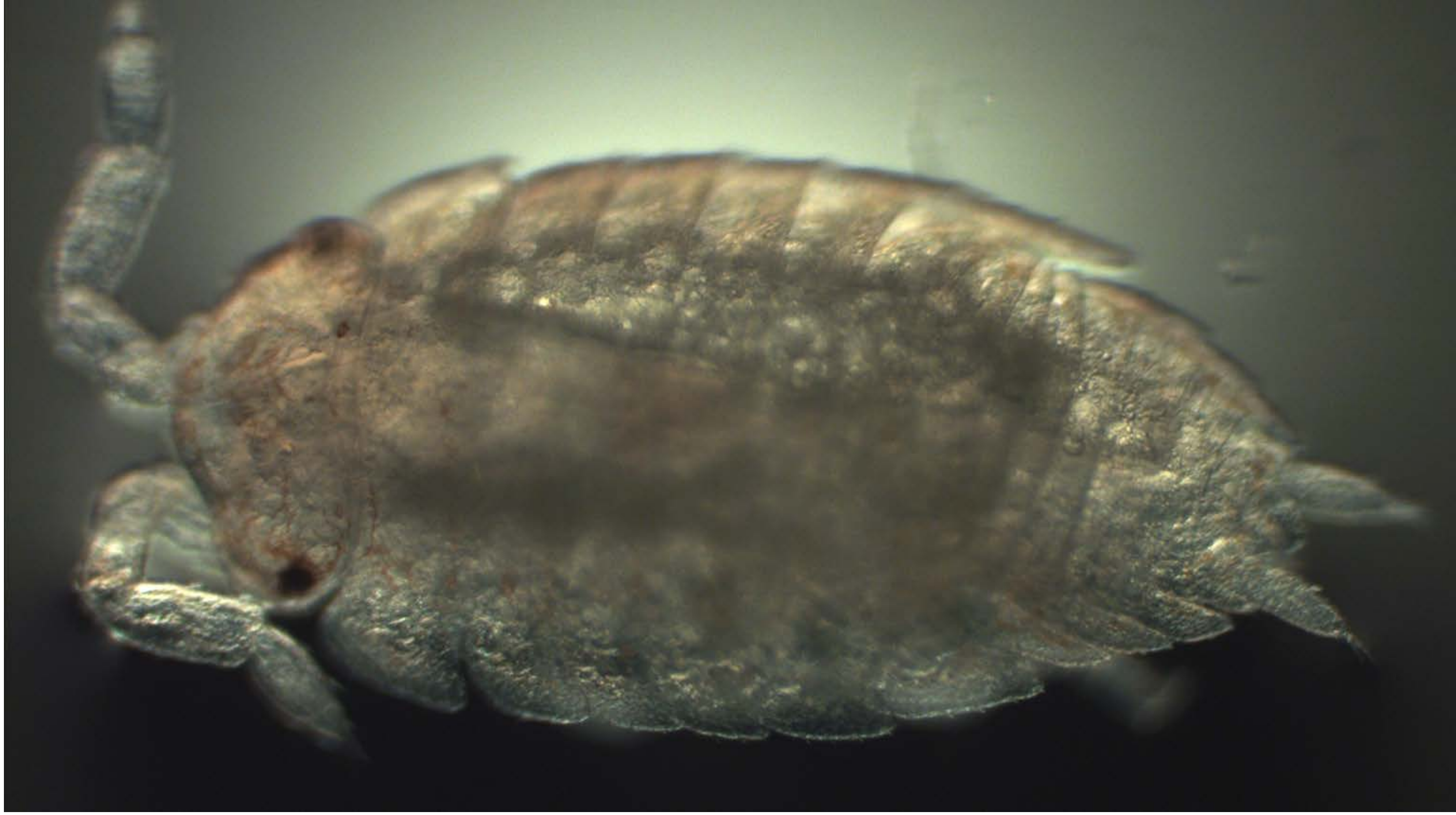
POLONA MRAK

Tvorba kutikule med razvojem kopenskega raka enakonožca

pod mentorstvom
prof. dr. Jasne Štrus
doc. dr. Nade Žnidaršič

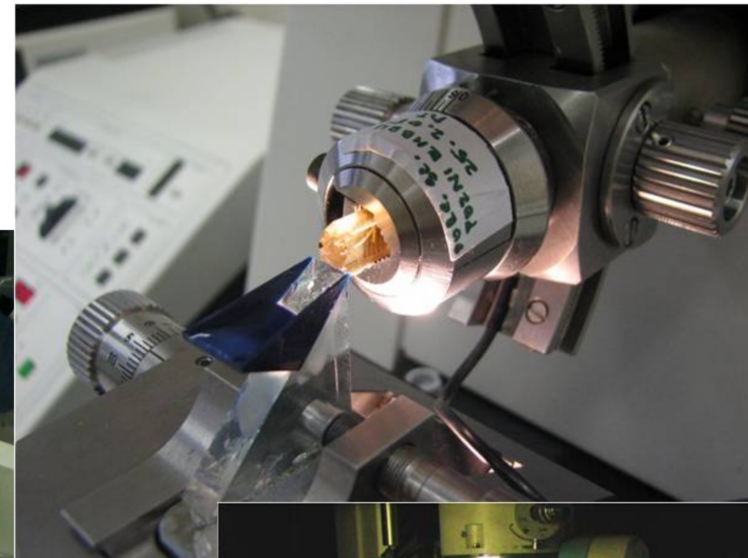


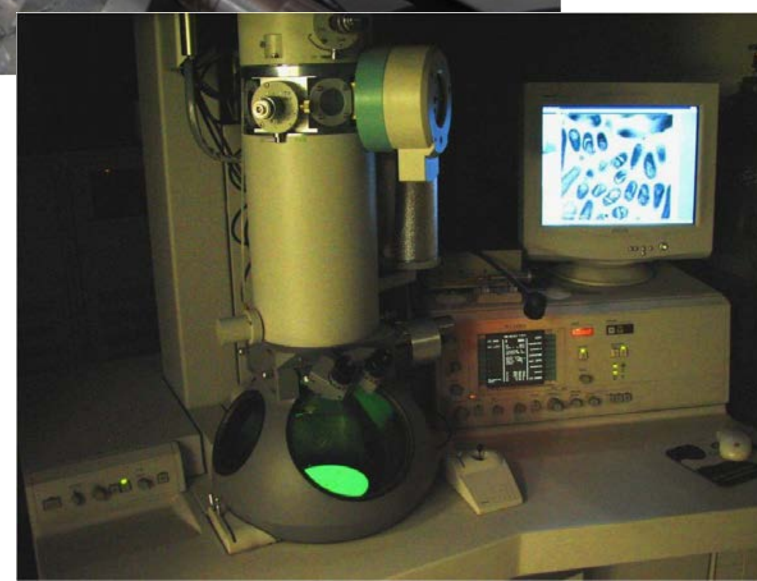
KUTIKULA
zunanji skelet rakov, zunajcelični hitinski matriks epidermalnih celic

CELIČNA BIOLOGIJA MINERALIZIRANIH ORGANSKIH MATRIKSOV
dinamika med nastajanjem in razgrajevanjem

KOPENSKI RAKI ENAKONOŽCI - Isopoda (*Porcellio scaber*)
prilagoditve na kopensko življenje

Delo v laboratoriju…





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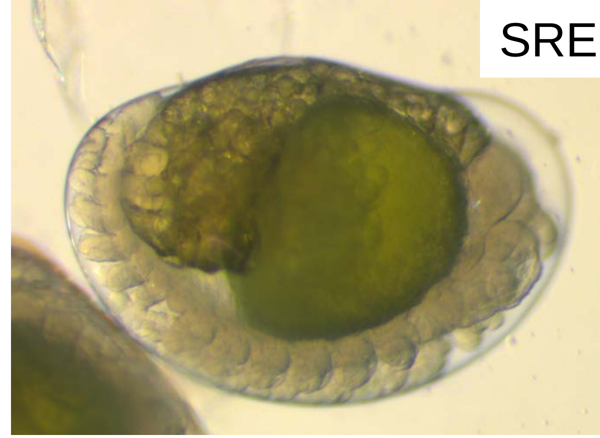

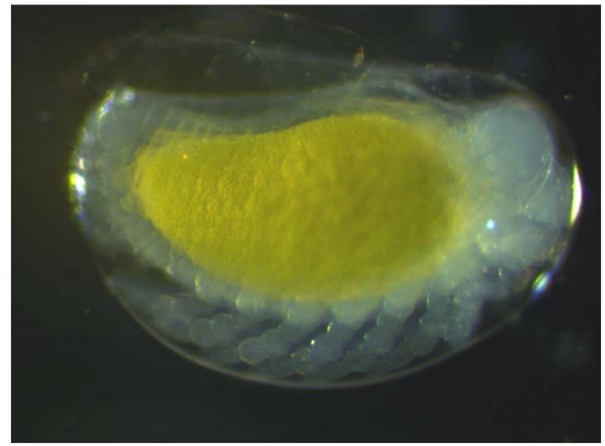

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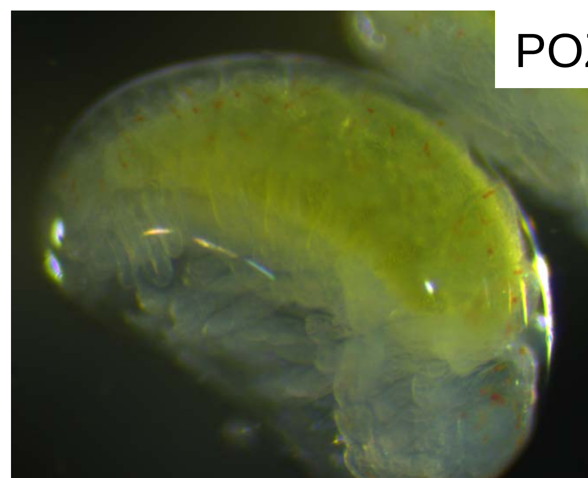


