

Ultrastructural studies on egg envelopes surrounding the miracidia of *Mediogonimus jourdanei* Mas-Coma et Rocamora, 1978 (Digenea, Microphalloidea, Prosthogonimidae)

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Abstract

The intrauterine, mature and fully embryonated eggs of the prosthogonimid trematode *Mediogonimus jourdanei* Mas-Coma et Rocamora, 1978 were examined by means of transmission electron microscopy (TEM), using high pressure freezing, freeze substitution and infiltration with resin techniques. Each embryonated egg is composed of a miracidium surrounded by three envelopes: (1) the egg shell, (2) the outer and (3) inner envelopes. Egg envelopes play an important role in the protection, metabolism, storage of nutritive reserves and the general biology of the *M. jourdanei* egg. The inner envelope is characterised by large, flattened nuclei, and its syncytial cytoplasm contains a heavy accumulation of glycogen, lipid droplets, mitochondria and large vesicles. These traits indicate that this layer has the features of a metabolically-active syncytial layer with an energy storage capability. In the infective eggs observed before the hatching of the miracidium, areas of so-called “focal cytoplasmic degradation” were frequently observed, which may be involved in the autolytic process of all components of this envelopes.

Keywords

Trematoda, Prosthogonimidae, *Mediogonimus jourdanei*, egg envelopes, ultrastructure

Introduction

Little information is available on the differentiation and transmission electron microscopy (TEM) of egg envelopes in digenean trematodes. There are several scanning electron microscopy (SEM) studies on the surface structure of trematode eggs, but 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. Almost all of these TEM studies have been done on three species of schistosomes of medical and veterinary importance: namely on *Schistosoma mansoni*, *S. haematobium* (Świderski *et al.* 1980; Eklun-Natey *et al.* 1982a, b; Świderski 1984, 1985, 1988, 1993, 1994); *S. mattheei* (Świderski 1986) and *S. japonicum* (Jones *et al.* 2008).

The Prosthogonimidae represents a family of microphaloid trematodes (Jones 2008) found essentially in birds, but some of its few genera and species are known from mammals. The anatomy of individuals of this family members is unremarkable, except that typically the genital pore is placed close to the oral sucker. This family is particularly interesting because its members may be located in a wide variety of unusual sites, such as: (1) the intestine, cloaca, bursa Fabricii, oviduct and under the nictitating membrane in birds; and (2) the intestine, peritoneal cavity and liver in mammals (Cribb and Spratt 1990, Jones 2008).

The aim of the present study is to describe the ultrastructure of egg envelopes in the trematode *Mediogonimus jourdanei* Mas-Coma et Rocamora, 1978 (Digenea, Microphalloidea, Prosthogonimidae), a parasite of naturally infected bank

