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VERSITA

Ultrastructure of the ovary, ovicapt and oviduct of the spathebothriidean tapeworm *Didymobothrium rudolphii* (Monticelli, 1890)

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Abstract

The ultrastructural details are presented of the ovary, ovicapt and oviduct of the spathebothriidean tapeworm *Didymobothrium rudolphii* (Monticelli, 1890) from the intestine of the sand sole *Solea lascaris*. Oogonia, maturing oocytes and mature oocytes are surrounded by a syncytial interstitial cytoplasm, one of the distinctive traits of which is the presence of numerous myelin-like bodies. Oocyte inclusions comprise cortical granules and a small number of lipid droplets. The thickened, nucleated epithelium of the ovicapt lacks any apical structure and is a prolongation of the narrow ovarian epithelium. The muscular sphincter of the ovicapt is formed by a band of longitudinal muscles and bands of radial muscles at right angles to the longitudinal layer, and numerous myocytes surround the ovicapt wall. The oviduct of *D. rudolphii* is subdivided into three regions: (1) the proximal oviduct; (2) the fertilization chamber – the region distal to the point of entry of the duct from the seminal receptacle; and (3) the ovovitelline duct – the region distal to the point of entry of the duct from the vitelline reservoir. A comparative analysis is made between the structures of the ovary, ovicapt and oviduct of *D. rudolphii* and those of two other spathebothriideans, *Cyathocephalus truncatus* and *Diplocotyle olrikii,* with a discussion of ultrastructural traits that might be used as taxonomic criteria within the order Spathebothriidea.

Key words

Didymobothrium rudolphii, Spathebothriidea, Cestoda, ovary, ovicapt, oviduct, ultrastructure

Introduction

The female reproductive system of spathebothriidean tapeworms has been the subject of several ultrastructural studies in two species: adult *Cyathocephalus truncatus* (Pallas, 1781) from the intestine of fish and mature, progenetic *Diplocotyle olrikii* Krabbe, 1874 from the body-cavity of gammarid crustaceans (see Poddubnaya *et al*. 2005a, b, c; Bruňanská *et al*. 2005). These two cestodes exhibit variation in the detailed morphology of the ovary, oocapt and oviduct. In the most recent taxonomic accounts of the Spathebothriidea, these species have been either included in a single family, the Acrobothriidae Olsson, 1872 (see Gibson 1994), or in two different families, the Cyathocephalidae Lühe, 1899 (*C. truncatus*) and the Diplocotylidae Monticelli, 1892 (*D. olrikii*) (see Protasova and Roytman 1995). Due to the relatively small number of spathebothriidean species which have been investigated by transmission electron microscopy (TEM), there is a need for detailed ultrastructural studies of other members of the order. It is also important to assess both new and previously published information on the ultrastructural features of the reproductive system of this group as possible characters by which to help estimate the phylogeny of the Eucestoda (see Mariaux 1996, Olson and Caira 1999, Olson *et al*. 2001).

Few studies have attempted to elucidate the utrastructure of the ovary in tapeworms (Douglas 1963, Poddubnaya *et al*. 2005b), and such data on the ovicapt (oocapt) of cestodes have been presented only for the caryophyllidean species *Caryophyllaeus laticeps* (Pallas, 1781) (see Davydov *et al*. 1994) and the spathebothriidean *Cyathocephalus truncatus* (see Poddubnaya *et al*. 2005b). The present study was undertaken to obtain information on the fine structure of the ovary, ovicapt and oviduct of *Didymobothrium rudolphii* (Monticelli, 1890) in order to search for features that might be pertinent to our understanding of both common and special traits of spathebothriidean species.

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Materials and methods

Adult *Didymobothrium rudolphii* were recovered from the intestine of the sand sole *Solea lascaris* (Risso) off Aveiro on the Atlantic coast of northern Portugal during September, 2005. All specimens examined correspond to the 'common' form of *D. rudolphii,* as defined by Marques *et al*. (in press). The worms were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 20 days at 5°C. The material was then dehydrated in a graded series of alcohol and acetone, and embedded in Araldite and Epon. Ultrathin sections were stained with uranyl acetate and lead citrate and examined using a JEM-100 C transmission electron microscope operating at 80 kV.

