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VERSITA

Utrastructural observations on *Goussia metchnikovi* (Laveran, 1897) in the spleen of gudgeon, *Gobio gobio* L.

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Abstract

The ultrastructure of gamonts and sporulated oocysts of *Goussia metchnikovi* in the spleen of gudgeon, *Gobio gobio* from the river Lee, England is described. In developing microgamonts, small amylopectin granules were grouped centrally and nuclei were often arranged peripherally, close to the surface membrane. Nuclear chromatin condensed into peripheral dense portions that became the nuclei of flagellated microgametes, released to the parasitophorous vacuole. The cytoplasm of macrogametes had larger, scattered amylopectin granules, lipid globules and small electron-dense bodies, but no obvious wall forming bodies; peripheral vesicular structures with the appearance of mitochondria were also present and the parasitophorous vacuole contained flocculent material, but was otherwise free of structures. Sporulated oocysts contained four sporocysts and oocyst walls appeared to consist of a single membrane. Sporocyst walls showed a dehiscence suture, characteristic of the genus *Goussia*, which had filamentous extensions in places. The sporocyst wall comprised a dense inner layer and a thin outer layer with a fuzzy coat, separated by an electron lucent layer. Groups of oocysts were encapsulated by fibrous layers and inflammatory cells, and many sporocysts and their contained sporozoites showed evidence of elimination by the host.

Keywords

Coccidia, *Goussia metchnikovi*, *Gobio gobio*, spleen, ultrastructure

Introduction

Several coccidia (Apicomplexa, Coccidia, Eimeriorina) are reported from gudgeon (*Gobio gobio* L.). These records include two found only in this fish host, namely *Eimeria molnari* Jastrzebski, 1982 and an *Eimeria* sp. Lukeš, 1994, both from the intestine. A further five species are known from both *G. gobio* and a variety of cyprinids. These species are: *Goussia alburni* Stankovitch, 1920 from adipose tissue surrounding the intestine; *Goussia carpelli* (Léger et Stankovitch, 1921) Dyková et Lom, 1983 from the intestine and possibly the gall-bladder; *Eimeria cheissini* Shul'man et Zaika, 1962 in the mesentery, walls of the intestine, the gall bladder and swim-bladder; *Goussia metchnikovi* (Laveran, 1897) Dyková et Lom, 1983 (syn. *Eimeria macroresidualis* Shul'man et Zaika, 1962) from the intestine, kidney, liver and spleen; and *Goussia siliculiformis* (Shul'man et Zaika, 1962) Dyková et Lom, 1981 from the intestine, kidneys, liver, swimbladder and possibly testes.

Goussia metchnikovi occurs in the spleen of *G. gobio* in South East (SE) England (Ball 1983, Davies and Ball 1993, Davies and Stewart 2000). Lom and Dyková (1992) also recorded it from the liver and kidney of *G. gobio* and in white-finned gudgeon, *Gobio albipinnatus* Lukasch, 1933 in Europe. Further observations are from Kessler's gudgeon, *Gobio kessleri* (Dybowski, 1862) and the Amur whitefin gudgeon, *Romanogobio tenuicorpus* (Mori, 1934), both quoted by Duszynski *et al*. (2000). Lom and Dyková (1992) reported merogony and gamogony of *G. metchnikovi* in splenic tissue and sporogony to be endogenous. An inflammatory host response culminates in encapsulation of oocyst clusters (Pellérdy and Molnár 1968, Lom and Dyková 1992, Davies and Ball 1993, Dyková and Lom 2007) and this occurs in summer and autumn (Pellérdy and Molnár 1968).

