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Ultrastructural studies on the reproductive system of progenetic *Diplocotyle olrikii* (Cestoda, Spathebothriidea): Ovarian tissue

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Abstract

The ultrastructure of the ovary and oogenesis are described from the immature and sexually mature female reproductive system of the progenetic spathebothriidean tapeworm, *Diplocotyle olrikii* from the body cavity of *Gammarus oceanicus*. Two types of cells are described: germinal (oogonia, oocytes) and interstitial. A comparison is made of the fine structure of oogonia, early and advanced maturing oocytes and mature oocytes. Two types of inclusions, cortical granules and lipid droplets, are produced by maturing oocytes, and remain in the cytoplasm of mature oocytes within the ovovitelline duct lumen while only lipid droplets are evident in the oocyte cytoplasm of intrauterine eggs. The fate and possible functions of both inclusions are discussed. The interstitial component of the ovary is a syncytium. The maturing oocyte surface is prolonged into lamellae, forming a lamellar mesh with adjacent germ cells and close association of interstitial mitochondria. Deep invaginations of the ovarian basement layer between numerous folds of ovarian lobules facilitate close contact of the interstitium and sarcoplasmic glycogen-rich processes with maturing oocytes. Synchronism in maturity among all of the oocytes in the ovary is shown at different stages of oogenesis. Such a pattern of oogenesis results in the production of many eggs at the same stage of development and is considered an adaptation for the dissemination of fertilized eggs that occurs only at the death of the gammarid host.

Key words

Cestoda, *Diplocotyle olrikii*, reproductive ultrastructure, progenetic pattern of oogenesis, oocyte inclusions, interstitial syncytium

Introduction

The general pattern of oocyte development in parasitic platyhelminths is well known: oogonial cells at the periphery of the ovary divide to form primary oocytes that enlarge to mature cells that fill the interior of the ovary. The structure and development of these oocytes is basically similar in cestodes as well as in digeneans and monogeneans (Gresson 1964; Spence and Silk 1971; Erasmus 1973; Halton *et al*. 1976; Grant *et al*. 1977; Holy and Wittrock 1986; Orido 1987, 1988; Awad and Probert 1990; Tappenden *et al*. 1993). Rybicka (1966) has reviewed her own and earlier studies of oogenesis of tapeworms done at the light microscope level. According to her, the first meiotic division occurs after the oocyte leaves the ovary and is completed with entry of sperm into the oocyte. More recently, there have been brief descriptions of the structure at the TEM level of the ovary sheath and the localization of the different stages of oocyte development of *Caryophyllaeus laticeps* (see Davydov *et al*. 1994), *Gangesia parasiluri* (see Korneva and Davydov 2001), *Proteocephalus torulosus* and *P. exiguus* (see Korneva 2001), *Triaenophorus nodulosus* (see Korneva 2002), *Diphyllobothrium latum* (see Poddubnaya 2002), *Eubothrium rugosum* (see Poddubnaya 2003a), *Archigetes sieboldi* (see Poddubnaya 2003b; Poddubnaya *et al*. 2003) and *Cyathocephalus truncatus* (see Poddubnaya *et al*. 2005). Except for the last study, little is known of oogenesis in spathebothriidean cestodes, especially in progenetic polyzoic species.