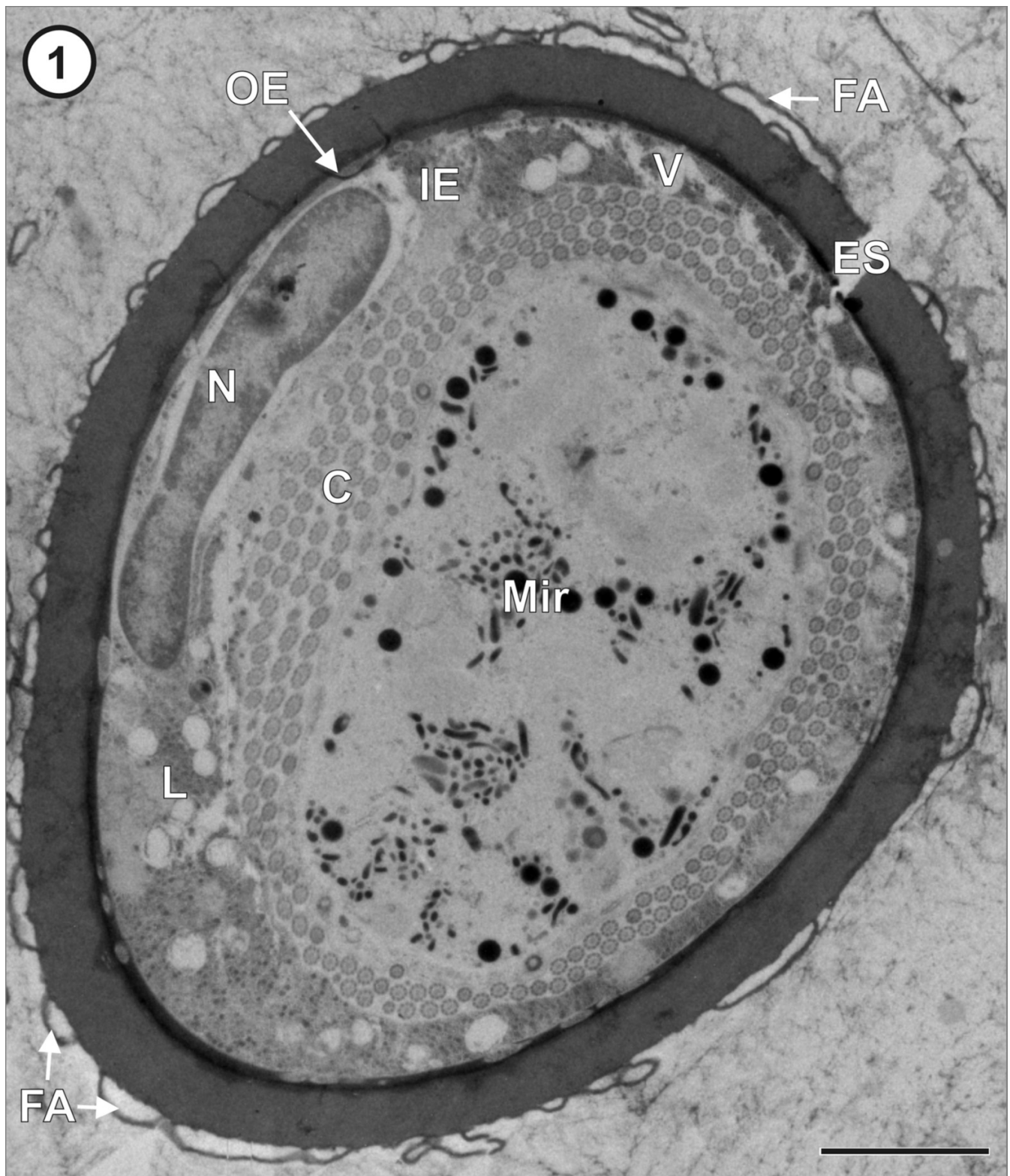


Fig. 1. The general topography of the mature egg of *M. jourdanei*. Note: (a) peeling of the outer surface of the egg shell, resulting from fixation artefacts; (b) flattened nucleus of the mesomere and numerous spherical lipid droplets in the inner envelope cytoplasm; and (c) a great number of cilia which occupy all the space between the egg envelopes and miracidium. Bar = 2 μ m. **Abbreviations to all figures:** Alpha-gl and Beta-gl – two types of glycogen, AFC – areas of focal cytoplasmic degradation, C – cilia, ES – egg shell, FA – fixation artefacts, L – lipid droplets, Mi – mitochondria, Mir – miracidium, N – nucleus, NP – nuclear pores, OE – outer envelope, IE – inner envelope, V – vesicles

voles, *Myodes glareolus* (Schreber, 1780), captured in the French Pyrenees. In order to avoid the above-mentioned technical difficulties with fixation and tissue penetration, currently recommended methods of fixation by means of freeze substitution and tissue infiltration with resin were used in this study. It allows a better preservation of proteins and some ultrastructural details, producing much fewer artefacts than associated with conventional techniques of material preparation for TEM examination.

Materials and methods

Materials

Naturally infected bank voles, *Myodes glareolus*, were captured in the Nature Reserve of Py (Pyrenean Mountains, France) during June, 2009. Live mature specimens of *Mediogonimus jourdanei* Mas-Coma et Rocamora, 1978 were col-