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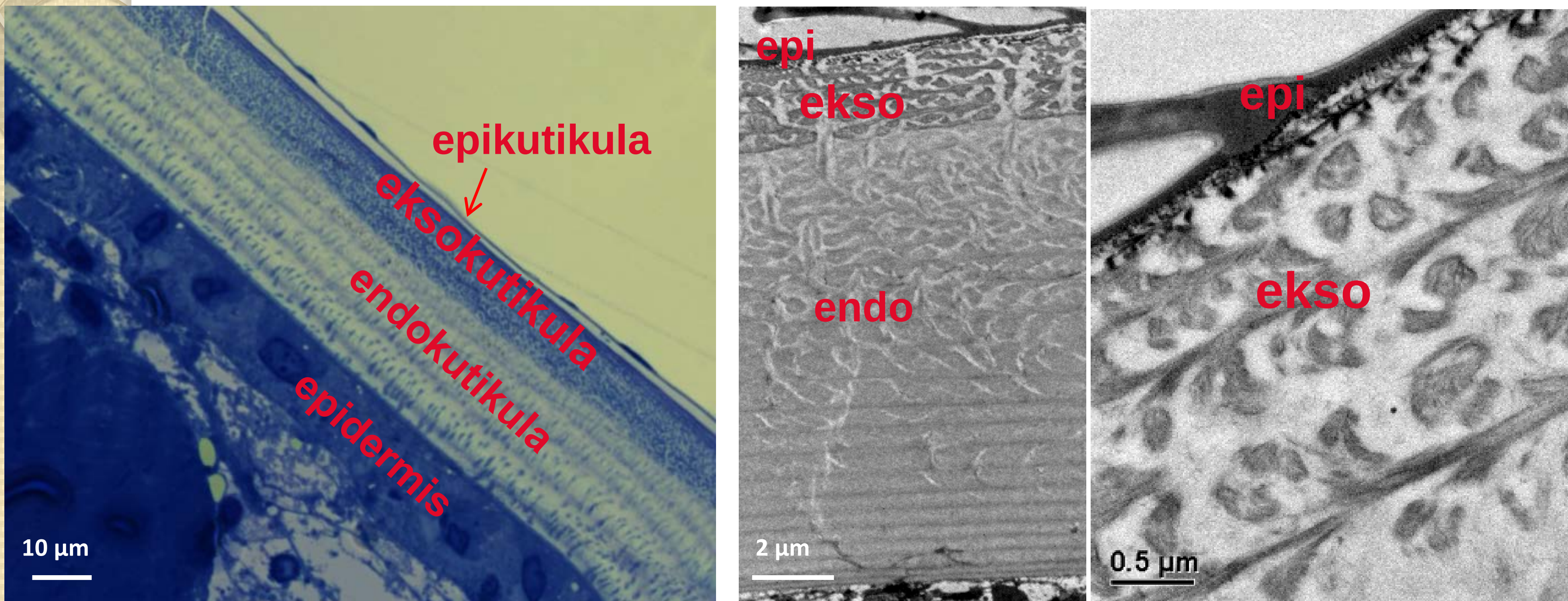
Mikroskopija

Kutikula odraslega *P. scaber*



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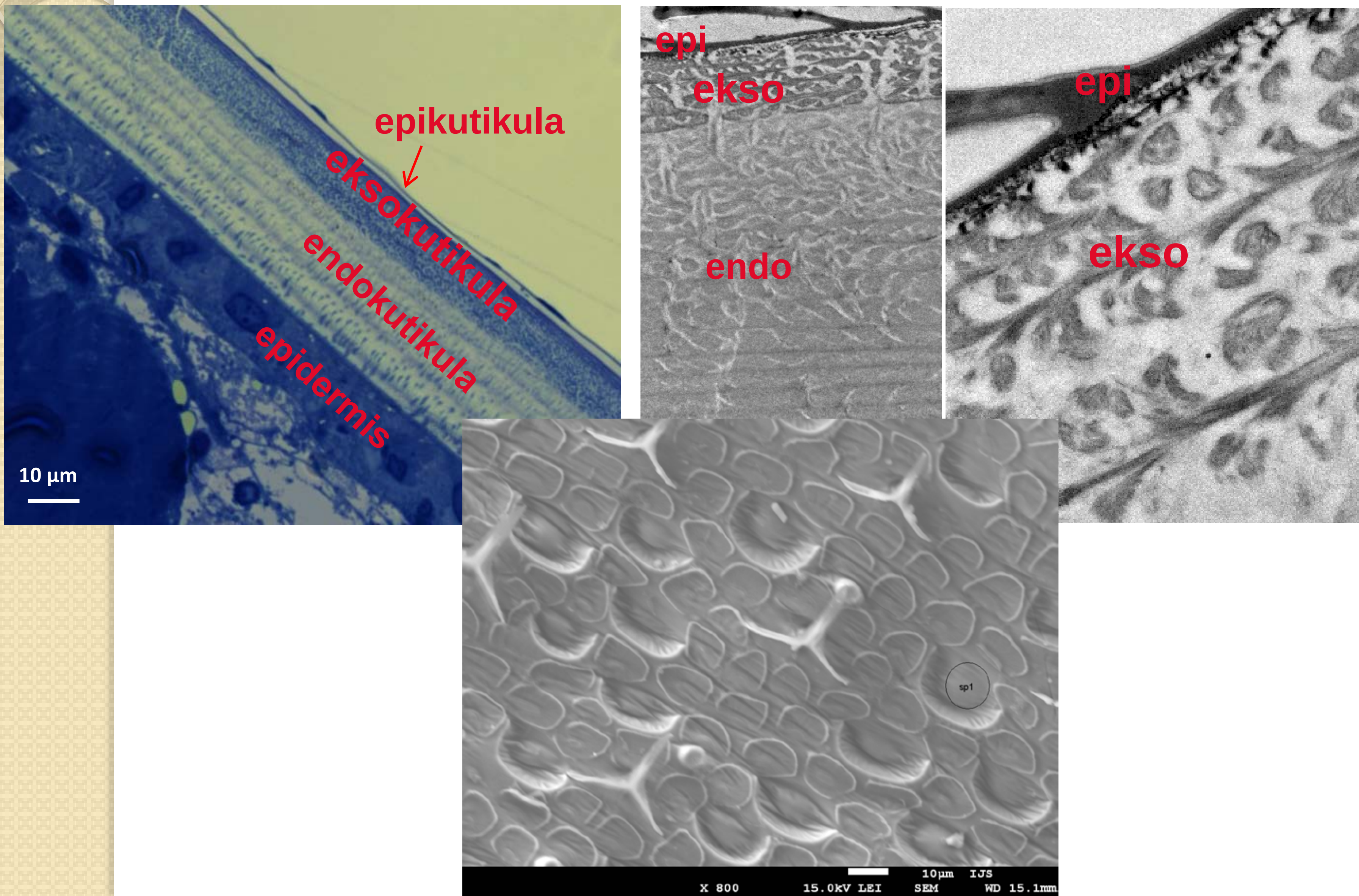
Kutikula odraslega *P. scaber*



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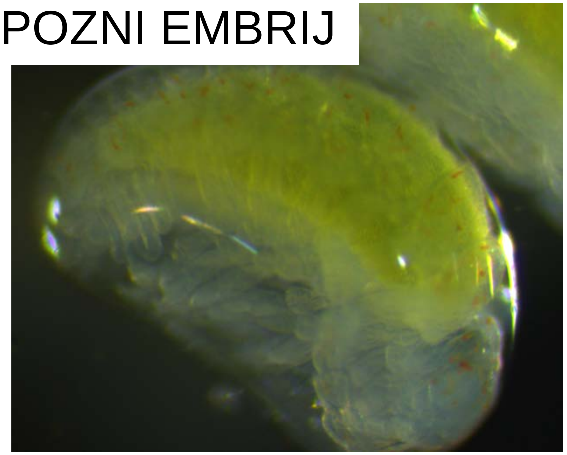

