

## Structure and composition of the pleoventral calcium phosphate deposits of the beach isopod *Tylos europaeus* (Crustacea)

A. Ziegler<sup>1</sup>, S. Jenes<sup>1</sup>, S. Hild<sup>2</sup>

1. Central Facility for Electron Microscopy, University of Ulm, 89069 Ulm, Germany

2. Institute for Polymer Science, Johannes Kepler University Linz, A-4020 Linz Austria

[andreas.ziegler@uni-ulm.de](mailto:andreas.ziegler@uni-ulm.de)

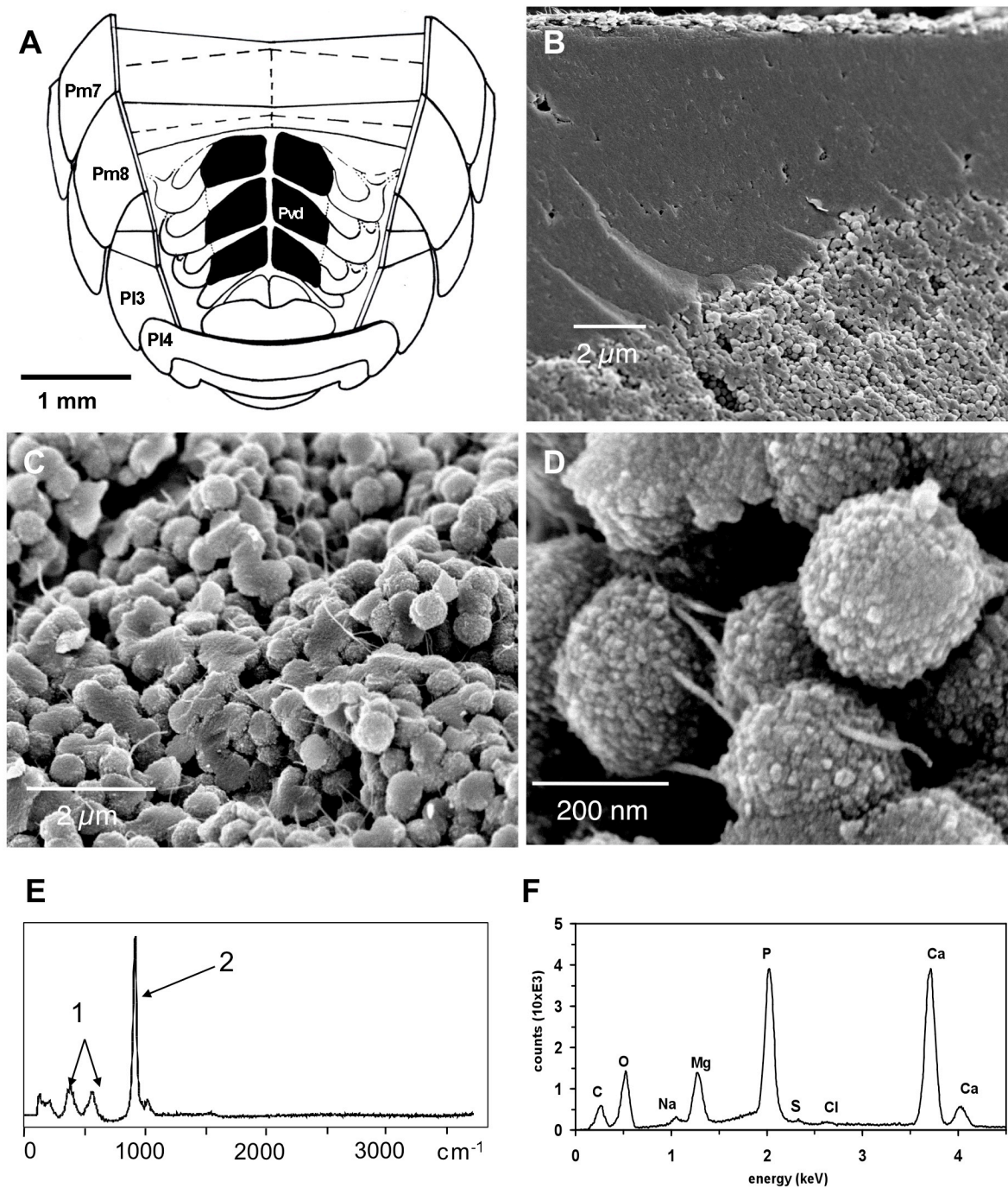
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Terrestrial isopods are unique in moulting first the posterior and then the anterior half of the body. Before the moult they store cuticular calcium within large calcium carbonate deposits within the ecdysial space between the first four anterior sternites. The calcium carbonate is in its amorphous form (ACC) and is used to mineralise the new posterior cuticle after the old cuticle is shed. Besides ACC the cuticle of most isopods contains considerable amounts of phosphate [1]. Recently we discovered that some terrestrial isopods store calcium phosphate in separate reservoirs in addition to the sternal ACC-deposits [2]. The storage sites for calcium phosphate vary among species. The sand burrowing beach isopod *Tylos europaeus* forms three pairs of quite thick calcium phosphate deposits within the ventral integument of the first three pleomeres (Fig. 1 A). We have analysed the structure and composition of these deposits by FESEM, Raman spectroscopy and EDX.

The calcium phosphate deposits consist of two layers, a distal layer that appears rather smooth and a proximal layer that is composed of numerous spherules of about 250 nm in diameter that are frequently fused together (Figs. 1 B, C). The spherules appear interconnected by strands belonging to an organic matrix (Figs. 1 C, D). The spherules are composed of nanoparticles with diameters between 10 and 20 nm (Fig. 1 D). Raman spectroscopy and EDX analysis indicate that the deposits consist mainly of calcium phosphate. The strong band at 958 cm<sup>-1</sup> (Fig. 1 E 2) derives from the symmetric stretching mode of the phosphate group and the presence of a broad, weak HPO<sub>4</sub> band with a maximum at about 420 cm<sup>-1</sup> as well as the PO<sub>4</sub> band at 590 cm<sup>-1</sup> (Fig. 1 E 1) suggest the presence of either nanocrystalline octacalcium phosphate or amorphous calcium phosphate [3]. EDX analysis indicates the presence of considerable amounts of magnesium.

The calcium phosphate deposits are shed with the posterior cuticle. However, *T. europaeus* ingests the exuviae immediately after ecdysis, with a high preference to the calcium phosphate deposits. This suggests that the animals recycle the lost calcium phosphate for the mineralization of the new cuticle. Mobilisation and uptake of calcium and phosphate ions is probably facilitated by the high solubility of nanocrystalline or amorphous calcium phosphate.

1. F. Neues et al., Cryst. Eng. Comm. **9** (2007) p1245.
2. Ziegler, Crustaceana Monographs, **2** (2003) p299
3. Sauer et al., Calcif. Tissue Int. **54** (1994) p414



**Figure 1.** Structure and composition of the pleoventral deposits of *Tylos europaeus*. (A) Schematic presentation of a dorsal view of the exuviae presenting pleoventral calcium phosphate deposits (pvd). (B-D) FESEM (Hitachi S-5200, 4kV) of the deposits revealing its spherular structure composed of nanoparticles. Raman spectroscopy (E) and EDX analysis (F) indicate that the deposits consist mainly of calcium phosphate. (Figures A and F modified from [2]).