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Ultrastructure of the digestive system and the fate of midgut during embryonic development in *Porcellio scaber* (Crustacea: Isopoda)

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Abstract

Microscopic anatomy of the digestive system in embryos and larvae of the terrestrial isopod crustacean *Porcellio scaber* was investigated by light bright field, fluorescence and electron microscopy. During marsupial ontogenetic development the event-dependent staging was used to discriminate the various embryonic stages. At the late embryo stage the differentiation of the ectodermal part of the gut into the complex filtering foregut and the hindgut with absorptive and transporting functions is accomplished. The gut of the marsupial manca larva is fully developed and similar to that of the adult. In early embryos the endodermal midgut gland primordia are filled with yolk and lipid globules. In late embryos the epithelium of paired midgut gland tubes is composed of two cell types; one of them exhibits orange autofluorescence. The endodermal cells located between the foregut and the midgut glands of late embryos form the prospective midgut. The cells have electron dense cytoplasm, abundant glycogen fields, endoplasmic reticulum, dictyosomes and numerous vesicles. In the adults the endodermal cells of the midgut remain only in the midgut gland ducts which connect the midgut glands and the foregut. Details of the cellular ultrastructure and morphogenesis of the ectodermal and endodermal parts of the digestive system during embryonic development of *Porcellio scaber* provide data for further phylogenetic and comparative studies in peracaridan crustaceans and other arthropods.

Keywords: Woodlice; Gut morphogenesis; Foregut; Midgut; Midgut glands; Hindgut

1. Introduction

In terrestrial isopods ontogenetic development from the egg to the marsupial larva takes place in the ventral brood pouch (marsupium) formed after the parturial moult of sexually mature females (Hoese, 1984). Embryos hatch in several weeks. Marsupial larvae grow and moult, and leave the marsupium approximately in a month. After several moults larvae reach the juvenile stage. Embryos feed mostly on components of the marsupial fluid which is secreted by the female during the breeding period (Warburg and Rosenberg, 1996). The feeding of larvae and juveniles depends on the development of specific and very complex structures present in the digestive tract of the adult woodlice (Storch, 1987; Štrus and Blejec, 2001). The development of the digestive system in woodlouse embryos and larvae has not been studied recently. Several authors described the various stages of embryonic development either based on the nervous system or limb development in woodlice (Hejnol, 2002; Meschenmoser, 1996; Whitington et al., 1993). Goodrich (1939) described the differentiation of the endodermal elements of the digestive system during embryogeny of *Porcellio laevis* in great detail. In a detailed histological study he described cellular processes underlying formation of the stomodeum and proctodeum and their association with the endodermal midintestine lobes (midgut glands). Our previous research of the digestive system in the amphibious isopods of the family Ligiidae determined the presence of a ring of cells between stomodeum and proctodeum forming a true midgut in the adult animals (Štrus et al., 1995). However, in strictly

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terrestrial isopods the true midgut is absent and the endodermal part of the digestive system is restricted to the four tubules of the midgut glands (Holdich, 1973; Bettica et al., 1987; Wägele, 1992). Ultrastructural analysis of the endodermal parts of the digestive system in woodlice embryos and larvae will help to understand the origin and the fate of the midgut during the development of these terrestrial crustaceans.

The way terrestrial isopods have adapted to the terrestrial lifestyle makes them unique among crustaceans. Marsupial development is an important adaptation for woodlouse because it helps them to cope with the lack of water in the terrestrial environment. Many of their life strategies such as feeding, moulting and reproduction, can be compared to the ones adopted by insects, the most successful group of terrestrial arthropods.

Campos-Ortega and Hartenstein (1985) and Costa et al. (1993) described the origin of the midgut epithelium in the terrestrial model organism *Drosophila*. The midgut develops from the anterior and posterior parts of the endoderm, initially contiguous with the mesoderm, originating from the primordia at both ends of the embryonic blastoderm. The endodermal midgut primordia are closely apposed to the ectodermal foregut and hindgut primordia. The prospective midgut cells become mesenhymal, migrate toward each other and form the midgut. Recent investigations provide molecular genetic data on the developmental program of the endodermal midgut in the *Drosophila* model organism. The authors present results on activities of different zygotic genes that act in the early determination of the digestive canal (Weigel et al., 1989, 1990). Reuter (1994) investigated the function of the gene *serpent* in the development of the midgut. Cooperation between HNF3/Fork head and GATA transcription factors is described as important for endoderm specification in *Drosophila* embryos (Nakagoshi, 2005). The evolutionarily conserved mechanism seems to be crucial for the development of endoderm in arthropods.

This paper presents an ultrastructural study of the digestive system in embryos and marsupial larvae of the terrestrial isopod *Porcellio scaber*. Using event-dependent staging the various stages of embryonic development in *Porcellio scaber* are described. A detailed staging of the embryonic development in the amphipod *Parhyale hawaiensis* was used as reference (Browne et al., 2005). Reports including morphogenesis of the digestive tract are sparse among existing reports on embryonic development in crustaceans. Our purpose was to: (1) provide ultrastructural evidence of digestive system morphogenesis including ectodermal and endodermal parts; and (2) describe the ultrastructural features of cellular domains of the endodermal midgut in embryos and larvae of *Porcellio scaber*. As a result, these studies will be followed by further molecular investigations and compared with the other peracaridan crustaceans and terrestrial arthropods.

2. Materials and methods

2.1. Animals used in the experiments

Breeding females of *Porcellio scaber* ($N = 34$) were maintained under laboratory conditions: (1) at 20 °C; (2) daylight cycles; and (3) fed with fish food and carrot slices. At different stages of development eggs, embryos and larvae were taken from the marsupium. Embryonic and larval stages were described according to gross morphological and anatomical features.

Prior to fixation embryos and larvae isolated from the marsupium of breeding females were anaesthetized with carbon dioxide.