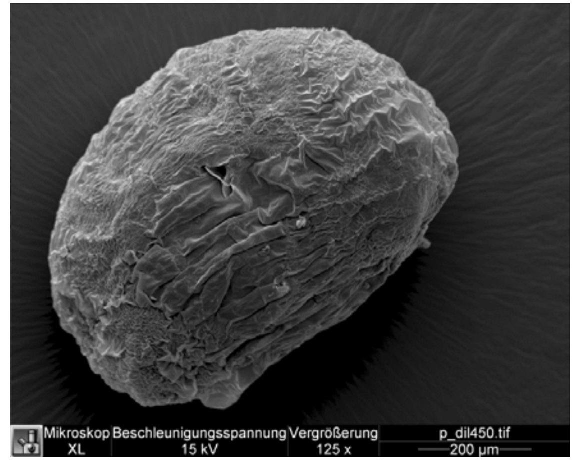

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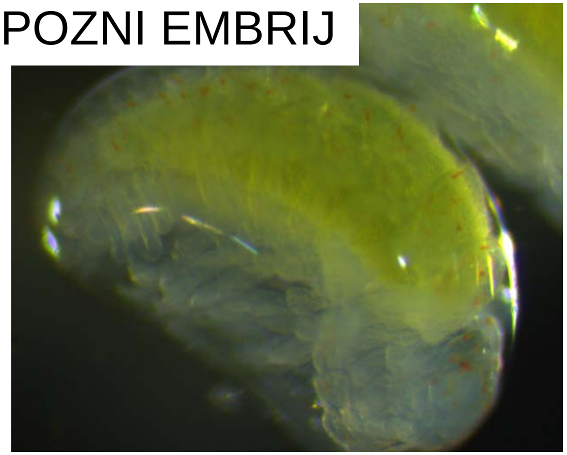

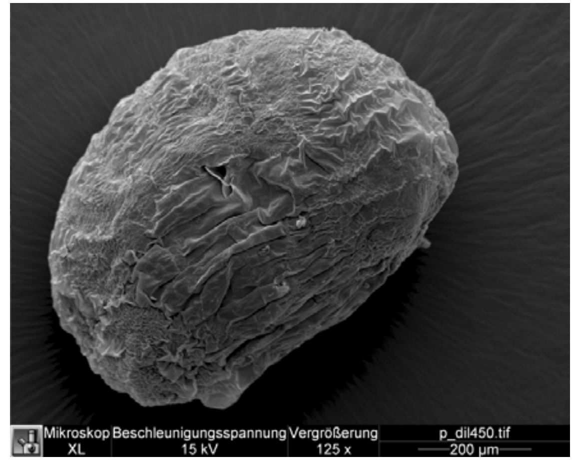

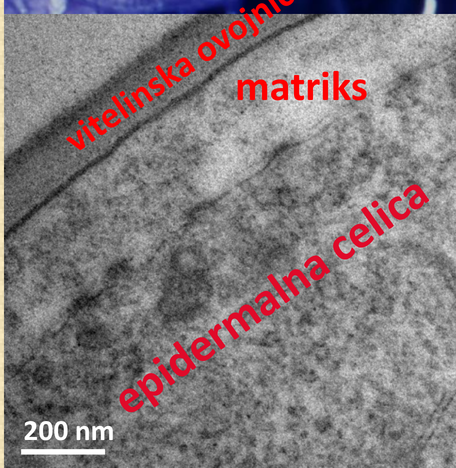

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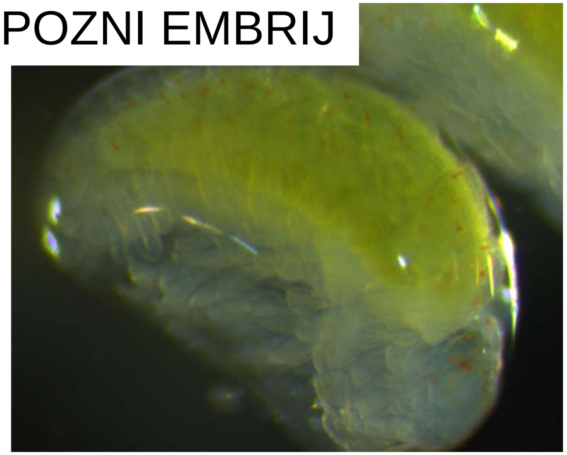

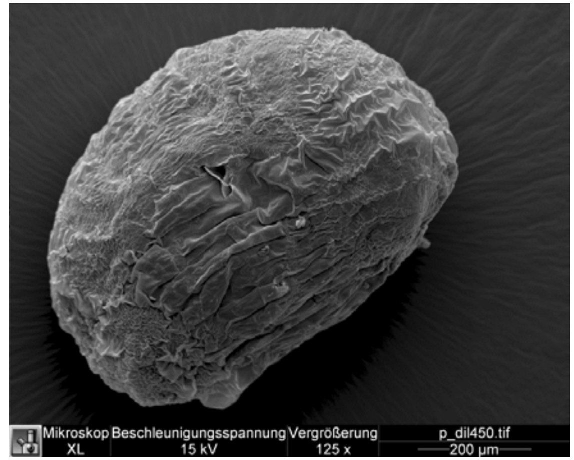

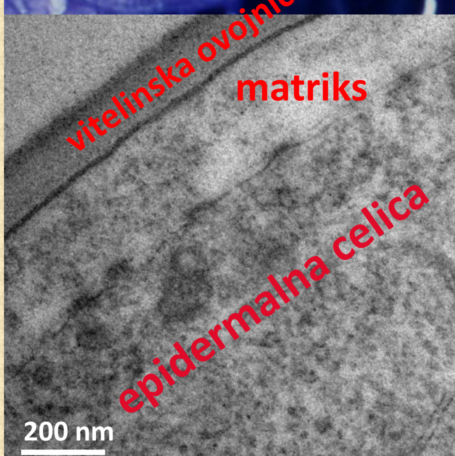

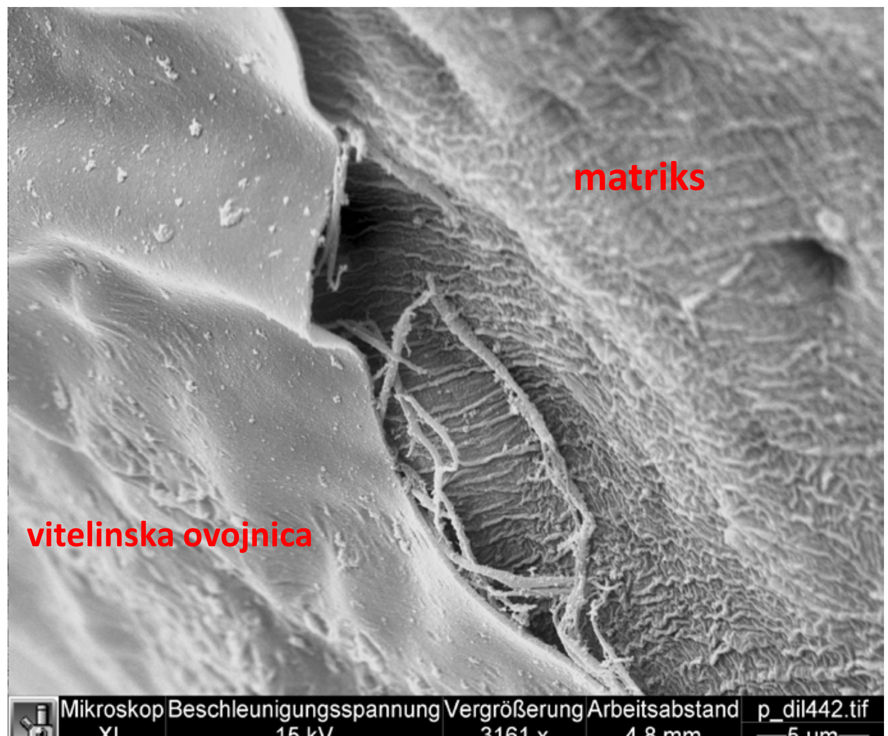

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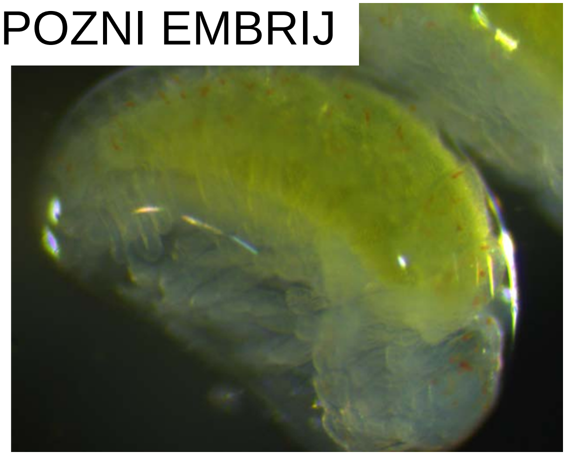