Results

Each hermaphroditic set of reproductive organs (i.e., proglottides) aligned along the strobila of *D. rudolphii* includes a lobed ovary situated medially and posterior to each genital pore. In the centre of the ovarian isthmus there is a muscular ovicapt, which forms the junction between the ovary and the

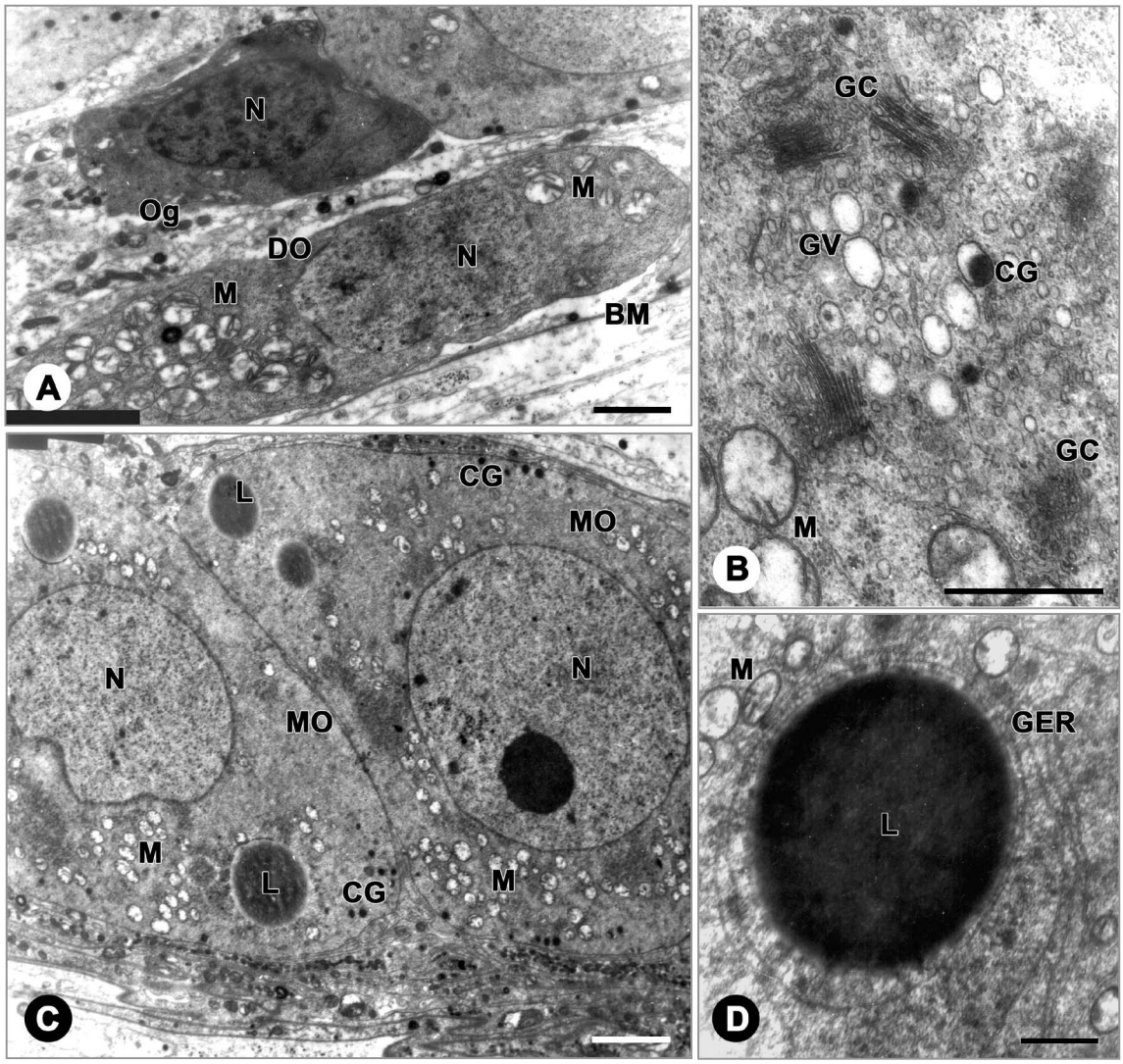

Fig. 1. Oocyte development of *Didymobothrium rudolphii*. **A.** Oogonium and developing oocytes at the periphery of the ovary. **B.** Formation of cortical granules inside vesicular cisternae of the Golgi complexes. **C.** Mature oocytes near the ovarian isthmus. **D.** Formation of lipid droplet associated with GER. Scale bars =1 μm (A), 0.5 μm (B, D), 3 μm (C). **Abbreviations to all figures:** BM – basal matrix, CG – cortical granule, DO – developing oocyte, EF – epithelial folds, EO – epithelium of ovary, EOc – epithelium of ovicapt, EOv – epithelium of oviduct, ES – eggshell, GC – Golgi complex, GER – granular endoplasmic reticulum, GV – Golgi vesicle, IC – interstitial cytoplasm, L – lipid droplet, Lm – lamellae, LM – longitudinal muscles, M – mitochondrion, MB – myelin-like body, Mc – myocyton, ML – muscle layers, MO – mature oocyte, N – nucleus, NP – nerve plexus, O – ovum, Og – oogonium, Ov – oviduct, RM – radial muscles, SE – syncytial epithelium, VC – vitelline cytoplasm, VD – vitelline duct