To date, the later stages of development, namely gamonts and sporulated oocysts, have been observed in wild-caught gudgeon in SE England, and these only in the spleen. Since existing reports of the ultrastructure of this parasite are lim-

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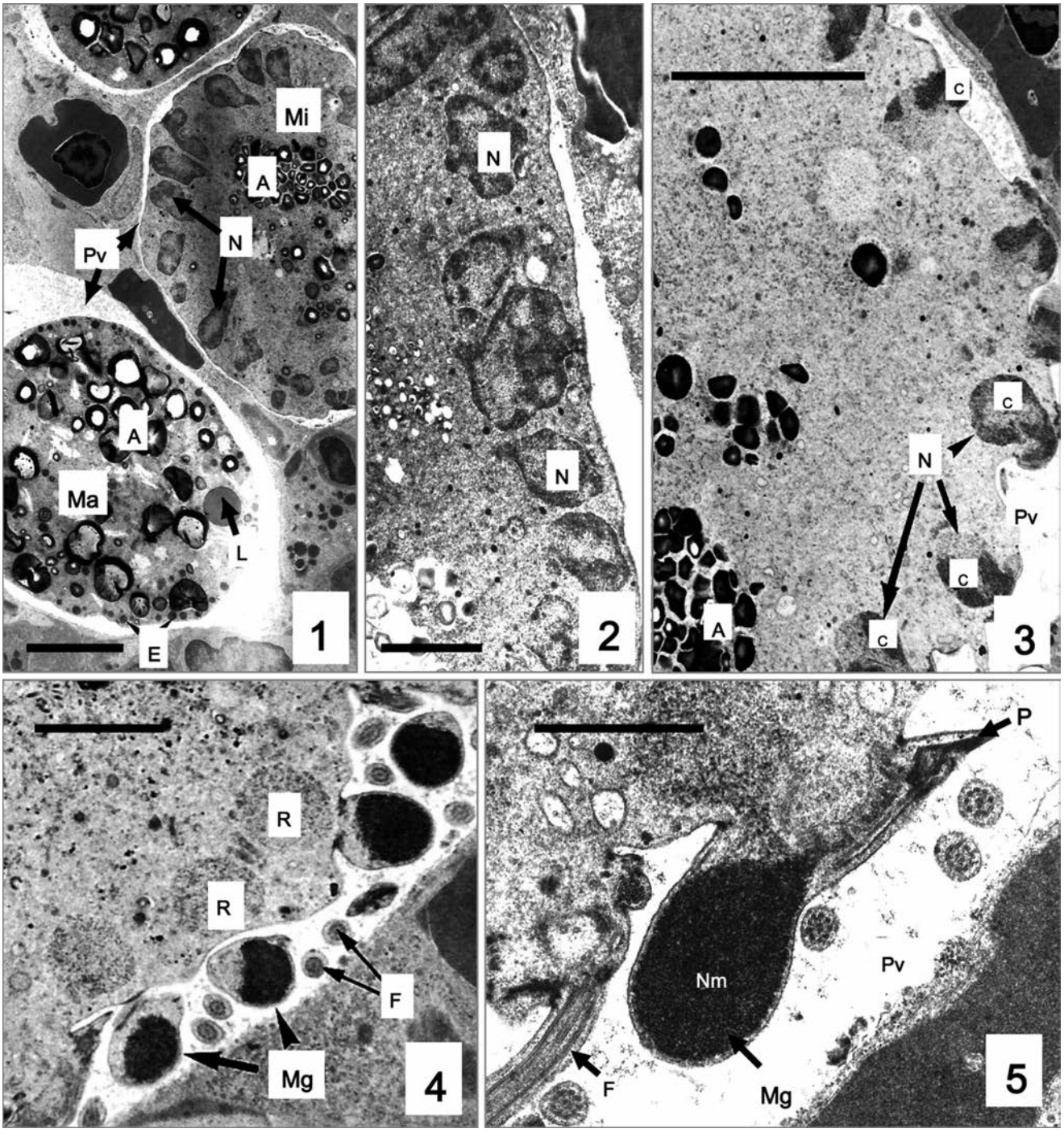
ited (Lom and Dyková 1992), we have investigated by transmission electron microscopy (TEM), the gamogonic stages, with some observations on the sporulated oocysts, sporocysts and the host tissue reaction, especially since the histopathology of coccidian infections in fishes is little understood (Gjurčević *et al*. 2008).



