

Ultrastructure of Vitellocytes in *Electrotaenia malopteruri* (Fritsch, 1886) (Cestoda: Proteocephalidae) A Parasite of *Malapterurus electricus* (Siluriformes: Malapteruridae) from Egypt

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Abstract: This study describes the Ultrastructure of mature Vitellocytes of the Proteocephalidae Cestode *Electrotaenia malopteruri* (Fritsch, 1886) a parasite of the common catfish *Malapterurus electricus* using transmission electron microscopy (TEM). The vitellocyte is characterized by the perinuclear cytoplasm that contains numerous parallel cisternae of granular endoplasmic reticulum (GER), several Golgi complex, its peripheral cytoplasm contains, lipid droplets, shell globule clusters, proposed glycogen like particles. The most characteristic feature of the mature vitellocyte of this Cestode species is the concentric arrangement of shell globule clusters.

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Introduction

Cestode class is known to be one of classes that has the highest reproductive capacities of all animal classes (Conn, 2000). Several TEM studies have been published on the ultra structure and differentiation of vitellocytes in cestodes, (Swiderski and Xylander, 2000). Vitellocytes in cestodes have two important functions, i.e. egg shell formation and the nourishment of the early embryo (Swiderski, *et al.*, 1970 a, b; Swiderski and Xylander 1998 and 2000). So many authors studied the characteristic features of mature vitellocytes in different cestode groups

On Vitellogenesis in the cestode *Inermicapsifer madagascariensis*, (Swiderski, 1973); on Bothriocephalidea *Bothriocephalus clavibothrium*, (Swiderski and Mokhtar, 1974); on *Progyllotia pastinace*, (Mokhtar-Maamour and Swiderski, 1976); on *Rhinebothriidea Echeneibothrium beaulchampi* (Swiderski and Mackiewicz, 1976); on *Proteocephalus longicollis* (Swiderski and Subilia, 1978); on *Grillotia erinaceus*, (McKerr, 1985); on Amphilinidea, *Gyrocotyle urna*, (Xylander, 1987) and on Amphilinidea, *Amphilina foliacea*, (Xylander, 1988); on Proteocephalidea *Proteocephalus exiguus* (Bruňanská, 1997); on Cyclophyllidea *Catenotaenia pusilla* (Swiderski *et al.*, 2000); on *Triaenophrus nodulosus*, (Koenewa, 2001); on *Moniezia expansa* and *Mosgovoyia ctenoides*, (Li *et al.*, 2003); on *Caryophyllaeus laticeps* (Swiderski *et al.*, 2004a); on *Khawia armeniaca*, (Swiderski *et al.*, 2004b); on Spathebothriidae *Cyathocephalus truncates*, (Bruňanská *et al.*, 2005); on *Trypanorhynchea Dollfusiella spinulifera*, (Swiderski *et al.*, 2006a); on

Progyllotia pastinace (Swiderski *et al.*, 2006b); on *Didymobothrium rudolphii*, (Podubnaya *et al.*, 2006); on *Paraechinophallus japonicus*, (Levron *et al.*, 2007); on *Paracharistianella trygonis*, (Swiderski *et al.*, 2007); on Caryophyllidea *Atractolytocestus huronensis*, (Brunanska *et al.*, 2009); on *Wenyonia virllis*, (Swiderski *et al.*, 2009); on Diphyllidea *Echeneibothrium euterpes* and *Clestobothrium crassiceps*, (Swiderski *et al.*, 2011).

In spite of all of these accumulated studies on different cestode groups, the work on Proteocephalidae still fragmented and less research has been done on the ultrastructure of reproductive system among proteocephalideans.

The aim of the work is to describe the aspect of Vitellocytes ultrastructure of *Electrotaenia malopteruri* a parasite of *Malapterurus electricus* to compare it with the results of previous reports of vitellocyte structure in other cestode species.

Materials and Methods:

Materials:

Mature specimens of *Electrotaenia malopteruri* were obtained from the intestine of the infested catfish, *Malapterurus electricus*, River Nile, Egypt. Living cestodes were dissected in a 0.6% NaCl solution and different portion of mature proglottids containing laterally the vitellaria and reproductive system were routinely processed for TEM examination.

Methods:

Specimen were fixed in cold (4°C) 3% glutaraldehyde in a 0.1M sodium cacodylate buffer at PH 7.2, for 2 h., washed in a 0.1M sodium cacodylate

buffer at PH 7.2, post fixed in cold (4°C) 1% Osmium tetroxide in the same buffer for 1 h., dehydrated in an ethanol series, and finally embedded in Epon resin. Specimens were sectioned for histological study at 0.5-1µm using a Reichert-jung Ultracut E.ultramicrotome, mounted on glass slides, stained with 1% Toluidine Blue O.

