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Vitellogenesis in *Mosgovoyia ctenoides* (Railliet, 1890) Beveridge, 1978 (Cyclophyllidea, Anoplocephalidae)

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Abstract

Vitellogenesis in *Mosgovoyia ctenoides* was examined by means of transmission electron microscopy. Mature vitelline follicles consist of cells in various stages of development, progressing from immature cells of gonial type near the periphery to mature vitellocytes towards the centre. Maturation is characterized by: (1) increase in cell volume; (2) extensive development of large parallel cisternae of granular endoplasmic reticulum (GER), the vitelline material producing units; (3) development of Golgi complexes engaged in vitelline material package; (4) continuous fusion of small vesicles into larger vitelline vesicles and fusion of these into a single very large vesicle, which is characteristic for mature vitellocytes of this tapeworm. Vitellogenesis in *M. ctenoides* is compared with that in other cestodes. Some conclusions concerning the interrelationship between the vitellogenesis pattern and the type of embryogenesis are drawn and discussed.

Key words

Mosgovoyia ctenoides, Cestoda, Anoplocephalidae, vitellogenesis, vitellocyte ultrastructure

Introduction

To date, a small amount of information is available on the ultrastructural aspects of vitellogenesis in cestodes (Swiderski *et al*. 1970a; Swiderski and Mokhtar 1974; Mokhtar-Maamouri and Swiderski 1976; Swiderski and Mackiewicz 1976; Swiderski and Xylander 1998, 2000; Świderski *et al*. 2000; Bruňanská *et al*. 2005). In tapeworms, the vitellocytes perform two important functions during their embryonic development; they supply materials for capsule formation and nutritive reserves for developing embryos (Swiderski 1968, 1972; Swiderski *et al*. 1970b; Swiderski and Subilia 1978). The embryology and in particular embryonic envelope formation of this species was described in separate papers (Młocicki 2004, Młocicki *et al*. 2005). The purpose of the present study is to describe the ultrastructural aspects of vitellogenesis in the anoplocephalid cestode *Mosgovoyia ctenoides* with particular emphasis on vitellocyte differentiation and the origin and development of a single large vitelline vesicle which apparently is characteristic not only for this species but for the vitelline cells of the family Anoplocephalidae (Swiderski 1973, Swiderski and Xylander 2000).

Materials and methods

Adult specimens of the cestode, *Mosgovoyia ctenoides* (Railliet, 1890) Beveridge, 1978 were obtained from naturally infected wild rabbits (*Oryctolagus cuniculus*). Tissue samples from mature proglottids were fixed in cold (4°C) 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4). After overnight washing in the same buffer and postfixation for 1 h in cold (4°C) 1% osmium tetroxide in the same buffer, they were dehydrated in an ethanol series, infiltrated with propylene oxide and embedded in Spurr epoxy resin. Ultrathin sections double stained with uranyl acetate and lead citrate, were examined with a JEM-100 B electron microscope operated at an accelerating voltage of 80 kV.

Results

General topography of vitellaria

The vitellaria of *M. ctenoides* are reniform, lobulate and posterior to the ovary. Mature vitelline follicles are surrounded by a thick external basement membrane. They consist of cells in

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various stages of development, progressing from immature cells of gonial type near the periphery to mature ones toward the centre. Although cytodifferentiation of vitellocytes constitutes a continuous process, in order to facilitate its description, the process was subdivided into four discrete stages in a somewhat arbitrary manner. These stages are the immature, early and advanced maturation phase and the mature vitellocyte phase (Fig. 1 I–IV). In fact, the gradual changes involved in this process clearly occur within each of the stages herein delineated.

