Ultrastructure and mineral phase distribution in the exoskeleton of the beach isopod *Tylos europaeus* (Crustacea)

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Crustaceans have an exoskeleton, the cuticle, composed of an organic matrix and a mineral phase of mostly calcium carbonate. The cuticle of isopods consists of four main layers: the distal thin epicuticle that is unmineralised, the mineralised exo- and endocuticles in the middle, and the proximal unmineralised membranous layer. The organic phase is organized in a characteristic twisted plywood structure, which is built by stacks of planar arrays of complex chitin-protein fibres [1]. In most isopods the mineral phase consists of calcite, amorphous calcium carbonate (ACC) and minor amounts of amorphous calcium phosphate (ACP) [2]. Calcite occurs in a distinct distal layer that contains little ACC and that corresponds to the exocuticle, whereas the endocuticle contains ACC only [3]. Composition and spatial distribution of organic and inorganic materials within the cuticle of isopods vary between species. These variations are related to the behaviour and habitat of the animal [2]. The nocturnal terrestrial isopod *Tylos europaeus* lives on beaches and burrows into the sand during daytime. Compared to many other isopods like the common woodlouse *Porcellio scaber*, *T. europaeus* has a very thick and hard dorsal cuticle.

We therefore investigated the relation between the structure of the mineral phase and the organic matrix in dorsal cuticular segments (tergites) of *T. europaeus* and *P. scaber* using low voltage STEM, FESEM and Raman spectroscopic imaging.

The tergite cuticle of *T. europaeus* has a very thick calcite layer that contains polyhedral structures (Fig. 1A, B, F). The exocuticle that contains these unusual structures contains only little organic matrix consisting of horizontal strands (Figs. 1 C, D). At the border to the endocuticle, however, the exocuticle forms a rather dense layer of fibres (Figs. 1 E). Raman spectroscopic imaging suggests calcite domains of varying crystal orientation (Figs. 1 F, G). The exocuticular structures differ markedly from those in other isopods, like *P. scaber*. Here, the calcite in the exocuticle forms a smooth and rough layer (Figure 2 A). STEM analysis reveals that the protein fibres are tube-like structures 13 nm in diameter with a 3nm thick core probably consisting of chitin crystals (Figure 2 B, C). [4]

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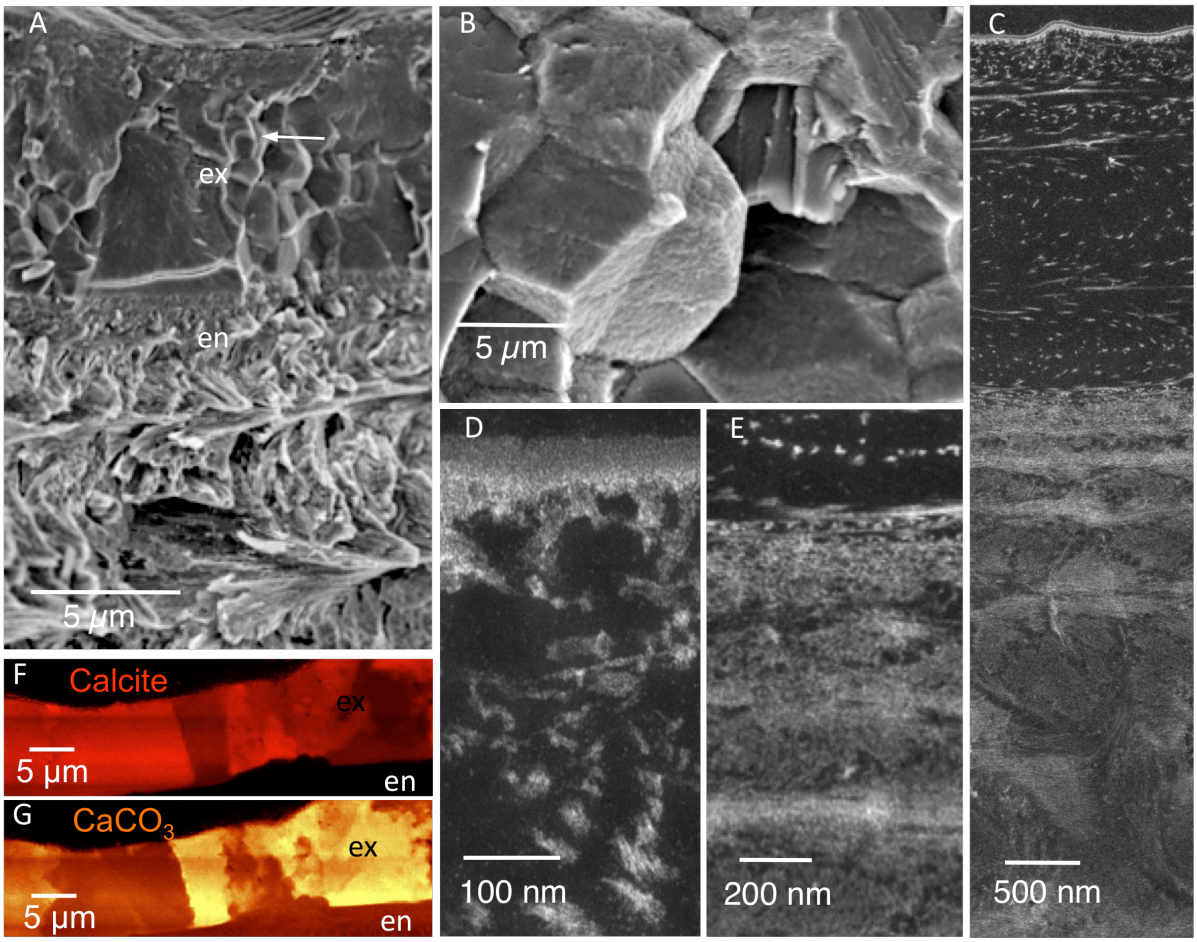