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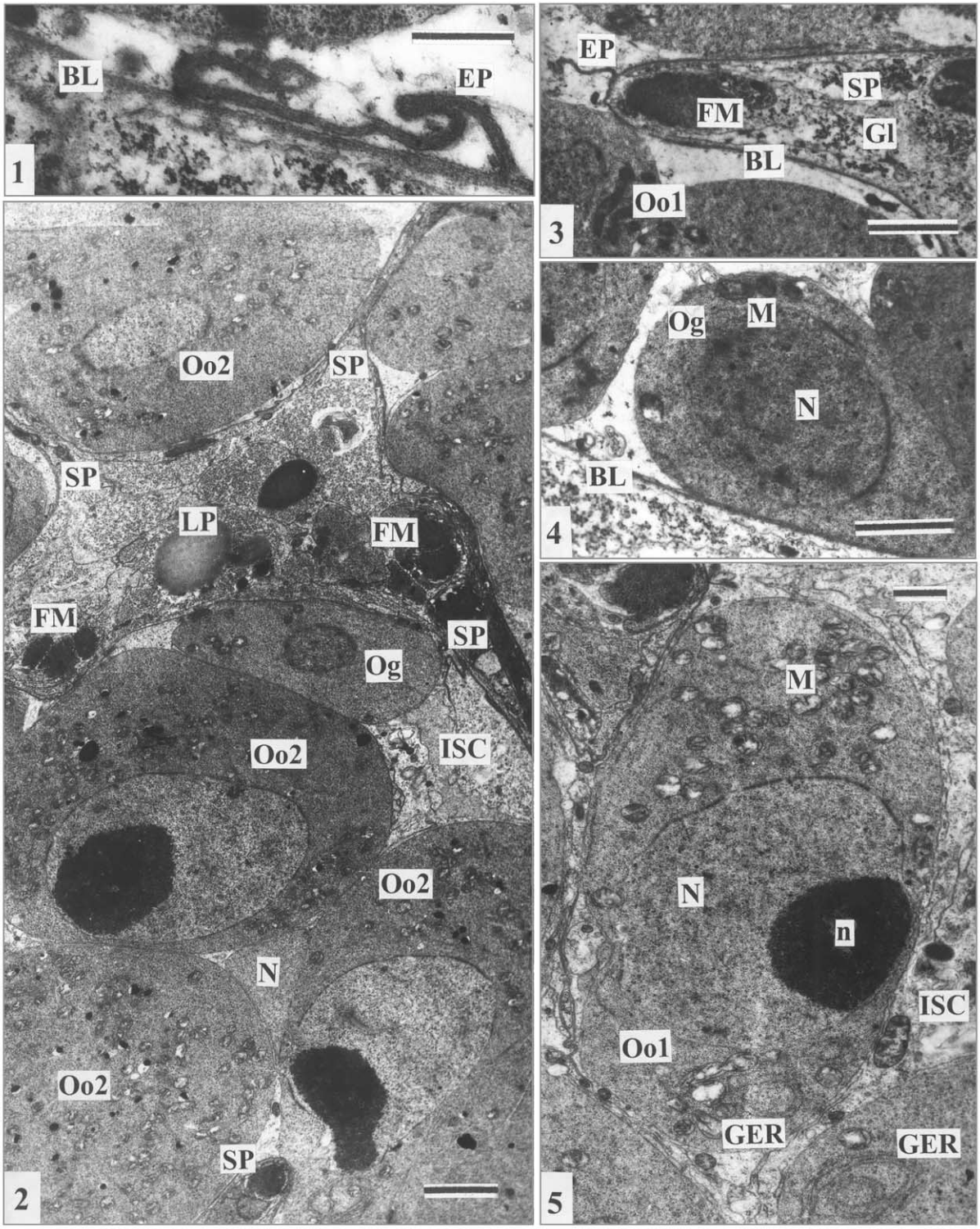


Figs 1–5. Ovarian histology and oocyte development of *Diplocotyle olrikii*. **Fig. 1.** Ovary epithelial layer with narrow projection. **Fig. 2.** Ovarian tissue with deep penetration of sarcoplasmic projections between oocytes. **Fig. 3.** Close relationship between oocyte plasmalemma and sarcoplasmic projection. **Fig. 4.** Oogonia within ovary of immature reproductive system. **Fig. 5.** Early maturing oocyte. Scale bars = 0.5 μm (Fig. 1), 3 μm (Fig. 2), 2 μm (Figs 3 and 4) and 1 μm (Fig 5). **Abbreviations to all figures:** BL – basement layer, CG – cortical granules, ED – epithelial wall of the ovovitelline duct, EP – epithelial projection, FM – fibrous muscle, GC – Golgi complex, GER – granular endoplasmic reticulum, Gl – glycogen, GV – vesicular Golgi cisternae, ISC – interstitial syncytium cytoplasm, L – lamellae, LM – lamellar mesh, LP – lipid droplet, M – mitochondria, MO – mature oocyte (ovum), N – nucleus, n – nucleolus, Og – oogonium; Oo1 – early maturing oocyte, Oo2 – advanced stage of oocyte maturation, OT – ovarian tissue, PU – proximal uterus, SP – sarcoplasmic projection, VM – vitelline material

The aim of this study is to: (a) present more detail of the fine structure of ovarian tissue in another spathebothriidean cestode, progenetic *Diplocotyle olrikii*, with particular attention to the types of granule inclusions within mature oocytes, (b) compare oogenesis between cestode species with progenetic development to those without it, and (c) consider the adaptive significance of nutritional and developmental characteristics associated with oogenesis in this species.

Materials and methods

The large progenetic procercoids or "progenetic caudate plerocercoid" in the system of Chervy (2002) of *Diplocotyle olrikii* (Krabbe, 1874) were obtained from the body cavity of amphipods, *Gammarus oceanicus* from the White Sea. According to Leontovich and Valovaya (1989), the life cycle of this cestode from Kandalaksha Bay of the White Sea, where our worms were collected, is limited to gammarids, and does not involve any sea fishes as definitive hosts. The worms were cut into small pieces, fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 6 h at 4°C, and postfixed in 1% OsO_4 in 0.1 M cacodylate buffer for 1 h at 4°C. The material was dehydrated in a graded series of acetone, and embedded in Araldite and Epon. Semithin sections were cut on a Reichert ultramicrotome, stained with methylene blue and examined under light microscope for identification of reproductive organs. Ultrathin sections were stained with uranylacetate and lead citrate. They were examined in a JEOL-100 C transmission electron microscope (TEM).