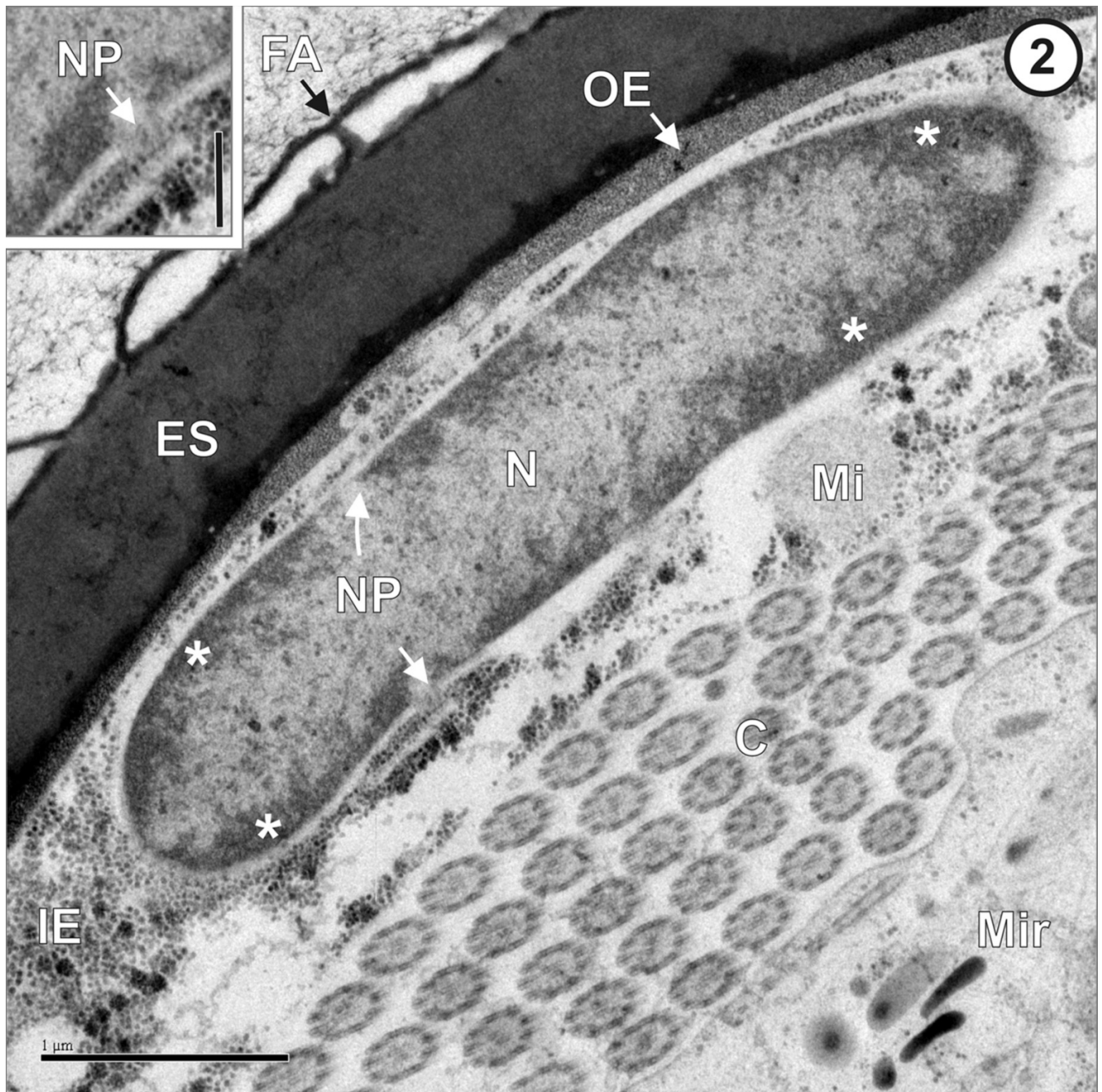


Fig. 2. Details of three egg envelopes with the nucleus and perinuclear region of the inner envelope. Note: (a) a thick electron-dense egg shell; (b) a thin, granular and almost continuous layer of the outer envelope; and (c) the inner envelope with an elongated, flattened nucleus of the mesomere, with several heterochromatin islands (stars) and nuclear pores marked by arrows and enlarged on the inset. Bar = 1 µm; inset bar = 0.2 µm

lected from the liver upon necropsy and dissection of voles at the laboratory of “Serveis Científics i Tècnics” of the University of Barcelona.

High pressure freezing

Live digeneans were cut open and pieces of uterus were selected in small Petri dishes under a stereomicroscope in PBS with 20% BSA. The sections of uterus were transferred to the

cavity of a 200 μm -deep flat specimen carrier. The specimen holder was then inserted into the rapid transfer system, high-pressure frozen using a Leica EM PACT and stored in liquid nitrogen.

Freeze substitution and infiltration with resin

For freeze substitution, sample holders were transferred to pre-cooled cryovials (-120°C) and freeze substitution was per-

