Inspection of gastro-intestinal system of crustacean embryos

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Keywords: embryo, development, gut, microscopy,

INTRODUCTION

Porcellio scaber is a representative of Oniscidea, terrestrial isopods, which have evolved specific structural and physiological adaptations to land habitats, unique among crustaceans. Digestive system of strictly terrestrial isopods is very complex compared to more primitive amphibious isopods. Due to good knowledge of its biology and easy breeding in laboratory, *P. scaber* is proposed as a model organism for developmental, evolutionary and ecotoxicological studies. Recent works on *P. scaber* embryogenesis base mainly on a simple staging system provided by Whitington et al. [1] which does not include descriptions of digestive system. Recently, an ultrastructural study on morphogenesis of digestive system has been published [2] and we further aim to prepare an upgraded staging system of *P. scaber* ontogenetic development with emphasis on differentiation of digestive system.

MATERIALS AND METHODS

To describe ontogenetic development of *P. scaber*, embryos at various developmental stages were isolated from brood pouches of gravid females and bred in artificial marsupial saline [3]. Embryonic development of live embryos was observed by stereomicroscope and differential interference contrast microscopy at daily intervals. Embryos at various stages of development were stained with DAPI and examined with fluorescent microscopy. Early and late embryos were also fixed for transmission electron microscopy. Semithin sections were stained with toluidine blue and ultrathin sections were contrasted with uranyl acetate and lead citrate.

RESULTS AND DISCUSSION

Ontogenetic development up to release from the brood pouch was divided into 30 well defined stages. Embryogenesis of *P. scaber* lasts 25 days, starting with egg phases (Fig.1a), followed by early (Fig.1b) and late (Fig.1c) embryos, and ending with hatching of late embryos from the vitelline membrane. Hatched marsupial manca larvae (Fig. 1d) stay in brood pouch for an additional week. Specific characteristics of digestive system such as typhlosole canal and hindgut differentiation into anterior and papillate regions enable very effective water retention which is prerequisite for survival of terrestrial isopods in dry conditions [4]. Results show that these differentiations are already present in late embryos still enveloped in vitelline membrane (Fig. 2a). A significant adaptation of terrestrial isopods to cellulose-rich diet is also a stout and complex foregut [2]. Complex cuticular structures of stomach, namely masticatory apparatus, primary and secondary filters, are also well developed already in late embryos (Fig. 2b, 2c, 2d). Presence of specific and very complex structures in the digestive tract of woodlice indicates that digestive system of manca larvae can be fully functional even before their release from the brood pouch.

References:

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DOI: 10.3217/978-3-85125-062-6-274,

This research was supported by Slovenian Research Agency, grant no. 1000-05-310053.

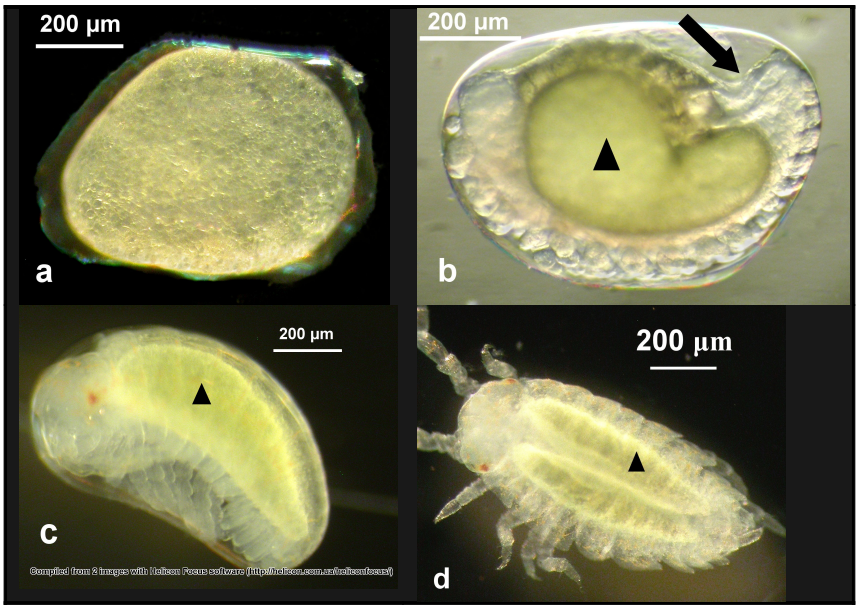

Fig 1. Stages of development of *P. scaber*: **a)** egg; **b)** early embryo; **c)** late embryo (arrow points to the hindgut); **d)** hatched marsupial manca larva. Arrowheads mark hepatopancreatic gland development.

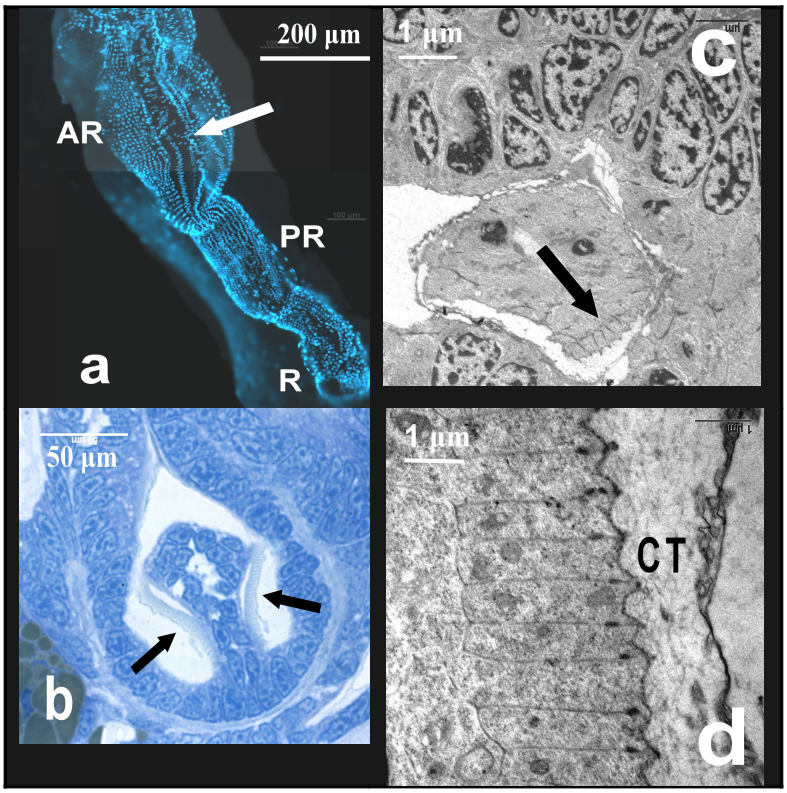

Fig 2. Gastro-intestinal tract of late embryos of *P. scaber*: **a)** DAPI staining of a hindgut with typhlosole (arrow) in the anterior region (AR); papillate region (PR); rectum (R); **b)** toluidine-stained semithin section of a stomach with secondary filter apparatus (arrows); **c)** transmission electron micrograph of a stomach with secondary filter apparatus (arrow); **d)** higher magnification of secondary filter apparatus with cells secreting cuticle (CT).

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