Light and electron microscopic study on vitellogenesis of the caryophyllidean cestode *Monobothrioides chalmersius* (Woodland, 1924) Hunter, 1930 (Lytocestidae) from the catfish *Clarias gariepinus*

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**Abstract**
Transmission electron microscopy (TEM) has been applied for the first time to study vitellogenesis in the caryophyllidean tapeworm *Monobothrioides chalmersius* (Woodland, 1924) Hunter, 1930 from the catfish *Clarias gariepinus* inhabiting Nile Delta water in Egypt. During development of vitellocytes, cell size and nuclear surface are increased. Meanwhile, parallel cisternae of granular endoplasmic reticulum (GER) and Golgi complexes develop, but the nucleo-cytoplasmic ratio is restored, shell-globules (vitelline granules) are formed, small shell-granules fuse into larger shell-globules and large shell-globules fuse into larger shell-globule clusters, cytoplasmic and nuclear glycogen accumulate. Mature vitellocytes of *M. chalmersius* contain five kinds of vitelline material: shell globules clusters (vitelline granules), glycogen, few probably lipid droplets, many peripheral translucent vesicles and electron-dense vesicles. The interstitial tissue consists of cells from which many cytoplasmic processes extend to be in close contact with maturing and mature vitellocytes. The cytoplasm of the interstitial cells contains some electron-dense vesicles. The possible function of the interstitial cells inclusions is discussed. The vitellogenesis of *M. chalmersius* follows the basic pattern of the process in caryophyllidean cestodes but some differences were recorded particularly in the ultrastructure of the mature vitellocytes and interstitial tissue. Vitellogenesis and ultrastructure of the mature vitellocytes and interstitial tissue of *M. chalmersius* are compared particularly with those in other monozoic cestodes.

**Introduction**
Caryophyllidean tapeworms (Platyhelminthes: Eucestoda) are unique among “true” cestodes (Eucestoda) in their possession of a monozoic non-segmented body type, which contains only a single set of male and female organs. They may be pathogenic for their fish hosts (Bauer et al., 1973;
Williams and Jones, 1994; Hamada and El-Naggar, 2003; Oros et al., 2009). In Egypt, *Monobothrioides chalmersius* was recorded from the intestine of the catfish *Clarias gariepinus* inhabiting Nile water (Hamada and El-Naggar, 2003; Hamada et al., 2004). The authors studied the surface topography, mode of attachment, histopathological effects and the detailed anatomy of this parasite. Moreover, spermatogenesis and ultrastructure of the spermatozoa of *M. chalmersius* were previously studied (Arafa and Hamada, 2004).

The vitellocytes of cestodes and trematodes play two important roles in their developmental biology: (1) Production and secretion of proteins for egg shell or capsule formation (Świderski et al., 1970a; Świderski and Xylander, 2000; Xylander, 1988). (2) Nourishment of the developing embryos with nutritive reserves stored in their cytoplasm (Świderski and Xylander, 2000; Świderski and Mackiewicz, 2004). Vitellogenesis of polyzoic cestodes has been studied by many authors (see for example, Świderski et al., 1970a,b; Świderski and Mackiewicz, 1976; 2003; Ortner-Scho¨nbach, 1913; Świderski et al., 2004a; Mokhtar, 1974; Korneva, 2001; Levron et al., 2007). However, limited data are available on vitellogenesis and vitellocytes ultrastructure of *M. chalmersius*. Therefore, the aim of the present study is to provide new data on vitellogenesis and ultrastructure of mature vitellocytes of the caryophyllidean cestode *M. chalmersius*. Examination of the cytological changes accompanying development of vitellocytes may contribute a better understanding of its biology.

**Materials and methods**

Specimens of the freshwater fish *C. gariepinus* (syn. *C. lazera*) were obtained from Damietta branch of the river Nile, near Mansoura, Daqahliya Province, Egypt. Fishes were kept alive in tanks containing aerated Nile water until required. They were dissected and the alimentary canals were opened in saline solution. The caryophyllidean cestodes *M. chalmersius* were recovered from the alimentary canal by the help of a fine needle using stereomicroscope. Some living specimens were flattened between two glass slides, fixed in 10% formalin, washed in distilled water and stained in alum carmine. After staining, specimens were washed in distilled water, dehydrated in an ascending series of ethanol, cleared in terpeniol or xylene and mounted in Canada balsam or DPX. The vitelline follicles were examined using phase-contrast and bright field microscopy.