2.2. Histological and ultrastructural analyses

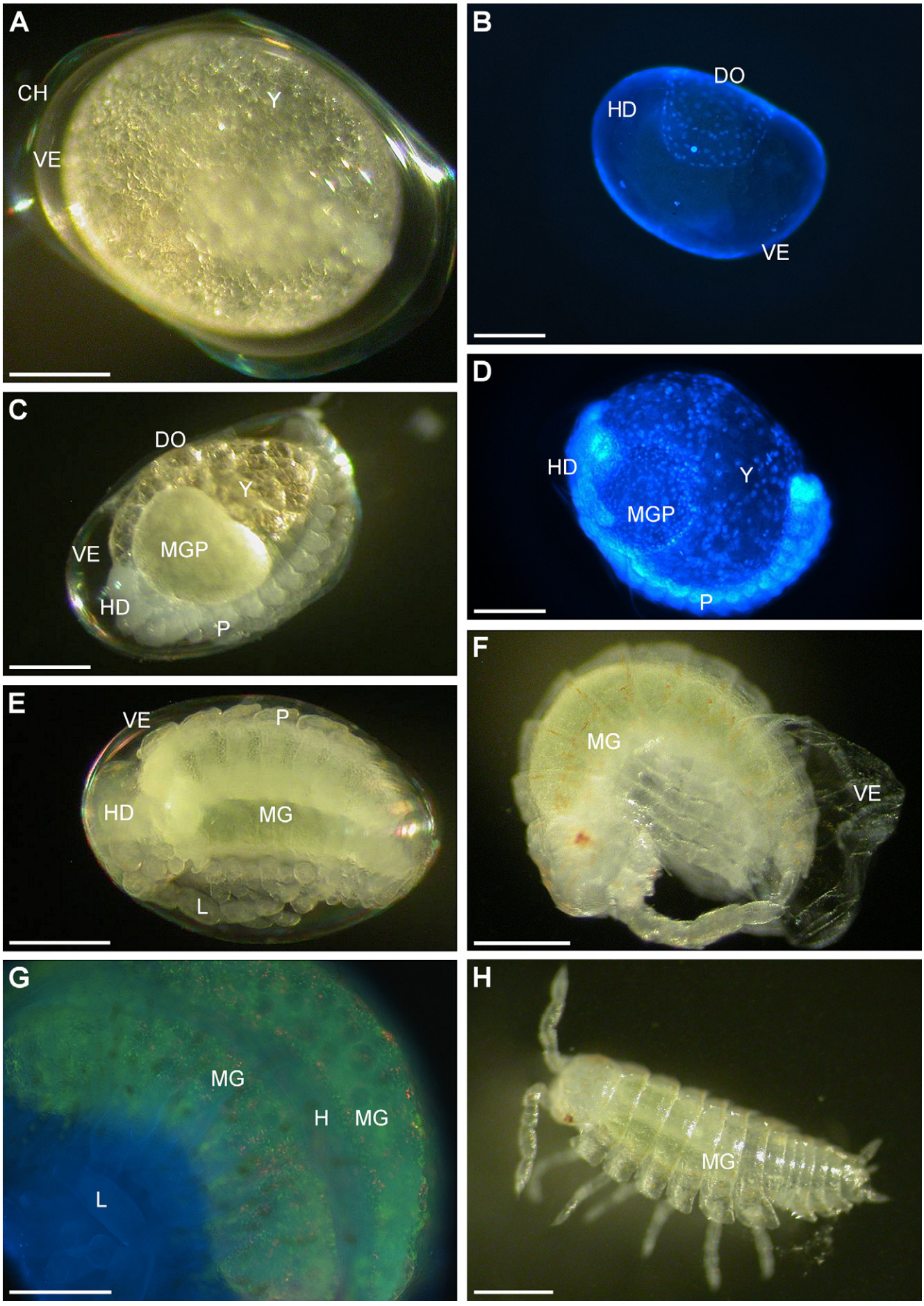
Following the procedure described by Browne et al. (2005) whole embryos were prepared for observation with the stereomicroscope Leica MZFLIII and the fluorescence microscope Zeiss AxioImager Z.1. First the outer embryonic membrane (chorion) of individual embryos was removed. Then embryos were washed for 1 min with methanol and incubated overnight at 4 °C in 50% (vvV) glycerol/1 × PBS containing 1 mg/ml DAPI for visualisation of cell nuclei by fluorescence microscopy. For better penetration of the fixatives the embryonic envelopes were removed prior to fixation. Embryos and larvae were fixed in Carnoy and Karnovsky fixatives and prepared for light and electron microscopy. Paraffin sections were stained with hematoxylin and eosin stains or Pollak Rapid Method (Pollak, 1997); semithin sections were stained with methylen blue (Richardson et al., 1960) and examined with the Axioskop Opton light microscope. Contrasted ultrathin sections of tissues embedded in Spurr were examined with the transmission electron microscope Philips CM 100. Images were taken with a Gatan Bioscan 972 digital camera. Whole embryos and larvae were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, dried in HMDS, postfixed in 1% osmium tetroxide and observed with a Jeol 840 A scanning electron microsope.

3. Results

3.1. Description of embryonic and larval stages

Animals reared in the laboratory breed all year-round in optimal conditions. Duration times for embryonic and

Fig. 1. Embryonic developmental stages in *Porcellio scaber*. Fertilized eggs contain centrally distributed yolk and are protected by a chorion membrane and vitelline envelope (A, light bright field image). Chorion was removed from the early embryos to expose the body with distinct pereomeres, midgut gland primordia and the dorsal organ (C, light bright field image; B, D, fluorescence micrographs stained with DAPI, the vitelline membrane was removed from the embryo on image D). Light bright field images of the late embryo surrounded by a vitelline envelope (E) and an embryo hatching from the vitelline envelope (F). Fluorescence micrograph of a midgut gland tubule in late embryo. Two types of cells can be distinguished by the orange autofluorescence of one cell type and the presence of lipid globules in the other cell type (G). Paired tubular midgut glands are visible through the transparent cuticle in the marsupial manca larva (H, light bright field image). All scale bars 0.2 mm. Chorion (CH), dorsal organ (DO), hindgut (H), head (HD), limb (L), midgut gland (MG), midgut gland primordia (MGP), pereomere (P), vitelline envelope (VE), yolk (Y).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postembryonic stages in the marsupium are from 30 to 35 days (34 females were investigated). In the marsupium of individual females asynchronous development as well as feeding and moulting were observed. Hatched embryos and marsupial larvae feed on eggs and embryos that failed to develop. On average breeding females produced 55 eggs. In our experiment, in different stages of development the marsupium of breeding females contained on average 40 early embryos or 27 late embryos or 23 larvae. The description of stages during embryogenesis in *Porcellio scaber* is based on morphological and anatomical characteristics determined by light bright field and fluorescence microscopy.

3.1.1. Fertilized eggs

Centrolecithal eggs, measuring up to 0.7 mm, contain large amounts of yolk and are protected by a chorion membrane and vitelline envelope (Fig. 1A). Egg cleavage is superficial. The first two cleavages are meridional; they are followed by an equatorial cleavage generating an eight cell oocyte. At the 16-cell stage the nuclei migrate to the egg surface and form the germ band at the egg border. Mesendoderm is discernible at the 64-cell stage (Gerberding and Patel, 2004). The blastula stage with a centrally-segregated yolk and 128 cells forming the blastodisc at the animal pole of the egg develops in several days. Following several waves of cell divisions, thoroughly described by Gerberding (1999), Gerberding and Patel (2004) and Hejnol (2002), gastrulation begins with the formation of the gastrulation center. The endomesodermal layer, which extends laterally into the yolk, forms the lobes of the midgut glands. The mesoderm and endoderm separate after gastrulation. It takes a fertilized egg approximately 7 days to develop into gastrula.

3.1.2. Early embryos

The size of early embryos ranges from 0.8 to 1.0 mm. They are recognized by well developed pereomeres and ventrally located limb buds (Fig. 1C,D). The head region is distinct and the dorsal organ is well developed (Fig. 1B). In unstained embryos an extensive dorsally located yolk sac and the bilateral outgrowths of midgut glands primordia are visible. During the development the vitelline envelope hardens and chorion desintegrates. This stage takes up to 7 days. The complete development, from fertilized egg to early embryo lasts 14 days.

3.1.3. Late embryos

The size of the late embryo ranges from 1.0 to 1.3 mm. The head, with paired antennae, mandibles and lightly pigmented eyes, is well defined (Figs. 1E and 2A). Pigmentation of distal parts of tergites is visible and the pereopods are well developed. The yolk sac is reduced, paired midgut gland tubes extend along the anterior hindgut (Fig. 1G). At the end of this stage the embryo hatches from the vitelline envelope (Fig. 1F). This stage takes up to 10 days. The whole embryonic development can last up to 24 days.

3.1.4. Marsupial manca larvae

Larvae range from 1.4 to 1.7 mm in size. The head, with prominent mouthparts, antennae and dark pigmented eyes, is well developed (Fig. 1H). The hindgut is clearly visible and the midgut glands extend posteriorly. The maxillipeds and six pairs of pereopods are completely developed (Fig. 2B).

Our laboratory experiments show that embryonic development in *Porcellio scaber* lasts less than a month, with most larvae hatching after 24 days and leaving the marsupium after 33 days of development. After the first moult the manca with a pair of maxillipeds and six pairs of pereopods develops into the larva which resembles the adult in the number of segments and legs. The development to the juvenile stage can last up to 8 months.

3.2. Morphology and ultrastructure of the digestive system in embryos and larvae

Early developmental stages of *Porcellio scaber* contain large amounts of yolk; therefore parts of the prospective digestive system are difficult to analyze ultrastructurally. In the early embryonic stage of *Porcellio scaber* the digestive system is differentiated into the ectodermal alimentary canal and the endodermal midgut glands. The foregut is differentiated into the esophagus and the stomach without distinct filtering and mechanical structures; the hindgut consists of prismatic cells covered with a thin cuticle. Tissues are loosely arranged; dividing cells with no prominent cuticular structures are present. Large amounts of yolk are concentrated in the paired tubular sacs of the midgut gland region in the early embryo (Fig. 3A). Flattened cells contain lipid droplets and encircle the yolk and lipid globules which accumulate in the tubular sac. The cells of the dorsal wall of the hindgut form the primordia of the typhlosolis (Fig. 3B).