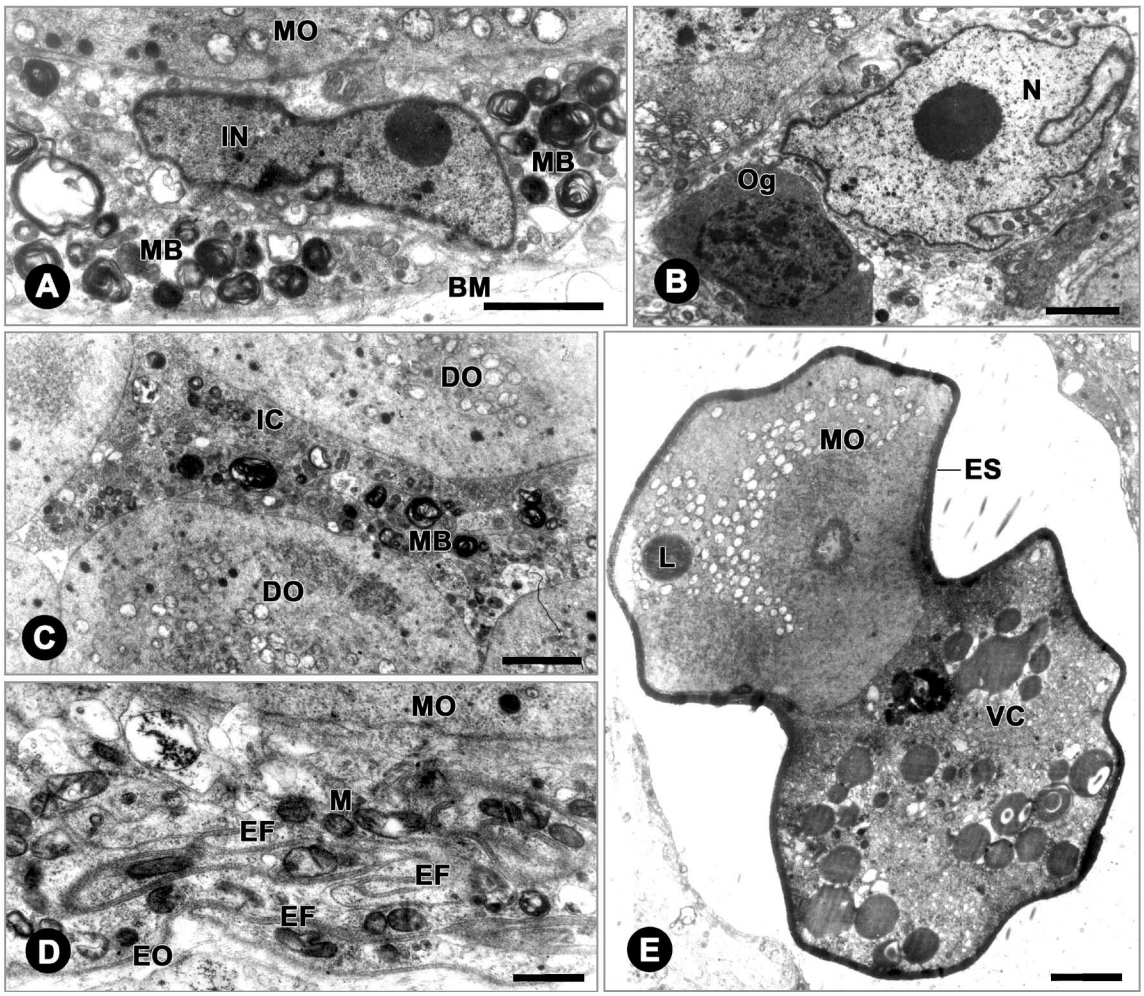

Fig. 2. Ovarian interstitial tissue and intrauterine egg of *Didymobothrium rudolphii*. **A.** Interstitial nucleus surrounded by numerous myelin-like bodies at the ovarian periphery. **B.** Oogonium and an interstitial nucleus in the central region of the ovary. **C.** Interstitial cytoplasm with myelin-like bodies between the developing oocytes. **D.** Deep infoldings in the epithelial lining near the isthmus of the ovary. **E.** Intrauterine egg containing an ovum and vitelline material. Scale bars = 1 μm (A, C), 2 μm (B), 0.5 μm (D), 4 μm (E)

oviduct. In approximately the middle of the oviduct it receives a short duct from the seminal receptacle (formed from an expansion of the vagina); as mature oocytes move through this region of the oviduct they are fertilized. Vitelline cells reach the oviduct from the vitelline reservoir via a short duct, which unites with the oviduct to form the ovovitelline duct close to the ootype, within which the eggs are formed.