Some toluidine blue-stained sections for light microscopy and ultrathin sections for transmission electron microscopy (TEM) were prepared as follows. Specimens of *M. chalmersius* were washed using saline solution and then fixed in 2.5% glutaraldehyde buffered to pH 7.3. Each specimen was cut into three regions; anterior, middle and posterior regions. The middle regions, containing most of the vitelline follicles, were left in the fixative for about 2 h and washed for about 1 h in several changes of cold buffer (0.1 M sodium cacodylate containing 3% sucrose and 0.1 M CuCl2). Post-fixation was carried out for about 1 h at 4 °C in freshly prepared 1% osmium tetroxide in 0.1 M sodium cacodylate-HCl. The specimens were left in the washing buffer overnight and were then dehydrated in ethanol solutions and propylene oxide. They were then orientated and embedded in capsules containing Spurr resin. The capsules were placed in an oven overnight at 60 °C. Sections were cut using glass knives at a thickness of 1 μm using an LKB ultramicrotome and were stained in a solution of 1% toluidine blue in 1% borax. Sections were mounted in DPX and examined using phase-contrast and bright field microscopy. Ultra-thin sections were cut in the middle regions of *M. chalmersius* at 70–90 nm using the ultramicrotome and diamond knife. The sections were mounted on multiple-hole coated grids and stained in a solution of uranyl acetate for about 30 min followed by lead citrate for about 5 min. They were then examined using a JEOL transmission electron microscope operating at 80 kv in the TEM unit, the University of Dammam (previously King Faisal University) Al-Dammam, Saudi Arabia.

**Results**

**Light microscope observations**

The female reproductive system of the caryophyllid *M. chalmersius* consists mainly of vitelline follicles, follicular ovary, ootype, receptaculum seminis, vagina and uterus. The ootype connects the uterus, receptaculum seminis and vitelline reservoir which receives vitellaria from the vitelline follicles via two common vitelline ducts.

The vitellaria of *M. chalmersius* extend from the anterior region of the body just posterior to the neck to the posterior region just anterior to the ovary. No post ovarian vitelline follicles were found in *M. chalmersius*. The follicles vary in their size, the smallest ones occur toward the neck, while the largest ones occur near the ovary. For more detailed description of this species, see Hamada et al. (2004).

Vitellaria of *M. chalmersius* are numerous oval or lobate follicles (Fig. 1). They occupy the cortical region of the parenchyma of their non-segmented monozoic strobila (Fig. 1). Examination of toluidine blue-stained sections revealed that each vitelline follicle contains vitellocytes at different stages of development and interstitial cells (Figs. 2–6). Four developmental stages of vitellocytes could be detected and described as immature (stage I), early stage of maturation (stage II), advanced stage of maturation (stage III) and mature vitellocyte (stage IV). The immature vitellocytes contain no vitelline granules (shell globules), while maturing and mature ones contain shell globules of different sizes and numbers (Figs. 2–6). The immature vitellocytes are often located at the periphery of the follicle (Fig. 2). Some maturing and mature vitellocytes are situated toward the center of the follicle (Fig. 3), while others are situated at the periphery (Figs. 4–6). It should be mentioned that the development of vitellocytes is a continuous process and its division into four different stages of development is done here to facilitate description. The interstitial tissue appears to be cellular and located among and in close contact with immature and maturing vitellocytes (Figs. 5 and 6). Some of the interstitial cells are located toward the center of the follicle (Fig. 6).
Electron microscope observations

TEM examination of the vitelline follicles revealed that each follicle is surrounded by an external cytoplasmic sheath (Fig. 7). The four developmental stages of vitellocytes were detected as immature, early stage of maturation, advanced stage and mature vitellocyte.