Figs 1–5. *Goussia metchnikovi* in the spleen of *Gobio gobio*. Ultrathin sections of gamogonic stages. **1.** Developing microgamont (Mi) with peripheral nuclei (N) and a cluster of small amylopectin granules (A). Developing macrogamete (Ma) with large amylopectin granules, lipid droplets (L) and small electron dense bodies (E). Pv is parasitophorous vacuole; scale bar = 5 μm. **2.** Nuclei of early microgamont; scale bar = 2 μm. **3.** Microgamont with nuclei beginning to protrude into the parasitophorous vacuole (Pv). Chromatin (c) condensing inside nuclei; scale bar = 5 μm. **4**. Transverse sections of microgametes (Mg) and flagella (F) free in the parasitophorous vacuole. R is residual nucleus; scale bar = 2 μm. **5.** Developing microgamete (Mg) showing nucleus (Nm), flagellum (F) and perforatorium (P); scale bar = 1 μm

Materials and methods

The spleen of 5/19 gudgeon collected in March from the river Lee in SE England were infected with *G. metchnikovi* as shown by macroscopic, whitish nodules and microscopically by the presence of gamonts and oocysts in wet preparations. Small portions of each infected spleen were fixed in 2.5% (v/v) glutaraldehyde in cacodylate buffer (pH 7.2), post fixed in 1% (w/v) osmium tetroxide and processed by standard electron microscope techniques (Pittilo and Ball 1979). Ultrathin sections were stained with uranyl acetate and lead citrate, and examined with a Philips 301 transmission electron microscope.

Results

All samples examined by TEM showed similar development stages. Microgamonts and macrogamonts occurred in groups within splenic tissue (Fig. 1) and their host cells appeared to be those of the connective tissues, although the specific identity of the cells was not obvious.

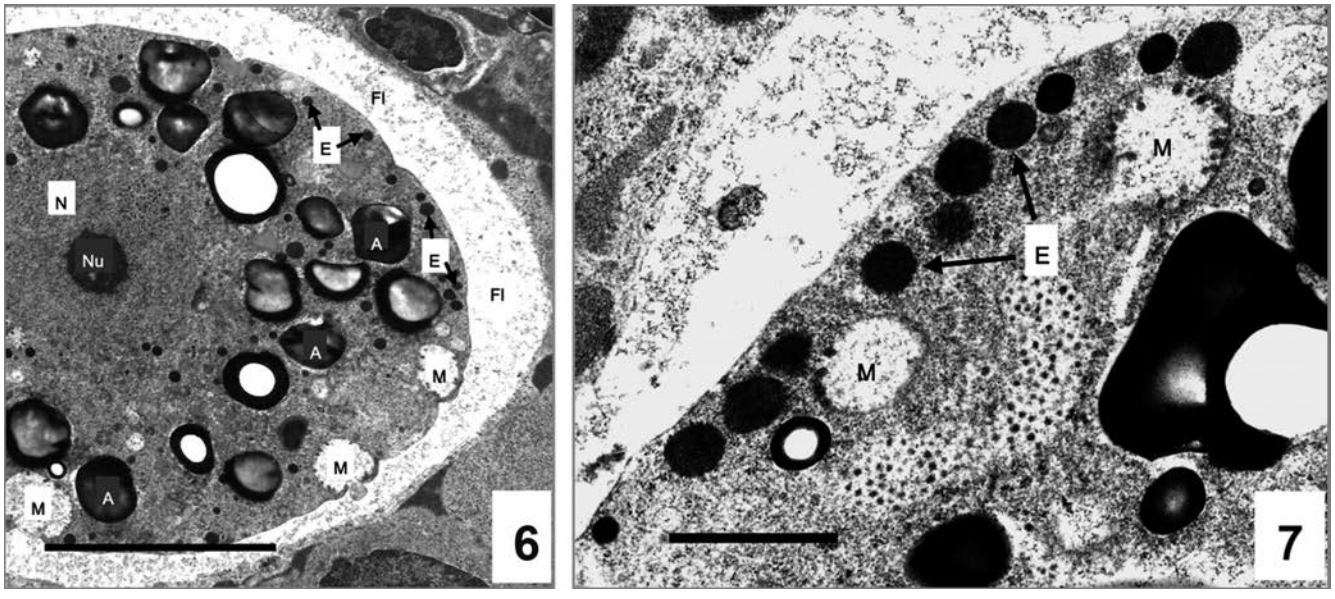
Microgamonts developed within a parasitophorous vacuole leaving a narrow margin between the parasite and host cell (Fig.1) and were rounded, irregularly oval, or somewhat elongate in shape. The earliest stages seen in TEM sections had nuclei that were randomly dispersed throughout the cytoplasm of the microgamont, or were located towards its periphery (Figs 1 and 2). Usually 20–30 nuclei, or occasionally more than this, were located in a single section through the microgamont. In these early stages, heterochromatin was concentrated near the nuclear envelope of each nucleus (Figs 1 and 2). As well as nuclei, numerous small dense amylopectin granules were prominent inclusions in the microgamont cytoplasm (Fig. 1).

