Subcellular Processes in Plants and Animal Cells

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Stomach and hindgut cuticular lining differentiation during embryogenesis of the crustacean *Porcellio scaber*

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Crustaceans possess a cuticle that covers body surface and ectodermal parts of the digestive system. Exoskeletal cuticle in adult terrestrial isopod crustaceans is a thick apical extracellular matrix secreted by epidermis, characterized by organization into several layers and displaying helicoidal arrangement of fibers [1]. Cuticle lining the lumen of the hindgut is thinner, consisting of a proximal lucent homogenous matrix and electron dense distal layer [2] (Figure 1). Cuticular structures inside the stomach are very diverse and complex, forming grinding and filtering devices. Both, exoskeletal cuticle and cuticle of the digestive tract are based on the chitin-protein network, but differ in architecture and functions. So, general as well as specific features are expected in the *de novo* formation of these matrices during embryogenesis. Here we present a microscopic characterization of the cuticle formation in the stomach and hindgut during embryonic and larval development of *Porcellio scaber*. The results are discussed with respect to data obtained on exoskeletal cuticle formation during embryogenesis [3].

Embryos and mancae of the isopod crustacean *Porcellio scaber* were isolated from the marsupium. Staging was performed as reported in developmental staging system of *Porcellio scaber* distinguishing twenty different morphological stages, from fertilized egg, through early-, mid- and late-stage embryos to hatched larva manca [4]. Specimens were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2), postfixed in 1% OsO_4 and embedded in Agar 100 resin. Semithin and ultrathin sections were made with Ultracut S (Leica), stained with Azure II – Methylene Blue or contrasted with 4% uranyl acetate and 10% lead citrate, respectively.

In late embryo stomach differentiation into a grinding and filtering device is observed and the typhlosole formation is noticeable in the anterior hindgut [5]. Cuticular lining is discerned in the stomach and hindgut, consisting of homogenous lucent matrix and a thin intensely ruffled dense layer facing the lumen (Figure 2). It morphologically resembles the embryonic epidermal matrix, that is formed before formation of typical exoskeletal cuticle [3]. In early marsupial manca formation of the typhlosole throughout the anterior hindgut region is evident, appearing as a ridge of cells in dorsal wall, protruding in the lumen (Figure 3a). In late marsupial manca the ridges and folds of the hindgut epithelium forming typhlosole channels are fully developed and similar to adults. The cuticle lining the lumen of the hindgut is not ruffled as it is in embryos and ultrastructurally resembles the adult hindgut cuticle. It is composed of the thick homogenous lucent layer overlaid with a thin trilaminar sheet (Figure 3b). The thickness of manca hindgut cuticle is only about one third of the adult one. The exoskeletal cuticle in the same specimen is divided in principal layers and displays characteristic pattern of chitin-protein fibers arrangement [3]. Our results show that formation of the gut and exoskeletal cuticles occurs already in late embryos and in this stage both are composed of structurally similar matrices. In marsupial mancae specialization of both cuticles is evident, hindgut cuticle acquires smooth outline and more prominent surface dense layer and exoskeletal cuticle differentiates in several layers displaying chitin-protein fibers patterns.

1. B.H.M. Seidl, A. Ziegler, Zookeys 176 (2012), p. 73-85.
2. J. Štrus, D. Drobne, P. Ličar in "Terrestrial isopod biology, Crustacean issues 9", ed. M. A. Alikhan, (A. A. Balkema, Rotterdam) (1995), p. 15-23.
3. P. Mrak, N. Žnidaršič, M. Tušek-Žnidarič, W. Klepal, D. Gruber, J. Štrus, Zookeys 176 (2012), p. 55-72.
4. M. Milatovič, R. Kostanjšek and J. Štrus, Journal of Crustacean Biology 30(2) (2010), p. 225-235.
5. J. Štrus, W. Klepal, J. Repina, M. Tušek-Žnidarič, M. Milatovič, Ž. Pipan, Arthropod Structure and Development 37 (2008), p. 287-298.

