Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 23(4): 189- 200 (2019)
www.ejabf.journals.ekb.eg

Ultra structural study of the Embryonic Development in the Intrauterine Eggs of *Acanthostomum* (*atrophocaecum*) *aswaninesis* Wannas, 1977 (Digenea, Acanthostomatidae) with evidence of early miracidium development

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ARTICLE INFO
Article History:
Received: Sept. 21, 2019
Accepted: Oct. 6, 2019
Online: Oct. 9, 2019

Keywords:
Intrauterine eggs
Acanthostomum
Miracidium
Digenea
Ultrastructure

ABSTRACT

Intrauterine embryonic development of the eggs of *Acanthostomum (atrophocaecum) aswaninesis* was investigated by transmission electron microscopy. The study revealed the successive stages of embryonic development of the eggs. The proximal uterus contains unembryonated eggs and eggs with early embryos while the distal regions of the uterus were occupied with eggs containing fully developed embryos, each was composed of a fertilized oocyte and few vitelline cells; which were fused forming the vitelline syncytium that characterized the early embryonated eggs. With egg development, the eggshell progressively thickened with the appearance of the operculum after shell formation at the narrower pole of the eggshell surface. Three types of differentiated blastomeres are formed as a result of the continued cleavage divisions; macromeres, mesomeres and micromeres, with nuclei of different shapes and sizes. They occupy different places within the embryo. As a result of the following cleavage divisions, early degeneration or apoptosis of some micromeres take place. Numerous fully developed cilia are easily visible around the differentiated embryo (miracidium) of the advanced stages. The cilia have a typical 9+2 (nine peripheral and two central) arrangement of microtubules. The germinative cells of miracidia have easily appeared in the medioposterior parts of their ciliated larvae. The results of the present study were compared with those previously reported for other parasitic Platyhelminthes.

INTRODUCTION

The ultra-structural data have to be interpreted in relation to parasite biology to understand the structural requirements of specific parasite strategies (Born-Torrijos *et al*. 2017).

Helminthic eggs play a critical role in movement of the parasite from definitive to intermediate host (Swiderski *et al*. 2015). Egg envelopes play an important role in the protection, metabolism, and storage of nutritive reserves with an energy storage capability in the cestodes (Swiderski *et al*. 2017).

The ultra-structural aspects of embryonic envelope and associated developmental stages of intrauterine eggs of trematodes (Fujino *et a.l* 2000, Ashton *et al*. 2001, Jones *et al*. 2008, Pinhero *et al*. 2015, Swiderski *et al*. 2010a, 2013a, b, 2014, Khampoosa *et a.l* 2012), aspidogastreans (Swiderski *et al*. 2011, 2012), cestodes (Burt 1986, Conn 2008, Mlocicki *et al*. 2005, 2006, 2010a, 2010b, 2011

Swiderski *et al*. 2004, 2005, 2010b, 2017) and trematodes with cestodes (Conn 2007, Swiderski 1996, Swiderski and Conn 2000, 2001) were studied.

TEM studies have been impeded by technical difficulties in getting the egg contents well fixed and infiltrated with embedding media, and in cutting the thick, hard egg shells (Swiderski *et al*. 2010a).

Details of egg ultrastructure among the digenean trematodes has been studied rather little (Conn *et al*. 2018). Many more studies are needed to explore egg ultrastructure in other digenean taxa, to explore potential phylogenetic patterns in egg development and structure, and to correlate structure with function in the life cycle.

Because trematode eggs are very small – usually microscopic – little can be seen using light microscopy methods. Thus, electron microscopy is necessary to reveal the essential features of the structure of the eggs and the embryos or larvae they contain. To further complicate their study, because the highly resistant trematode egg shells are designed to protect the embryo and larva from harsh environmental conditions outside the host and outside the parent worm, preparing trematode eggs for ultrastructural studies is technologically challenging. Thus, egg ultrastructure in this important group of parasites has received relatively little study, even when compared to cestodes, another diverse and widespread class of parasitic Platyhelminthes (Burt 1986; Swiderski 1996; Swiderski and Conn 2000, 2001; Conn 2007, 2016).