Immature vitellocyte

The undifferentiated cells of gonial type (Figs 1 I and 2), situated at the periphery of vitelline follicles, represent the precursors of vitellocytes. They have a high nucleo-cytoplasmic ratio, small vitelline granules and a large concentration of free ribosomes, well developed GER and Golgi complexes in their cytoplasm (Fig. 2 and inset). Their large nuclei measure about 4 μm in diameter while the diameter of the entire cell is approximately 6 μm. The nucleus usually contains irregular clumps of dense heterochromatin dispersed in the karyoplasm (Fig. 2).

Early differentiation stage

Early cytodifferentiation of vitellocytes (Figs 1 II and 3) is characterized by: (1) a small increase in cell size; (2) an increase in the number of mitochondria and GER cisternae; (3) initiation of secretory activity of the GER; and (4) appearance of the first secretory granules within membrane bound vesicles of Golgi origin similar to that in Figures 2–4 but sometimes larger. Fusion of small secretory vesicles into larger ones is also initiated in this stage (Figs 1 II, 7 and 8).

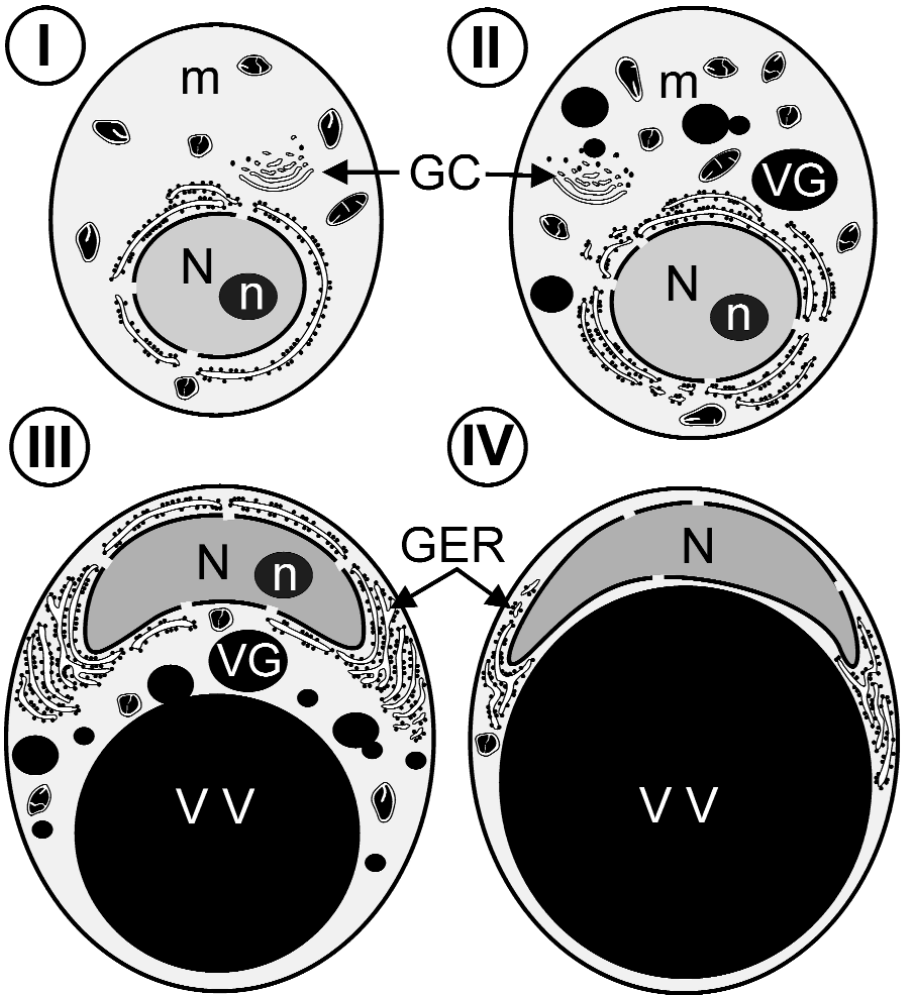


Fig. 1. Schematic diagram of four consecutive stages of vitellogenesis in *Mosgovoyia ctenoides*. **Abbreviations to all figures:** Ch – heterochromatin islands, GC – Golgi complex, GER – granular endoplasmic reticulum, m – mitochondria, n – nucleolus, N – nucleus, np – nuclear pore, VG – vitelline granules, VV – vitelline vesicle, I – immature vitellocyte, II – early differentiation, III – advanced maturation stage, IV – mature vitellocyte