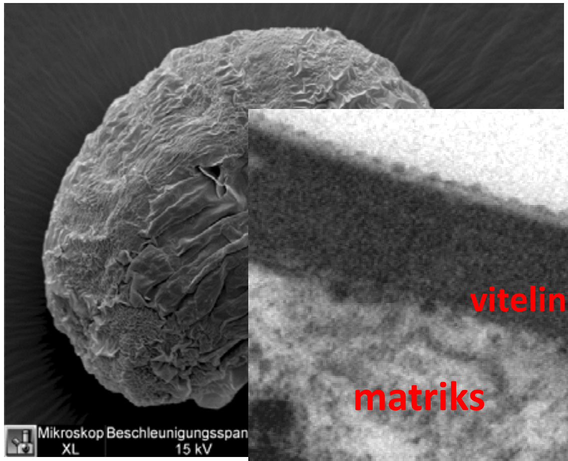

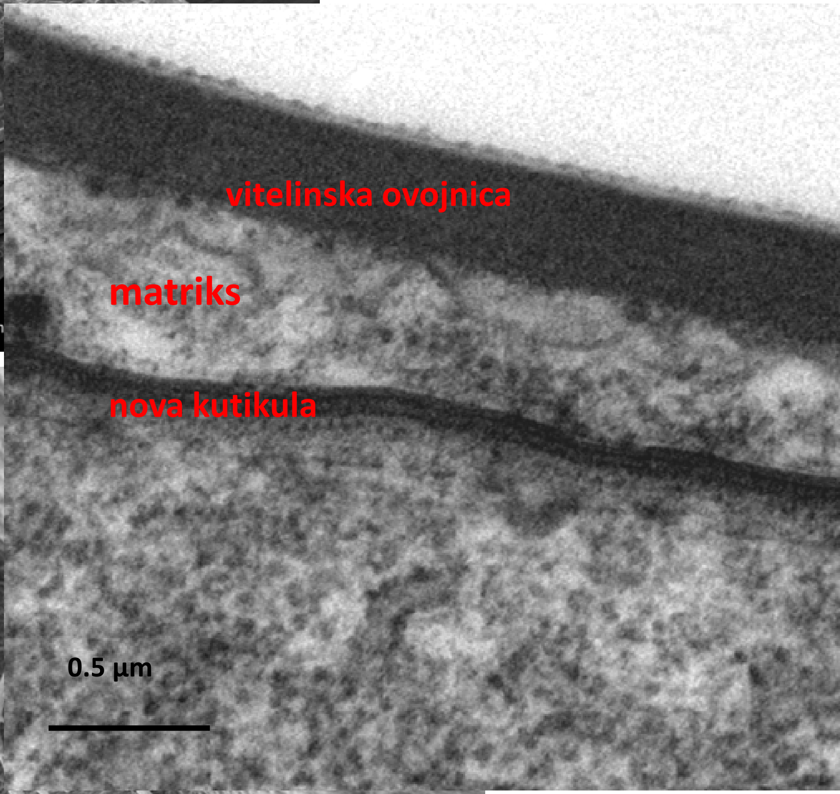

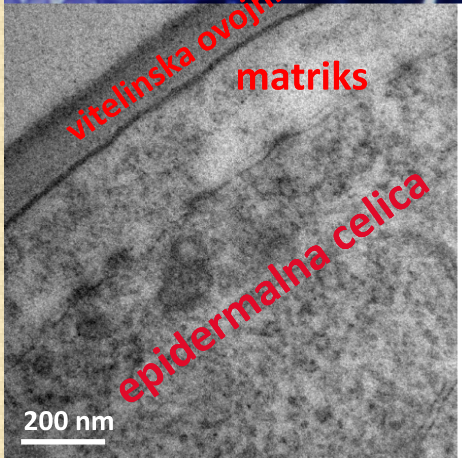

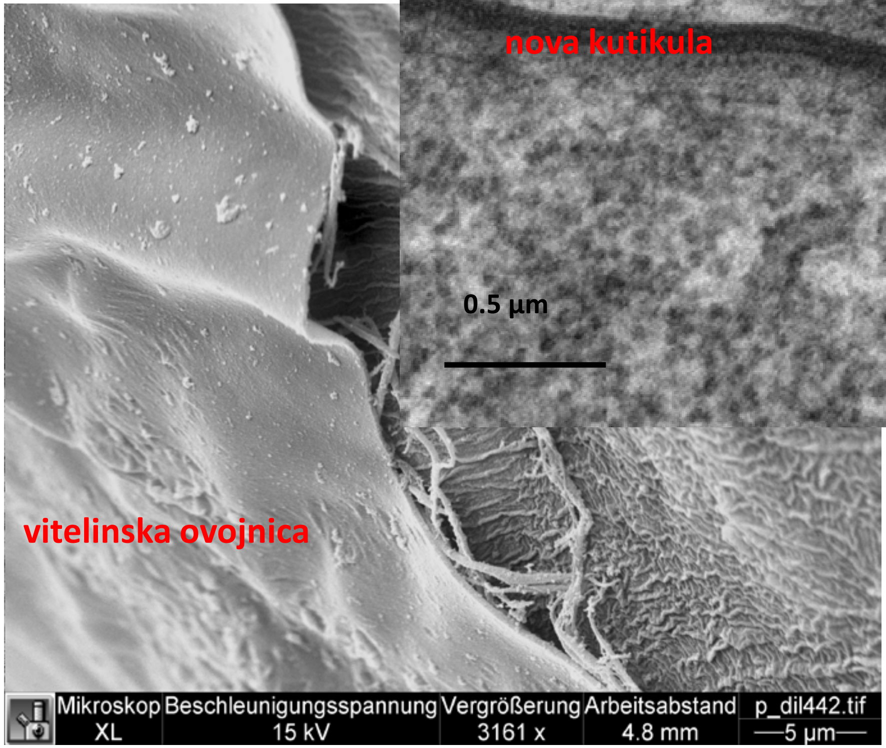

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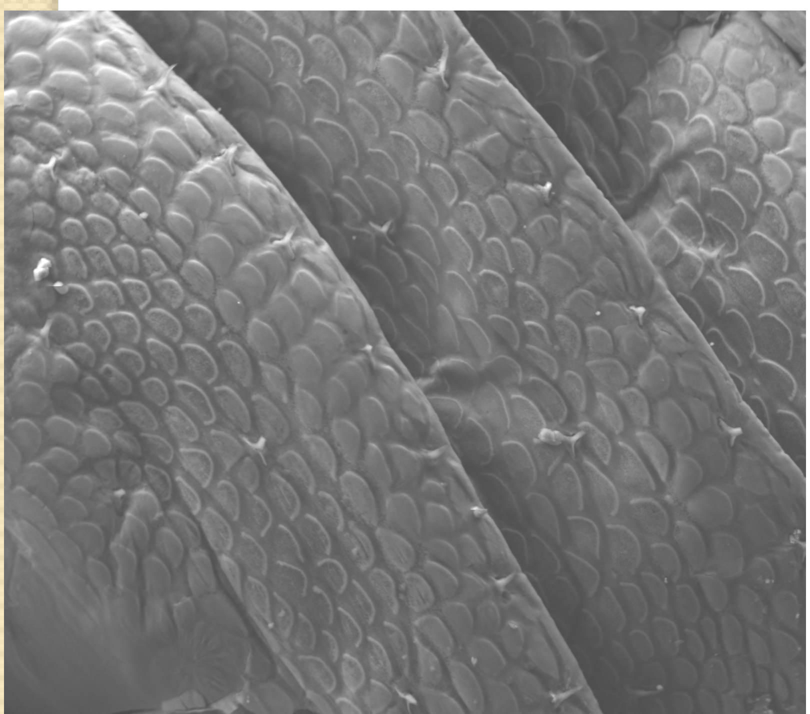

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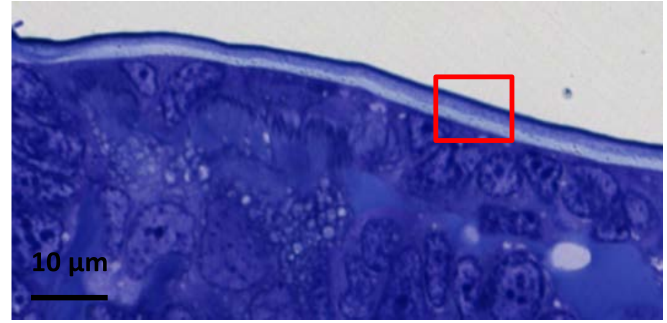