Ovary

The ovary contains the oocytes in various stages of development, with the typical localization of younger cells in the peripheral regions of the ovarian lobes. Maturing oocytes are situated more centrally, and mature oocytes occur close to the ovicapt (Figs 1A, C; 3A, C). Oogonia are often irregular in shape and possess little cytoplasm and a large nucleus containing dense chromatin patches. Their cytoplasm is dense and filled with free ribosomes; a few mitochondria are also present (Figs 1A; 2 B).

Developing oocytes are larger than oogonia and contain an abundance of mitochondria, which are dispersed evenly in the cytoplasm (Fig. 1A). The enlarged volume of cytoplasm in maturing oocytes contains Golgi complexes and rows of granular endoplasmic reticulum (GER); cortical granules and lipid droplets are also formed (Fig. 1B, D). The first cortical granule material can be observed within the vesicular cisternae of the Golgi complexes (Fig. 1B), and lipid droplets occur in close association with GER (Fig. 1D).

The mature oocytes possess a large, spherical nucleus with a prominent nucleolus and reticular, dispersed chromatin (Fig. 1C). In their cytoplasm are abundant mitochondria, a few large lipid droplets and infrequent peripheral cortical granules. The cortical granules are ovoid, electron-dense and ca. 0.1 μm in diameter and the lipid droplets occur singly and vary in size from 0.5 to 1.5 μm in diameter (Fig. 1C, D).

The cytoplasm of the fertilized ovum of intrauterine eggs contains only lipid droplets as cell inclusions (Fig. 2E).