Advanced maturation stage

During the advanced phase of vitellocyte maturation (Figs 1 III and 4), the cell doubles in size and the nucleus starts changing into a semilunar shape. The homogeneous type of nucleolus (Figs 1 I, II and 3) observed in the immature cells and early maturation stage gradually disappears. Vitellocyte mat-

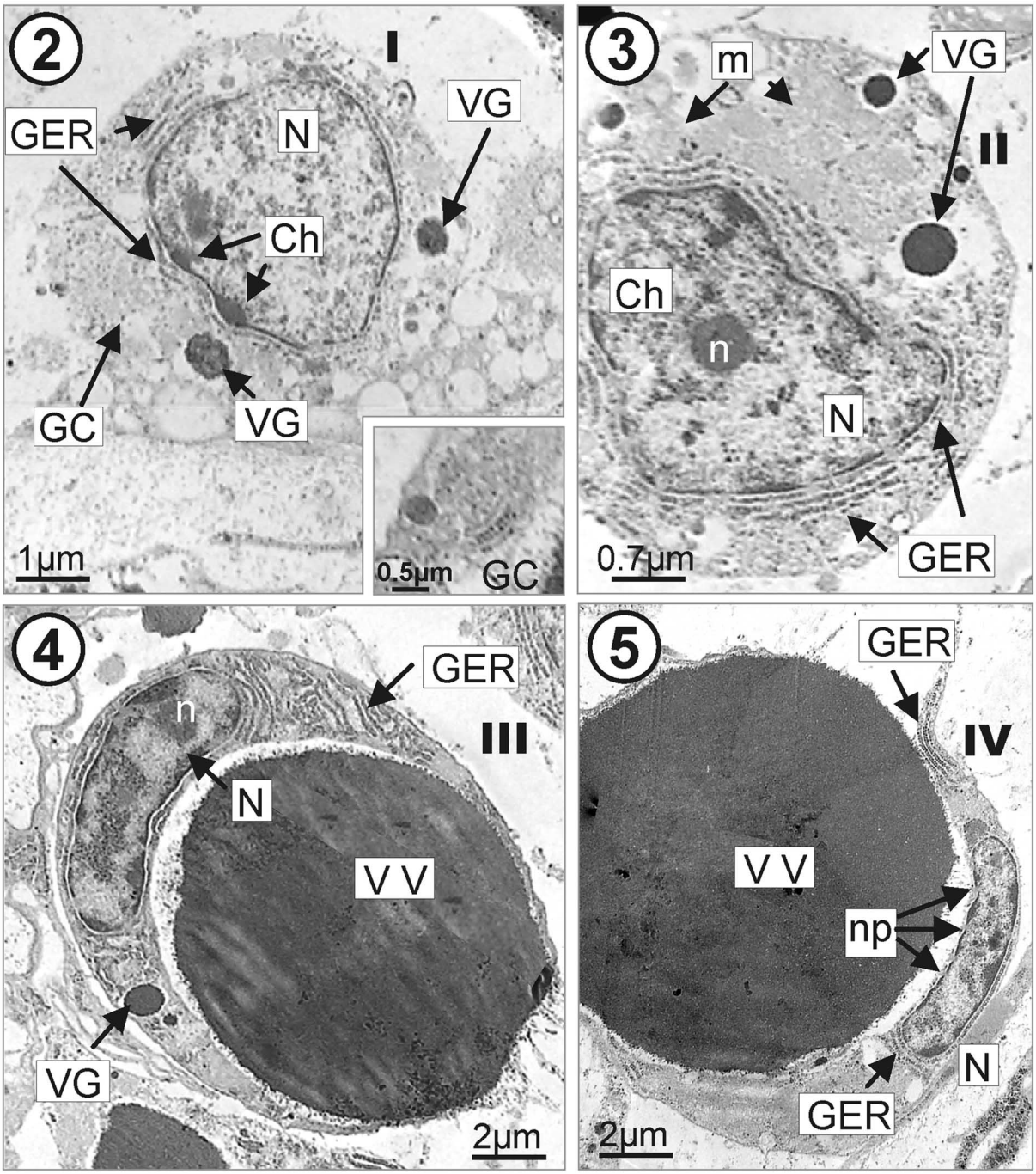
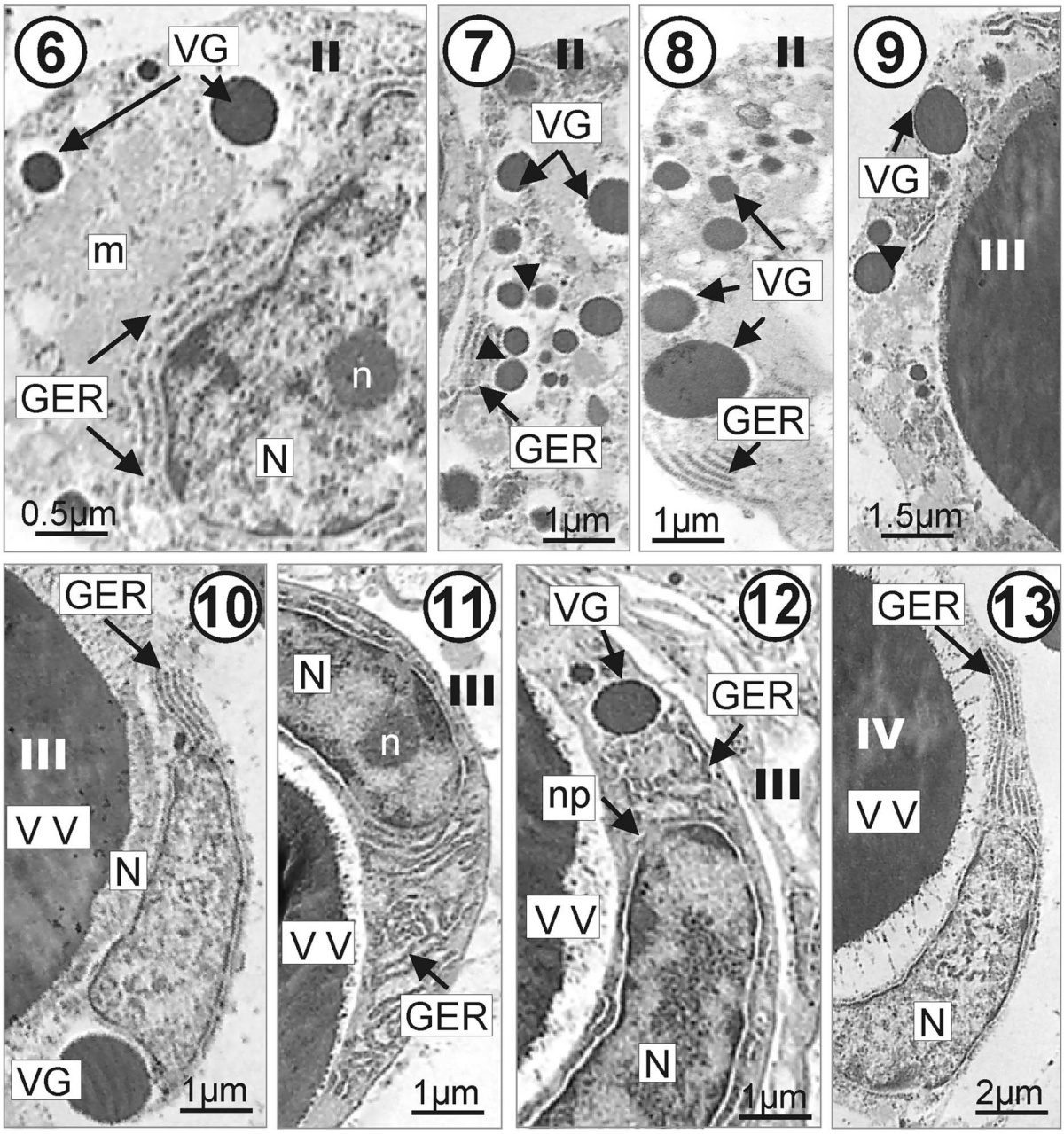