3.2.1. Ectodermal part of the digestive system in late embryos

The ultrastructure of the digestive system in the late embryo in which the yolk is mostly resorbed is described next. The ectodermal part of the digestive system consists of a stomach differentiated into a grinding and filtering device and a hindgut with a dorsal typhlosolis (Fig. 4A,B). The foregut extends from the mouth to the stomach-hindgut junction. It is covered with a finely elaborated cuticle. Tightly packed ectodermal cells of the filtering regions have large oval nuclei rich in heterochromatin and apical protrusions which secrete the components of the filter cuticle (Fig. 5A,B). Cell cytoplasm is electron-lucent and contains large amounts of glycogen, endoplasmic reticulum, and numerous mitochondria. Various protrusions of the stomach wall are present, including lateralia which direct large food particles to the hindgut, while fluids are filtered into the midgut glands. The digestive system of the larvae is fully differentiated and similar to that in an adult (Fig. 6A,B).

The hindgut of adults and larvae is a long straight tube lined with a cuticle and connected to the stomach by valve-like structures (Figs. 6A,B and 7A). The division of the hindgut into the anterior chamber and papillate region, typical for adults is not clear in embryos. However different cell types

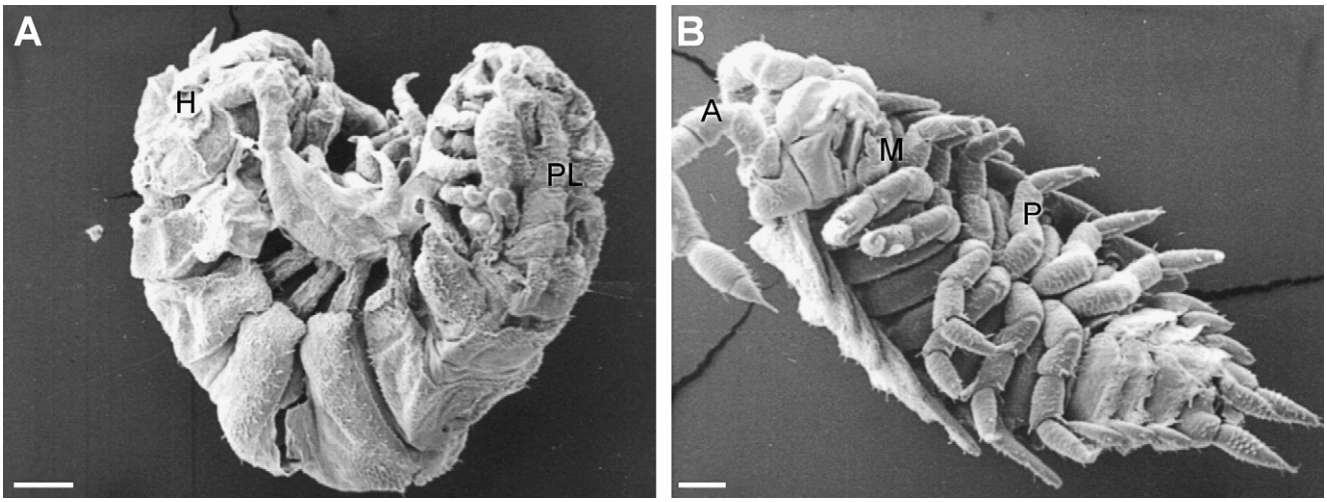

Fig. 2. SEM micrographs of the late embryo removed from the vitelline envelope (A) and of the marsupial manca larva with a paired maxilliped and six pairs of pereopods (B). Scale bars 100 μm. Antenna (A), head (H), maxilliped (M), pereopod (P), pleon (PL).

are present in the dorsal and ventral parts of the tubular hindgut (Figs. 7B and 8A). The dorsal rows of cells have large oval nuclei with abundant heterochromatin. Large intercellular spaces were observed between the cells of the dorsal wall of the anterior hindgut epithelium (Fig. 8B). Cells located ventrally have large nuclei with a net-like pattern (Fig. 8C,D).

3.2.2. Endodermal part of the digestive system in late embryos

Cells without cuticular lining were observed at the foregut-hindgut junction of an adult *Porcellio scaber* in the atrium area where ducts of the midgut glands merge with the stomach (Figs. 6A and 7A). In embryos the region between the ectodermal part of the digestive system and the midgut glands consists of clusters of endodermal cells with electron dense cytoplasm (Fig. 7B,C). The cells have large nuclei, abundant glycogen fields, endoplasmic reticulum, vesicles and dictyosomes. The endodermal part of the digestive system in late embryos contains different cells with microvilli which are either connected to the atrium of the stomach or organized into midgut gland-tubes. Midgut glands are paired tubular structures composed of a monolayered epithelium and filled with lipid globules and yolk. The epithelium is composed of two cell types which can be distinguished by fluorescence microscopy. The cells

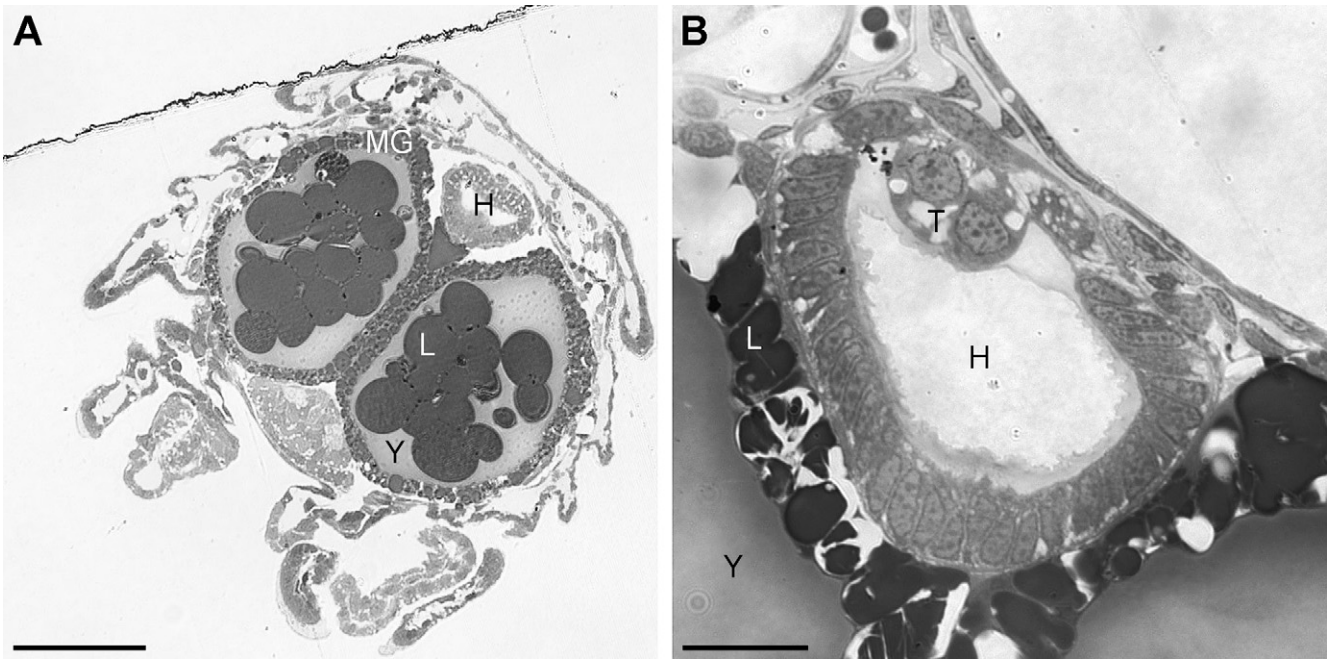

Fig. 3. Histological section of the early embryo with paired tubular midgut glands and the hindgut (A, scale bar 100 μm) and a larger magnification of the hindgut with primordia of the typhlosolis (B, scale bar 20 μm). Hindgut (H), lipids (L), midgut glands (MG), typhlosolis (T), yolk (Y).