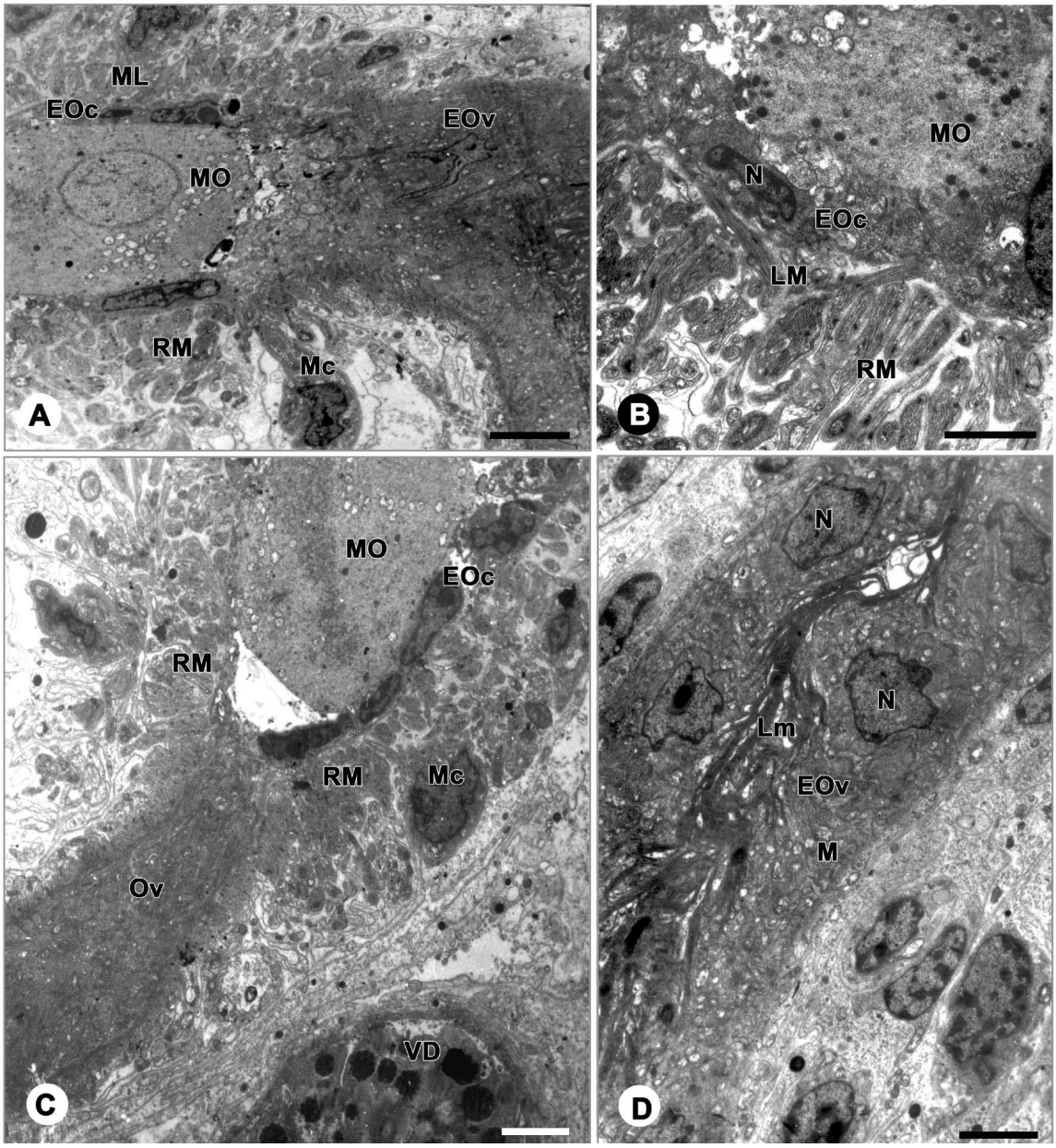

Fig. 3. Fine morphology of the ovicapt of *Didymobothrium rudolphii*. **A** and **C.** The junction between the ovicapt and the oviduct, with mature oocytes in the ovicapt lumen, numerous nuclei in the ovicapt epithelium, and longitudinal and radial muscles with myocytons beneath the ovicapt epithelium. **B.** Part of the ovicapt epithelial wall with longitudinal and radial muscles beneath it. **D.** The portion of the proximal oviduct with numerous lamellae projecting into the lumen. Scale bars = 5 μm (A), 2 μm (B), 4 μm (C), 3 μm (D)

Interstitial tissue contains several irregular nuclei which occupy positions in both the peripheral and in central regions of the ovarian lobes (Fig. 2A, B). The syncytial cytoplasm surrounds the germ cells (Fig. 2C) and is directly attached to the epithelial surface of the ovary (Fig. 2D); it is rich in mitochondria (Fig. 2D) and myelin-like bodies (Fig. 2A, C).

The epithelial wall of the ovary delimits the lumen of this organ. The epithelial lining of most portions of the wall is thin (ca. 0.08 μm in thickness) and rests on the fibrous basal matrix (Figs 1A; 2D). Not far from the isthmus of the ovary, the epithelial lining forms deep folds that encroach on the ovarian lumen. Between these folds are numerous mitochondria (Figs 2D; 4).

Ovicapt

The ovicapt wall is a prolongation of the epithelium of the ovarian isthmus (Fig. 4). The thickened wall (ca. 1.5 μm in thickness) includes numerous nuclei, many of which have lost their nucleoli, with dense areas of heterochromatin (Figs 3B, C; 4). The epithelial cytoplasm is dark and contains abundant

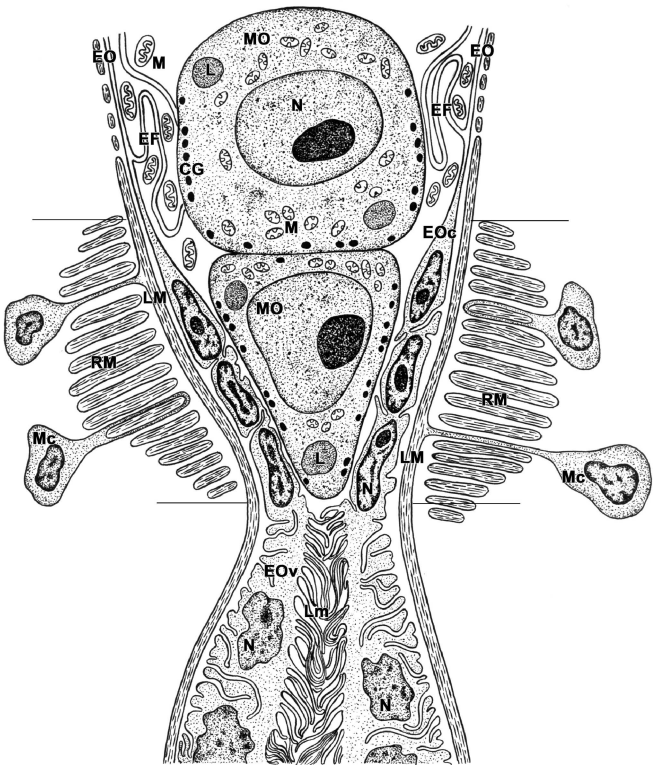

Fig. 4. Diagrammatic representation of the ovicapt of *Didymobothrium rudolphii*. The ovicapt region has been indicated as between the upper and lower horizontal lines

free ribosomes. No apical structures occur on the surface membrane of the epithelial lining. The ovicapt wall is surrounded by a distinct, well-developed musculature comprising a single layer of longitudinal muscles immediately beneath the epithelial basal matrix, external and at right-angles to which are layers of radial muscles. Myocytons of both muscle layers occur here (Figs 3A-C; 4).

Oviduct

The oviduct wall extends from the ovicapt epithelium (i.e., the proximal extremity of the oviduct) as a syncytial epithelial lining measuring ca. 4.5 µm in thickness (Figs 3D; 4; 5A). The surface lamellae reach their greatest length and density in the proximal region of the oviduct, forming a highly plicate 'net' within the lumen (Figs 3D; 4; 5A). The epithelial lining of the oviduct has irregularly shaped nuclei (Fig. 3D), and the epithelial cytoplasm contains mitochondria, free ribosomes and vesicles, and is broken by deep invaginations of both the surface and basal epithelial membranes (Fig. 5A). A layer of longitudinal muscles surrounds the proximal region of the oviduct (Fig. 5A).