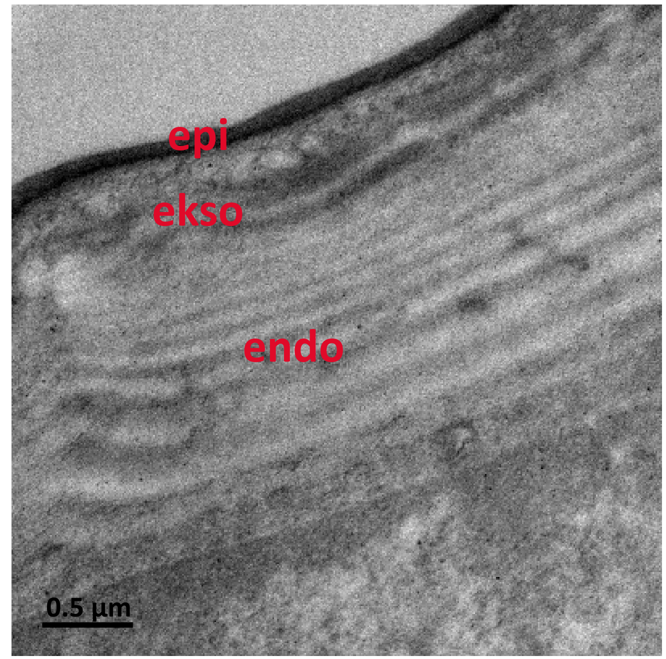

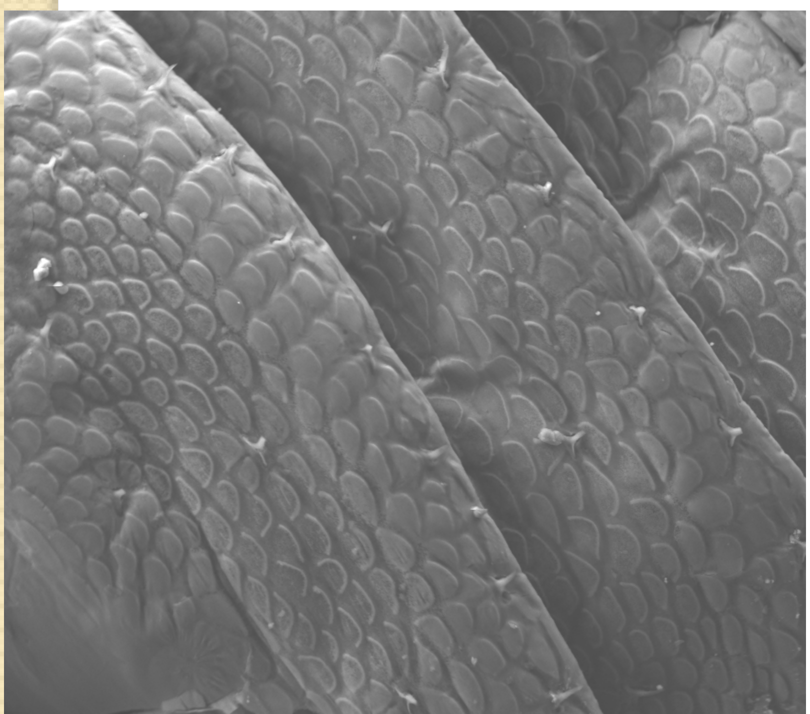

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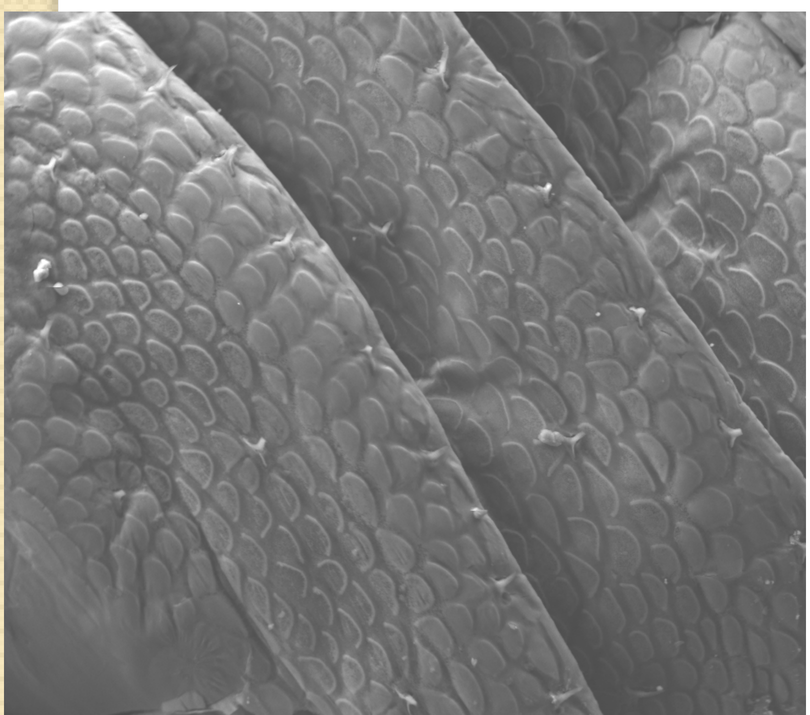

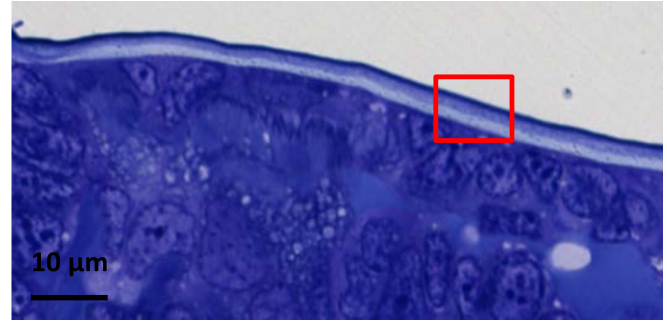

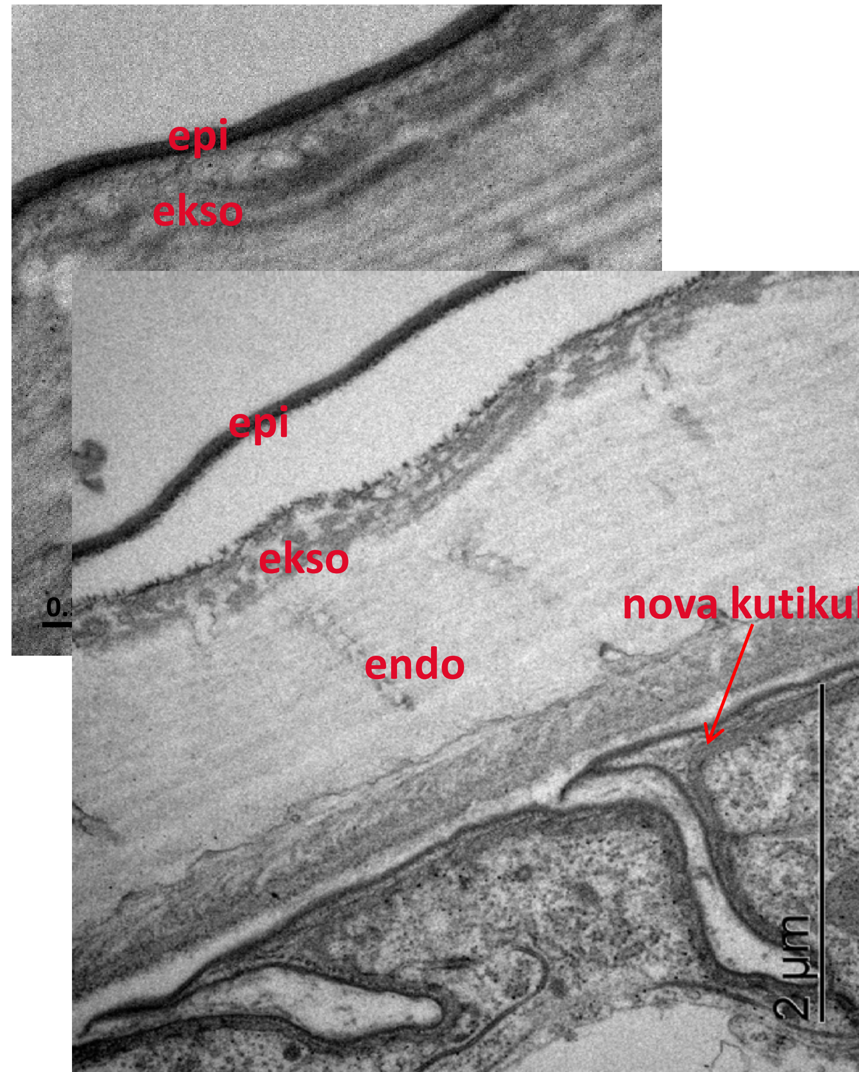

Analize, lokalizacije

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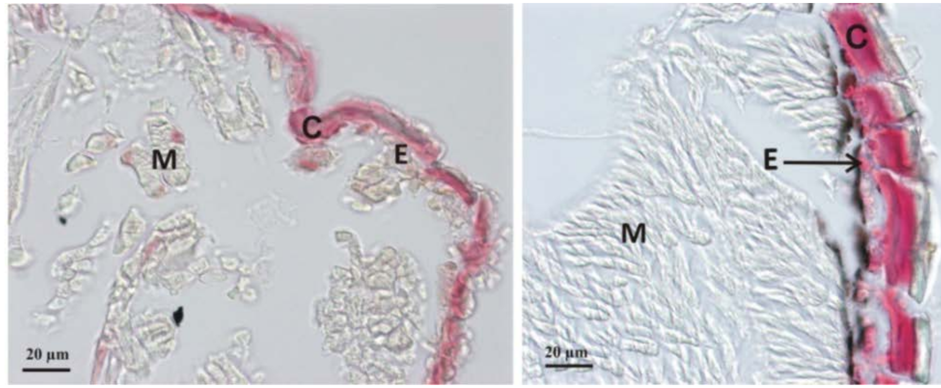
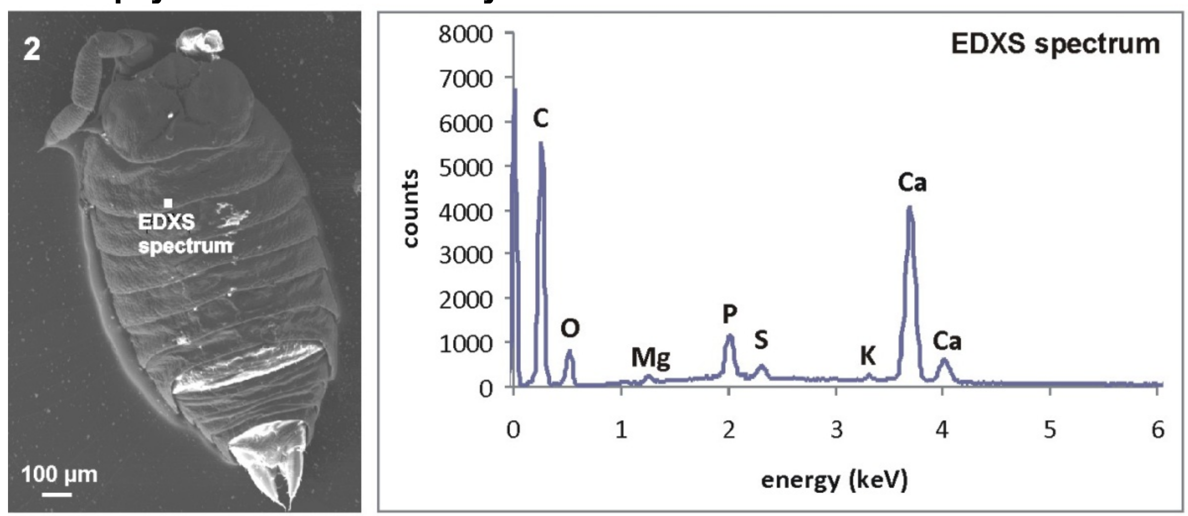