Results

Ovary

The strongly bilobed ovary of *Diplocotyle olrikii* has each lobe subdivided into smaller folded lobules. The two main lobes are connected by a narrow isthmus from which arises the oviduct. At the TEM level, the lumen of the ovary is lined by an ovarian epithelial layer that is thin, compressed and has narrow projections (Fig. 1). The ovary is surrounded externally by a thin basement layer and sarcoplasmic projections which are filled with glycogen, mitochondria, solitary lipid droplets and fibrous muscles (Figs 2 and 3). Deep invaginations of the ovarian basement layer between numerous folds of ovarian lobules bring the glycogen-rich sarcoplasmic projections in contact with the maturing oocytes. A thin ovarian wall (Figs 2 and 3) separates the oocyte plasmalemma from the glycogen-containing projections. There are two types of cells within the ovary: germinal (oocytes) and interstitial.

Oocytes

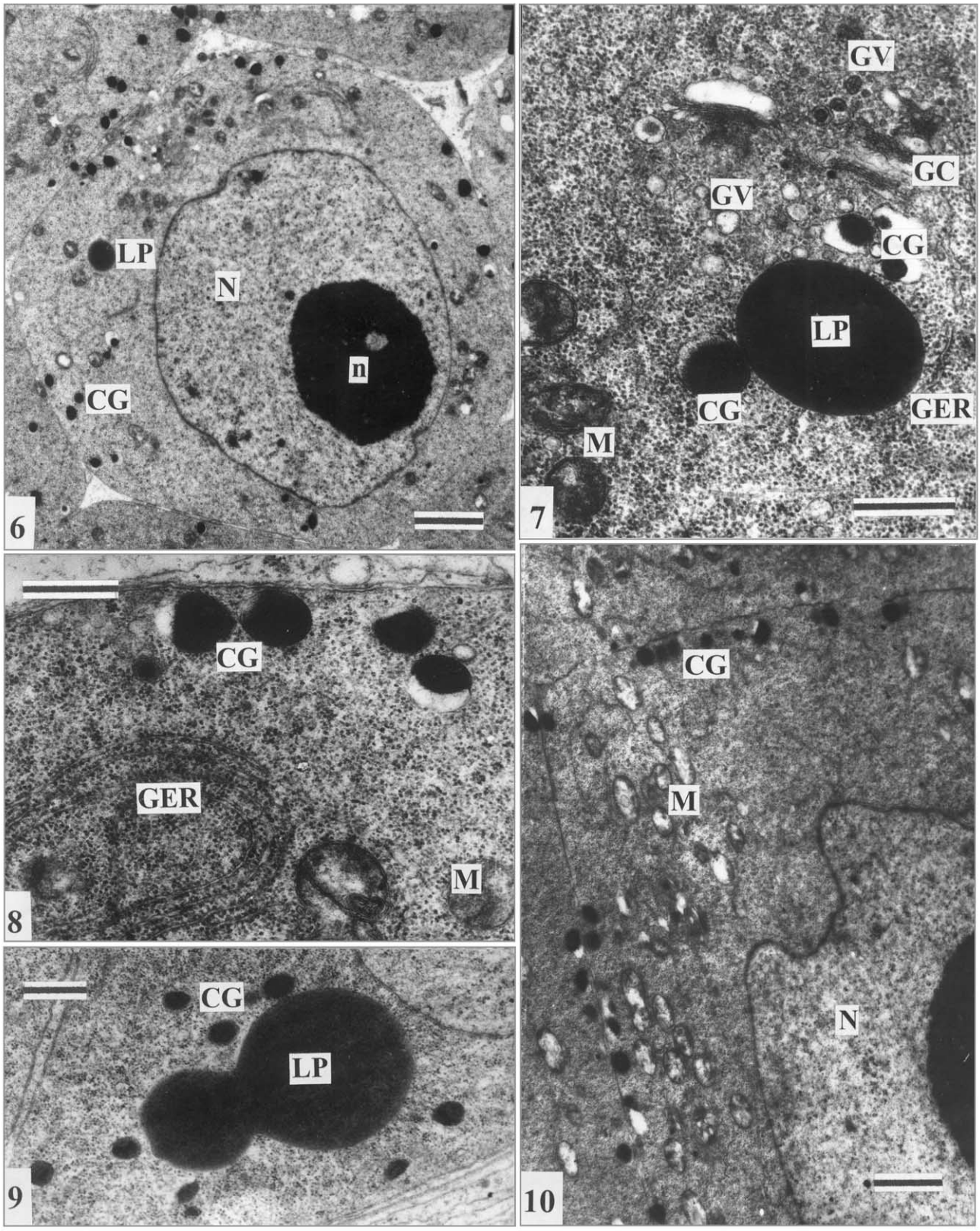
The ovary of immature *D. olrikii* is filled with the first type of germinal cell or oogonium. Oogonia have little cytoplasm and a nucleus that lacks a nucleolus but has dense chromatin patches. The cytoplasm is filled with free ribosomes and contains a few mitochondria (Figs 2 and 4). Oogonia are up to 9 μm in length and to 4.5 μm wide. As the ovary matures, the oogonia mature into oocytes. The early maturing oocytes measure approximately 12 μm in length and 7.5 μm in width. Their cytoplasm is marked by the appearance of profiles of endoplasmic reticulum with attached ribosomes. Mitochondria increase in number and are scattered throughout the cytoplasm. In contrast to the oogonia, the nuclei of oocytes usually have a well defined nucleolus (Fig. 5) and do not contain dense patches of chromatin.