Fig. 2. Late immature vitellocyte of gonial type. Note the large nucleus containing numerous heterochromatin islands, profiles of granular endoplasmic reticulum and Golgi complexes. Inset: High power magnification of the Golgi complexes. **Fig. 3.** Early differentiation stage of vitellocyte in a phase of active synthesis of vitelline material. Note the electron-dense nucleolus and heterochromatin islands in the karyoplasm of the nucleus. The cytoplasm contains numerous profiles of granular endoplasmic reticulum and several vitelline granules. **Fig. 4.** Detail of advanced maturation stage of vitellocyte differentiation. Note: semilunar nucleus and large vitelline vesicles inside the cytoplasm as well as very well developed granular endoplasmic reticulum. **Fig. 5.** Section through the nucleated pole of a mature vitellocyte. Note the presence of: (1) numerous pores in the nuclear envelope and heterochromatin islands in the karyoplasm of the semilunar nucleus; (2) large single vitelline vesicle in the cytoplasm; and (3) degenerated profiles of granular endoplasmic reticulum



Figs 6–13. Consecutive stages of vitellocyte maturation. **Fig. 6.** High power magnification of part of the early differentiation stage showing early vitelline granules of different sizes and accumulation of mitochondria inside the cytoplasm. **Fig. 7.** Vitellocyte showing increase in number and diameter of the vitelline granules. Note the presence of membrane around the vitelline granules and their progressive fusion. **Fig. 8.** Part of the cytoplasm near the Golgi complex region. Note the vesicles of different sizes and electron densities. **Fig. 9.** Part of the vitelline cell in advanced maturation stage. Note the decrease in the number of vitelline granules, into large single vitelline vesicles and reduction of the cytoplasmic layer. **Fig. 10.** Late stage of vitelline cell maturation. Note the changes in nucleus shape. **Fig. 11.** Section through the nucleated part of the advanced maturation stage showing part of the semilunar nucleus with electron-dense nucleolus. Note the presence of well developed profiles of granular endoplasmic reticulum. **Fig. 12.** Progressive degradation of granular endoplasmic reticulum profiles and wide nuclear pores in the semilunar nucleus. Note the increase in the number of heterochromatin islands and electron density of the karyoplasm. **Fig. 13.** Part of the mature vitellocyte showing degenerating granular endoplasmic reticulum profiles inside the thin cytoplasmic layer reduced by the large vitelline vesicle

uration may be characterized as a period of a very high secretory activity. The GER rapidly becomes the most predominant organelle in the cell (Figs 1 III, 4, 11 and 12). In addition, free ribosomes are also abundant in the cytoplasm.

An intimate association between GER and Golgi complexes is developed with the extensive development of both complexes during the late maturation period. In this stage, the enlarged Golgi complexes appear to be composed of vesicles

of different sizes (Figs 1 III and 9). These secretory granules of Golgi origin represent the earliest stage of vitelline granule formation or their precursors and contain the required materials for capsule formation. The membrane bound granules (Figs 1 III, 4 and 9) now undergo changes in size and electron density as they become larger granules, apparently as a result of the fusion of minute secretory granules migrating from the perinuclear region. In the advanced stage of vitellocyte maturation, all the vitelline granules fuse to form a single large vitelline vesicle in each cell, which is characteristic for mature vitellocytes of *M. ctenoides* (Figs 1 IV and 5). The beginning of this fusion is shown on Figures 1 III and 7.

