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Orientation of Organic Fibers and the Presence of Amorphous Calcium Phosphate in Elongated Crustacean Skeletal Elements

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Arthropods are perspective models for the study of biomechanics. Various regions of their mobile exoskeletons are structurally optimized in relation to different functions they perform. The exoskeleton of terrestrial crustaceans is particularly interesting as it is a composite material, incorporating organic fibers embedded in mineralized matrix, mainly containing calcite. Furthermore, life on land demands a sturdier exoskeleton, as air does not offer the buoyancy encountered in the aquatic environment.

We analyzed the large, spine-like sensory setae on the legs of the terrestrial crustacean *Porcellio scaber*, which are likely mechanoreceptors. These are small and elongated exoskeletal elements a few tens of micrometers in size subjected to substantial loading. This necessitates great fracture resistance and their nanoscale structural features may thus inspire biomimetic design of small machine elements subjected to similar loading. In order to elucidate structural and compositional adaptations of the exoskeletal cuticle forming these elements, they were analyzed with scanning electron microscopy (SEM) and (scanning) transmission electron microscopy ((S)TEM) combined with analytical methods. This enable the analysis of the architecture of the organic fibers in the cuticle as well as the composition and structure of the mineralized matrix surrounding them.

To this end, legs were rapidly dehydrated in methanol, embedded in Spurr's resin and sectioned for observations in (S)TEM. The Zeiss SESAM, equipped with an electrostatic Ω-type monochromator and the MANDOLINE filter [1] and JEOL ARM200F STEM microscopes were used to perform electron energy-loss spectroscopy (EELS) in the low-loss range and of the energy-loss near-edge structures (ELNES) at high energy resolution as well as for energy-dispersive X-ray (EDX) analyses. Selected area electron diffraction (SAED) experiments were performed with a Zeiss 912 Omega TEM with an in-column omega energy filter. Alternatively, methanol-fixed samples were air-dried, sputter-coated with platinum and observed with a JEOL JSM-7500F field emission SEM.

The setae are elongated structures with a round cross-section, a hollow core that accommodates the dendrites of sensory cells, and are linked to the leg by a flexible joint membrane. Interestingly, the setal cuticle incorporates several concentric layers with contrasting architectures: the innermost endocuticle, in which the organic fibers are aligned axially, and two layers of the external exocuticle. In the inner exocuticle, fibers shift their orientation helicoidally, whereas in the outer exocuticle, they are axially aligned (Fig. 1). This organization of organic fibers is also reflected in the architecture of the mineral particles, which follow the orientation of the fibers (Fig. 1). Combination of SAED and analytical STEM demonstrated that the joint membrane of the seta is not mineralized and contains very small amounts of Ca. In the shaft of the seta, on the other hand, presence of large amounts of Ca and P was detected. As further demonstrated by the combination of SAED and EDX, as well as by low-loss EELS, the Ca-$L_{2,3}$ and the O-K ELNES of the mineral in the shaft, the predominant mineral polymorph is amorphous calcium phosphate.

The observed structural features together with the presence of amorphous calcium phosphate can be interpreted as adaptations that may increase the fracture-resistance of setae as elongated and thin exoskeletal elements. Layers of the cuticle in which organic fibers are aligned in the axial direction likely increase the axial strength of the setae, preventing buckling, whereas the quasi-isotropic layers increase the perpendicular strength, preventing failure in cases when the setae are loaded from the side. The presence of amorphous calcium phosphate may prevent fracture, as this mineral is less stiff, yet more fracture resistant as compared to calcite [2]. A similar interchange of layers with contrasting organization of fiber orientations has been reported in spider fangs [3]; however, in the case of isopod setae, mineral components are also involved in optimizing the structure's performance.

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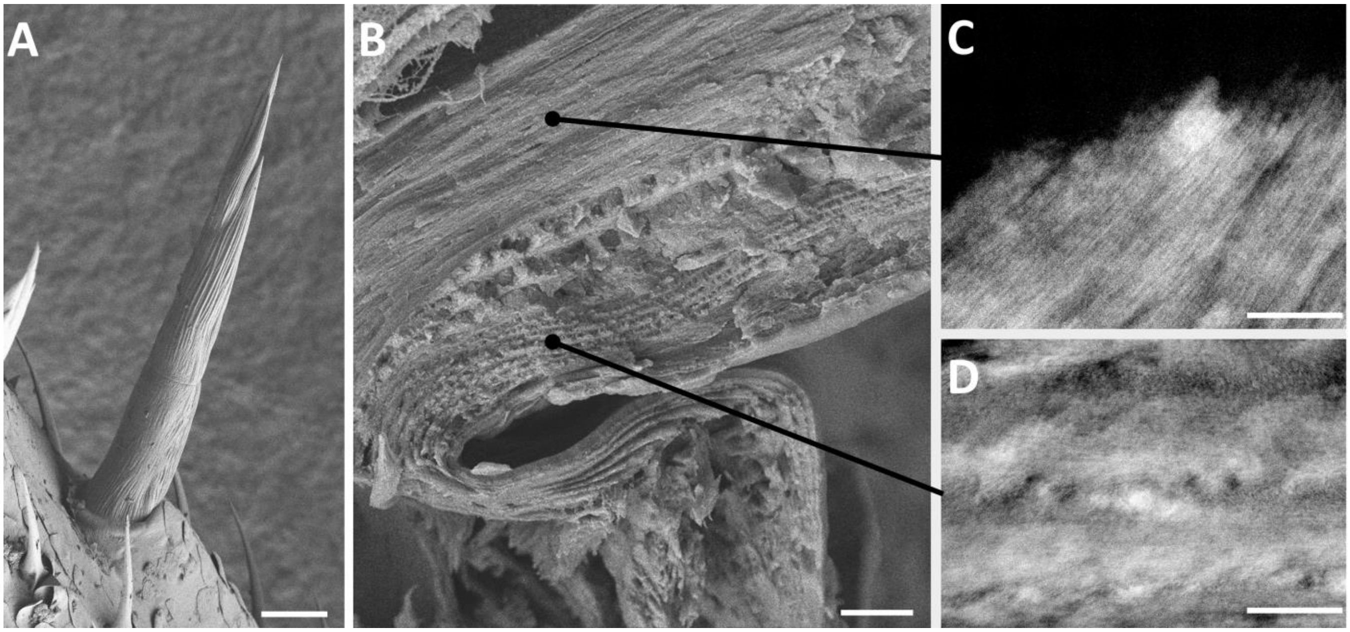

Figure 1. A: SEM image of a seta on the leg of *Porcellio scaber*. **B:** SEM image of a longitudinally fractured seta at its base. **C:** HAADF-STEM image of the mineral particles in the endocuticle, which follow the axial alignment of the organic fibers. **D:** HAADF-STEM image of the mineral in the inner exocuticle. Mineral particles follow the shifting orientation of the helicoidally arranged organic fibers. Scale bars: 20 µm (A); 2 µm (B); 200 nm (C, D).