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LS-13-P-2365 FEG-SEM and TEM imaging combined with EDXS analyses of cuticle differentiation during early ontogenetic development in terrestrial crustacea

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Crustacean exoskeletal cuticle is an epidermal apical extracellular matrix, based on chitin-protein fibers and hardened by calcification. Mature cuticle is organized in three principal layers: the outermost epicuticle, exocuticle and the inner endocuticle. In adult isopods exo- and endocuticle are mineralized by calcite and by amorphous calcium carbonate and calcium phosphate. In isopods the cuticle is formed during ontogenetic development in the female brood pouch - marsupium and periodically renewed during molting of adults. The integrative part of cuticle formation is calcification, which is still poorly investigated in the forming cuticle of terrestrial isopods.

In our study a combination of complementary microscopic methods was applied to investigate the exoskeletal cuticle differentiation in sequential developmental stages of marsupial larvae mancae of isopod Porcellio scaber in comparison to adults. Ultrastructure and elemental composition of methanol fixed intact and transversely fractured cuticles were analysed by FEG-SEM imaging in LEI mode, supplemented by EDXS analyses. Next, methanol fixed resin embedded cuticles were prepared for correlative LEI imaging and EDXS analyses of the sample block face in combination with TEM of the corresponding ultrathin section. Methanol fixation was performed to preserve the mineral phases in the cuticle as suggested in the literature. Data were compared to conventionally aldehyde-fixed and resin embedded samples.

Our results suggest that exoskeletal cuticle calcification occurs already in marsupial larvae mancae. In advanced marsupial mancae the cuticle displays all three principal layers, lamellar sublayers in exo- and endocuticle and a network of pore canals (Fig.1). The cuticle elemental composition resembles that of adults, indicating prominent cuticle calcification in this stage (Fig.2). In earlier developmental stage, newly hatched marsupial manca, the exoskeleton displays elaborated structure of epicuticle and no distinctive structural division in exo- and endocuticle (Fig.3). EDXS analyses suggest initial calcium sequestration in the cuticle in this stage. Cuticle is a non-homogenous and dynamic matrix, thus different approaches are required for examining the cuticle during morphogenesis. Combination of applied microscopic techniques is suitable to obtain data on cuticle structure and calcification in the same sample and thus follow cuticle differentiation in progressive developmental stages. The results suggest the important role of calcification during cuticle formation in developing larvae, contributing to its support and mobility which was observed within marsupium. Elaborated cuticle also protects the larvae against physiological stress after having shed the egg envelopes.
Fig. 1: Exoskeletal cuticle in advanced marsupial manca of P. scaber. (a) TEM image of aldehyde-fixed specimen shows cuticle differentiation into epicuticle (ep), exocuticle (ex) and endocuticle (en), with lamellar sublayers and pore canals. ec-epidermal cell. (b) TEM image of methanol-fixed specimen shows lamellae of chitin-protein fibers in endocuticle.

Fig. 2: EDXS spectrum obtained from the cuticle surface in methanol-fixed advanced marsupial manca of P. scaber shows conspicuous calcium peaks, in addition to phosphorus, magnesium, sulphur, potassium, carbon and oxygen peaks. Ca peaks are evidently higher than P peaks.

Fig. 3: Ultrastructure of exoskeletal cuticle in the newly hatched marsupial manca. TEM image of aldehyde-fixed cuticle (a) and methanol-fixed cuticle (b) show elaborated epicuticle (ep) and procuticle (pro). ec-epidermal cell. In the image (b) chitin-protein fibers arranged in characteristic helicoidal pattern are clearly evident in the cuticular matrix.