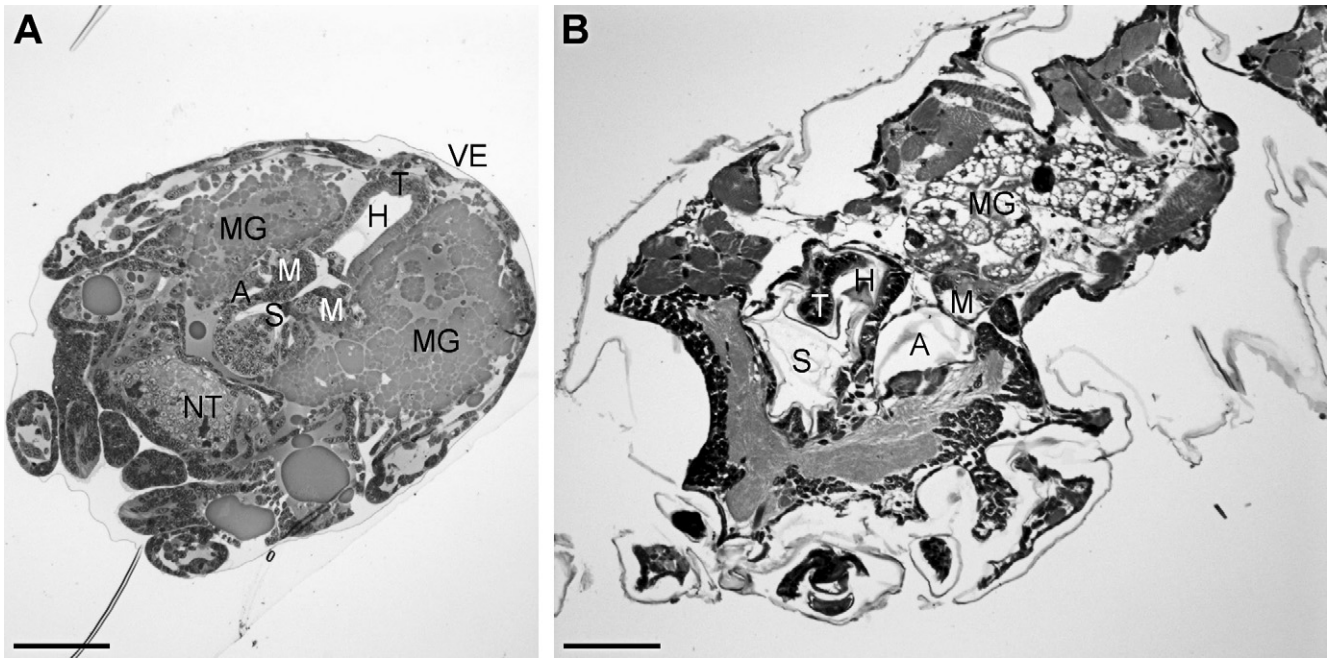

Fig. 4. Cross semithin section of the late embryo in the area of the foregut-midgut-hindgut junction (A, scale bar 100 μm). Longitudinal histological section of the late embryo stained with the Pollak rapid method (B, scale bar 100 μm). Atrium (A), hindgut (H), midgut (M), midgut glands (MG), neural tissue (NT), stomach (S), typhlosolis (T), vitelline envelope (VE).

which exhibit orange fluorescence under UV are interspersed among the cells with lipid droplets (Fig. 1G). Ultrastructurally midgut gland epithelium consists of: (1) large cells with microvillar surface and electron-dense cytoplasm containing lipid droplets and mitochondria (Fig. 9A); and (2) smaller cells with large nuclei (Fig. 9B). Occasionally intracellular bacteria were found in the cytoplasm of large cells, frequently apposed to nuclei and lipid droplets (Fig. 9C,D).

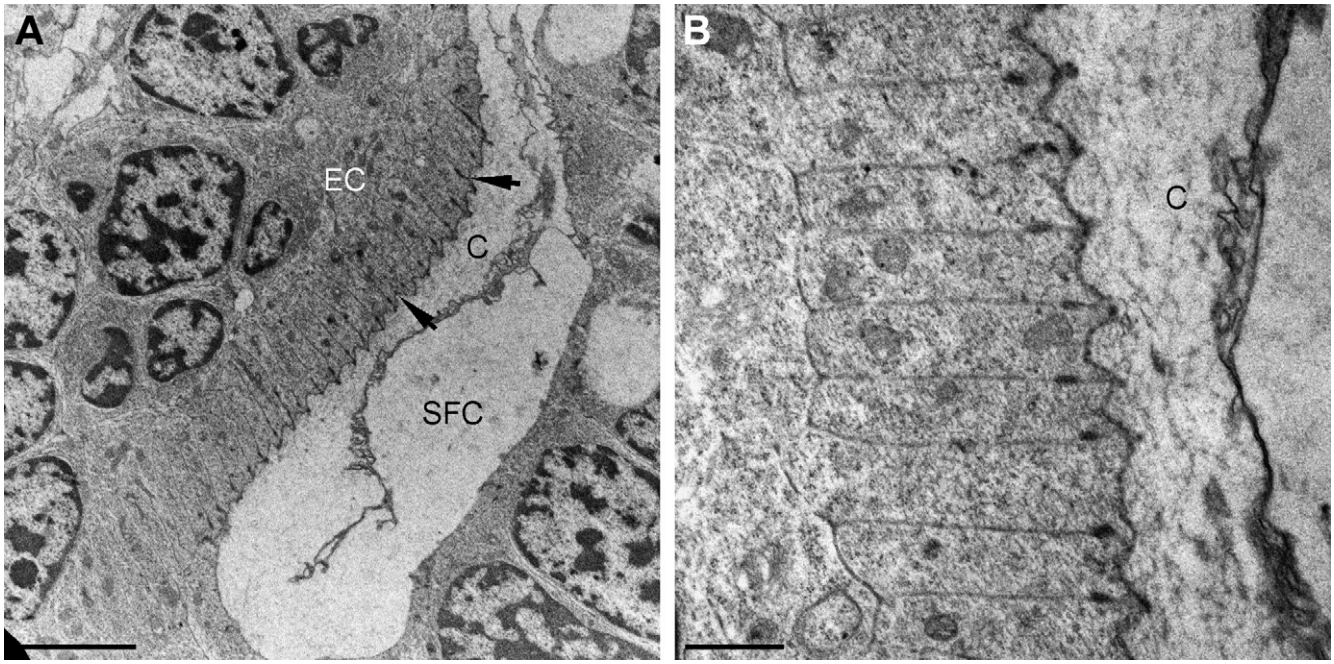

Fig. 5. TEM micrograph of the secondary filtering region of the stomach in the late embryo. Ectodermal cells (EC) secrete a new cuticle of the secondary filter (A, arrows point to the microvillar cell surface, scale bar 5 μm). Apical parts of tightly packed ectodermal cells with a microvillar surface (B, scale bar 1 μm). Secondary filter canal (SFC), cuticle (C).