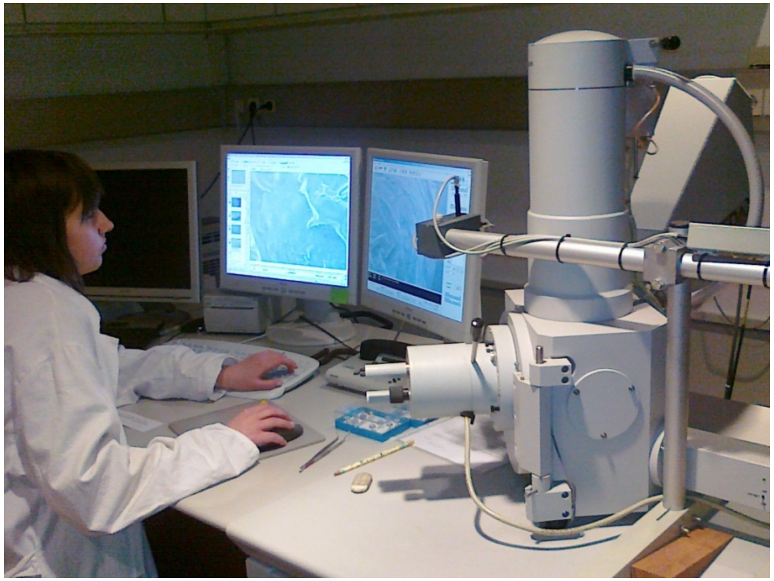
Analitska mikroskopija - sodelovanje z IJS

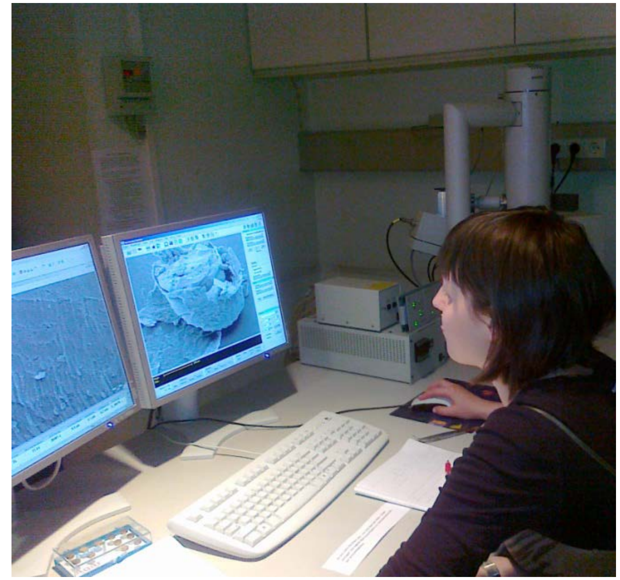


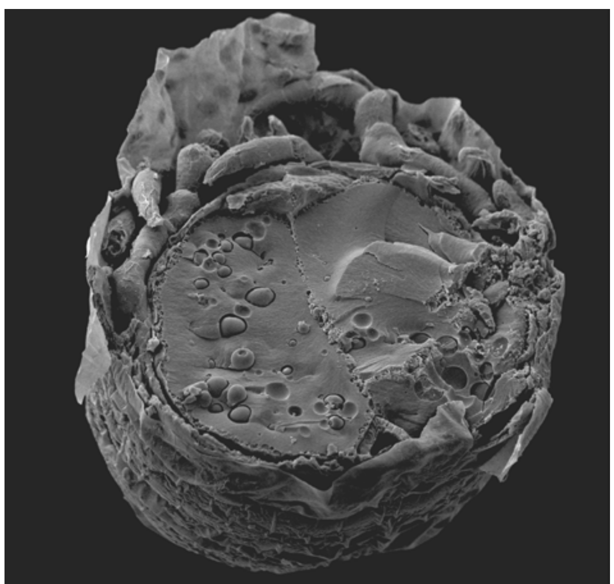
Lokalizacija hitina, imunolokalizacije celičnih komponent...

Kratek obisk na Dunaju

Department of Ultrastructural Research, University of Vienna







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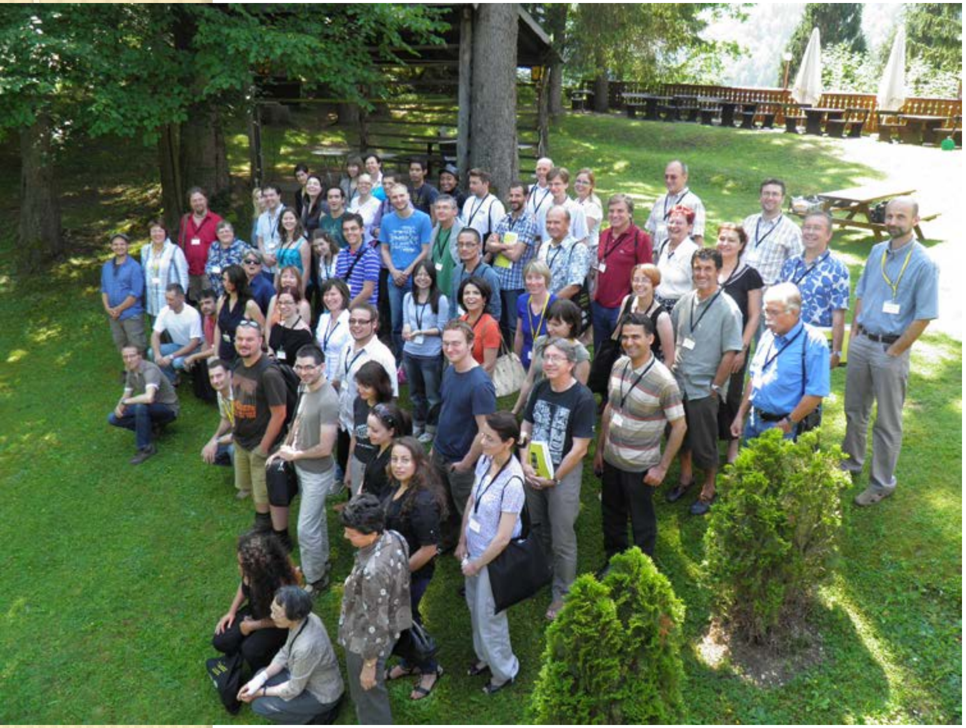
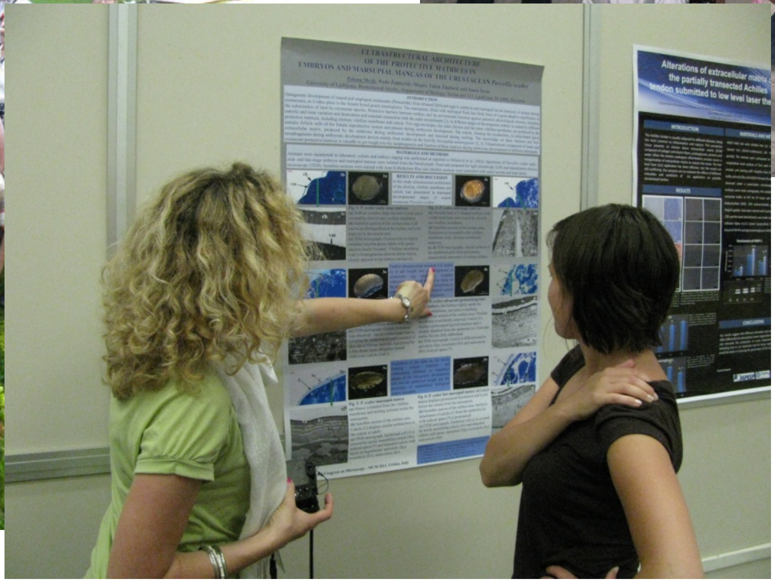


