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•LETTER TO THE EDITOR•

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Three-dimensional reconstruction of pore canals in the cuticle of the brown planthopper

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Dear Editor,

The insect cuticle is a multifunctional coat that defines and stabilizes the shape of the body, appendages and internal organs, serves as a barrier against water and chemical compounds, such as insecticides, prevents infection and protects against predators (Moussian, 2010). The surface of insects is covered by a lipid layer composed of diverse hydrocarbons, wax esters, fatty acids, fatty alcohols, sterols and triglycerides. Delivery of lipids to the body surface occurs through the cuticle by a nano-canal system consisting of pore canals (PC) and wax canals (WC). Starting from the epidermis, PC run through the inner chitinous layer (procuticle) and ramify in the adjacent layer (epicuticle) as thinner WCs that terminate underneath the surface layer (envelope). The ultrastructure of PCs and WCs has been inferred from 2D sections observed by electron microscopy. Hence, to date, their overall spatial architecture is sketchy. Here, we used focused ion beam scanning electron microscopy (FIB-SEM) to describe directly the 3D architecture of PCs and WCs in the brown planthopper *Nilaparvata lugens*. *N. lugens* lives in paddy fields and is active on the water surface and is therefore very well adapted to varying conditions of humidity. A complex network of genes is involved in producing the surface lipid barrier that enables the survival of *N. lugens* in its humid environment (Li et al., 2019b; Li et al., 2019a). Analyses of the structural basis of lipid barrier formation in this insect species, also in insects in general, are crucial for our understanding of how lipids are transported to the cuticle surface.

By microscopic observation, it is evident that *N. lugens* is covered with a white hydrophobic lipid or wax layer on the surface. To test whether the surface of the *N. lugens* abdominal cuticle is uniformly coated by a lipid layer, we applied SEM to examine the abdominal segments of *N. lugens* nymphs. SEM images show that the *N. lugens* surface was covered with a granular and fibrous material, presumably the hydrophobic lipid or wax layer. We did not see any difference between abdominal segments B to I (Figure S1 in Supporting Information). Therefore, we randomly selected the abdominal segment C as the region for FIB-SEM sample preparation in the following section.

Single FIB-SEM images show the ultrastructure of the abdominal ventral cuticles from *N. lugens* (Figure S2a in Supporting Information). The cuticle formed at the apical

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site of a monolayer of polarized epithelial cells is composed of the outermost envelope, the middle epicuticle and the innermost procuticle. Hence, the ultrastructure of *N. lugens* cuticle resembles the cuticle ultrastructure of other well-studied insect species including *Locusta migratoria*, *Drosophila melanogaster* and *Tribolium castaneum* (Yu et al., 2016; Noh et al., 2016; Sobala and Adler, 2016). Based on FIB-SEM results, we measured the thickness of the envelope, the epicuticle and the procuticle. On average, they were 0.03±0.006, 0.75±0.11 and 6.09±0.13 μm (n=30), respectively. Inside the procuticle, we observed PCs that were predominantly cut obliquely, appearing as fusiform structures of various lengths in single sections (Figure S2 in Supporting Information). The density of PCs is 1.56 μm^{-2}, and the average distance between PCs is 0.76±0.17 μm (n=24). In *Calpodes*, the distance between PCs was measured to be 0.016 μm (Locke, 1961). In *N. lugens*, PCs are composed of an electron-lucid lumen with a thin canal wall and a filament with an electron-dense margin and a less electron-dense core that is considerably narrower than the canal lumen (see below). We believe that the discrepancy between the lumen and the filament diameter may represent a fixation artefact. The overall organization of PCs in a broad lumen and a narrow filament is seemingly conserved in insects as described in classical work (Locke, 1961; Wigglesworth, 1985). The configuration of the helicoidal structure of *N. lugens* PCs shown in Figure S2 (in Supporting Information) also resembles the configuration of PCs analyzed in more recent work. In the migratory locust *L. migratoria*, for example, PCs with an electron-lucid lumen containing an electron-dense filament have been described in the nymphal and adult cuticle (Yu et al., 2016). Likewise, in the *T. castaneum* larval cuticle, there are a large number of somewhat twisted corkscrew-shaped PCs that cross the horizontal laminae in a transverse direction in an apparently helical path (Noh et al., 2014).

As in many other insect species (Wigglesworth, 1990; Locke, 1961), the filament probably consists of lipids and associated proteins. Analyses of consecutive sections revealed that the PC filament is continuous with the apical plasma membrane of epidermal cells (Figure 1J and K). Occasionally, we observed vesicle-like structures in the cytoplasm associated with the filament. Thus, in our model, PCs originate in the cytoplasm, cross the apical plasma membrane and run to the cuticle surface. In contrast to the filament, the electron-lucid lumen abuts on the plasma membrane without fusing with it (Figure 1L and M). In *T. castaneum*, the margin of chitin-containing PCs in the elytral cuticle is continuous with the apical plasma membrane, while the filament (chitin) is not (Noh et al., 2018). We conclude that the lipid-transporting and chitin-containing PCs are different structures.