In the ovary of fully mature worms, advanced maturing oocytes and mature oocytes predominate with only solitary cells in earlier stages of development (Fig. 2). The cytoplasmic volume of advanced maturing oocytes is larger than that of early oocyte stages (Fig. 6). They are approximately 17 μm in length and 14 μm in width and are characterized by the appearance of Golgi complexes and production of two types of inclusions: cortical granules and lipid droplets (Figs 6–9). The spherical nucleus is in the centre of the cell and measures about 11 × 9.5 μm in diameter (Fig. 6). Golgi complexes are in the form of short rods and small vacuole-like structures and lie in a compact mass adjacent to the endoplasmic reticulum and mitochondria (Fig. 7). Ribosomes are in a free state and also associated with the endoplasmic reticulum (Figs 8 and 14). Cortical, ovoid granules, about 0.35 × 0.26 μm in diameter, are membrane bound and homogeneously electron-dense. They are few in number and are localized in the central area of the cytoplasm (Figs 6–9). Dense material, resembling early cortical granules, form within the vesicular Golgi cisternae. The vesicles coalesce and their contents condense, producing fully formed granules (Fig. 7). The second type of inclusions, dense lipid droplets, varies in size (from 0.6 up to 1.6 μm in diameter) and occurs singly or in groups (Figs 6, 7, 9, 13 and 16). They increase in size by fusing with each other (Fig. 9).