8th International Symposium of Terrestrial Isopod Biology 2011, Bled

Kongresi

10th Multinational Congress on Microscopy 2011, Urbino



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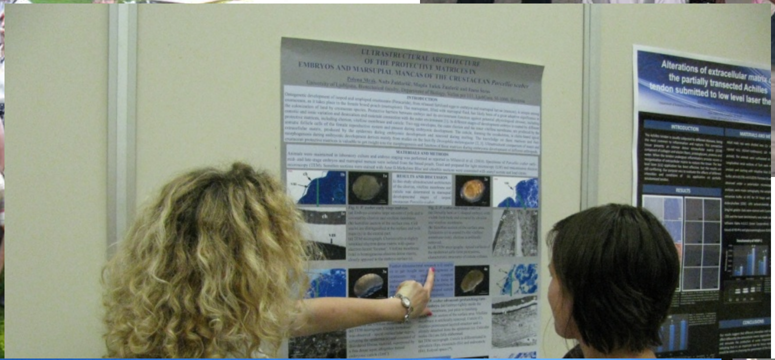

10th Multinational Congress on Microscopy 2011, Urbino

Microscopy Conference 2013, Regensburg

EMBO tečaj – EM in stereologija v celični biologiji (Češke Budejovice)

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Pisanje…

Pisanje…

Pisanje…

CUTICLE STRUCTURE IN *PORCELLIO* EMBRYOS AND

Mrak P.[1], Žnidaršič N.[1], Tušek-Žnidarič M.[1], Gruber D.[2], Klepal W.[2], Štrus J.[1]
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ABSTRACT: Crustacean exoskeleton is a chitin-based extracellular matrix, produced
epithelium during embryogenesis and during molting. Data on terrestrial crustacean cuticle d
embryonic development are very scarce. In this study we report on ultrastructural cha
exoskeletal cuticle of embryos in two developmental stages within the brood pouch of *Por*
cuticle of late embryos and marsupial mancas was already differentiated in three main
exocuticle and endocuticle, and displayed architecture similar to the cuticle of adults. Analy
earlier embryonic stages is underway to gain additional insight into crustacean cuticl
embryogenesis.

Keywords: cuticle, embryo, isopod, ultrastructure

1. Introduction

Cuticle is an apical extracellular matrix, comprising distinct horizontal layers, differing in structure and composition. It is produced by the integument, hindgut and foregut epithelia during embryonic development and it is renewed periodically during molting. Cuticle synthesis implies extensive polarized secretion at the apical side of epithelial cells and sequential elaboration of cuticular layers. The knowledge on cuticle differentiation during embryonic development derives mainly from studies on the fruit fly *Drosophila melanogaster*, as this model species offers wide opportunities for a genetic approach to address the cuticle

0.1M cacodylate buffer (pH=7.2
the vitelline membrane of emb
perforated with a thin needle.
cacodylate buffer, the samples w
osmium tetroxide for 2 hours,
dehydrated.
Specimens for LM and TEM
Agar 100 resin. Prior to embed
perforated with a thin needle for
resin. Semithin sections were cu
stained with Azur II-Methylene
Zeiss AxioImager Z.1 light mi
sections were cut by a Re
ultramicrotome (Leica), contrast

Cuticle formation during marsupial development of the crustacean *Porcellio scaber*: Imaging and analysis

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Keywords: embryo, marsupial manca, terrestrial isopods, exoskeleton, ultrastructure, mineralization, chitin

Introduction: Crustacean exoskeleton is a chitin-protein matrix, formed apically by a single-layered epidermis. Organic cuticle constituents are hierarchially ordered in horizontal layers and incorporate minerals, mostly calcium carbonate and phosphate. The data on *de novo* formation of crustacean cuticle during embryogenesis provide knowledge of cell biology of chitin-based mineralized matrices, biomineralization processes and the role of hard biological materials. In this study several intramarsupial developmental stages of terrestrial isopod *Porcellio scaber* are examined, including analysis of cuticle ultrastructure, localization of N-acetylglucosamine (chitin monomer) and presence of mineral component. The embryonic development of terrestrial isopods takes place in the fluidic environment of the female marsupium. Embryos hatch into mancae and continue their development in marsupium for another week. Our results are compared with two model arthropod species, insect *Drosophila melanogaster* with non-mineralized exoskeleton and different reproductive strategy [1, 2] and aquatic amphipod crustacean *Parhyale hawaiensis* [3].

Methods: A detailed staging system through twenty progressive stages [4] was used to characterize the stages of *P. scaber* embryos and mancas, isolated from the marsupium. Conventional ultrastructural study and labelling of N-acetylglucosamine with WGA lectin were performed on ultrathin sections. The elemental composition of the cuticle was examined in methanol-fixed specimens by energy-dispersive x-ray spectroscopy (EDXS) in scanning electron microscope.

Results and discussion: Ultrastructural and compositional study of *Porcellio* epidermal extracellular matrices was performed in two sequential stages of late embryos and in two sequential stages of

CUTICLE STRUCTURE IN *Porcellio* EMBRYOS

Mrak Polona (1), Žnidaršič Nada (1), Tušek-Žnidarič Magda (1), Gruber Daniela (2), Klep
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CALCIFICATION OF LARVAL AND POSTMOLT ADULTS' EXOSKELETON IN ISOPOD CRUSTACEANS AS REVEALED BY EDX ANALYSIS AND HISTOCHEMISTRY

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INTRODUCTION

Crustacean exoskeletal cuticle is an epidermal apical extracellular matrix, based on chitin-protein fibers. Mature cuticle in adult isopod crustaceans is mineralized by calcite, amorphous calcium carbonate and calcium phosphate (Becker et al. 2005, Hild et al. 2008, 2009). The cuticle is formed *de novo* during ontogenetic development in the female brood pouch - marsupium (Mrak et al. 2012) and during molting in adults. Calcification is an integrative part of cuticle formation, but data on calcification of the forming cuticle is very limited.

AIM

In this study a combination of microscopic methods was applied to obtain data on ultrastructure and composition of the exoskeletal cuticle in different developmental stages of *Porcellio scaber* marsupial mancae, in comparison to adult isopod crustaceans in molting period.
Ultrastructure was determined by TEM of glutaraldehyde fixed and resin embedded tissue ultrathin sections. SEM – EDX analyses were performed in methanol fixed, air dried and carbon coated specimens. For histochemical calcified tissues screening paraffin and cryostat sections were used.

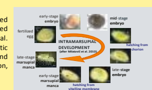

Figure 1: Embryos of *Porcellio scaber* develop in marsupium and hatch into larvae, named marsupial mancae. Further development of marsupial mancae, including exoskeleton formation, continues for approximately one week till release into external environment.

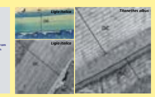

Figure 2: In adult isopods the exoskeletal cuticle is periodically renewed during molting. The new cuticle is elaborated underneath the old detached exoskeletal cuticle.
NC – new cuticle; DC – detached cuticle

RESULTS

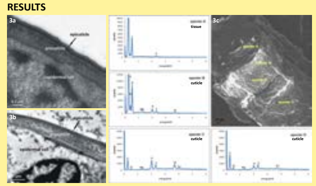

Figure 3: Early-stage marsupial mancae of *P. scaber,* soon after hatching from the vitelline membrane. Exoskeletal cuticle is up to 1 µm thick and differentiated into epi- and procuticle. **3a,b:** TEM images of glutaraldehyde fixed, osmicated (**3a**) and non-osmicated (**3b**) tergite. **3c:** SEM image of transversely cut manca and positions of EDX analyses. EDX spectra obtained on the manca surface show calcium and phosphorus peaks in the cuticle (**spectra B, C and D**). The Ca:P ratio is < 1 in all spectra. In addition, magnesium was detected in the cuticle. Specter of the internal tissues does not display Ca and Mg peaks (**specter A**).

Figure 4: Histochemical Alizarin red S staining of *Porcellio scaber.* **4a**: In early-stage marsupial mancae (soon after hatching from the vitelline membrane), the cuticle (C) is not stained. **4b, c**: The cuticles (C) of late-stage marsupial mancae (**4b**) and adults (**4c**) are differentially stained, intense red staining demonstrates calcified tissue. E – epidermis; m – muscles.

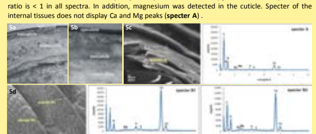

Figure 5: Advanced early-stage marsupial mancae of *P. scaber*. **5a, b**: Ultrastructure of the glutaraldehyde fixed tergites shows main features of adult crustacean cuticle. Three principal layers, epicuticle, exocuticle and endocuticle with a network of pore canals (arrows) are evident. **5c, d**: EDX spectra obtained from the transversely fractured tergite cuticle (**5c, specter A**) and surface (**5d, spectra B1, B2**) show conspicuous calcium peaks, similar as in the cuticle of adults. Magnesium was detected in the cuticle as well. In all spectra Ca peaks are much higher than P peaks.

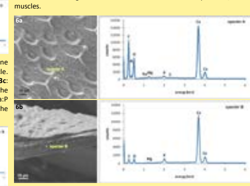

Figure 6: Tergite cuticle in postmolt adult *P. scaber* : The EDX spectra obtained in the surface (**6a, specter A**) and in the transverse fracture (**6b, specter B**) of tergite cuticle show prominent contents of calcium. Additional principal elements detected are phosphorus and magnesium.