The purpose of this paper is to provide a brief review of the known studies on digenean trematode egg ultrastructure, while providing up-to-date context by reporting new comparative data on eggshell formation, embryogenesis and egg development and a new synthesis of information of *A. (a.) aswaninesis* by transmission electron microscopy.

MATERIALS AND METHODS

Adult alive female worms of *A. (a.) aswaninesis* (Wannas, 1977) were collected alive from the intestine of naturally infected *Bagrus bayad*, caught from Timsah Lake, Egypt and treated for transmission electron microscopy (TEM) as follow. Alive specimens were rinsed in 0.9 Na Cl solutions and immediately fixed with 2.5% glutaraldehyde in 0.1M phosphate buffer, PH 7.3, for 2h at 4°C following a buffer wash, post fixed in 1% osmium tetroxide in 0.1M phosphate buffer at PH 7.3 for 1h. Samples were dehydrated in ethanol and propylene oxide, embedded in resin, at 60°C for 48h. Semithin sections were cut on a Leica ultramicrotome and stained with to-luidine blue for light microscopy. For TEM, ultrathin sections (60-90) nm at different levels in the body (testes and vas deferens) was cut using a diamond knife of an LKB 4800-Ultramicrotome. Sections were moun-ted on uncoated copper grids and double stained with alcoholic uranyl acetate and aqueous lead citrate for 20 min., ultrathin sections were examined in a Joel - JEM/ 1010 transmission electron microscopy made in Japan at 80 Kv.

RESULTS

The eggs of *A. (a.) aswaninesis* measured 27-32 μm long and 14-18 μm wide; they appear ovoid or slightly elongated in ultrathin cross- and/or oblique sections, operculated and have two poles; one pole is observed narrower than the second (Fig. 1a).

During the development; each egg is characterized by containing few vitellocytes supplementary with each fertilized oocyte (Figs. 1b-d); so they can be classified as oligolecithal type.

The proximal regions of the uterine lumen contains unembryonated eggs (Fig. 1b) and eggs with early embryos (Fig. 1c) while the posterior (distal) regions of the uterus were occupied with eggs containing fully developed embryos (Figs.1h, 2a).

The eggs of *A.* (*a*.) *aswaninesis* show different successive stages of the embryonic development. The unembryonated egg appears composing of a fertilized oocyte (ovum) and few vitelline cells. The fertilized oocyte is formed of a large nucleus, surrounded by a thick layer of granular cytoplasm rich in numerous mitochondria and cisternae of granular endoplasmic reticulum (GER) (Fig.1c). The cytoplasm of the vitellocytes usually contained spherical balls of concentrically arranged cisternae of GER bodies (Fig.1d). Then the vitellocytes appearing fused forming vitelline syncytium that situated directly beneath the newly-formed egg shell (Fig. 1e).