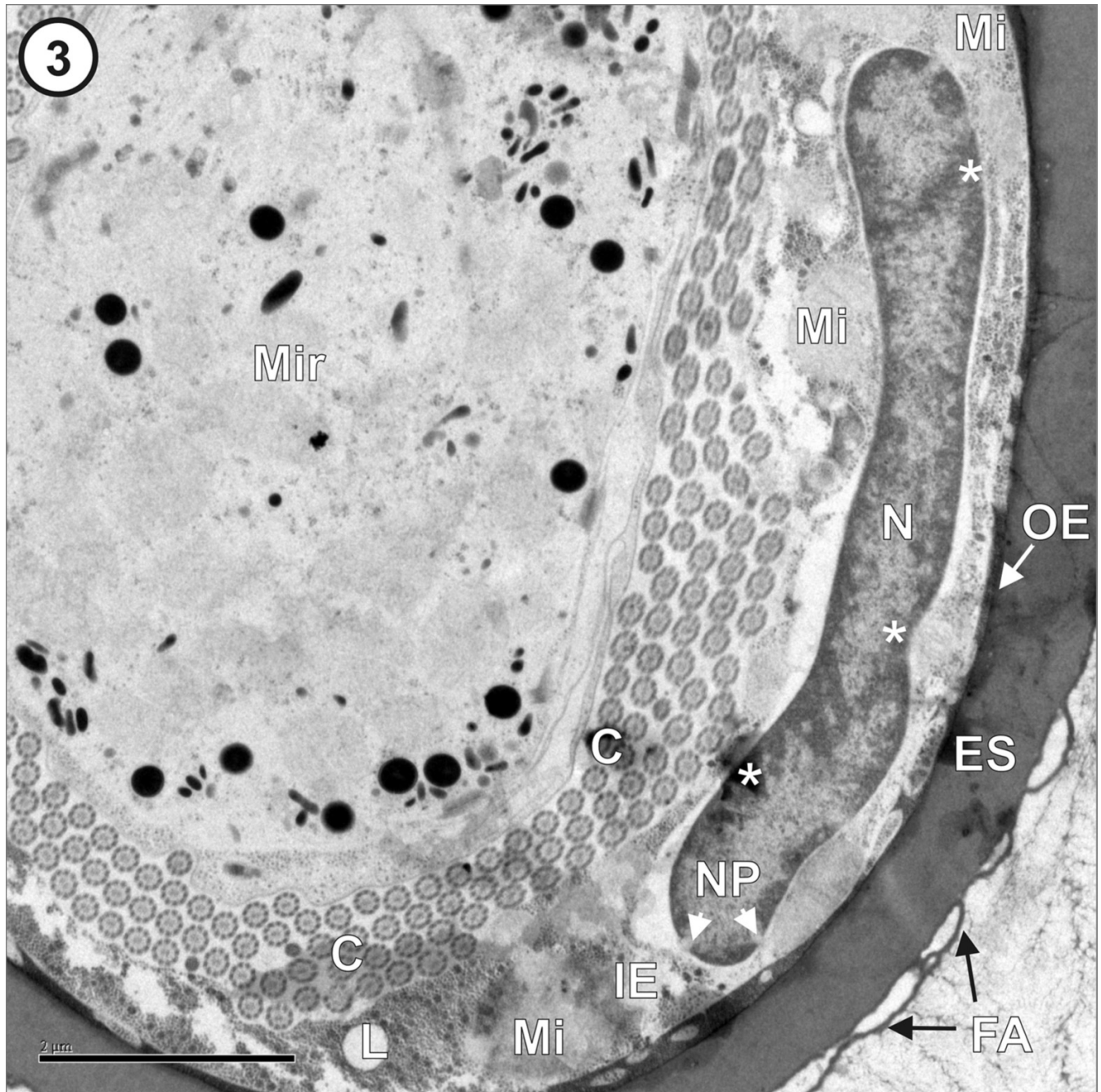


Fig. 3. Part of the egg showing a more flattened and elongated nucleus of the mesomere, with several heterochromatin islands (stars) and nuclear pores. Note several, large mitochondria and lipid droplets in the cytoplasm of this envelope. Bar = 2 μm

formed in anhydrous acetone containing 2% osmium tetroxide. Using a Leica EM AFS, samples were maintained for 24 h at -90°C . Hereafter, the temperature was raised at a rate of 2°C/h to -60°C and then to -30°C . Samples were kept at each level for 9 h in the original substitution medium. Specimens were then washed three times for 10 min in fresh anhydrous acetone. After the washes, the temperature was gradually raised to room temperature and the specimens were infiltrated with Spurr resin (one part resin/three parts acetone) overnight; 1:1 for 4 h; 3:1 for 4 h and 100% resin for 4 h and then overnight. Polymerization was carried out by heat at 60°C for 72 h. Ultrathin sections were cut using a Reichert-Jung Ultracut E ultramicrotome, placed on copper grids and post-stained with uranyl acetate (2%) in methanol for 5 min and lead citrate for 4 min. Finally, ultrathin sections were examined using a JEOL 1010 transmission electron microscope operated at an accelerating voltage of 80 kV.

Results

General topography of mature infective eggs

The life cycle of *M. jourdanei* remains unknown, but the embryonated eggs are certainly passed into an aquatic environment, each containing a fully-developed ciliated larva, the miracidium.