Figure 1. Tergite cuticle of *Tylos europaeus*. (A, B Field-emission SEM of exocuticle). (C-E) STEM micrographs (Hitachi S-5200, 30 kV). (C) Overview of epi- and exocuticle. (D) Detail of distal cuticle (E). Detail of dense layer. (F, G) Raman spectroscopic images showing the distribution of calcite and $CaCO_3$ and total $CaCO_3$.

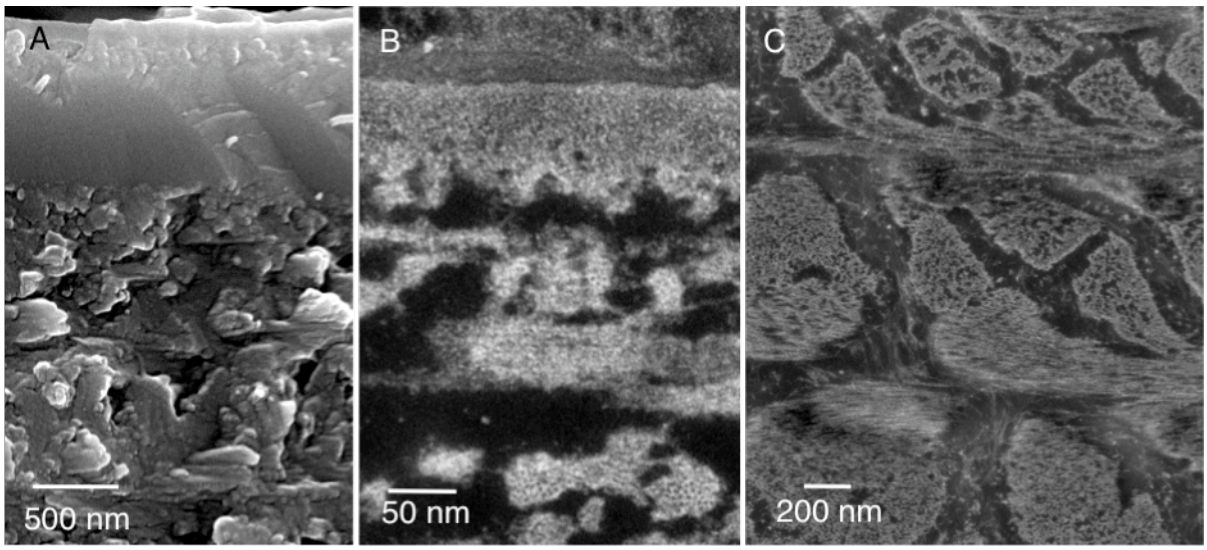

Figure 2. Tergite cuticle of *Porcellio scaber*. (A) Field-emission SEM of exocuticle (modified from [3]). (B, C) STEM micrographs (Hitachi S-5200, 30 kV) of epicuticle, distal exocuticle (B), and proximal exocuticle (C).

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