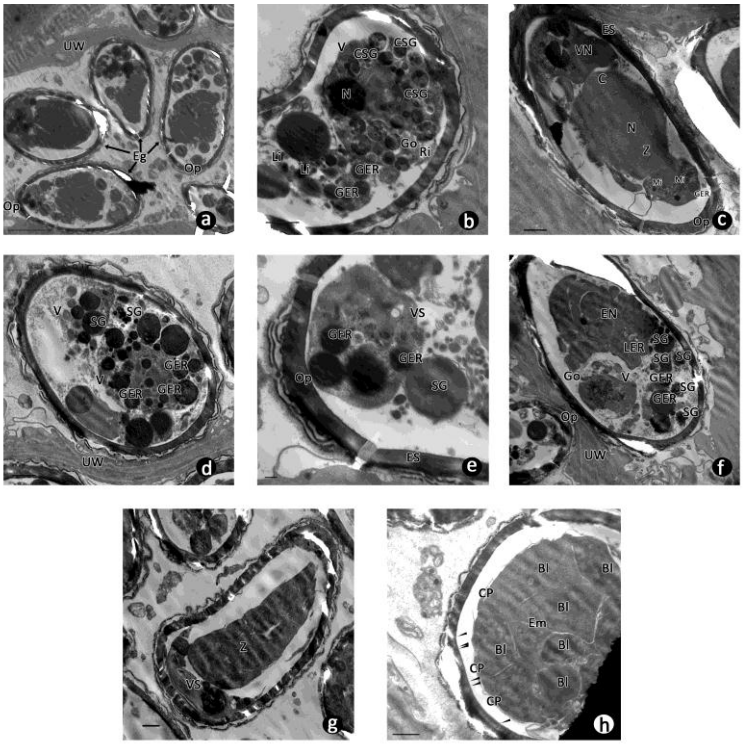

Fig. 1: TEM micrographs of ultrathin cross- and/or oblique sections of proximal and distal parts of the uterus of *A. (a.) aswaninesis*.

a. Low magnification shows four eggs (Eg), uterine wall (UW), operculum (Op) bar= 10µ.

b. Proximal part of the uterus with unembryonated eggs, vitellocyte (V), vitellocyte nucleus (N), free ribosomes (Ri), Golgi complex (Go), granular endoplasmic reticulum (GER), clusters of shell globules (CSG), lipid droplets (Li) bar= 2µ

c. Proximal part of the uterus early embryo (zygote) (Z), egg shell (ES), zygote nucleus (N), cytoplasm (C), vitellocyte nucleus (VN), endoplasmic reticulum (GER), mitochondria (Mi) bar =500nm.

d. Proximal part of uterus with unembryonated egg, vitellocytes (V), uterine wall (UW), granular endoplasmic reticulum (GER), shell granules (SG) bar= 2µ.

e. High magnification shows vitelline syncytium (VS), egg shell (ES), granular endoplasmic reticulum (GER), shell granules (SG), operculum (Op) bar =500nm.

f. Proximal part of uterus with newly formed embryo, embryo nucleus (EN), vitellocytes (V), uterine wall (UW), granular endoplasmic reticulum (GER), shell granules (SG), lamellar endoplasmic reticulum (LER), Golgi complex (Go) bar= 2µ.

g. Proximal part of uterus with newly formed embryo, collapse vitelline syncytium (VS), zygote (Z) bar= 2µ.

h. Posterior (distal) region of uterus with eggs containing developed embryo (Em), numerous blastomeres (Bl), cytoplasmic processes (CP), dark depositions (arrow heads) bar= 2µ.

The newly-formed electron dense egg shell of *A. (a) aswaninesis* , consisted of a tanned protein looked very thick which differ slightly in thickness in different regions. The egg shell has no pores or channeles (Figs. 1a-e). With egg development, the egg shell progressively thickened with the appearance of the operculum after shell formation at the narrower pole of the egg shell surface (Figs.1a, c, e, f), whereas the opposite pole is covered by a continuous thick and solid layer of electron -dense shell.

The early embryonated egg is characterized by containing of the vitelline syncytium which is gradually disappeared, and the remnants of the vitelline material are granular endoplasmic reticulum, Golgi complex and numerous shell globules with different size and electron-density. Whereas the nuclei of the vitellocytes appear degenerated and located beneath the embryonic envelope (Fig. 1f). Then the vitelline syncytium becomes collapse (Fig. 1g) and not appears especially in the eggs containing numerous blastomeres (Fig. 1h).

Internally and externally electron dark depositions appear on the egg shell; these depositions seem during different developmental stages of the embryos (Figs. 1h, 2b).