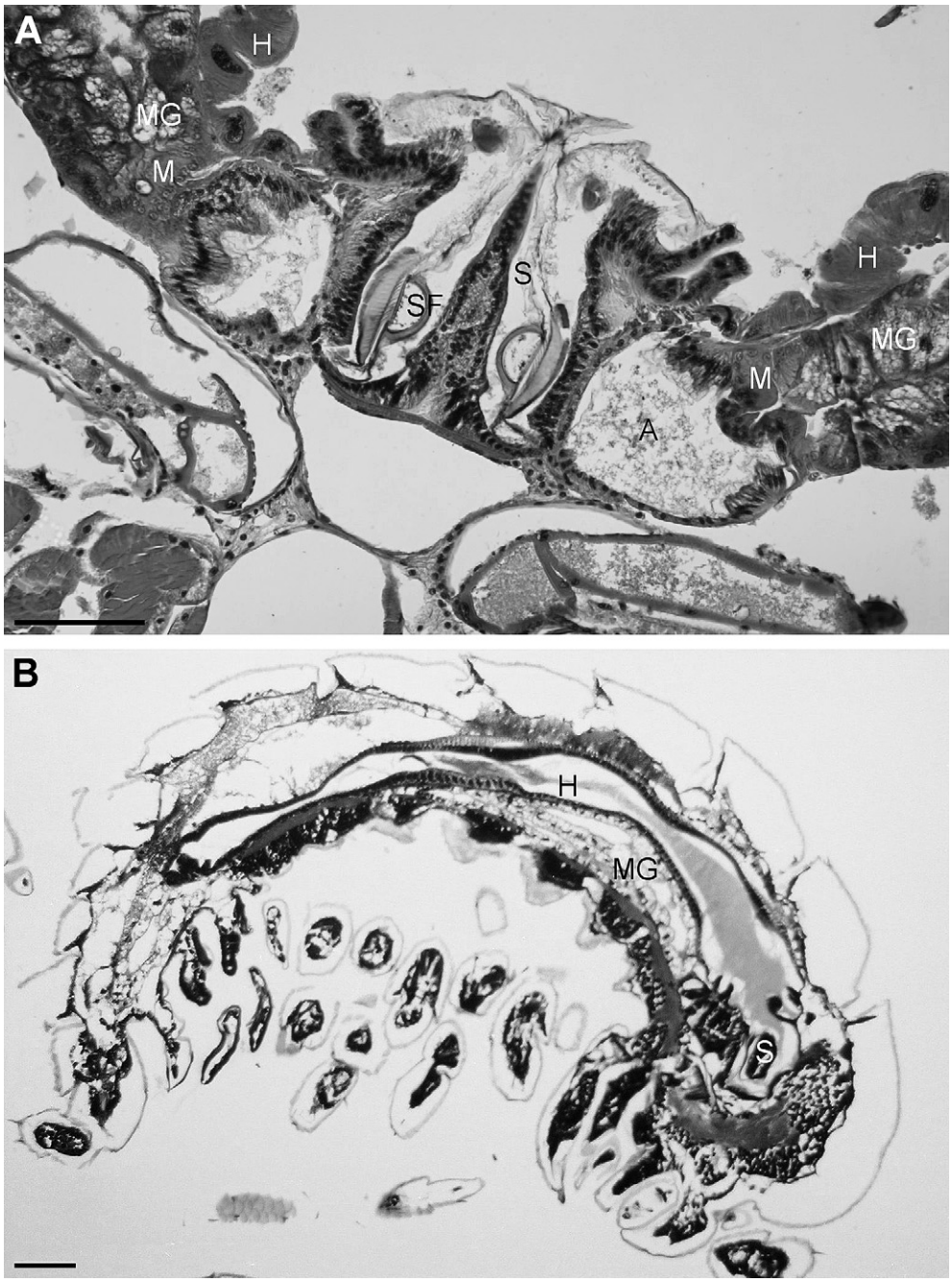

Fig. 6. Histological cross section of the foregut-midgut-hindgut junction in the adult *Porcellio scaber* (A, scale bar 100 μm). Longitudinal section of the larva exposing the digestive system. Tissues were stained with the Pollak rapid method (B, scale bar: 100 μm). Atrium (A), hindgut (H), midgut (M), midgut glands (MG), stomach (S), secondary filter (SF).

4. Discussion

4.1. Embryonic and larval development in the marsupium of breeding females

Morphogenesis of the digestive system was studied in several crustaceans but there are few data on gut development in terrestrial isopods (McMurrich, 1895; Goodrich, 1939). Age-dependent staging of embryonic development in *Porcellio scaber* was suggested by Whitington et al. (1993). According to his results the development from the egg to hatching takes approximately 26 days. After two weeks of development, when the embryo hatches from the chorion, the hindgut begins to extend anteriorly. The early ontogenetic development of peracaridan crustaceans and the functional morphology of the embryonic dorsal and lateral organs in the terrestrial isopod *Oniscus asellus* were described by Meschenmoser (1996). Since the embryos within the envelope are ‘‘comma’’ shaped, size measurement is not a very accurate staging mechanism. Because of the asynchronous development of the embryos in

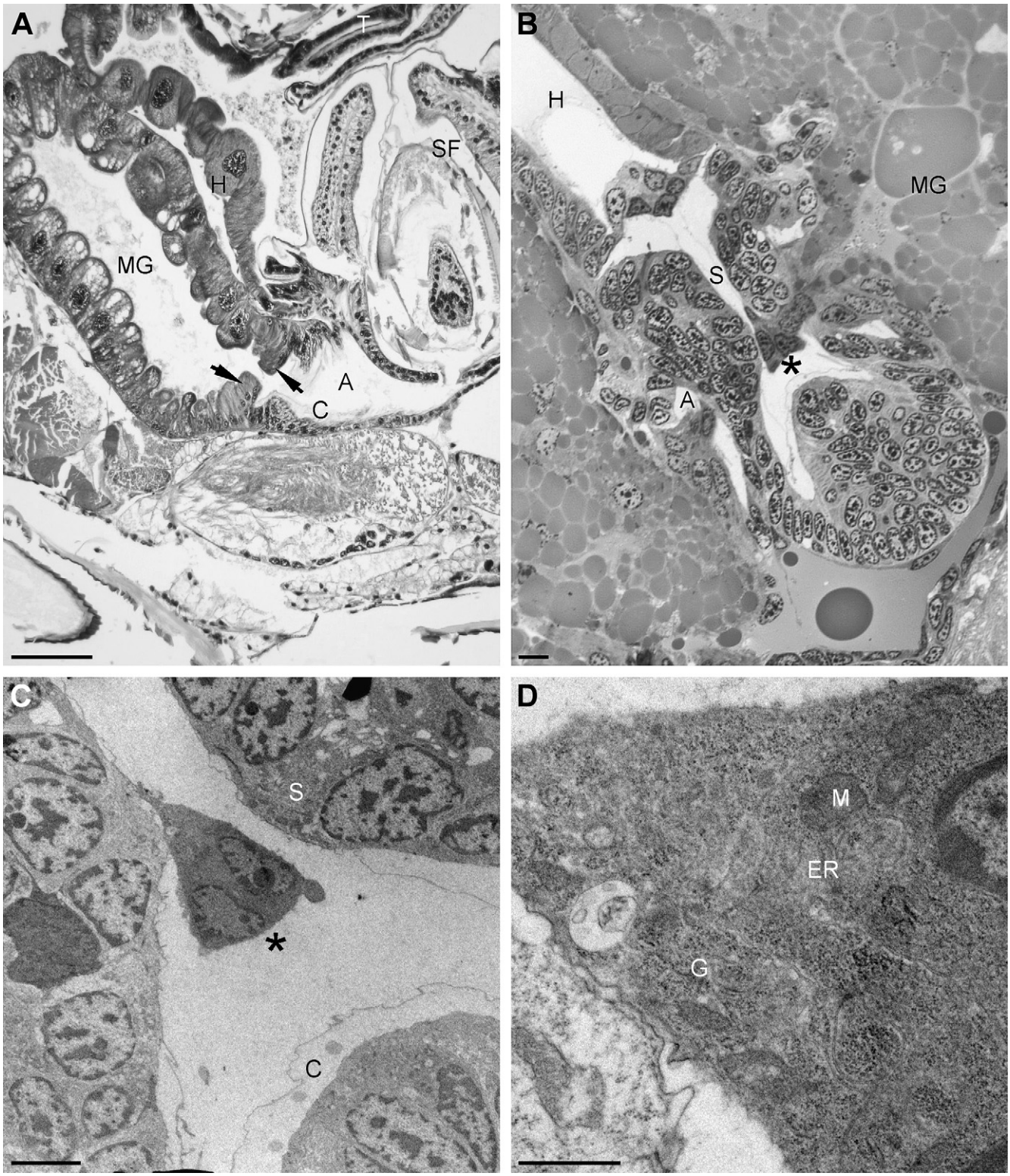

Fig. 7. Histological cross section of the stomach in adult *Porcellio scaber* stained with the Pollak rapid method. The atrium of the stomach is covered with cuticle. A ring of endodermal cells forming the midgut gland ducts (arrows) connects the atrium and the midgut glands (A, scale bar 100 μm). A semithin section of the foregut-midgut-hindgut junction in late embryos. Endodermal cells in the midgut area are marked by an asterisk (B, scale bar 10 μm; C, scale bar 5 μm). TEM micrograph of a midgut cell with dark cytoplasm and abundant glycogen fields (D, scale bar 1 μm). Atrium (A), cuticle (C), endoplasmic reticulum (ER), glycogen (G), hindgut (H), mitochondrion (M), midgut glands (MG), stomach (S), secondary filter (SF), typhlosolis (T).