Mature vitellocyte

The mature vitelline cells (Figs 1 IV, 5 and 13) are ovoid or spherical in shape and measure approximately 11 μm in diameter. Their nuclei are semilunar and measure approximately 8 × 1 μm in diameter (Figs 1 IV and 5). The nuclear envelope possesses numerous nuclear pores (Fig. 1 IV and 5). The karyoplasm contains irregularly shaped heterochromatin islands of moderate electron density. The cytoplasm (Figs 5 and 13) contains: (1) a single large vitelline vesicle; (2) randomly dispersed free ribosomes; and (3) short dilated GER cisternae, all situated mainly in the perinuclear region. The large vitelline vesicle is always membrane bound (Figs 5 and 13) and contains a homogeneous material of moderate electron density. Glycogen particles and lipid droplets are absent from the mature vitellocytes.

Discussion

Vitellogenesis pattern and embryogenesis in M. ctenoides

As previously mentioned, cestode vitellocytes play a double role for the developing embryos, namely, secretion of materials for capsule formation and accumulation of nutritive reserves. Both functions can be intensified or reduced to different extents in different taxa, depending on the type of embryogenesis, mode of egg and larval stage survival and peculiarities of life cycles. The type of vitellogenesis in *M. ctenoides* corresponds to the intrauterine type of embryogenesis pattern and viviparous development observed in this species (Młocicki 2004, Młocicki *et al*. 2005). As in other cyclophyllideans producing oligolecithal eggs (a single vitellocyte per fertilized oocyte), in *M. ctenoides* both nutritive and capsule forming functions of vitellocytes are greatly reduced. There are two important features characteristic for this species: the (1) "viviparous"-type (Jarecka 1975) of intra-uterine development of the embryo, entirely depending upon the parent for nutrition, with no trace of glycogen or other nutritive material in vitellocytes; and (2) retention *in utero* of fully differentiated infective oncospheres within the gravid proglottids ensuring egg protection. This protection of *M. ctenoides* embryos and infective oncospheres at the end of embryonic development is taken over progressively by two important protective structures of different origin: (1) a thick and hard outer coat or "shell" resulting from incrustation of the initially delicate membranous capsule by the electron-dense secretion of the uterine epithelium; and (2) very thick and robust embryophore forming the so-called "pyriform apparatus" characteristic of anoplocephalid eggs. These features are compatible with the reduction in nutritive and protective functions of *M. ctenoides* vitellocytes described above.

Comparison of vitellogenesis pattern in M. ctenoides *and other cestodes*

The process of vitellogenesis of *M. ctenoides* generally resembles that described in other cyclophyllideans (Swiderski *et al*. 1970b) and is particularly similar to that in another anoplocephalid, *Inermicapsifer madagascariensis*, where a large single vitelline vesicle occupies the vitelline cell. It differs from the patterns observed in pseudophyllideans (Swiderski and Mokhtar 1974), proteocephalideans (Swiderski *et al*. 1978, Bruňanská 1997), spathebothriideans (Bruňanská *et al*. 2005), and representatives of 3 groups of monozoic cestodes: the caryophyllidean, *Glaridacris catostomi* (Swiderski and Mackiewicz 1976), the gyrocotylidean, *Gyrocotyle urna* (Xylander 1987) and the amphilinidean, *Amphilina foliacea* (Xylander 1988). Those differences can be summarized as follows: vitellocytes of pseudophyllideans and monozoic cestodes are responsible for the formation of a thick and hard eggshell originating from shell globules; they are tightly packed with glycogen and/or lipid reserves for embryo nutrition; about 20 to 30 vitellocytes, containing a high accumulation of shell globules of heterogeneous type, surround each fertilized egg.