The early embryo composed of several blastomeres with different sizes, which formed as a result of cleavage divisions for the eggs. The cleavage divisions for the eggs are continued; giving rise the appearance of increase in numbers of blastomeres of the early embryo; resulting in constituting different progressive developmental stages of the embryo (Fig. 1h).

As a result of the continued cleavage divisions; three types of blastomeres are differentiated; macromeres, mesomeres and micromeres (Fig. 2a).

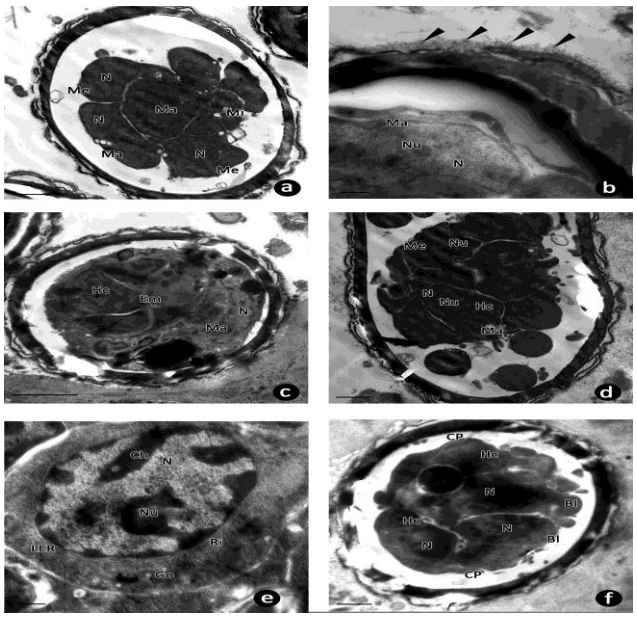

Fig. 2: TEM micrographs of ultrathin cross- and/or oblique sections of distal parts of the uterus of *A. (a.) aswaninesis.*

a.Posterior (distal) region of the uterus with eggs containing fully developed embryo; macroomere (Ma), mesomere (Me), micromere (Mi), nucleus (N) bar= 2µ.

b. High magnification shows macroomere (Ma), nucleus (N), nucleolus (Nu), external dark depositions (arrow heads) bar= 500nm.

c. Posterior region of the uterus with eggs containing fully developed early embryo (Em), macroomere (Ma), crescent-shape nucleus (N), heterochromatin (Hc) bar= 10µ.

d. Posterior region of the uterus shows macroomere (Ma), mesomere (Me), nucleus (N), nucleolus (Nu), heterochromatin (Hc) bar= 2µ.

e. High magnification of mesomere shows nucleus (N), nucleolus (Nu), heterochromatin (Hc), ribosomes (Ri), Golgi complex (Go), lamellar endoplasmic reticulum (LER) bar= 500nm.

f. Posterior region of the uterus with eggs containing fully developed embryo shows differentiated blastomeres (Bl), their nucleus (N), heterochromatin (Hc), cytoplasmic processes (CP) bar= 2µ.

They seem having nuclei with different shape and size, and also they occupy different places within the embryo. Their nuclei and cytoplasm are gradually changed during their differentiation. The early cleavage divisions of the zygote produce a few numbers of large macromeres that are usually appearing underneath the egg shell near the surface of the early embryo. Each macromere has a flatten nucleus, the nucleus sometimes appears with crescent-shape form localized at one pole of the egg and has a spherical nucleolus (Figs. 2b, c). Gradually, the macromeres moved to the central part of the embryo (Fig. 2d), they exhibited a large spherical nucleus. Their nuclei exhibit islands of heterochromatin which appears irregularly scattered within the nucleoplasm. Sometimes their nuclei exhibit an electron dense spherical nucleolus (Figs. 2b-d). Mesomeres appeare to be large cells where their nuclei and cytoplasm exhibit large size. Their nucleus contained numerous irregular electron dense islands of heterochromatin and spherical nucleolus, their cytoplasm appeared granulated and contains ribosomes, Golgi complex and lamellar endoplasmic reticulum (Fig. 2e).