The lumen of the middle region of the oviduct (the fertilization canal) is filled with spermatozoa (Fig. 5C) which enter through a short duct uniting the oviduct with the lumen of the seminal receptacle (Fig. 5B). Three layers of longitudinal muscle enclose the thin epithelial lining of this region of the duct (Fig. 5B). Both the thickness of the oviduct lining and the length and density of the lamellae on the luminal surface gradually diminish from the proximal to the distal regions of the oviduct.

Figure 5D shows the more distal oviduct lumen with a fragment of mature oocyte cytoplasm. The epithelial lining of this region of the oviduct contains numerous nuclei and is surrounded by 2–3 muscle layers. The most distal region of the oviduct (the ovovitelline duct) contains vitelline material which reaches the lumen of this duct via a short duct from the vitelline reservoir. The epithelial wall of this region of the oviduct is lined by short, sparsely distributed lamellae (Fig. 5E).

Discussion

Oogenesis has been carefully studied in cestodes (Douglas 1963, Poddubnaya *et al*. 2005b), flukes (Gresson 1964; Holy and Wittrock 1986; Orido 1987, 1988) and monogeneans (Halton *et al*. 1976, Tappenden *et al*. 1993). Ultrastructural changes in the oocytes accompanying the initial phases of oogenesis in the ovary are basically the same in all platyhelminths, including those involving the oogonia, maturing oocytes and fully mature oocytes at different stages of their development. The ovarian interstitial tissue has a syncytial structure with several nuclei which may occur in any region of the ovary in spathebothriidean tapeworms (Poddubnaya *et al*. 2005b) and in lung flukes (Orido 1987). Oocyte inclusions of the Spathebothriidea are cortical granules and a few lipid droplets. In previous morphological observations of another spathebothriidean species, *Diplocotyle olrikii*, cortical granules were observed in ova from the ovovitelline duct; this, and the discovery of free cortical granules within the ovovitelline duct lumen, would tend to support the idea that the union of vitelline material and the ovum is dependent on the discharge of cortical granules from the ovum, at least in the case of the Spathebothriidea (Poddubnaya *et al*. 2005b, c). It is of interest that oocytes of other cestode orders studied to date do not contain cortical granules, a point discussed by Świderski and Conn (1999) and Świderski *et al*. (2004). Conn (1988) suggested that vitellocyte vesicles in cyclophyllidean cestodes, whose oocytes lack cortical granules, may play a role in fertilization events analogous to the cortical granules of other animal phyla. Lipid droplets are present in the ovum of intrauterine eggs of spathebothriidean species. The probable functional significance of these lipid droplets is as an additional energy reserve for the subsequent development of the embryo following the deposition of the egg in water, where it may remain for a long period of time. The presence of lipid droplets in the oocytes of some trematodes has been considered as an energy reserve, as their vitelline cells lack both lipid droplets and glycogen (Holy and Wittrock 1986).

Ultrastructural characteristics of the ovarian components in the Spathebothriidea

The present TEM observations of the ovary, ovicapt and oviduct of the spathebothriidean tapeworm *Didymobothrium*