The proteocephalideans occupy an intermediate position in this respect. In these tapeworms, despite the heterogeneous nature of eggshell globules, generally only one vitellocyte attaches to a fertilized oocyte, forming a thin, delicate capsule (Swiderski *et al*. 1978, Bruňanská 1997).

Origin of vitelline material and development of vitelline vesicles

The general pattern of vitelline material formation and storage in vitellocytes of *M. ctenoides* is similar to that described by Fawcett (1966) and Harris and Reid (1969) in a majority of other protein secreting cells. In *M. ctenoides*, as in *Bothriocephalus clavibothrium* (Swiderski and Mokhtar 1974) and *G. catostomi* (Swiderski and Mackiewicz 1976), vitellocyte maturation can be characterized as a period of very high secretory activity, accompanied by the rapid development of an extended network of GER and Golgi complexes and also an increase in the number of free ribosomes and mitochondria. Vitelline material is secreted within GER cisternae and packed within the Golgi complexes. As observed in the earliest stages of vitellocyte maturation and what is common for these three cestode species at the beginning of vitellogenesis, the larger vitelline vesicles result from the progressive fusion of minute vesicles containing condensed vitelline material with other

larger vesicles. However, the final product of small vitelline granule fusion is completely different in the three above mentioned species (Swiderski and Xylander 2000).

The vitelline material of *M. ctenoides* vitellocytes is stored within a single large vitelline vesicle which is characteristic not only for this species but probably for anoplocephalid cestodes in general (Swiderski 1973, Swiderski and Xylander 2000). Histochemical research shows the presence of acid mucopolysaccharides in the vesicles of cyclophyllidean vitellocytes (for review see Rybicka 1966). At the ultrastructural level, the vitelline material appears as a homogeneous and moderately electron-dense secretion and differs from that of *G. catostomi* or *B. clavibothrium.* In those species, the vitelline material is in the form of the so-called "shell-globules" of heterogeneous type, composed probably of two different substances: an electron-dense phenolic protein and electron-lucid phenolase, the enzyme that brings about the tanning of the shell material (Smyth and McManus 1989).

Functional significance

The oncosphere of *M. ctenoides* develops into the second larval stage a cysticercoid, in oribatid mites (Oribatidae), which act as the intermediate host. The homogeneously electron-dense vitelline material, accumulated within the large electron-dense vitelline vesicle of *M. ctenoides* vitellocytes, resembles that of other cyclophyllideans (Swiderski *et al*. 1970a, b; Swiderski and Xylander 2000) and is used exclusively for the formation of a very thin and delicate vitelline capsule surrounding the embryo. No traces of glycogen or lipids, which could represent nutritive reserves for the developing embryos and larval stages, were noticed in the vitellocytes of this cestode species. This lack of nutritive reserves can be explained by the fact that developing embryos are retained and nourished *in utero* until mature and infective. Despite the fact that it has not been so far demonstrated experimentally and conclusively how this nutritive role for the *in utero* developing cestode embryos is achieved, the specialized review articles and textbooks (Roberts 1980, Smyth and McManus 1989), generally take it for granted. Such passage of nutrients into the infective hexacanths appears even more enigmatic, when in concerns the mature, embryonated eggs where the infective hexacanths remain surrounded by a thick and hard outer coat or "shell" and very thick and robust embryophore forming the so-called "pyriform apparatus" when mites eat the shed proglottids. It appears, however, that the eggs are allowing the continuous transfer of nutrients from parent organism to offspring. Once the eggs are eaten, the cysticercoid type metacestode has a very rapid development within the intermediate host (Smyth and McManus 1989). The lack of egg nutritive reserves is therefore closely connected with the post-embryonic larval development and life cycle of this parasite.

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