Through the progressive stages of the development of *A.* (*a.*) *aswaninesis*, the embryo rapidly increased in size giving rise a large number of different developmental blastomeres. Then the growing blastomeres extend containing cytoplasmic processes (Figs. 1h, 2f) which fused forming a common syncytial layer of embryonic envelope situated beneath the egg shell.

As a result of occurrence the following cleavage divisions, the early degeneration or apoptosis of some micromeres take place. The micromeres seem contain a spherical nucleus with a pycnotic type that has highly condensed electron dense karyoplasm containing large heterochromatin islands at its periphery (Fig. 3a) also large electron-dense regions of focal cytoplasmic degradation appear in the their thin layer of cytoplasm (Fig. 3b).

Some late mature eggs; have a well-developed egg shell under which a cellular material mass was observed between the egg shell and the embryo. This mass was observed as an incompletely envelop enclosed the embryo especially at the opercular region. This envelop contains a cytoplasm in which mitochondria, Golgi complex and other organelles (Figs. 3c, g).

The mature embryo (miracidium) enclosed in the intrauterine eggs show the presence of almost some of the larval organelles and cells; the body wall of the pyriform mature embryo (miracidium) is composed of ciliated epithelium. The anterior most end of the miracidium appeared elongated forming an anterior pointed cone like part directed towards the opercular pole beneath of the eggshell (Fig. 3c).

Within these ciliated larvae; near the anterior end; characteristic secretory granules with different sizes were observed. The granules appeared have oval and spherical shapes (Fig. 3d). These granules have different electron density, as secretion of larval glands. The large nuclei of these glands show numerous irregularly shaped electron-dense islands of heterochromatin. Their granular cytoplasm, rich in numerous glycogen particles, lamellar endoplasmic reticulum and large elongated mitochondria (Figs. 3d, e).

Numerous fully developed cilia are easily visible around the differentiated embryo (miracidium) of the advanced stages (Figs. 3c-g). The cilia (Fig. 3f), have a typical 9+2 (nine peripheral and two central) arrangement of microtubules with long striated ciliary rootlets (Fig. 3e) penetrating deep into the cytoplasm of the epithelial layer.

The germinative cells of miracidia are easily appeared and localized in the medioposterior parts of their ciliated larvae. They characterized by having large nuclei which occupy nearly one half of each cyton, and by the numerous electron-dense

heterochromatin islands accompanied by their nucleoli of different sizes and electron density (Fig. 3g).

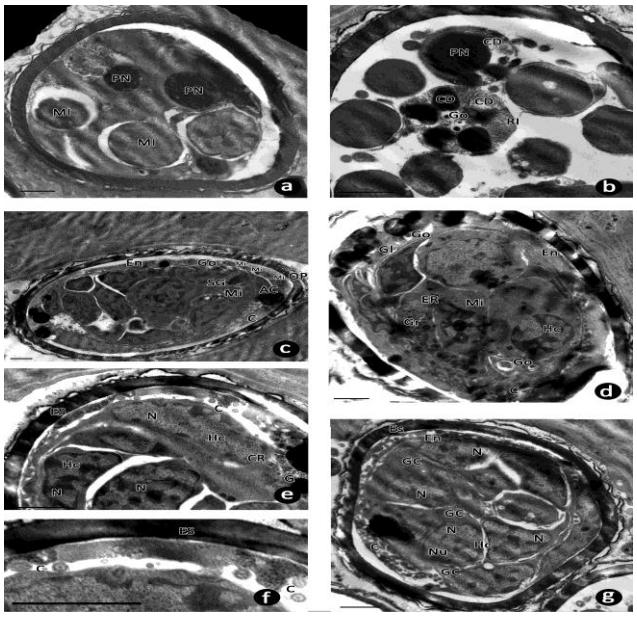

Fig. 3: TEM micrographs of ultrathin cross- and/or oblique sections of distal parts of the uterus of *A. (a.) aswaninesis.*

a. Posterior region of uterus shows microomere (MI), pycnotic nucleus (PN) bar= 2µ.