The morphology of the eggs is very important for the correct identification, but it is difficult to characterise precisely their structural details at the light microscope level as eggs of this species are very small. Eggs have a smooth, hard shell that is transparent and yellow-brown, and a more conventional, ovoid, but somewhat elongated shape. All mature eggs are of about the same size, usually measuring $30\text{--}37.5\text{ }\mu\text{m}$ in length and $12\text{--}15\text{ }\mu\text{m}$ in width, with an average size of $33.5 \times 14\text{ }\mu\text{m}$. The egg also has a very slight opercular shoulder,

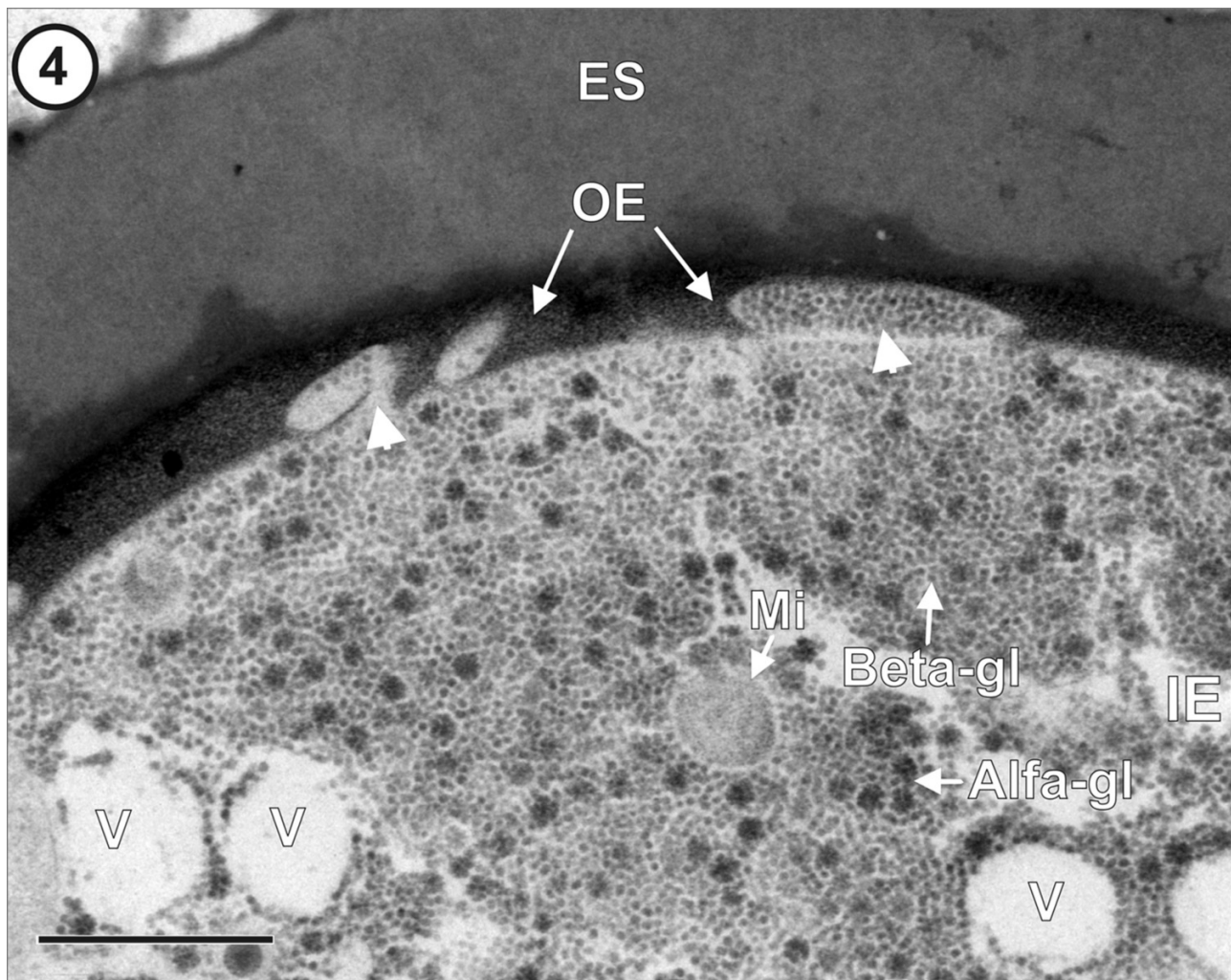


Fig. 4. High power TEM-micrograph showing details of the thick, homogeneously dense egg-shell, the border line between the outer and inner envelopes with characteristic pockets (arrow heads) of the inner envelope cytoplasm embedded in a very dense, granular cytoplasm of the outer envelope. In the cytoplasm of the inner envelope note: (a) heavy accumulation of glycogen in the form of alpha-glycogen rosettes and single beta-glycogen particles; (b) numerous large, electron-lucent vesicles; and (c) several mitochondria. Bar = $0.5\text{ }\mu\text{m}$

marking the line of cleavage between the shell and operculum or “escape hatch” for the miracidium.