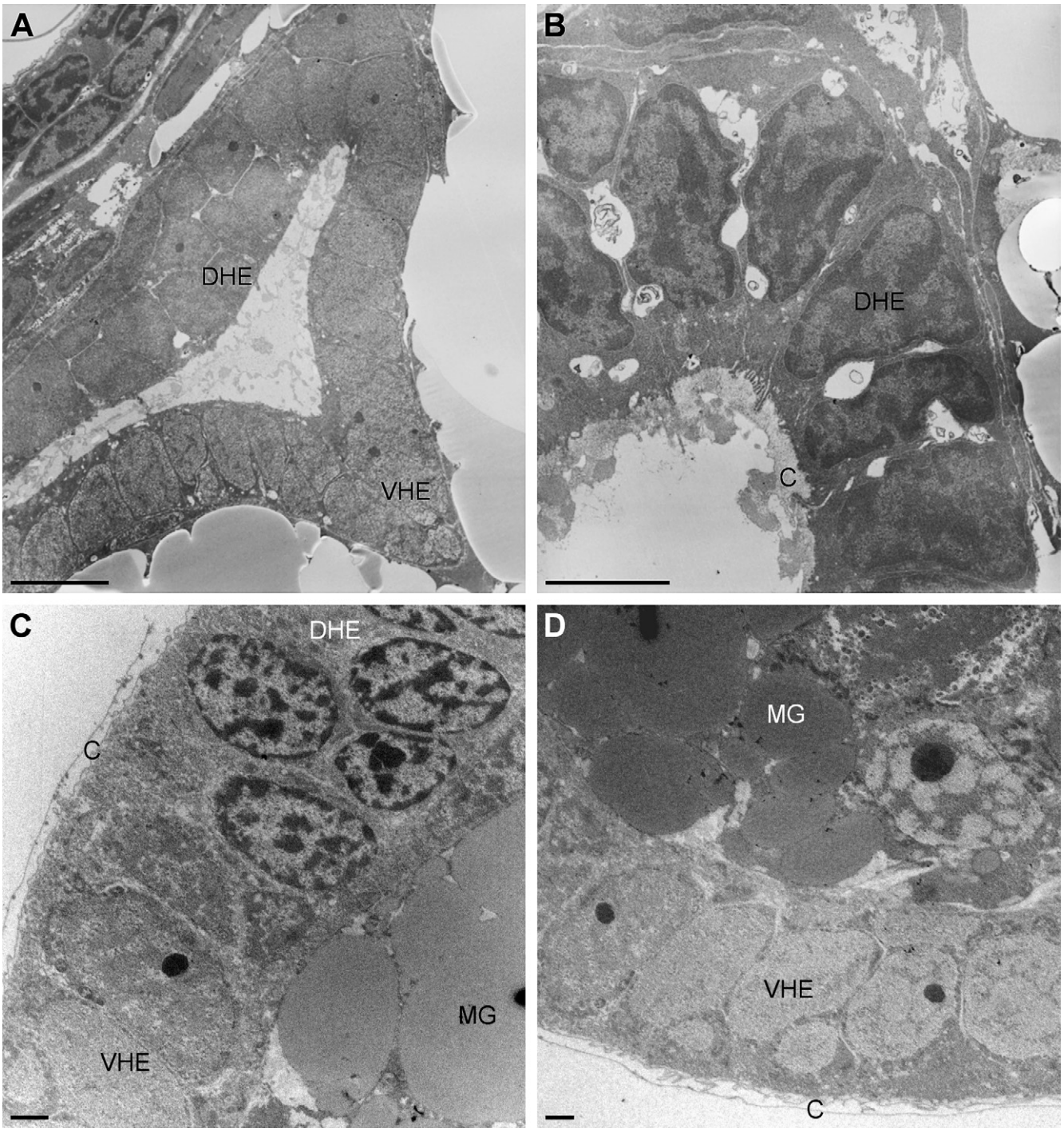

Fig. 8. A semithin section of the hindgut epithelium composed of two cell types in the early embryo (A, scale bar 10 μm). The dorsally located cells have large intercellular spaces (B, scale bar 5 μm). TEM micrographs of the hindgut epithelium with the two different cell types arranged dorsally and ventrally (C, scale bar 2 μm). Cells of the ventral part of the hindgut are closely apposed to the cells of the midgut glands which contain lipid droplets (D, scale bar 2 μm). Cuticle (C), dorsal hindgut epithelium (DHE), midgut glands (MG), ventral hindgut epithelium (VHE).

the marsupium, it is impossible to describe the stages in the age-dependent way accurately. For the purposes of our study we used event-dependent staging and present only approximate durations of the described embryonic stages. Fertilized eggs and early embryonic stages in *Porcellio scaber* were described in detailed studies of early cell line differentiation in limb morphogenesis and axonogenesis (Hejnol, 2002; Hejnol and Scholtz, 2004; Hejnol et al., 2006). The early embryo is described as a stage with limb buds, prominent yolk sac and midgut glands primordia and an embryonic dorsal organ which is involved in osmoregulation. After developing for two weeks the early embryo hatches from the chorion. The late embryo is surrounded by the vitelline envelope which is an embryonic cuticle. The head region with mouthparts, antennae and pigmented eyes is prominent, maxillipeds and six pairs of pereopods are fully developed. The embryo rotates dorsoventrally