b. High magnification shows microomere (MI), pycnotic nucleus (PN), cytoplasmic degradation (CD) bar= 2µ.

c. Posterior region of uterus containing late mature egg shows; envelop (En), mitochondria (Mi), Golgi complex (Go), apical cone (AC), cilia (C), operculum (Op) bar= 2µ.

d. Posterior region of uterus containing late mature egg shows; mitochondria (Mi), Golgi complex (Go), endoplasmic reticulum (ER), granules (Gr), glycogen (G) heterochromatin (Hc), cilia (C) bar= 2µ.

e. Posterior region of uterus with late mature egg containing meracidium; egg shell (ES), glycogen (G), glandular nucleus (N), heterochromatin (Hc), cilia (C), cilliary rootlets (CR) bar= 2µ.

f. High magnification shows enlarged cilia (C), egg shell (ES) bar= 2µ.

The unembryonated oligolecithal egg of *A.* (*a*.) *aswaninesis* is contained a fertilized oocyte and few vitelline cells. This result was resembled with numerous previously studies on digenean species (Swiderski *et al*. 2013a,Taeleb and Gamil 2015) on other hand it is not resembled with other like in a cestode, *Didymobothrium rudol phii* (Swiderski *et al*. 2010 b). Vitelline cells contribute for eggshell formation and possibly serve a minor yolk function (Irwin and Threadgold 1972, Smyth and Halton 1983, Jones *et al*. 2008) while (Schmidt 1998) revealed that vitellocytes enclosed in the eggs provide nutrients for the embryo and the glycan vesicles retain until late embryogenesis. The present study revealed that; vitellocytes composed of clusters of shell globule, numerous lipid droplets and a few amount of glycogen particles that represent the nutritive reserves for the developing embryos, this is similar to various reports of digenean species *Maritrema feliui* (Swiderski et al 2013 and *A.a. aswaninesis* (Taeleb and Lashein, 2013), for cestode *Wenyonia virilis* (Młocicki *et al*. 2010b) and Aspidogasterians *Aspidogaster limacoides* (Swiderski *et al*. 2011, 2012).

DISCUSSION

In trematodes, eggshell formation results from the combined action of Mehlis' gland secretion and shell globules from the vitellocytes (Swiderski *et al*. 2013a). It has been assumed that Mehlis' gland secretion is responsible for the release of shell globules from the vitellocytes; in addition, it forms a primary membrane which later becomes reinforced by the merging of vitelline cell globules during the passage of newly formed eggs through the ootype (Irwin and Threadgold, 1972, Burton, 1963, Swiderski *et al*. 2004). The secretion of Mehlis' gland also serves to both the ootype and the luminal surface of the uterine wall and takes part in shell tanning and/or hardening as the eggs pass through the uterus (Swiderski, *et al*. 2011). The present results revealed that the egg shell of *A. a.aswaninesis* appeared compact and thick , this is resemble to different trematodes like *Orientocreadium batrachoides* (Taeleb & Gamil, 2015) and *Aspidogaster limacoides* (Swiderski *et al*. 2011) where they reported that ,the egg shell consists of compact ,thick and tanned protein.

In the present study through the progressive egg developmental stages of *A. a. aswaninesis* the embryo was rapidly increased in size giving rise a large number of different developmental blastomeres which were showed in three types; macromeres, mesomeres and micromeres. This result is similar to that reported previously in *Maritrema feliui* (Swiderski *et al*. 2013a) and in *Orientocreadium batrachoides* (Taeleb and Gamil, 2015).