Each embryonated egg is composed of a miracidium with an average length of 27 μm and width of about 10 μm and is surrounded by three main egg envelopes: (1) the smooth, hard egg-shell, (2) the remnants of the granular layer of an outer envelope, and (3) the thick layer of the inner syncytial envelope. Details of their ultrastructural characteristics are pre-

sented below, whereas their functional ultrastructure is analysed in the “Discussion” and compared with similar data described for other digeneans.

Ultrastructural characteristic of the egg envelopes

The mature eggs appear ovoid or slightly elongated in ultrathin cross- and/or oblique sections (Fig. 1). The thickness of the

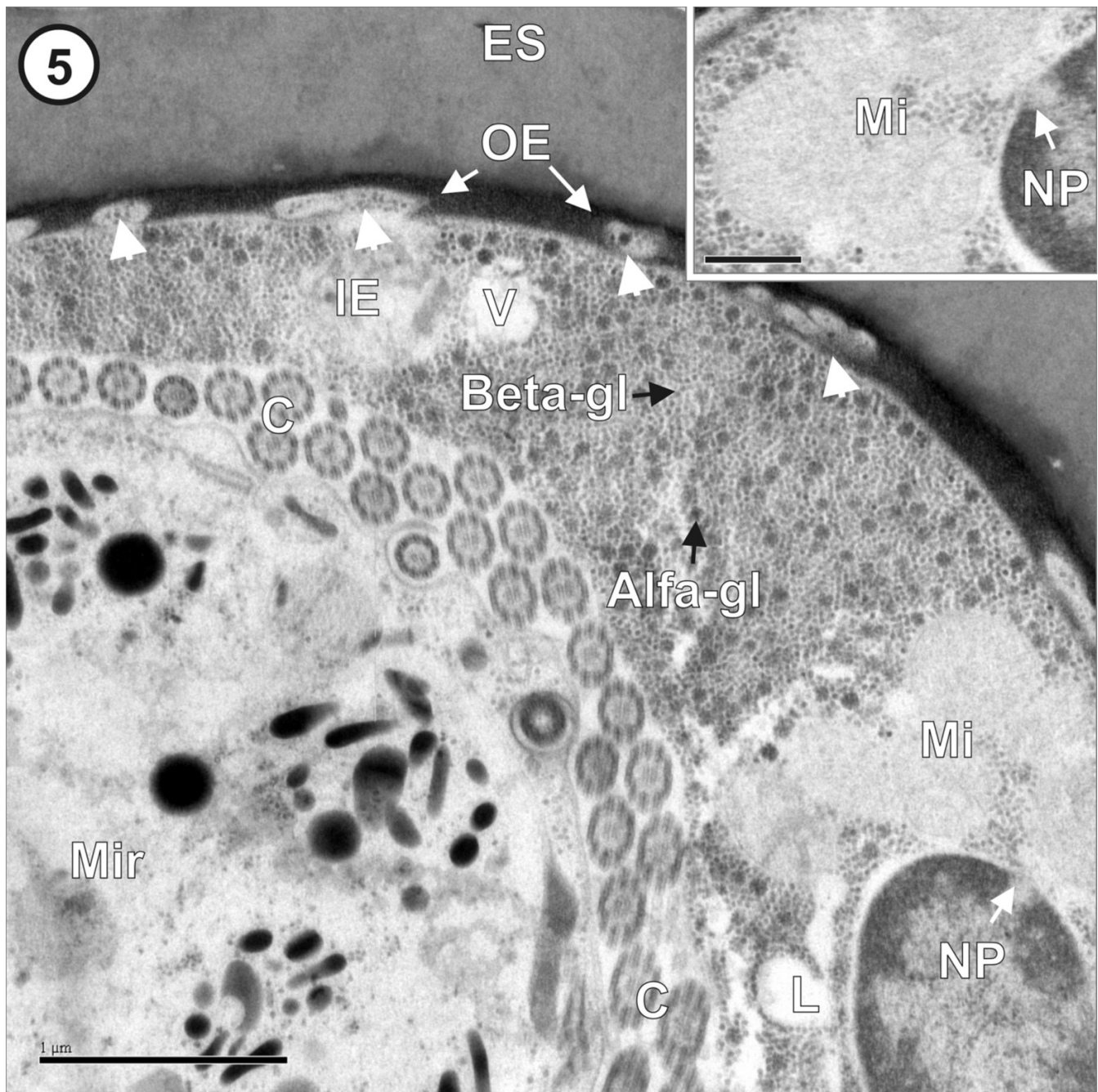


Fig. 5. Perinuclear cytoplasm of the inner envelope showing several large mitochondria, heavy accumulation of alpha-glycogen rosettes and beta-glycogen particles, and numerous pockets (arrow heads) of the inner envelope cytoplasm embedded in a very dense, granular cytoplasm of the outer envelope. Inset: enlarged detail of mitochondria closely adjacent to the nuclear envelope with a nuclear pore. Bar = 1 μm ; inset bar = 0.25 μm

egg-shell and the two envelopes depends to a great extent on the orientation of the egg and level of the section through the egg (Figs 1–3).