Ultra thin sections were cut at thickness of 20-70 nm, placed on copper grids and double-stained with uranyl acetate and lead citrate. Sections were examined using a Joel 1010 Transmission electron microscopy (TEM) operated at 80 KV.

Results

The numerous vitelline follicles are arranged as a continuous series in the cortical parenchyma with some solitary follicles in the medullary parenchyma. In the centre of each follicle extend a small vitelline duct (plate 1,A,B,C)

Examination of ultrathin sections showed that the vitellaria of *Electrotaenia malopteruri* (Fritsch, 1886) is an extensive system of numerous oval or

elongated vitelline follicles enclosed by the parenchyma (Plate 2,A). The characteristic arrangement of concentric distribution of shell globules clusters which is diagnostic to *E. malopteruri* (Plate2,A).

Vitellocytes generally have high Nuclei /cytoplasmic ratio (Plate2, A). The nucleus is round and contains large clumps of heterochromatin and the narrow cytoplasm contains a large number of lipid droplets, proposed glycogen like droplets, (Plate. 2,B) and concentric rough endoplasmic reticulum (RER). Differentiation of vitellocytes was characterized by the increase of RER. Cisterna which is filled with an electron-dense material (Plate 2, F), Golgi vesicle gave rise to large membrane-bound inclusions (Plate 2, F). When completely mature they had a multigranular and were delimited by the smooth membrane. Oocytes are accompanied by the vitellocytes which have shell globules (Plate 2, D, E), few mitochondria scattered in the parenchyma (Plate 2, C).