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CONCLUSIONS

In early stage marsupial mancae soon after hatching from the vitelline membrane the initial cuticle calcification is indicated as Ca, Mg and P were determined by EDX analysis, but strongly calcified tissue was not evident in histochemical staining.
The elemental composition of cuticle in advanced early marsupial mancae is similar to that in adult specimens, with prominent Ca, Mg and P peaks.
In late stage marsupial mancae histochemical staining and ultrastructure reveal that exoskeleton is similar to adults.

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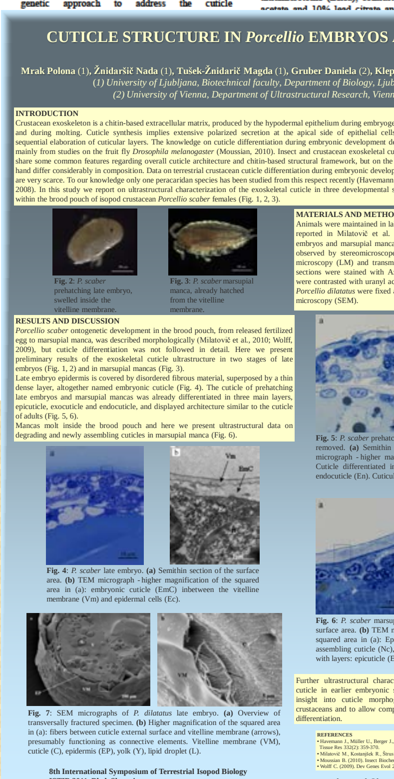

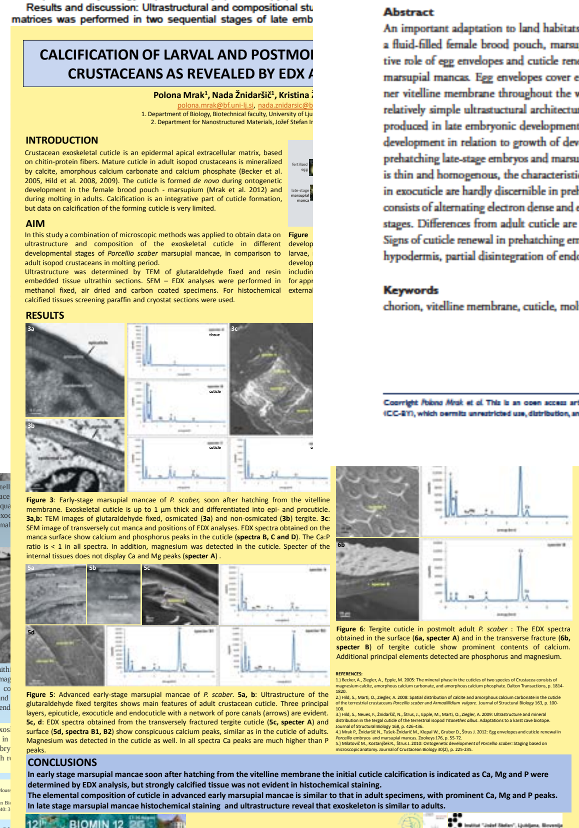

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RESEARCH ARTICLE

Egg envelopes and cuticle renewal in *Porcellio* embryos and marsupial mancas

Polona Mrak[1], Nada Žnidaršič[1], Magda Tušek-Žnidarič[1], Waltraud Klepal[2], Daniela Gruber[2], Jasna Strus[1]

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An important adaptation to land habitats in terrestrial isopod crustaceans is development of embryos in a fluid-filled female brood pouch, marsupium. The study brings insight into the structure and protective role of egg envelopes and cuticle renewal during ontogenetic development of *Porcellio* embryos and marsupial mancas. Egg envelopes cover embryos, the outer chorion until late-stage embryo and the inner vitelline membrane throughout the whole embryonic development. Egg envelopes of *Porcellio* have relatively simple ultrastuctural architecture compared to *Drosophila* egg envelopes. Exoskeletal cuticle is produced in late embryonic development by hypodermal cells of the embryo and is renewed in further development in relation to growth of developing embryos and mancas. Cuticle structure and renewal in prehatching late-stage embryos and marsupial mancas exhibit main features of cuticle in adults. Epicuticle is thin and homogenous, the characteristic arrangement of chitin-protein fibers and the dense distal layer in exocuticle are hardly discernible in prehatching embryo and distinct in marsupial mancas. Endocuticle consists of alternating electron dense and electron lucent sublayers and is perforated by pore canals in both stages. Differences from adult cuticle are evident in cuticle thickness, ultrastructure and mineralization. Signs of cuticle renewal in prehatching embryo and marsupial mancas such as detachment of cuticle from hypodermis, partial disintegration of endocuticle and assembly of new cuticle are described.

Keywords

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RESEARCH ARTICLE
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Exoskeleton anchoring to tendon cells and muscles in molting isopod crustaceans

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Keywords

cuticle, chitin, microtubules, anchoring junctions, extracellular matrix, embryo

CUTICLE STRUCTURE IN *PORCELLIO* EMBRYOS AND…

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[2] Department of Ultrastructural Research, University of Vienna, Vienna, Austria.

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Cuticle formation du… *Porcellio scaber*: Im…

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CUTICLE STRUCTURE IN *Porcellio* EMBRYOS…

CALCIFICATION O… CRUSTACEAN…

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AIM

RESULTS

CONCLUSIONS

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8th International Symposium of Terrestrial Isopod Biology
ISTIB 2011, Bled, Slovenia

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Pisanje.

CUTICLE STRUCTURE IN *PORCELLIO* EMBRYOS AND

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(1) University of Ljubljana, Biotechnical faculty, Department of Biology, Ljub
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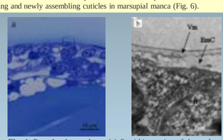
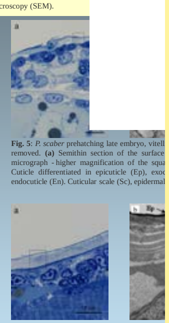
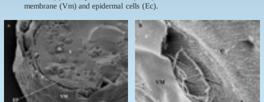
RESULTS AND DISCUSSION







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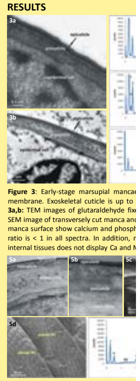

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ABS ACTA BIOLOGICA SLOVENICA
LJUBLJANA 2013 Vol. 56, Št. 2: 51–62

Alizarin red S staining of the crustacean cuticle: implementation in the study of *Porcellio scaber* larvae

Histokemijska analiza kutikule rakov z barvilom alizarin rdeče S: uporaba v proučevanju ličink raka enakonožca vrste *Porcellio scaber*

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Abstract: Exoskeletal cuticle of crustaceans is a chitinous matrix, produced apically by epidermis and stiffened by sclerotization and calcification. Embryos of terrestrial isopod crustacean *Porcellio scaber* develop within the female brood pouch, marsupium, and after hatching larvae mancae continue their development in the marsupium for another week. This study was performed to reveal at the histochemical level whether the exoskeletal cuticle of marsupial mancae is already calcified. Fifteen different procedures of histochemical staining with alizarin red S (ARS), established for calcified tissue localization primarily in vertebrate histology, were evaluated on mancae and adult *P. scaber* specimens. The best differential staining of the exoskeletal cuticle was obtained with neutral buffered formaldehyde fixation, followed by paraffin sections staining with ARS 1 (pH 9) or ARS 2 (pH 6.4) or ARS 3 (pH 4.8) solution. Clear differential staining was achieved also in cryosections of formaldehyde fixed samples, stained with ARS 1 solution (pH 9). Our results suggest that prominent calcification of exoskeletal cuticle is present during postembryonic development of *P. scaber* mancae in the marsupium. Exoskeleton hardening is likely important also for body movements, that we observed in mancae before they were released from marsupium. The proposed procedures of ARS method are presumed to be applicable for histochemical studies of other calcified chitinous matrices.

Keywords: calcification, histochemistry, larval development, terrestrial isopods, Crustacea

Izvleček: Eksoskeletna kutikula rakov je hitinski matriks na apikalni strani epidermisa. Trdnost kutikule je posledica sklerotizacije in kalcifikacije organskega matriksa. Embriji kopenskega raka enakonožca *Porcellio scaber* se razvijajo v vrečastem valilniku samice, marzupiju, kjer po izleganju nadaljujejo svoj razvoj približno en teden tudi ličinke manke. S histokemijsko metodo smo ugotavljali, ali je eksoskeletna kutikula marzupijskih mank že kalcificirana. Na vzorcih mank in odraslih rakov *P. scaber* smo ovrednotili petnajst različnih postopkov histokemijske analize z barvilom alizarin rdeče S (ARS), ki se uporabljajo za lokalizacijo kalcificiranega tkiva pri vretenčarjih. Eksoskeletna kutikula se je izrazito diferencialno obarvala z primerno fiksacijo z nevtralno raztopino formaldehida, ki ji je sledilo

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~Carl Sagan