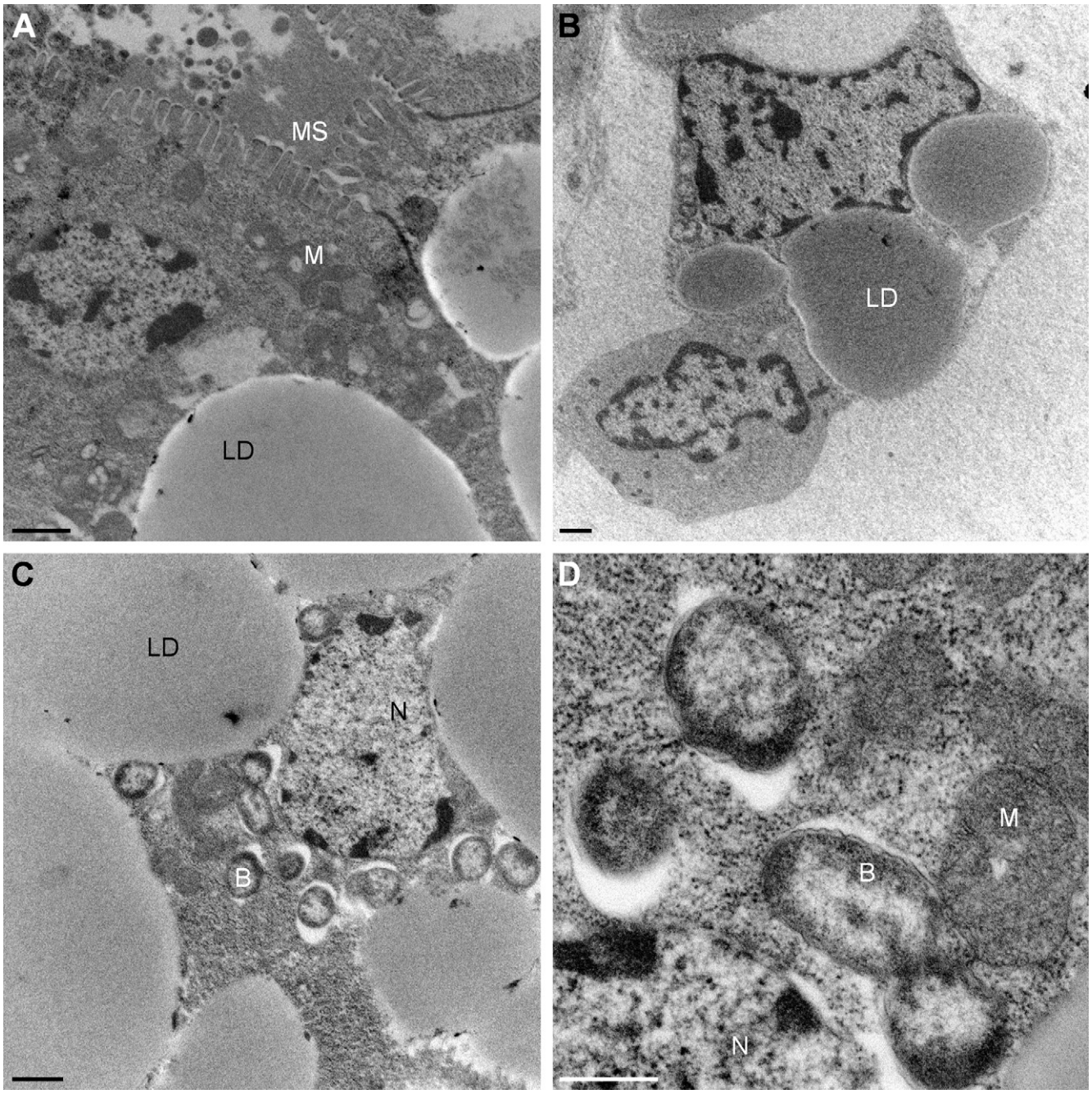

Fig. 9. TEM micrographs of the two cell types present in the midgut gland epithelium of late embryos. Large cells with microvillar border are filled with lipid droplets (A, scale bar 1 μm). Smaller cells with large nuclei and lipid droplets are interspersed between the large cells (B, scale bar 1 μm). Intracellular bacteria are present in the large cells (C, scale bar 1 μm). Bacteria are frequently apposed to nuclei and lipid droplets (D, scale bar 0.5 μm). Bacteria (B), lipid droplet (LD), microvillar surface (MS), mitochondrion (M), nucleus (N).

within the envelope and hatches into manca larva. The latter remains in the marsupium for up to ten days and feeds on non-developed eggs and embryos. The manca larva lacks the seventh pereomere and the seventh pair of pereopods. They develop following the first moult after leaving the marsupium. Postembryonic development in *Porcellio scaber* was thoroughly described by Tomescu and Craciun (1987).

We recommend this simple event-dependant staging of embryonic development in *Porcellio scaber* for further comparative, ecological and ecotoxicological studies where embryos are used as model organisms.

4.2. Ultrastructure of the digestive system and fate of the midgut in embryos and larvae of Porcellio scaber

The structure and function of the digestive system in adults of *Porcellio scaber* has been described by several authors (Bettica et al., 1987; Storch, 1987; Hames and Hopkin,

1989; Štrus et al., 1995). A complex digestive tract is lined entirely by cuticle. The four midgut gland tubules are the only endodermal part of the digestive system in the adult. The cuticle of the alimentary canal is renewed by frequent moulting. The morphogenesis of the digestive system in embryos and larvae of *Porcellio scaber* is described in this study for the first time. We investigated: (1) the ultrastructural differentiation of the cuticle in the masticatory and filtering parts of the stomach; (2) the formation of the typhlosolis in the hindgut; and (3) the differentiation of the endodermal midgut. Goodrich (1939) presented a detailed histological study of the early development of the gut in *Porcellio laevis*. During gastrulation the mesendodermal cells migrate into the yolk area and form the primary endoderm from which midgut glands develop. Most mesendodermal cells are arranged in epithelial sheets surrounding the yolk ventrolaterally. They form the epithelial midintestine, while some of the cells migrate dorsally and form a temporary yolk sac between the stomodeum and the proctodeum. When the yolk sac is completely absorbed, the midintestine is divided into bilateral lobes. They evaginate anteriorly, forming paired hepatic diverticula, which send mesial outgrowths toward the stomodeum. The outer wall of the lip of stomodeum envelops the mesial outhgrowths of hepatic diverticula. After their desintegration it forms the epithelium of the hepatic ducts. The proctodeum grows forward and fuses with the stomodeum. Thus the endoderm remains only in the annexes of the digestive canal and the hepatic diverticula.

Our study confirmed and further explained the development of the gut at the ultrastructural level. In the early embryos the alimentary canal is a simple tube formed by the fusion of stomodeum and proctodeum. The cells with large nuclei secrete a thin cuticle and divide frequently. In late embryos the stomach exhibits complex structure with masticatory and filtering parts. The cells of the filtering region with microvillar projections contain large amounts of glycogen. They secrete the cuticle of the filters. The cells of the dorsal and ventral hindgut wall are structurally different which suggests their functional variety. The dorsal typhlosolis is present in the anterior hindgut. Large intercellular spaces observed between the cells of the dorsal hindgut epithelium suggest that the cells are frequently rearranged during the formation of typhlosolis and/or cuticle secretion. After hatching of the embryo, the alimentary canal of marsupial larvae is fully developed and they can feed on the debris from the marsupium.

The purpose of the present study was to describe the ultrastructure of different types of ectodermal and endodermal cells during the embryonic development of a strictly terrestrial isopod *Porcellio scaber*. We also wanted to provide evidence of endodermal cells at the foregut-midgut-hindgut junction for future molecular studies of midgut differentiation in embryos. In most peracaridan crustaceans and arthropods the endodermal midgut (mesenteron) is a long tube like the structure located between the stomodeum and the proctodeum (Schmitz and Scherrey, 1983; Friesen et al., 1986; Wirkner and Richter, 2007). In isopods this part of the digestive tract is reduced (Wägele, 1992). In adult amphibious species of the family Ligiidae a ring of cells forming a true midgut between the stomach and the hindgut was described (Štrus and Drašlar, 1988; Štrus et al., 1995). The ultrastructure of the cells with a dense microvillar border, numerous dictyosomes, vesicles and large amounts of glycogen suggests their secretive function. In embryos of *Porcellio scaber* cells with apical microvilli, electron dense cytoplasm, abundant rough endoplasmic reticulum and numerous vesicles were observed in the region between midgut glands and the atrium of the stomach. The cell ultrastructure is similar to that described in the midgut of *Ligia italica* and other peracaridan crustaceans (Graf and Michaut, 1980; Štrus et al., 1995). The cells with a similar structure were observed in the midgut gland ducts of adult *Porcellio scaber*. Our results suggest that the endodermal cells described in late embryos of *Porcellio scaber* are the precursors of the epithelium of the midgut gland ducts. This remains to be confirmed by immunocytochemical studies.

The flattened mesendodermal cells that surround the yolk in early embryos develop into a monolayered midgut gland epithelium. The pattern of scattered orange autofluorescence indicates that the midgut gland epithelium of late embryos is already differentiated into two cell types. The orange autofluorescence is characteristic for the S cells in the midgut glands in adults (Žnidaršič et al., 2005). Most of the cells in the midgut gland epithelium of late embryos have a dense microvillar border and contain numerous lipid droplets and mitochondria. Among them small cells with large nuclei were interspersed. Presumably the two cell types described are the precursors of the large B cells and small S cells in the midgut glands of the adults (Hames and Hopkin, 1989).