The present study revealed the absence of pores and canals in the operculated egg shell in *A.* (*a.*) *aswaninesis* that is similar to the basic patterns of embryogenesis of different described digeneans (Swiderski *et al*. 2011, Taeleb and Gamil 2015, Born-Torrijos *et al*. 2017), while dissimilar to schistosomes where the egg shell is shown to be richly porous (Swiderski 1994a, Ashton 2001, Jones *et al*. 2008). The prescenc or absence of pores in shell may be therefore correlate with the diverging life cycle strategies of the two majour digenean groups (Khampoosa *et al*. 2012). Although the impermeable outer membrane of the inner envelope may be changed into a porous membrane by digestion of granules packed in the pores (Sakamoto 1981). Our opinion was in agreement with those reported previously by Born-Torrijos *et al*. 2017 who reported that, in case of the egg shell impermeability the developing embryos completely isolated from the supplies of the nutritive substances by the uterine wall and the miracidium's dependency on glycogen nutritive reserves contained in vitellocytes in early embryos. Also authors concluded that ultrastructural data (like lack of pores in eggshell) have to be interpreted in relation to parasite biology to understand the structural requirements of specific parasite strategies.

In the present study the vitelline syncytium of early embryonated eggs (their embryos composed of several blastomeres) of *A. (a.) aswaninesis* was degenerated and gradually disappeared; this is similar to the results of (Swiderski *et al*. 2010b) who report that the cytoplasm of the degenerating vitellocytes exhibits the presence of so-called ‘foci of cytoplasmic degradation’, which appear to be involved in the autolytic process of the vitellocyte cell components and inclusions, such as a high accumulation of lipids and glycogen. This progressive degeneration of the vitellocytes, considered as an example of programmed cell death or apoptosis. Our opinion is agreed with his conclusion that likely contributes towards the resorption of nutritive reserves by the developing embryo.

In *A. a.aswaninesis* the cytoplasmic processes which developed from the blastomeres and fused forming a common syncytial layer of embryonic envelope were situated just beneath the egg shell. These processes were similar to those of

Maritrema feliui and *Orientocreadium batrachoides* (Swiderski *et al.* 2013a, Taeleb and Gamil, 2015). Swiderski *et al*. 2017,discussed that, the functional ultrastructure and cytochemistry of egg protecting envelopes in taeniid cestodes may contribute to discovering more effective ovicidal substances against extremely resistance parasite eggs that so far are resistant to tested drugs and physicochemical factors. In *Aspidogaster limacoide* (Swiderski *et al*. 2011) as in all other aspidogastreans examined (Rohde 2001) only a characteristic single-layered embryonic envelope in a close contact with the egg shell has been described. On the other hand two embryonic envelopes were reported for the majority of digeneans and eucestodes examined previously (Swiderski 1994a, Swiderski *et al*. 2010a, b 2017 Mlocicki *et al.* 2005). Moreover these embryonic envelopes undergo further differentiation into secondary envelopes (Egg-envelopes) which surround the fully developed larva of both groups (Swiderski1994 a, b, c).

The present observations revealed degeneration and apoptosis was visible at early stages of developmental embryos in several micromeres of *A.* (a.) aswaninesis; this was showed as pycnotic nuclei and dense bodies may represent the remnants of the autolysed nuclei and cytoplasm of micromeres undergoing apoptosis. This is similar to the common feature for lower cestodes (Świderski 1994b, Świderski and Mackiewicz 2004, Młocicki *et al.* 2010 a, b), higher cestodes (Conn and Świderski 2008), aspidogastreans (Świderski *et al*. 2011, 2012) and digeneans (Świderski 1984, 1985, 1994 a, c, Świderski *et al*. 2013a, b, 2014 and Taeleb, and Gamil, 2015). Swiderski *et al*. 2013a reported that micromeres degeneration or apoptosis show that the embryonic development of *Maritrema feliui* starts in utero and represents an example of early stage ovoviviparity. After autolysis and re-absorption, appear to represent an important source of nutritive reserves for the embryo. Our opinion is agreed with this conclusion.