**Immature vitellocyte**

Immature vitellocyte of *M. chalmersius* is nearly oval in shape and has a large nucleus and little amount of cytoplasm (high nucleo-cytoplasmic ratio) (Fig. 7). The nucleus contains a conspicuous electron-dense nucleolus and irregular clumps of dense heterochromatin which are dispersed in the nucleoplasm (Fig. 7). The cytoplasm of these cells is moderately electron-dense in which few mitochondria could be detected in the cytoplasm.

**Early stage of maturation**

During early stages of maturation, the nuclear surface area increases. Lobe-like processes project from the nuclear envelope.
The nucleus has a conspicuous nucleolus and dispersed clumps of heterochromatin (Fig. 8). The nucleo-cytoplasmic ratio decreases. The vitellocyte is also characterized by the presence of long cisternae of the granular (rough) endoplasmic reticulum (Figs. 8 and 9) and many mitochondria. Golgi complexes produce vesicles (the precursors of shell globules). Subsequently, small electron-dense granules (shell globules) are formed first in a translucent matrix (Fig. 9). Many small spherical shell globules are found in the cytoplasm. The small shell globules fuse to form larger ones (Fig. 8 Inset). During this stage of development, a few translucent vesicles were also detected in the cytoplasm of the vitellocyte (Fig. 8). A single probably lipid inclusion is associated with concentric rows of the endoplasmic reticulum (Fig. 10).
Advanced stage of maturation

During advanced stages of vitellocyte maturation, the cell size and the volume of the cytoplasm increase. The nucleus of the vitellocyte has an irregular shape with dispersed heterochromatin, some of which become very close to the nuclear envelope (Figs. 11 and 12). Granular endoplasmic reticulum, Golgi complexes and mitochondria are more abundant in the cytoplasm of the vitelloocytes (Figs. 11 and 12). Single shell globules fuse to form shell globules clusters (Figs. 11 and 12). The number of the shell globule clusters increases in the cytoplasm. Occasionally, a small translucent vesicle is found in this matrix (Fig. 12). A considerably electron lucent large probably lipid droplet is incorporated with shell globules clusters in moderately electron-dense region of cytoplasm.

Fig. 9  Electron micrograph of a vitelline follicle of *Monobothrioides chalmersius* showing part of a vitellocyte at early stage of maturation. g, region intranuclear glycogen particles; GER, granular endoplasmic reticulum; m, mitochondria; n, nucleus; sg, shell globule. Note that the small shell globules are formed first in a translucent matrix (arrow).

Fig. 10  Electron micrograph of a vitelline follicle of *Monobothrioides chalmersius* showing a vitellocyte at early stage of maturation. A single probably lipid inclusion (l) is associated with concentric rows of the endoplasmic reticulum (ER). n, nucleus; sg, shell globules.

Fig. 11  Electron micrograph of a vitelline follicle of *Monobothrioides chalmersius* showing part of a vitellocyte at advanced stage of maturation. g, region of nuclear glycogen particles; m, mitochondria; n, nucleus; sg, shell globule; sgc, shell globules cluster.

Fig. 12  Electron micrograph of a vitelline follicle of *Monobothrioides chalmersius* showing part of a vitellocyte at an advanced stage of maturation. g, nuclear glycogen; GER, granular endoplasmic reticulum; m, mitochondria; l, probably lipid droplet; n, nucleus; sgc, shell globules cluster. Note the electron lucent vesicle (arrow) incorporated with shell globules clusters in moderately electron-dense region of cytoplasm.
Mature vitellocyte

The size of mature vitellocytes increases five to six times and the nucleo-cytoplasmic ratio is restored. The nucleus has a spherical shape and is bounded by a conspicuous double nuclear envelope (Fig. 13). More accumulations of cytoplasmic and intranuclear glycogen particles could be detected. Intranuclear glycogen occupies the central region of the nucleus, while the heterochromatin is found at the periphery adjacent to the nuclear envelope (Fig. 13). The shell globule clusters are abundant in the cytoplasm of the mature vitellocytes (Fig. 13). They vary in size and each one appeared to be formed of 2 to 8 single shell globules. Only one kind of shell globule clusters is found in the cytoplasm of mature vitellocytes (Figs. 13–15). In some sections through the mature vitellocytes, considerably large areas of cytoplasmic glycogen were detected (Figs. 14 and 15). Many translucent vesicles and electron-dense granules were also detected in the cytoplasm (Fig. 13). The translucent vesicles vary in their size and situated at the periphery surrounding the cytoplasmic glycogen (Figs. 14 and 15). A few probably lipid droplets were found in the cytoplasm of mature vitellocytes (Fig. 15).