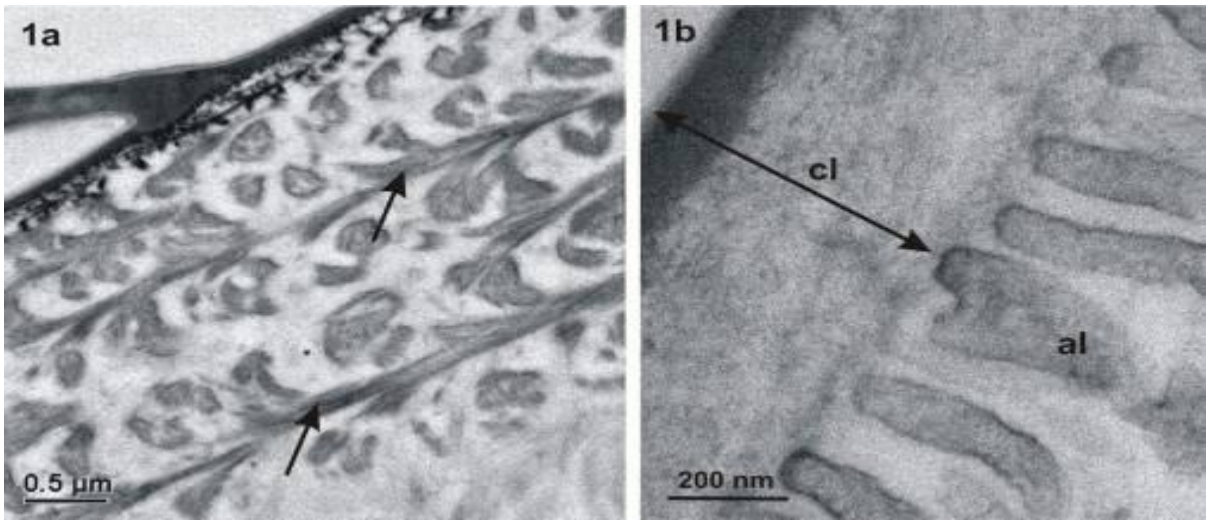

Figure 1: Adult *Porcellio scaber* (a) Distal part of exoskeletal cuticle. Bundles of chitin-protein fibers (arrows) are clearly resolved and are arranged in helicoidal layers. (b) Cuticular lining (cl) of the hindgut consists of a non-patterned proximal layer and a dense layer facing the lumen. Epithelial cells have a distinctive apical labyrinth (al).

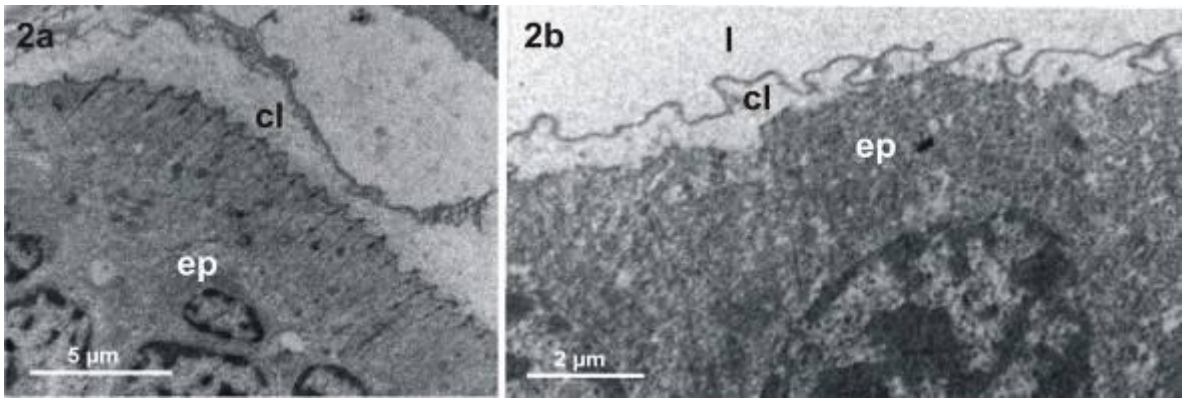

Figure 2: Late embryo of *Porcellio scaber*. Cuticular lining (cl) is distinctive in the filtering region of stomach (a) and the hindgut region (b). The matrix is intensely ruffled and consists of homogenous lucent material, lined distally by a thin dense layer. l – lumen of hindgut; ep – epithelium.

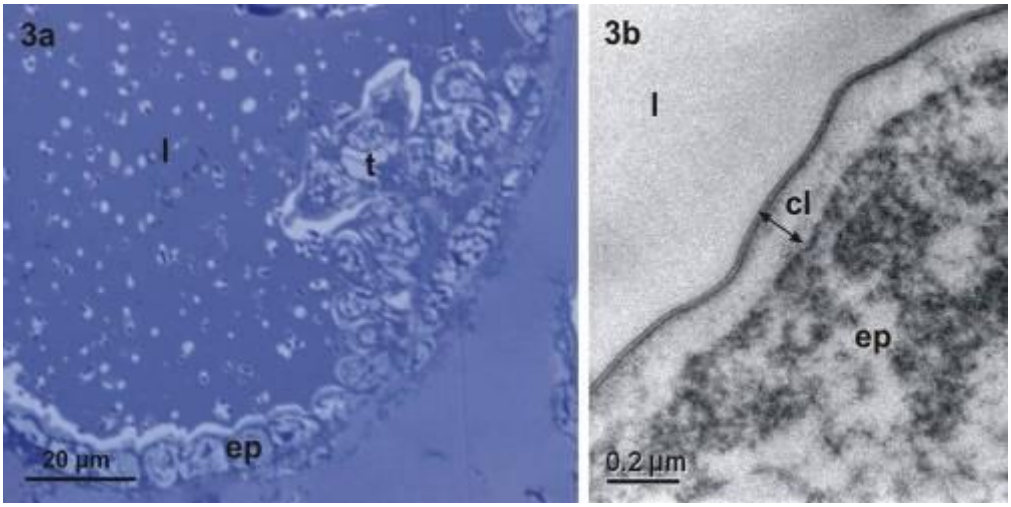

Figure 3: Early marsupial manca of *Porcellio scaber.* (a) Typhlosole formation (t) is evident in the anterior region of the hindgut. (b) Cuticular lining (cl) of the posterior hindgut region is an apical matrix, consisting of a homogenous lucent layer, lined by a trilaminar surface layer. l – lumen of hindgut; ep – hindgut epithelium.