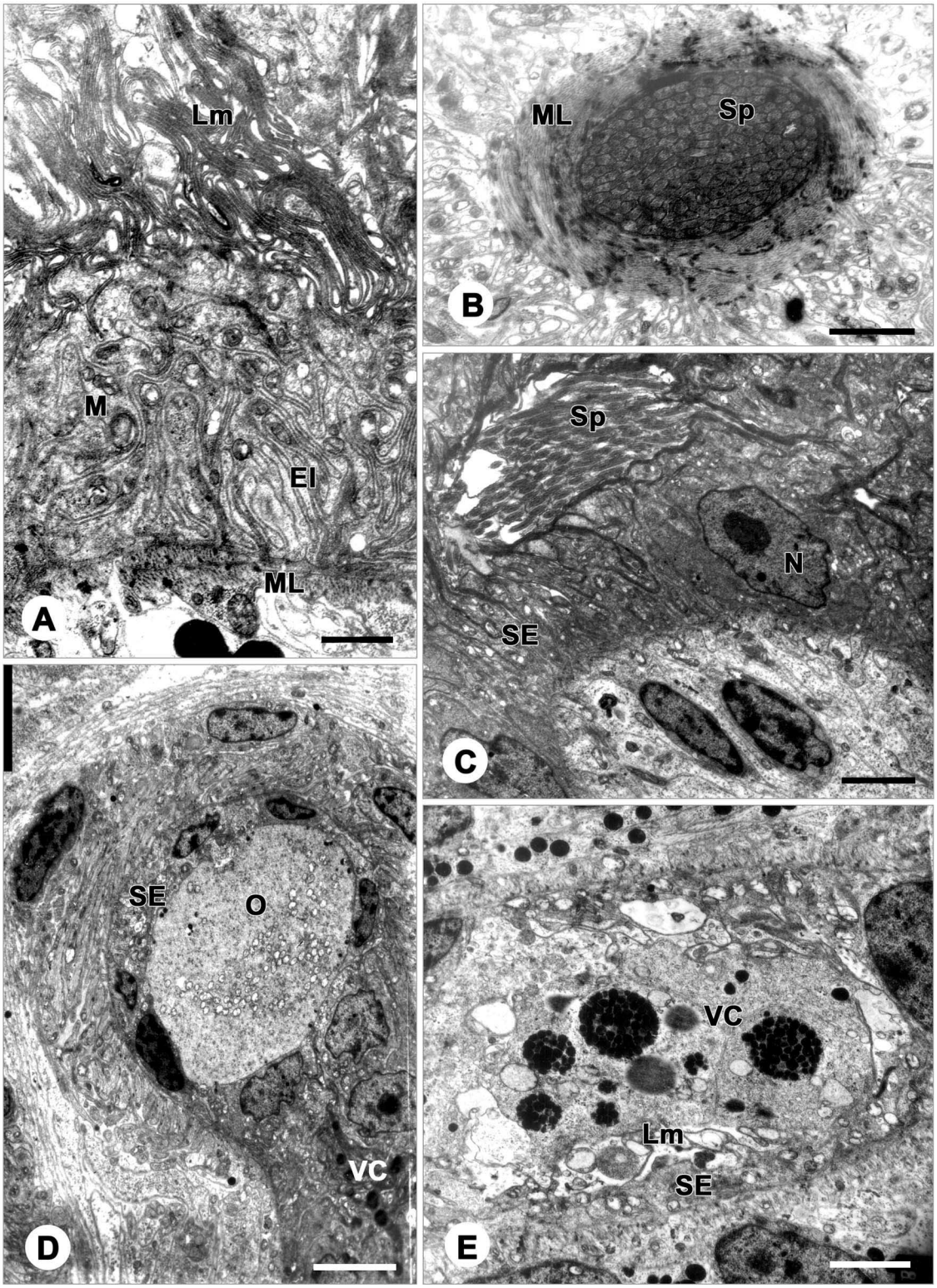

Fig. 5. Morphological variations in the oviduct of *Didymobothrium rudolphii*. **A.** Proximal portion of the oviduct lined by a lamellar brush which fills the lumen. **B.** Short duct joining the seminal receptacle with the oviduct. **C.** Middle region of the oviduct (fertilization chamber) with spermatozoa in the lumen. **D** and **E.** Distal region of the oviduct (ovovitelline duct) with ova (D) and vitelline cytoplasm (E) within the lumen. Scale bars = 1 μm (A, E), 3 μm (B, C), 5 μm (D)

rudolphii reveal ultrastructural features that can be used to distinguish it from *Cyathocephalus truncatus* and *Diplocotyle olrikii*, examined previously (Poddubnaya *et al*. 2005a, b, c). The presence of numerous myelin-like bodies in ovarian interstitial cytoplasm of *D. rudolphii* distinguishes it from the other two spathebothriidean species. These bodies may be considered as a lysosome variation which has a heterogeneous structure. The interstitial system of some organs and tissues of tapeworms is presumably concerned with the transport of materials and the supply of energy for intercellular exchange (Gresson 1964, Conn 1993, Świderski and Xylander 2000). This is a well-known regulative function (mechanism of intracellular renewal) of lysosomes during morphogenesis and the differentiation of animal organs and tissues (Zavarzin and Charasova 1982). Here we suggest a similar function for the myelin-like bodies which are components of the interstitial cytoplasm of *D. rudolphii*.

Another minor variation occurs in oocyte morphology, where the surface covering of the maturing oocytes of *D. olrikii* may be transformed into lamellae, resulting in a lamellar meshing with adjacent oocytes at the same stage of maturation and loosely packed within the ovary (Poddubnaya *et al*. 2005b). The surfaces of the maturing oocytes of *D. rudolphii* and *C. truncatus* do not have a pronounced lamellar surface, rather their surface is relatively smooth and the oocytes are tightly packed within the ovary, not segregated from other germ cells at different stages of maturation.