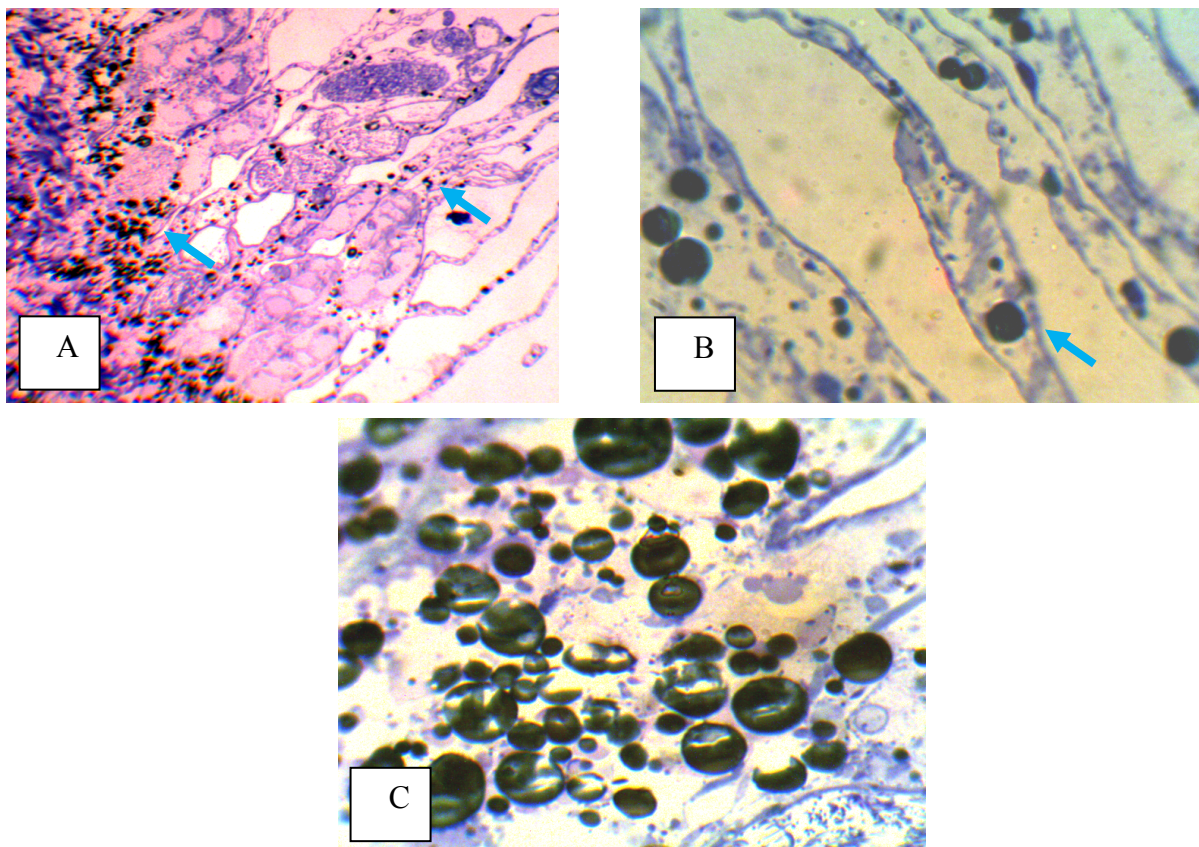


Plate 1: Semi thin sections of *Electrotaenia malopteruri* showing, A-Vitelline follicle arranged as a continuous series in the cortical parenchyma (arrow) with some solitary follicles in the medullary parenchyma (arrow head), 400 x. B-Branches of vitelline duct containing vitellocytes (Arrow), 800 x. C-Higher magnification of A showing Vitellaria, 2000 x.