Egg-shell

The normally smooth surfaced egg-shell of this species has fixation artefacts, caused by high pressure freezing visible as peel-

ing of a very thin layer of its entire outer surface (Figs 1–3). Deeper parts of the hard, electron-dense and homogeneous egg-shell are always well preserved and remain intact (Figs 1–6).

Outer envelope

The outer envelope degenerates very early and practically disappears in the mature eggs (Figs 1–6). It is only seldom vis-

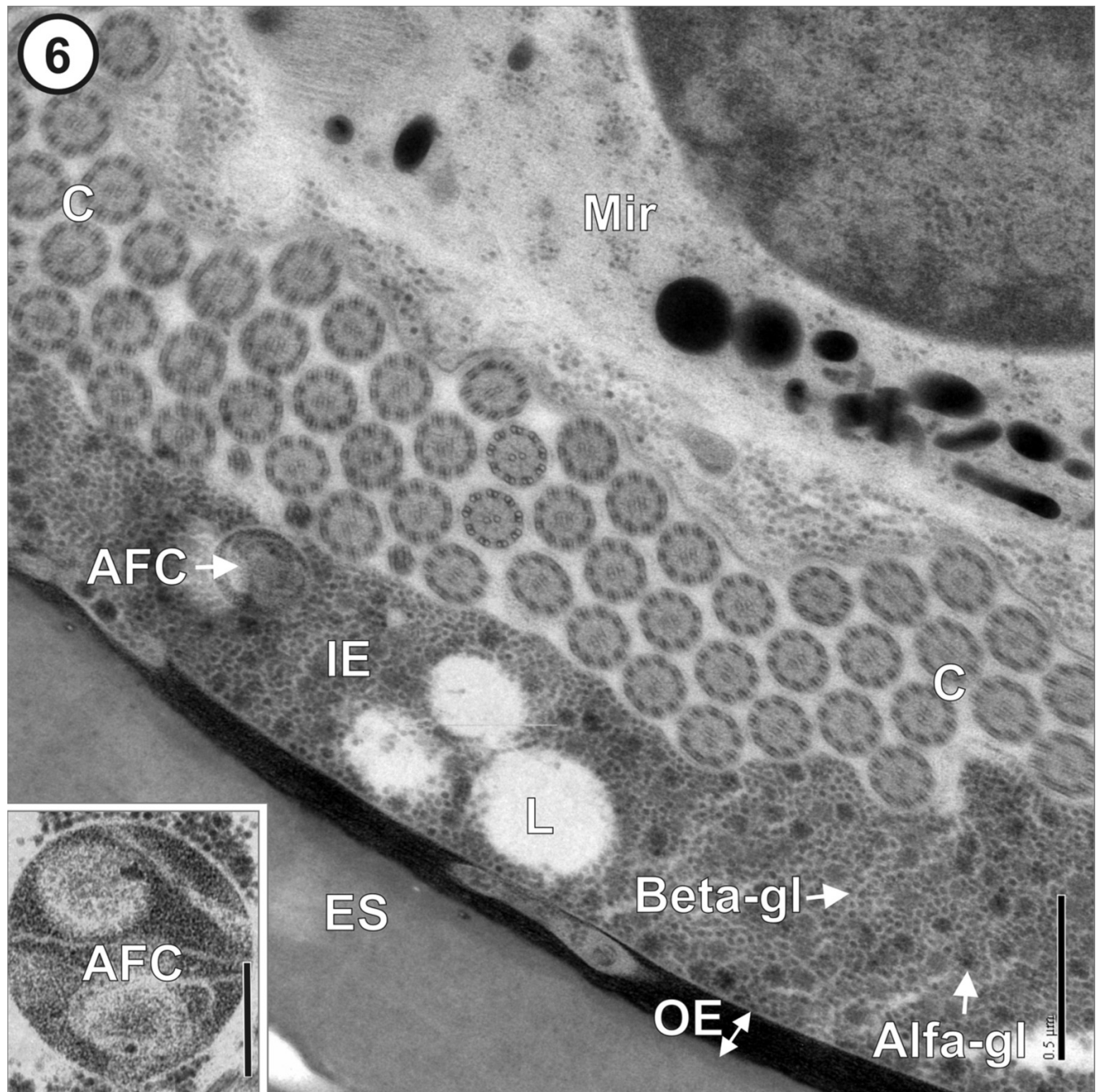


Fig. 6. Part of the mature, infective egg prior to the hatching of the miracidium. Note: (a) evident differences in the ultrastructure of three egg envelopes and their demarcation lines; (b) the presence of the so-called “areas of focal cytoplasmic degradation” and a few lipid droplets; and (c) apparent reduction in the volume of the glycogen and lipid accumulation in the inner envelope cytoplasm observed before hatching. Bar = 0.5 μ m. Inset: high magnification of the so-called “areas of focal cytoplasmic degradation”. Inset bar = 0.2 μ m

ible as a continuous granular layer (Figs 2–6). The compact granular and highly electron-dense residual layer of the outer envelope has numerous flattened pockets of the inner envelope which penetrate deep within the outer envelope. Small areas of electron lucent granular cytoplasm (Figs 4–6) are present at the border line between the outer and the inner envelopes (Figs 4–6).

Inner envelope

A thick syncytial layer of cytoplasm of the inner envelope contains two or three flattened nuclei of mesomeres which took part in formation of this structure (Figs 1–3). Much elongated nuclei of this envelope contain, in their granular nucleoplasm, numerous heterochromatin islands adjacent to the nuclear envelope, which frequently exhibit the presence of large nuclear pores (Figs 2 and inset, 3 and 5).

The syncytial cytoplasm, rich in free ribosomes and polysomes, contains a large amount of glycogen accumulated in this layer in the form of α -glycogen rosettes and individual β -glycogen particles (Figs 4–6). In addition, it contains also several mitochondria, saturated lipid droplets, large vesicles and structures resembling the so-called “areas of focal cytoplasmic degradation” (Figs 4–6 and inset). The lipid droplets observed in this layer appear very osmiophobic, showing a very low affinity for osmium, and therefore considered to be saturated lipids.

All spaces beneath the inner envelope are occupied by the cilia of the miracidial tegument (Figs 1–3, 5 and 6) that will be described in a separate paper on cellular organisation of *M. jourdanei* miracidia.

Discussion