This study provides ultrastructural evidence of different cellular lines of the digestive system in late embryos and larvae of *Porcellio scaber*. Cells of the ectodermal part are characterized by ultrastructural features, such as large glycogen fields, dense filaments at the apical part and large intercellular spaces, which are related to cuticle secretion. The mesendodermal cell lines surrounding the yolk differentiate into midgut gland epithelium. The prospective cells of the embryonic midgut form the epithelium of the midgut gland ducts which connect the glands to the atrium of the stomach in adults. The identification and ultrastructural description of the midgut cells in the embryos of terrestrial isopods provides data for further molecular genetic analyses of endodermal cell lines with specific markers, already applied in other arthropods.

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References

Bettica, A., Witkus, R., Vernon, G.M., 1987. Ultrastructure of the foregut-hindgut junction in *Porcellio scaber* Latreille. Journal of Crustacean Biology 7, 619–623.

Browne, W.E., Price, A.L., Gerberding, M., Patel, N.H., 2005. Stages of embryonic development in the amphipod crustacean, *Parhyale hawaiensis*. Genesis 42, 124–149.

Campos-Ortega, J.A., Hartenstein, V., 1985. The Embryonic Development of *Drosophila melanogaster*. Springer Verlag, Berlin.

Costa, M., Sweeton, D., Wieschaus, E., 1993. Gastrulation in *Drosophila*: Cellular mechanisms of morphogenetic movements. In: Bate, M., Martinez Arias, A. (Eds.), The Development of *Drosophila melanogaster*. Cold Spring Harbor Laboratory Press, NY, pp. 425–465.

Friesen, J.A., Mann, K.H., Willison, J.H.M., 1986. Gross anatomy and fine structure of the gut of the marine mysid shrimp *Mysis stenolepis* Smith. Canadian Journal of Zoology 64, 431–441.

Gerberding, M., 1999. Cell lineage und engrailed Expression in der Mittellinie der höheren Krebse *Orchestia cavimana* und *Porcellio scaber*. Dissertation, Inst. für Biologie der Humboldt-Universität zu Berlin.

Gerberding, M., Patel, N.H., 2004. Gastrulation in crustaceans: germ layers and cell lineages. In: Stern, C. (Ed.), Gastrulation: From Cells to Embryos. Cold Spring Harbor: CSHL Press, pp. 78–89.

Goodrich, A.L., 1939. The origin and fate of the entoderm elements in the embryogeny of *Porcellio laevis* Latr. and *Armadillidium nasatum* L. (Isopoda). Journal of Morphology 64, 401–429.

Graf, F., Michaut, P., 1980. Fine structure of the midgut posterior caeca in the crustacean *Orchestia* in intermolt: recognition of two distinct segments. Journal of Morphology 165, 261–284.

Hames, C.A.C., Hopkin, S.P., 1989. The structure and function of the digestive system of terrestrial isopods. Journal of Zoology 217, 599–627.

Hejnol, A., 2002. Der postnaupliale Keimstreif von *Porcellio scaber* und *Orchestia cavimana* (Crustacea, Peracarida): Zelllinie, Geneexpresion und Beginn der Morphogenese. Dissertation, Institut für Biologie der Humboldt-Universität zu Berlin.

Hejnol, A., Scholtz, G., 2004. Clonal analysis of *Distal-less* and *engrailed* expression patterns during early morphogenesis of uniramous and biramous crustacean limbs. Development Genes and Evolution 214, 473–485.

Hejnol, A., Schnabel, R., Scholzt, G., 2006. A 4-Dmicroscopic analysis of the germ band in the isopod crustacean *Porcellio scaber* (Peracarida, Malacostraca)-developmental and phylogenetic implications. Development, Genes and Evolution 216, 755–767.

Hoese, B., 1984. The marsupium in terrestrial isopods. Symposium of the Zoological Society London 53, 65–76.

Holdich, D.M., 1973. The midgut/hindgut controversy in isopods. Crustaceana 24, 211–214.

McMurrich, J.P., 1895. Embryology of the isopod crustacea. Journal of Morphology 11, 63–154.

Meschenmoser, M., 1996. Dorsal- und Lateralorgane in der Embryonalentwicklung von Peracariden (Crustacea, Malacostraca). Dissertation, Göttingen, Cuvillier-Verlag.

Nakagoshi, H., 2005. Functional specification in the *Drosophila* endoderm. Development Growth and Differentiation 47, 383–392.

Pollak, O.J., 1997. A rapid trichrome stain. In: Presnell, J.K., Schreibman, M.P. (Eds.), Humason's Animal Tissue Techniques, fifth ed.). The Johns Hopkins University Press, pp. 144–145.

Reuter, R., 1994. The gene *serpent* has homeotic properties and specifies endoderm versus ectoderm within the *Drosophila* gut. Development 120, 1123–1135.

Richardson, K.C., Jarett, L., Finke, E.H., 1960. Embedding in epoxy resins for ultrathin sectioning in electron microscopy. Stain Technology 35, 313–323.

Schmitz, E.H., Scherrey, P.M., 1983. Digestive anatomy of *Hyalella azteca* (Crustacea, Amphipoda). Journal of Morphology 175, 91–100.

Storch, V., 1987. Microscopic anatomy and ultrastructure of the stomach of *Porcellio scaber* (Crustacea: Isopoda). Zoomorphology 106, 301–311.

Štrus, J., Blejec, A., 2001. Microscopic anatomy of the integument and digestive system during the molt cycle in *Ligia italica* (Oniscidea). In: Kensley, B., Brusca, R.C. (Eds.), Crustacean Issues. "Isopod systematics and evolution", vol. 13. A.A. Balkema Publishers, Rotterdam, Netherlands, pp. 343–352.

Štrus, J., Drašlar, K., 1988. Ultrastructural evidence of midgut cells in the isopod *Ligia italica* (Isopoda, Crustacea). Institute of Physics ConferenceSeries 93, Institute of Physics, Bristol and Philadelphia 3, 149–150.

Štrus, J., Drobne, D., Ličar, P., 1995. Comparative anatomy and functional aspects of the digestive system in amphibious and terrestrial isopods (Isopoda: Oniscidea). In: Alikhan, M.A. (Ed.), Crustacean Issues. "Terrestrial Isopod Biology", vol. 9. A.A. Balkema Publishers, Rotterdam, Netherlands, pp. 13–23.

Tomescu, N., Craciun, C., 1987. Postembryonic ontogenetic development in *Porcellio scaber* (Crustacea. Isopoda). Pedobiologia 30, 345–350.

Wägele, J.W., 1992. Isopoda. In: Harrison, F.W., Humes, A.G. (Eds.), Microscopic Anatomy of Invertebrates, vol. 9. Wiley-Liss, Inc., Crustacea, pp. 529–617.

Warburg, M.R., Rosenberg, M., 1996. Brood-pouch structures in terrestrial isopods. Invertebrate Reproduction and Development 26, 45–62.

Weigel, D., Bellen, H.J., Jurgens, G., Jackle, H., 1989. Primordium specific requirement for the homeotic gene *fork head* in the developing gut of the *Drosophila* embryo. Roux's Archives of Developmental Biology 198, 201–210.

Weigel, D., Jurgens, G., Klingler, M., Jackle, H., 1990. Two gap genes mediate maternal terminal pattern information in *Drosophila*. Science 248, 495–498.

Whitington, P.M., Leach, D., Sandeman, R., 1993. Evolutionary change in neural development within the arthropodsaxonogenesis in the embryos of two crustaceans. Development 118, 449–461.

Wirkner, C.S., Richter, S., 2007. The circulatory system and its spatial relations to other major organ systems in Spelaeogriphacea and Mictacea (Malacostraca, Crustacea) – a three-dimensional analysis. Zoological Journal of the Linnean Society 149, 629–642.

Žnidaršič, N., Zdešar, K., Štrus, J., 2005. Microscopic characterization of hepatopancreatic S cells with regard to their role in copper homeostasis of isopods. In: Čeh, M., Dražić, G., Fidler, S. (Eds.), 7th Multinational Congress on Microscopy, Portorož, Slovenia. *Proceedings*. Ljubljana: Slovene Society for Microscopy: Department for Nanostructured Materials. "Jožef Stefan" Institute, pp. 251–252.