Our TEM observations confirm the development of ciliated miracidial larvae within the intrauterine eggs of *A. (a.) aswaninesis.* The results showed that the intrauterine eggs contain the larval miracidia with some larval organelles and cell types characteristic for the mature larvae. The egg shell enclosed miracidia composed of an anucleate layer of peripheral cytoplasm covered by a great number of cilia with long striated ciliary rootlets deeply embedded in the tegumental cytoplasm. The ciliated tegument of the larvae of *A.* (a.) *aswaninesis* generally resembles that of other digenean miracidial tegument as that reported in (Świderski *et al*. 2013b, 2014) for *Brandesia turgida*.

The germinative cells of digenean miracidia are easily recognized by their large nuclei which occupy nearly one half of each cyton, numerous electron-dense heterochromatin islands, nucleoli of different sizes and electron density in different species (Świderski *et al*. 2014). In *A.* (a.) *aswaninesis* there are germinative cells localized in the medioposterior parts of their ciliated larvae; their nuclei have numerous electron-dense heterochromatin islands and nucleoli of different sizes, these results are similar to those described in *Brandesia turgida* (Świderski *et al.* 2013b, 2014), *Schistosoma mansoni* (Swiderski 1984, 1985) and *Schistosoma haematobium* (Świderski *et al*. 1980; Eklu- Natey *et al*. 1982a, b). Both nuclear and cytoplasmic features observed in the ultrastructure of germinative cells within the miracidium may indicate their great developmental potential for further growth and multiplication. It is evident that they play an important role in digenean ontogenesis, as by definition they are involved in a growth and differentiation of the sporocyst, the following stage of the parasite life cycle (Świderski *et al*.2014).

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ARABIC SUMMARY

دراسة بالميكرسكوب الإلكتروني النافذ للتطور الجنيني في بيض داخل رحم طفيل
***Acanthostomum* (*atrophocaecum*) *aswaninesis* Wannas, 1977**
(Digenea, Acanthostomatidae) مع وجود دليل على تطور الميراسيديوم المبكر

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في هذه الدراسة تم فحص التطور الجنيني داخل الرحم لبيض أكانثوستوموم (أتروفوسيكم) أسوانينيسيس بواسطة المجهر الإلكتروني النافذ. كشف البيض المراحل المتتالية من التطور الجنيني. يحتوي الرحم القريب على بيض غير مدمج وبيض مع أجنة مبكرة بينما كانت المناطق البعيدة من الرحم مشغولة ببيض يحتوي على أجنة متطورة تمامًا ، وكان كل منها مكونًا من بويضات مخصبة وعدد قليل من خلايا المح ؛ التي تم اندماجها لتشكل المخليت المحيِّية التي تميزت البويضات الجنينية المبكرة بوجودها. مع نمو البويضة تصبح قشرة البيضة أكثر سماكة بشكل تدريجي مع ظهور الطبقة الزجاجية بعد تشكيل القشرة عند القطب الأضيق لسطح قشرة البيضة. تتشكل ثلاثة أنواع من الخلايا البلاستولية المتمايزة نتيجة للانقسامات المستمرة ؛ macromeres ، mesomeres و micromeres ، مع وجود نوى مختلفة الشكل والحجم تشغل أماكن مختلفة داخل الجنين. نتيجة للانقسامات المتتالية يحدث تنكس مبكر أو موت الخلايا المبرمج في بعض الميكروميرات. يمكن رؤية أهداب عديدة متطورة تمامًا حول الجنين المتمايز (الميراسيديوم) في المراحل المتقدمة. والأهداب لديها ٩ + ٢ (تسعة طرفية واثنين مركزية) من الأنابيب الدقيقة. من السهل ظهور الخلايا الإنباتية للميراسيديا في الأجزاء المتوسطة من يرقاتها الهدبية. وقد تم مقارنة نتائج هذه الدراسة مع تلك التي أبلغ عنها سابقا للديدان المفلطحة الطفيلية.