With the onset of microgametogenesis, chromatin in the microgamont nuclei became condensed and surface invaginations around the nuclei indicated that they were beginning to protrude into the parasitophorous vacuole (Fig. 3). Cytoplasm at this stage still contained small amylopectin granules which remained grouped in clusters towards the centre of the microgamont compared with those in the macrogamonts which were less numerous, larger and dispersed (Fig. 1).

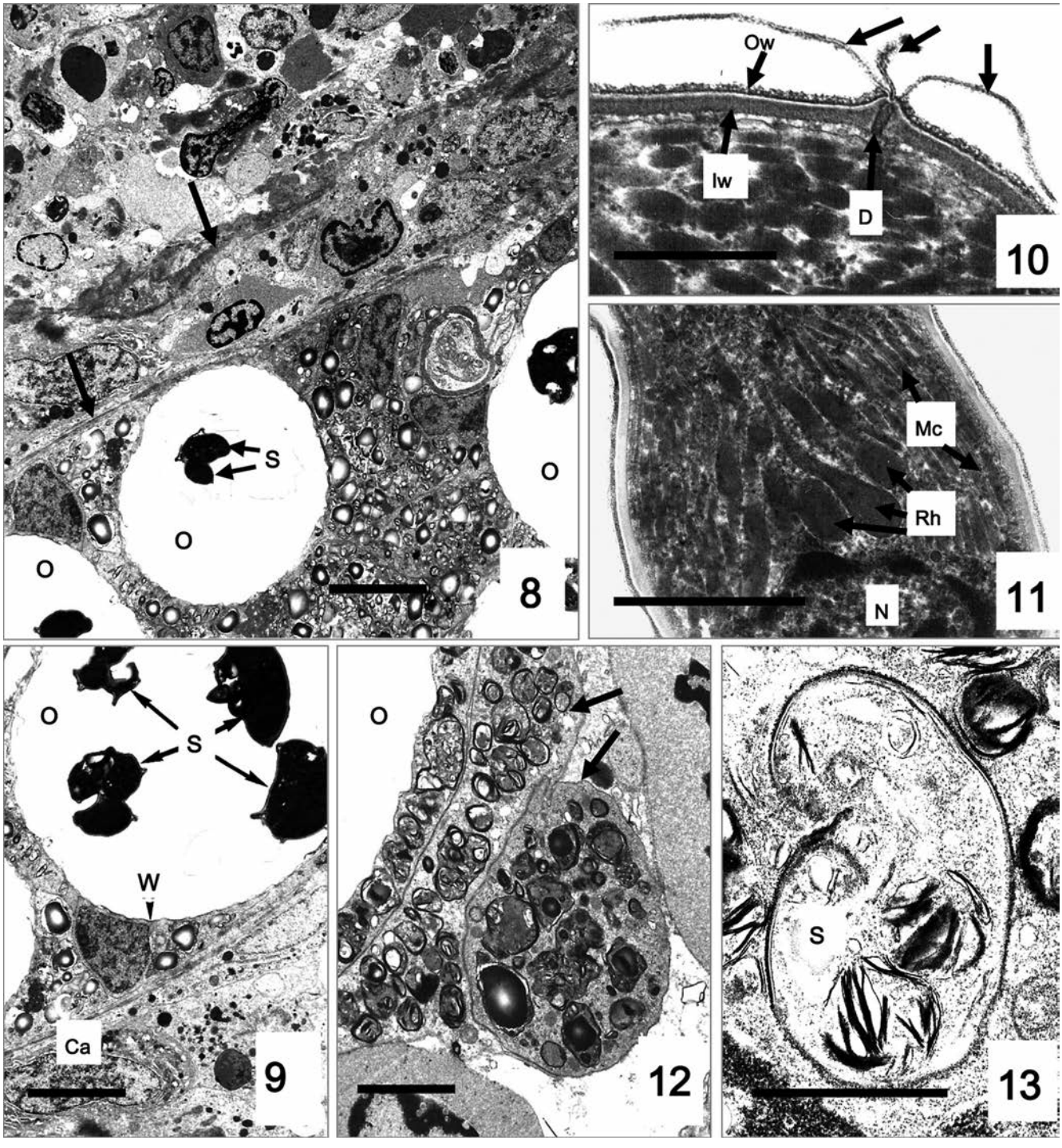
As development progressed, microgamete nuclei protruded more into the parasitophorous vacuole together with developing flagella (Figs 4 and 5). At this stage, each nucleus exhibited two distinct portions: the extruded, condensed section for the microgamete nucleus; and the inner, less electron-dense residual portion, retained in the cytoplasm of the microgamont (Fig. 4).