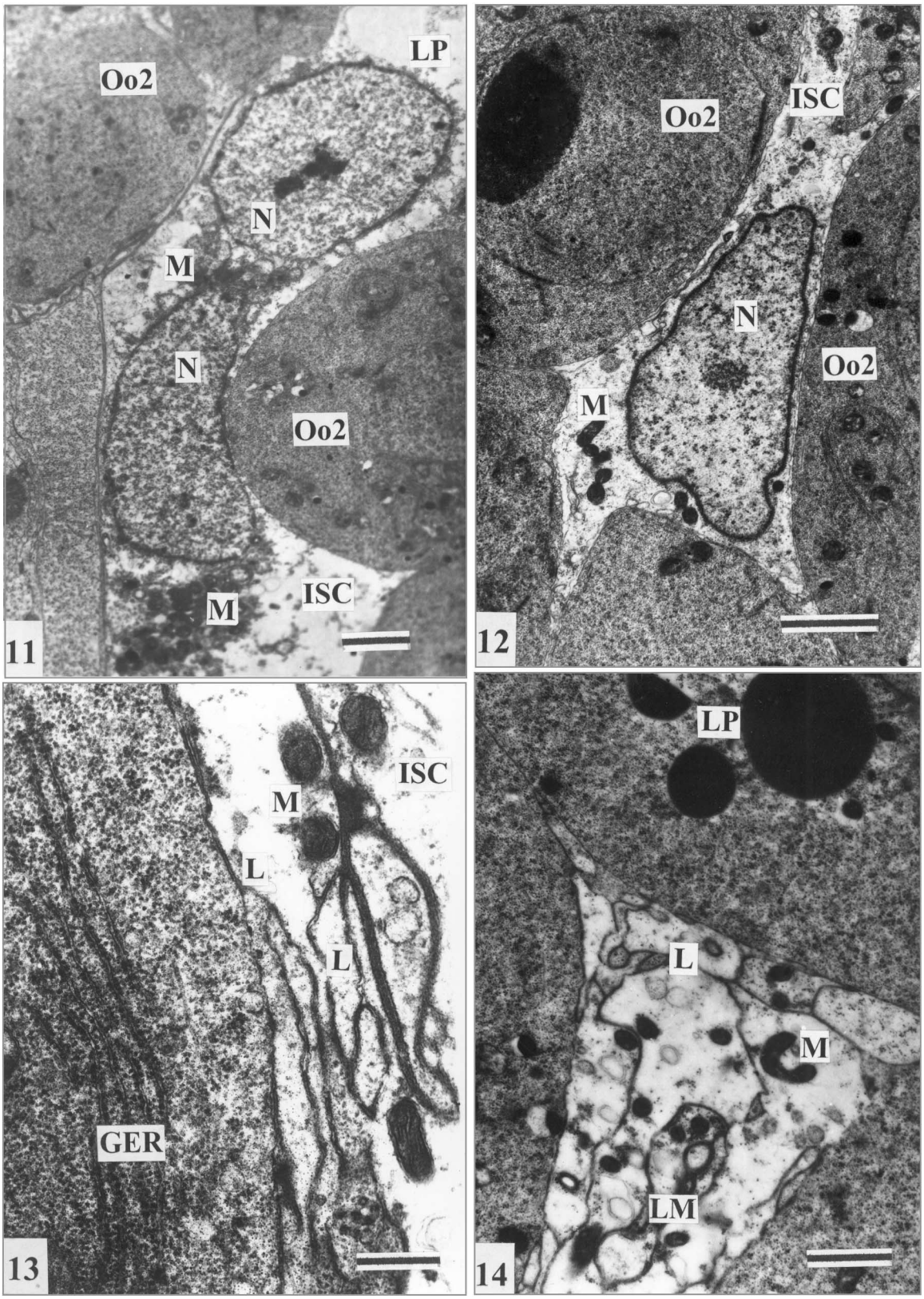
Mature oocytes are rounded when free from other cells, but may become deformed or hexagonal when in contact with other cells (Fig. 10). They display a subplasmalemmal layer made up of cortical granules and the plasmalemma (Fig. 10). Mature oocytes also contain lipid droplets and numerous small mitochondria evenly dispersed in the cytoplasm. Sometimes there are conspicuous aggregations of rough endoplasmic reticulum.

The surface of the plasma membrane of maturing oocytes becomes elevated to form stacks of lamellae (Figs 13 and 14). These thin lamellae are long, dendritic and consist of a two-layered plasma membrane. In some regions of the oocyte, lamellae lie close and parallel to the surface while in other places they are separate and elevated and intermixed with similar lamellae of adjacent oocytes. Where lamellae of adjacent cells mix there is supporting syncytial cytoplasm with numerous mitochondria (Figs 13 and 14).

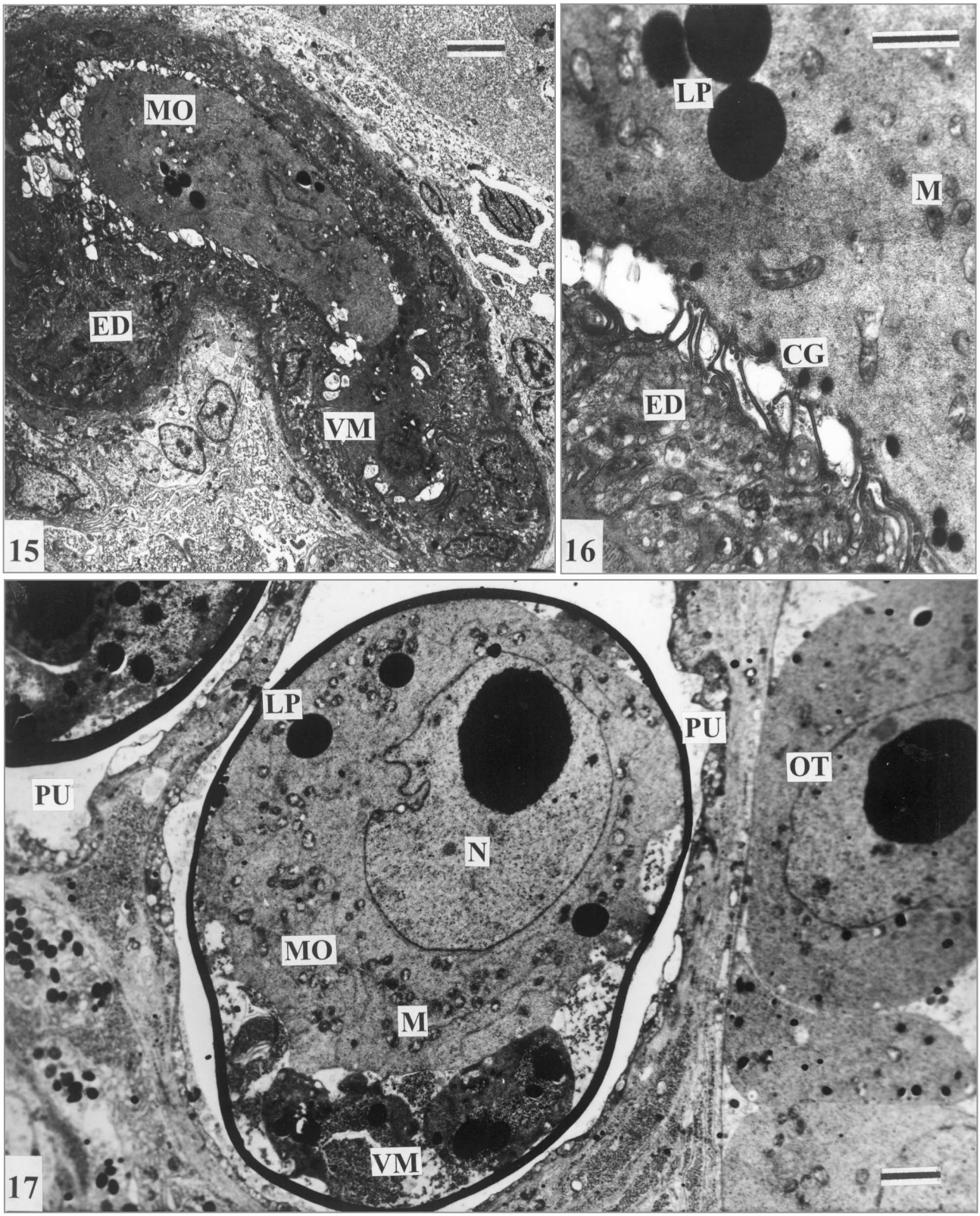
Mature oocytes break away from the ovary and enter the oviduct and pass to the ootype. The ovicapt (= sphincter of



Figs 6–10. Maturing and mature oocytes of *Diplocotyle olrikii*. **Fig. 6.** Advanced stage of oocyte maturation. **Fig. 7.** Golgi complexes and formation of cortical granules. **Fig. 8.** Cortical granules within cytoplasm. **Fig. 9.** Two types of inclusions within oocyte cytoplasm. **Fig. 10.** Fragment of mature oocyte cytoplasm with subplasmalemmal layers of cortical granules. Scale bars = 2 μm (Fig. 6), 0.5 μm (Figs 7–9) and 1 μm (Fig. 10)



Figs 11–14. Interstitial syncytium and surface of maturing oocyte of *Diplocotyle olrikii*. **Fig. 11.** Interstitial syncytium of ovarian tissue. **Fig. 12.** Interstitial nucleus and surrounding syncytial cytoplasm. **Fig. 13.** Elevated lamellae on the surface of adjacent maturing oocytes. **Fig. 14.** Surface lamellae associated with syncytial cytoplasm and mitochondria. Scale bars = 2 μm (Figs 11 and 12), 0.5 μm (Fig. 13) and 1 μm (Fig. 14)



Figs 15–17. Mature oocytes within lumen of reproductive ducts in *Diplocotyle olrikii*. **Fig. 15.** Ovovitelline duct with oocyte and fragment of vitelline cell cytoplasm. **Fig. 16.** Cortical granules and lipid droplets in cytoplasm of oocyte from ovovitelline duct. **Fig. 17.** Fertilized oocyte within the newly formed egg with a thick, electron-dense egg shell located in the proximal part of uterus. Scale bars = 5 μm (Fig. 15), 1 μm (Fig. 16) and 2 μm (Fig. 17)

oviduct) is not prominent and appears to lack any muscle layers, a characteristic that may indicate that there is little selection of oocytes by the ovicapt. Within the ovovitelline duct where oocytes and fragments of vitelline cytoplasm mix, oocytes contain lipid droplets and cortical granules (Figs 15 and 16). On the other hand, only lipid droplets, but no cortical granules, were seen (Fig. 17) in the cytoplasm of fertilized oocytes of intrauterine eggs.

Interstitial cells

The other type of cell forms the interstitial tissue that is in contact with all ovarian cells and forms an amorphous material of light electron density. The interstitial tissue is a syncytium that contains several nuclei scattered at the periphery as well as middle of the ovary (Figs 11 and 12). Syncytial cytoplasm contains numerous mitochondria, free ribosomes, myelin structures, endoplasmic reticulum, Golgi components and rarely lipid droplets (Figs 11–14). The nuclei are round or oval with or without a nucleolus, and their chromatin is dispersed (Figs 11 and 12).

Discussion

Progenesis

We consider *Diplocotyle olrikii* as a progenetic tapeworm because of the presence of a cercomer (Protasova and Roytman 1995) and gonopore closed by tegument (Leontovich and Valovaya 1989), described on the basis of light microscope observations from gravid *D. olrikii* with a monoxenous cycle from amphipods. Furthermore, a covering cyst-like structure on the worm's surface, similar to the glycocalyx layer of progenetic *Archigetes*, has been shown in progenetic *D. olrikii* from the body cavity of gammarids by Davydov *et al*. (1997).

Progenesis in *D. olrikii* truncates the life cycle and appears to be an adaptive strategy for maintaining the life cycle without a vertebrate host in a marine littoral zone.

Oogenesis

Oogenesis in the progenetic spathebothriidean tapeworm, *D. olrikii*, appears to follow the same general pattern observed in other parasitic flatworms (Gresson 1964; Rybicka 1966; Spence and Silk 1971; Erasmus 1973; Halton *et al*. 1976; Grant *et al*. 1977; Holy and Wittrock 1986; Orido 1987, 1988; Awad and Probert 1990; Tappenden *et al*. 1993; Davydov *et al*. 1994; Korneva and Davydov 2001; Korneva 2001, 2002; Poddubnaya 2002; Poddubnaya *et al*. 2005). That pattern has the stages: oogonia, primary oocytes, maturing oocytes, and mature oocytes. As in most helminths, oogonia and primary oocytes are at the periphery of the ovary and move to the central area of the ovary to complete their maturation. Also, there is no significant difference between the ultrastructure of developing cells during oogenesis of *D. olrikii* and other parasitic flatworms.

There are differences, however, in the pattern of oogenesis. Unlike other helminths where there is little synchrony among all of the oocytes, in *D. olrikii* most of them appear to be at closely related stages of oogenesis as the ovary matures. The same type of development has been observed in the ovary of the caryophyllidean *Archigetes sieboldi* and the pseudophyllidean *Eubothrium rugosum* (Poddubnaya 2003a, b; Poddubnaya *et al*. 2003). The net effect of this pattern of oogenesis is the production of many eggs at one time rather than a continuous production over time. This pattern of egg production would appear to be an adaptation for survival when eggs are not able to be gradually disseminated outside the host over a period of time. Such a pattern appears to be the case in progenetic cestodes living in parenteral sites in the invertebrate intermediate host. In *Archigetes*, for example, the cestode ruptures the body wall of the tubificid annelid and disintegrates in the environment, disseminating all of the eggs at the same time (Poddubnaya *et al*. 2003). Progenetic *D. olrikii* leaves the body cavity of gammarids in the same way, decays in the environment and disseminates clusters of fertilized eggs. There is thus an interconnection between the pattern of oogenesis and the production and dissemination of a modest number of fertilized eggs. Such eggs from progenetic cestodes become readily available for the infection of annelid or amphipod hosts and, over time, for the subsequent evolution of a monoxenic life cycle.

Similar synchronous development of oocytes has been shown also in the pseudophyllidean *E. rugosum* (Poddubnaya 2003a). In this case, eggs embryonate *in utero* and accumulate in an enlarged part of the distal uterus where, after egg maturation, the involutive uterine pore opens allowing the eggs to escape into the intestinal lumen. This type of egg development is in sharp contrast to the non-synchronous strategy of oocyte development illustrated for the pseudophyllidean, *Diphyllobothrium latum*, and spathebothriidean *Cyathocephalus truncatus* (see Poddubnaya 2002, Poddubnaya *et al*. 2005). In these tapeworms, oocytes are in various stages of maturation within the ovary and mature oocytes are gradually discharged from the ovary by ovicapt regulation. Consequently, egg production occurs over a period of time and eggs are gradually released into the lumen of the host's intestine and expelled into water, where embryonation is completed.

Cortical granules

The fine structure of cortical granules of *D. olrikii* closely resembles that of another spathebothriidean species, *C. truncatus* (Poddubnaya *et al*. 2005). The granules are ovoid, membrane bounded, and homogeneously electron-dense. On the other hand, Spence and Silk (1971), Erasmus (1973) and Awad and Probert (1990) described the presence of a dense core with a number of outer "lamellae" in cortical granules of some trematodes. This variation in morphology of cortical granules in different worms may indicate variations in chemical composition.

Little is known of the chemical composition of these granules. According to Guraya (1982), cortical granules in both invertebrate and vertebrate species are composed of protein and carbohydrates. Anderson (1968) and Boyer (1972) have

indicated the presence of proteins and acid or neutral polysaccharides in cortical granules of echinoid and polyclad turbellarians. Opinions vary as to the significance of the cortical granules in mature oocytes. Gresson (1964) suggested that they represent nutritive structures. However, more recent evidence indicates that they play a role in blocking polyspermy (Grey *et al*. 1976) and are involved in the formation of the fertilization membrane by cortical granule exocytosis (Orido 1988, Conn 2000). Functional significance of cortical granules is also discussed by Świderski and Conn (1999) and Świderski *et al*. (2004). Participation of these inclusions in the initial stages of fertilization had been suggested earlier by Anderson (1968) and Halton *et al*. (1976).

In our study, cortical granules were observed in oocytes in the ovovitelline duct but not in oocytes of intrauterine eggs. This observation indicates that cortical granules undergo changes as the oocytes pass through the ovovitelline duct or when they pass along the ootype. According to Nybelin (1922), the arrangement of the proximal oviduct and accessory ducts are similar in *Diplocotyle* and *Cyathocephalus*. As recently reported by Poddubnaya *et al*. (2005), oocytes are fertilized in the fertilization canal in *C. truncatus*. No doubt the same is true in *Diplocotyle*. It would appear that in *Diplocotyle*, the discharge of cortical granules may be initiated at fertilization but completed within the ovovitelline duct where they may be necessary for joining of vitelline material to oocytes. In cestodes of different orders, fertilization may occur in the oviduct or in the fertilization canal (Swiderski 1976, Bruňanská 1999, Świderski and Conn 1999, Świderski *et al*. 2004). This fact may help explain why the cortical granules appear to have a different function in different species.

Lipid droplets

Whereas cortical granules appear to be present within oocytes of all worms, the same is not true for lipid droplets. There can be variation in their occurrence between genera in the same order. For example, within the Spathebothriidea lipid droplets are present in the oocytes of the monoxenous *D. olrikii* (present study) but are absent from those of the dixenous *C. truncatus* (Poddubnaya *et al*. 2005). According to Holy and Wittrock (1986), lipid droplets in the oocytes of *Halipedus eccentricus* (Trematoda) compensate for the lack of energy reserves (lipid droplets and glycogen) in vitelline cells included within eggs. It seems unlikely that a similar situation is present with *D. olrikii* because lipid droplets and glycogen are present in the vitelline cells of both cestode species (Bruňanská *et al*. 2005). An egg of *Diplocotyle* therefore has abundant energy reserves in the form of lipid droplets in the zygote and lipid droplets and glycogen in the vitelline cells. The functional significance of such large amounts of lipid droplets within eggs of this species is not known. Whether embryogenesis in this species normally requires large amounts of energy reserves or that such reserves help extend the life of viable eggs disseminated in the environment or affects their specific gravity thus making them more available to amphipods, remains to be explored.

Interstitial cells

The interstitial system of the *D. olrikii* ovary is a syncytium with several nuclei, a large number of mitochondria and extensive cytoplasmic areas in contact with germ cells. This system, also associated with the tegument, excretory system, parenchyma, and vitellaria, is engaged in the transport of materials and supply of energy for intercellular exchange (Gresson 1964, Conn 1993, Swiderski and Xylander 2000). Supporting, interstitial tissue containing several nuclei has also been described in the ovary of the trematode *Paragonimus ohirai* by Orido (1987). In *D. olrikii*, the surface of maturing oocytes is prolonged into lamellae, thus increasing the oocyte transport surface for receiving nutrients from the interstitial syncytial cytoplasm. Prolongations of adjacent cells form a lamellar mesh with close association of mitochondria. Such a mesh, along with the numerous deep invaginations of the folded ovarian basal layer, probably functions to enhance the distribution of nutrients from the glycogen-rich sarcoplasma adjacent to the ovary wall to the developing germ cells. The intimate association of sarcoplasmic processes with the interstitial system and maturing oocytes is assumed to indicate a functional relationship between them, probably, by means of pinocytic movement of material from glycogen-rich processes. A similar association of actively developing cells, interstitial and nutrient-rich cells has previously been described for the high metabolism of vitelline cells in the vitellaria of some trematodes (Oshmarin 1978).

The distinctive structure of the ovarian tissue and oogenesis in two spathebothriidean species, *D. olrikii* (present study) and *C. truncatus* (Poddubnaya *et al*. 2005) is a consequence of progenetic development within the body cavity of the invertebrate host of *D. olrikii*. Our future ultrastructural studies on gravid *D. olrikii* from amphipods may provide still further evidence of adaptations for the progenetic development of this tapeworm.

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