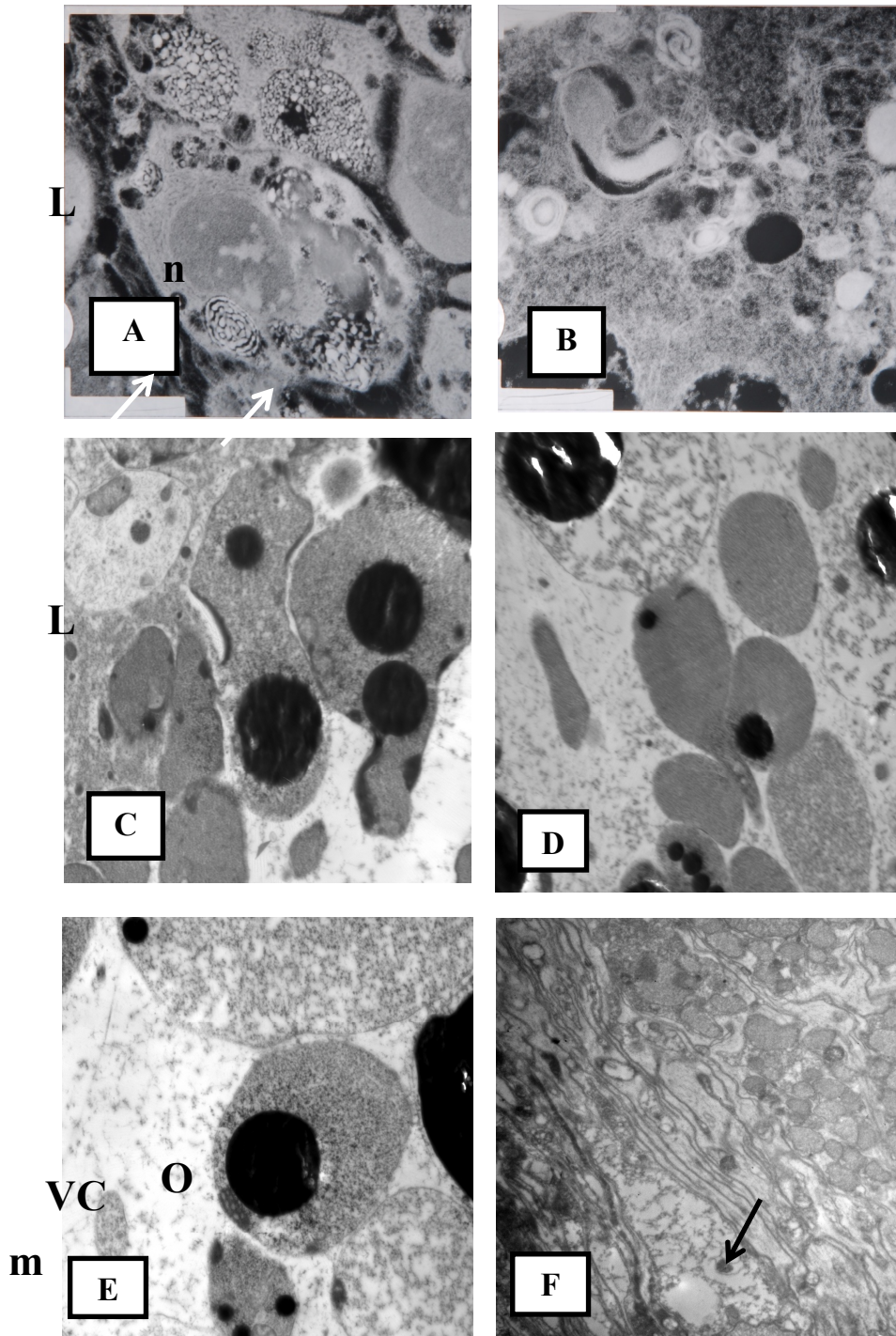


Plate (2): A-TEM micrograph illustrating the mature vitellocyte with the concentric shell globules (**arrows**), nucleus (**n**), Lipid droplets (**L**), 5000X. B- TEM micrograph illustrating the concentric arrangement of the endoplasmic reticulum (arrow, double arrow), proposed glycogen like-particles (**G**), lipid droplets, 7500X. C- TEM micrograph illustrating the developing Oocytes (**Oo**) adjacent to the vitellocyte (**vc**), note the mitochondria (**m**), 2500X. D-TEM micrographs illustrating the aggregation of shell globules (arrow), 2500X. E-TEM micrographs illustrating the scattered shell globules (arrows), 3000X. F-TEM micrograph illustrating the characteristic parallel cisternae of endoplasmic reticulum (white arrows), large membrane bound inclusions of Golgi complex (black arrow), 8000X.

Discussion

As summarized by Swiderski and Xylander (2000) in their extensive review, cestode vitellocytes are very important for egg formation and embryonic development. They play two significant functions including, the formation of hard egg shell or a delicate vitelline capsule and supplying nutritive reserves for the developing embryo.

Both roles are closely connected with the presence of two types of cytoplasmic inclusions in cestode vitelline cells such as:

Egg shell globules, vitelline vesicles or shell globules clusters that taking part in the egg shell or vitelline capsule formation.

Glycogen and/ or lipids (Sometimes mixtures of both in different proportion which represent recent various energy sources for the developing embryos).

In the present study lipid droplets were localized only in the vitellocyte cytoplasm this is in agreement with *Paracharistianella trygonis*, (Swiderski *et al.*, 2007). On the other side in Tetracystidae *Echeneibothrium beaulchampi* by the study of Mokhtar-Maamouri and Swiderski, (1976) and in *Didymobothrium rudolphii* studied by Poddubnaya *et al.*, (2006) it was reported inside the cell nuclei. Other studies on *Diplocotyle olrikii* by Bruňanská *et al.*, (2005) and on Caryophyllidea *Atractolytocestus huronensis* by Bruňanská *et al.*, (2009) their conclusion was that the lamellar heterogenous egg globules are represented in great amount, which was different from our studies that as we noticed that the egg shell globules have a characteristic concentric shape. Report of Swiderski *et al.*, (2004a) on *Caryophyllaeus laticeps* stated that lipid granules were absent in mature vitellocyte, while in our study lipid droplets were distributed through the cytoplasm of vitellocytes. Whereas, Swiderski and Mickiewicz (1976) work on *Glaediacris catostomi* found a great amount of cytoplasmic and nuclear glycogen. Study of Swiderski *et al.*, (2004b) on *Khawia armeniaca* reported the lamellar granules in the cytoplasm of this Caryophyllidean cestode. Swiderski *et al.*, (2009) work on *Wenyonia virllis* found moderate accumulations of cytoplasmic glycogen. In addition, Swiderski *et al.*, (2011) work on Diphyllidea *Echinobothrium euterpes* noticed a large amount of glycogen accumulations around the large, saturated lipid droplets of maturing and mature vitellocytes. Swiderski *et al.*, (2006a) work on *Trypanorhyncha Dollfusiella spinulifera* found very few glycogens in the cytoplasm. Many investigators; Swiderski and Mokhtar (1974); Mokhtar-Maamour and Swiderski (1976); Swiderski and Subilia (1978); McKerr (1985); Bruňanská, (1997); Swiderski *et al.*, (2000), Koeneva (2001), Swiderski *et al.*, (2006 b), Swiderski *et al.*, (2007) and Levron *et al.*, (2007) discussed the ultrastructure of the vitellocytes in the

following species: *Grillotia erinaceus*, *Paracharistianella trygonis*, *Echeneibothrium beaulchampi*, *Progillotia pastinance*, *Bothriocephalus clavibothrium*, *Paraechinophallus japonicus*, *Proteocephalus longicollis*, *Inermicapisifer madagascarinensis*, *Triaenophorus nodulosus*, *Catenotaenia pusilla*, *Moniezia expansa*, *Proteocephalus exiguous* and *Mosgovoyia ctenoides* and compared their contents.

The discovery that lipids vary in the vitellocytes of different families raised important questions regarding the factors determining lipid types, functional significance and what role they might have in assessing evolutionary relationships at any level. As the nutrient reserves are related to the ecology and life cycle in some species and its accumulation in the vitellocytes may deflect the adaptation to the parasitic way of life in different groups of cestodes, Swiderski and Xylander (2000).

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