Mature microgametes were elongated, eventually detaching from the cytoplasm to lie free in the parasitophorous vacuole and appeared biflagellate, these structures arising close to the apical perforatorium (Fig. 5).

Unlike microgamonts, all macrogametes observed appeared to be at a similar stage of development. They were largely subspherical in section, bounded by a plasma membrane and surrounded by a relatively wide parasitophorous vacuole (Figs 1 and 6). The nucleus was not well defined generally, but the nucleolus was prominent (Fig. 6). Within the cytoplasm were large amylopectin inclusions, lipid and small bodies of varying electron-density (Figs 1, 6 and 7). The parasitophorous vacuole contained flocculent material and this substance also appeared to be present within perimeter vacuoles, with mitochondrial appear-



Figs 6–7. *Goussia metchnikovi* in the spleen of *Gobio gobio.* Ultrathin sections. **6.** Macrogamete showing nucleus (N) with nucleolus (Nu) and parasitophorous vacuole containing flocculent material (Fl) similar to that in peripheral mitochondria (M). A is amylopectin. E is electron dense bodies; scale bar = 5 μm. **7.** Peripheral electron dense bodies of macrogamete. M is mitochondrion; scale bar = 1 μm



Figs 8–13. *Goussia metchnikovi* in the spleen of *Gobio gobio*. Ultrathin sections. **8.** Peripheral layer (arrows) encapsulating oocysts (O). S is sporocyst; scale bar = 5 μm. **9.** Oocyst (O) showing four sporocysts (S) within encapsulating layer (Ca). W is oocyst wall; scale bar = 5 μm. **10.** Detail of sporocyst wall consisting of inner (Iw) and outer (Ow) layers; outer layer has fuzzy coat. Dehiscence suture (D) and filamentous extensions (veils) (unlabelled arrows) are shown; scale bar = 0.5 μm. **11.** