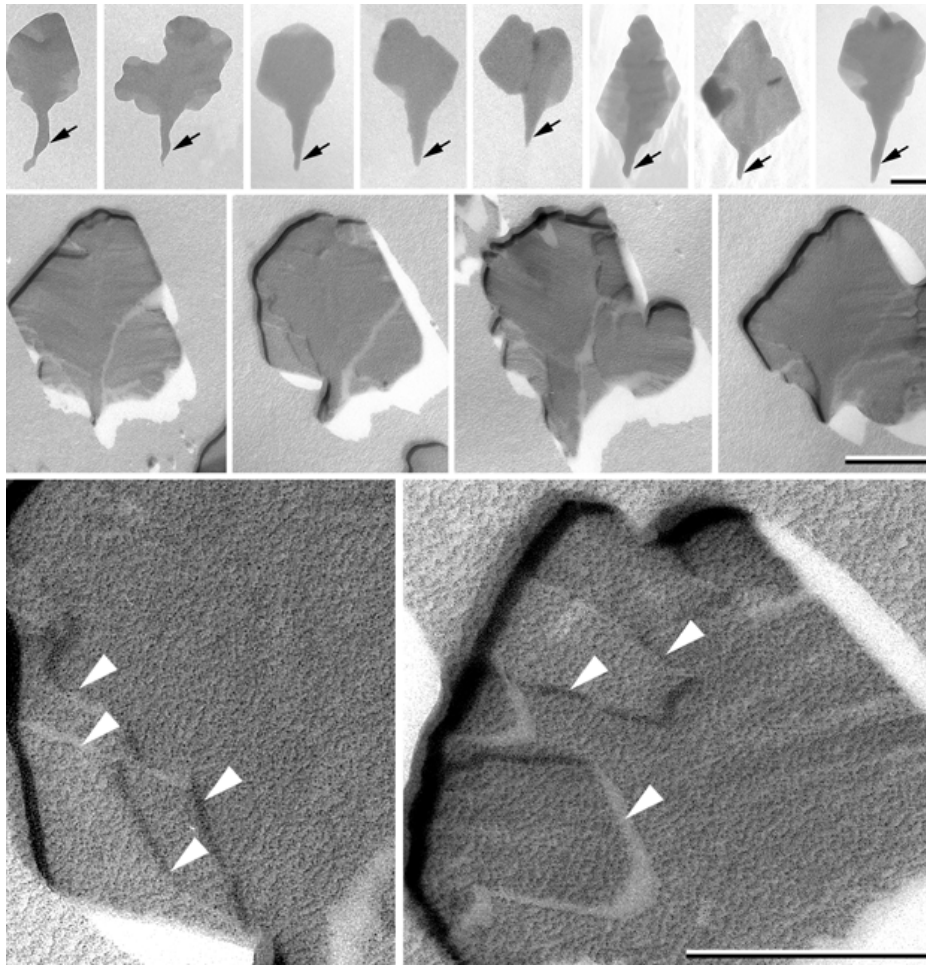
Visualization of isolated wax crystals of the upper wax layer in transmission electron microscope (TEM)

Mechanically detached crystals were also examined by the carbon-platinum coating technique^{S1}. Wax crystals were collected by wiping off the inner surface of the pitcher with a soft paintbrush. The crystals were then transferred onto the copper TEM slit, covered with thin transparent polyolefin film, by shaking the brush over the film. The preparations were coated with carbon-platinum at a shallow angle (about 15°). This method resulted in an increase of image contrast and better appearance of surface structures. The preparations were examined in a Philips CM10 TEM. This method enabled observations of wax crystals from the upper layer^{S2}.

On TEM preparations, crystals consist of multiple layers orientated parallel to each other and to the crystal plane (Supplementary Figure-S1). Each platelet bears a stalk situated in the same plane as the crystal plate. In many cases, tiny pieces broke off of the platelets. Some crystals are partly exfoliated or even broken into several parts^{S2}.

S1. Schwarz, H. & Gorb, S. Method of platinum-carbon coating of ultrathin sections for transmission and scanning electron microscopy: An application for study of biological composites. *Microsc. Res. Tech.* **62**, 218-224 (2003).

S2. Gorb, E., Haas, K., Henrich, A., Enders, S., Barbakadze, N. & Gorb, S. Composite structure of the crystalline epicuticular wax layer of the slippery zone in the pitchers of the carnivorous plant *Nepenthes alata* and its effect on insect attachment. *J. Exp. Biol.* **208**, 4651-4662 (2005).



Supplementary Figure S1. TEM micrographs of isolated wax crystals from the upper wax layer without coating (upper row) and after sputter-coating with carbon-platinum (middle and lower rows). In crystals, depicted in the upper row, stalks are clearly seen. The middle and lower rows show crystals or their fragments, respectively, with characteristic layered structure. Arrows point out stalks of wax crystals. Arrowheads indicate layers of wax in the platelet structure. Scale bars: 500 nm.