Interstitial tissue

Cytoplasmic extensions from the interstitial tissue ramify to surround the vitellocytes at different stages of maturation within the follicle (Figs. 7 and 8). The nucleus of the interstitium could not be detected in the TEM sections. Ramified cytoplasmic processes contain dense matrix, mitochondria, granular endoplasmic reticulum and some electron-dense vesicles (Figs. 6 and 7).

Discussion

As far as our knowledge, the present work is the first to study the ultrastructure of vitellogenesis in the caryophyllidean cestode *M. chalmersius*. Maturation of vitellocytes is characterized by: (1) Increase in cell size five to six times; (2) decrease and restoring of the nucleo-cytoplasmic ratio; (3) development of parallel GER cisternae; (4) development of Golgi complexes; (5) fusion of the small shell-granules into larger shell-globules and into larger shell-globe clusters; (6) synthesis and storage of glycogen in the cytoplasm and in the nucleus; (7) disintegration of the GER and accumulation of more shell-globe clusters within the vitellocyte cytoplasm; and (8) accumulation of more cytoplasmic and nuclear glycogen. In this respect, the vitellogenesis of *M. chalmersius* appears to follow the basic pattern of the process as previously described for many caryophyllidean cestodes (Mackiewicz, 1968; Świderski and Mackiewicz, 1976; Świderski et al., 2004a,b; Bruňanská et al., 2009). However, some differences were recorded particularly in the ultrastructure of the mature vitellocytes and interstitial tissue.

Generally, mature vitellocytes of caryophyllids possess two types of vitelline inclusions: Shell globule clusters and glycogen (Świderski and Xylander, 2000). However, Bruňanská et al. (2009) detected three types of vitelline material (shell globule clusters, “lamellar” granules, and glycogen) in the mature vitellocytes of the caryophyllid *Atractolytocestus huronensis*. The present study revealed that mature vitellocytes of *M. chalmersius* contain five kinds of the vitelline material (shell globules clusters, few probably lipid droplets, many peripheral translucent vesicles, electron-dense vesicles and glycogen).

A few translucent vesicles were detected in the cytoplasm of the vitellocytes during early and late stages of maturation. However, many peripheral translucent vesicles were detected in the cytoplasm of mature vitellocytes. These translucent vesicles were not detected in the mature vitellocytes of other caryophyllids (Świderski and Xylander, 2000). Histological
Randomly dispersed nuclear glycogen was recorded in the mature vitellocytes of Caryophyllidea, however, absence of cytoplasmic lipid droplets are the most characteristic features of vitellogenesis in the Caryophyllidea, however, nuclear glycogen was not detected in the mature vitellocytes of the caryophyllidean cestode *A. huronensis* (*Brunšanka et al.*, 2009) or cellular as in *W. virilis* (*Świderski et al.*, 2009).

The cytoplasm of the interstitial tissue in the vitelline follicles of *M. chalmersius* contains some electron-dense vesicles. These vesicles are supposed to be nutrients transported to the vitellocytes. The presence of such suggestion. The nutritive role of the interstitial tissue to vitellocytes has not been proved in Eucestoda. However, in the caryophyllidean cestode *A. huronensis*, *Brunšanka et al.* (2009) found evidences supporting the suggestion that the electron-dense vesicles in the interstitium may represent nutrients transported from the parenchyma toward the vitellocytes. In *Amphilina* (Cestoda) and some Monogenea, the parenchymal extensions are responsible for the selection and transport of the nutritive material toward the developing vitellocytes (Haltan et al., 1974; *Xylander*, 1988).

**References**


Mackiewicz, J.S., 1968. Vitellogenesis and egg shell formation in Caryophyllaeus laticeps (pallas) and Caryophyllaeoides fennica (Schneider) (Cestoidea: Caryophyllidea). Z. Parasitenkd. 30, 18–32.