The ultrastructure of egg envelopes of *M. jourdanei* resembles to great extent that described earlier in different species of schistosomes of medical and veterinary importance (Świdorski *et al.* 1980; Świdorski 1984, 1985, 1986, 1988, 1993, 1994; Jones *et al.* 2008). An important difference is a very early degeneration of the outer envelope in eggs of *M. jourdanei*, which disappears very rapidly, leaving only a very thin, frequently discontinuous layer or small debris of cytoplasmic remnants. In schistosome eggs, on the other hand, it remains in its residual form of a distinct, granular, membrane-delimited layer for much longer, until the hatching of the miracidium. Two other differences in egg-shells of *M. jourdanei* are: (a) lack of microspines on the egg-shell surface, (b) lack of pore canals piercing and perforating the egg shells; both of them remain characteristic for schistosome eggs, that are non-operculated.

Similarly, as described by Jones *et al.* (2008) for *S. japonicum*, in *M. jourdanei* eggs, small pockets of low electron-dense granular material of the inner envelope penetrate the very electron-dense material of the outer envelope at the border between these two layers.

The syncytial inner envelope in mature eggs of *M. jourdanei* is transformed from being metabolically active into a layer mainly adapted for food storage. Its cytoplasm is rich in ribosomes, alpha- and beta-glycogen and large lipid droplets which are associated with mitochondria. The lipid droplets demonstrate a low affinity for osmium, which may indicate that they represent saturated lipids. The elongated nuclei of the inner envelope contain numerous dense islands of heterochromatin, closely adjacent to the nuclear envelope that exhibits large nuclear pores. As indicated by the ultrastructure of the nuclei and cytoplasm, the inner envelope has features of a metabolically active layer with an energy storage capacity (glycogen, lipids).

The large areas of focal cytoplasmic degradation, which appear in this envelope before the hatching of the miracidium, resemble similar structures described in the eggs of *S. mansoni*, where their lysosomal activity was confirmed by a positive reaction for acid phosphatase (Świdorski 1994). Such areas of focal cytoplasmic degradation observed in *M. jourdanei* and schistosome eggs, which appear before the hatching of the miracidium, seem to be involved in the process of autolysis. It appears, therefore, that, as in schistosome eggs (Świdorski 1994), the structural components and food storage reserves of the inner envelope are reabsorbed by the miracidium prior to hatching.

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