Variations of ovicapt structure

One of the distinctive features of the spathebothriidean genera examined is the differences in the morphology of the ovicapt. The ovicapt prevents immature oocytes from passing into the oviduct and allows through only mature oocytes. Relatively little is known of the fine structure of the ovicapt in other cestodes (Douglas 1963; Davydov *et al*. 1994; Poddubnaya 2002; Poddubnaya *et al*. 2005a, c), trematodes (Podvyaznaya 1990, Galaktionov and Dobrovolsky 2003) and monogeneans (Tappenden *et al*. 1993). What is known indicates that it acts as a muscular sphincter, as this has been indicated for many cestode species in taxonomic works based on light microscope observations. The present study of the ovicapt of *D. rudolphii* extends our observations on the fine morphology of this muscular organ. Cestode genital ducts have a syncytial structure and the different female and male ducts are modified into different regions or structures along their length without distinct borders, changing only the apical structures, cytoplasmic organelles and the thickness of the muscle layers. The epithelial wall of the ovicapt is a prolongation of the ovarian epithelium, which increases in thickness with the appearance of numerous nuclei in the cytoplasm. There is no apical structure on the surface of this region of the female genital duct. The longitudinal muscles, which are scarce in the wall of the ovary, become a continuous band formed by the myocytes of this layer. The well-developed bands of radial muscles, with their own numerous myocytes, are localized at right-angles to the longitudinal layer. In *C. truncatus*, the ovicapt lumen can be blocked by a syncytium with five or more nuclei through which mature oocytes pass into the oviduct (Poddubnaya *et al*. 2005a). A similar regulating structure for the passage of oocytes occurs at the proximal end of the intra-ovarian (intragermarial) tube of the monogenean *Entobdella soleae* (see Tappenden *et al*. 1993, Poddubnaya *et al*. 2005a). In addition to these two patterns, in progenetic *D. olrikii,* associated with the ovicapt region, there are only separate muscles surrounded by a thick myocyte cytoplasm with a well-developed neuronal plexus beneath it. Such an ovicapt structure in *D. olrikii* may be dependent on the presence of oocytes at approximately the same stage of maturity within the ovary (Poddubnaya *et al*. 2005b, c).

Oviduct structure

The oviduct of spathebothriideans may be subdivided into three regions: the proximal oviduct and the sections demarcated by the entry ducts from the seminal receptacle (forming the fertilization chamber) and vitelline reservoir (forming the ovovitelline duct), which enable the fertilization of mature oocytes and the subsequent association of fertilized ova with vitelline material. A comparison of our results from *D. rudolphii* with previous studies of the oviduct in *D. olrikii* and *C. truncatus* shows that the proximal part of the oviduct in the first two species is lined by long lamellae that fill the duct lumen, whereas in *C. truncatus* the oviduct is a long, narrow tube lined by a compressed epithelial layer with sparse lamellae (Poddubnaya *et al*. 2005a). The form of the proximal oviduct in *C. truncatus* resembles morphologically that of the pseudophyllidean *Diphyllobothrium latum* (see Poddubnaya 2002) and the intra-ovarian tube of the monogenean *Entobdella soleae* (see Tappenden *et al*. 1993). Conversely, the morphology of the proximal oviduct of *D. rudolphii* and *D. olrikii* is typical of many cestode species (see comparative results in Poddubnaya *et al*. 2005a). Comparison of the fine structure of the fertilization chamber and ovovitelline duct in the three spathebothriidean species reveals only minor differences in their morphology.

Taxonomic comments

The comparative analysis of the fine morphology of the ovary, ovicapt and oviduct of three genera supplements the characters available for the determination of phylogenetic relationships of these divergent taxa (Marques *et al*., in press) within the order Spathebothriidea. Two ultrastructural features would appear suitable for a character matrix: the structure of the ovicapt and the morphology of the proximal region of the oviduct. Taxonomic evaluation based on these criteria unites *Didymobothrium* and *Diplocotyle* to the exclusion of *Cyathocephalus*. According to Gibson (1994), the Spathebothriidea contains two families, the Spathebothriidae Yamaguti, 1934 and the Acrobothriidae Olsson, 1872, and he included all of the above three genera in the Acrobothriidae. However, Protasova and Roytman (1995) subdivided the order into three families, the Cyathocephalidae Lühe, 1899, the Diplocotyli-

dae Monticelli, 1892 and Spathebothriidae, with the inclusion of *Cyathocephalus* Kessler, 1868 in the Cyathocephalidae and both *Didymobothrium* Nybelin, 1922 and *Diplocotyle* Krabbe, 1874 in the Diplocotylidae. However, a recent molecular phylogenetic analysis (Marques *et al*., in press) has shown that two cryptic species of '*Didymobothrium rudolphii*' form the sister lineage to *Cyathocephalus*, and were thus separated from *Diplocotyle* and *Spathebothrium*. To fully evaluate interrelationships in this enigmatic order detailed molecular and ultrastructural studies of *Bothrimonus* Duvernoy, 1842 and *Spathebothrium* Linton, 1992 are needed.

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