Projection of FIB sections allowed reconstruction of continuous PCs (Figure 1; Figure S1 and Video S1 in Supporting Information). As in many examples in the literature (Locke, 1961; Wigglesworth, 1985; Noh et al., 2014), PCs in *N. lugens* traverse the cuticle helicoidally from the apical plasma membrane of the epithelial cell layer to the epicuticle. Their shape is not uniform along their vertical axis. At their basis where they emanate from the apical plasma membrane, they are thicker (398.83±113.62 nm, n=18) than at their tips (96.86±17.51 nm, n=18) (Table S1 in Supporting Information). The diameter of the electron-dense filament in the PC lumen follows these changes in diameter: we measured a

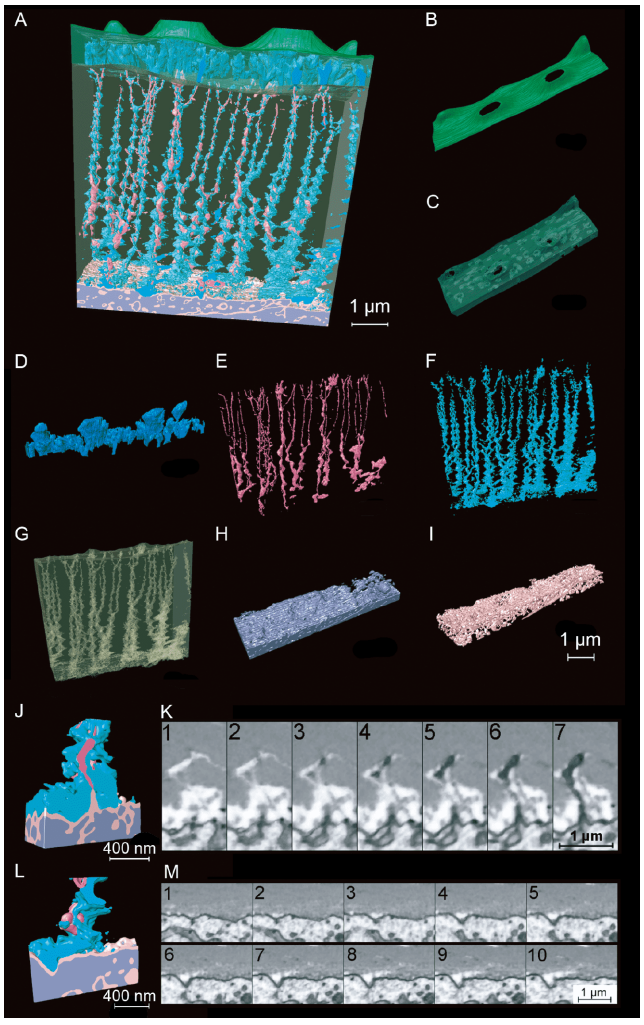

Figure 1 Ventral cuticle organization. A, Overview of the structural model. The structural components are in different colors, following the color key presented in Figure S2 in Supporting Information. B to I, Model of the separated components. In the assembled cuticle model, half of the envelope (B), the epicuticle (C), the wax canal (D), the pore canal filament (E), the pore canal (F), the procuticle (G), the epithelium (H) and the plasma membrane (I) were removed to clearly present the spatial relationships among the components. J, The connection of PC filament and epithelium. K, Lateral views of sequential, cross-sectional FIB-SEM images of a representative delivery of cuticular lipids from the vesicles in the epithelium. L, The connection of PC lumen and epithelium. M, Lateral views of sequential, cross-sectional FIB-SEM images of a representative connection of PC lumen and epithelium.

diameter of 248.06 nm (±111.41 nm, n=19) and 34.95 nm (±9.63 nm, n=19) at the basis and the tip of PCs, respectively. PCs ramify into numerous narrower WC filaments at the junction of the procuticle and the epicuticle. The WCs eventually terminate at the surface of the envelope that they perforate. These holes are probably the sites of lipid externalization. Locke (1961) reported that WCs fused to form fewer and larger canals as cuticle secretion proceeds. Consistently, WCs that penetrate the epicuticle to at least the outer ridge of the envelope appear in different sizes and shapes (Table S1 in Supporting Information; Figure 1D).

Here, the spatial relationships among the cuticular components and their relative positions within the cuticle were revealed by FIB-SEM and then reconstructed into a 3D model that represents the general structural features of the *N. lugens* cuticle. To the best of our knowledge, this is the first report on the fine structure of insect cuticle displayed in 3D reconstruction and in animated movies rather than in schematic drawings. This 3D structure may serve as a basis for further physico-chemical and mechanical studies on the properties and functions of especially PCs and WCs in an insect cuticle.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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SUPPORTING INFORMATION

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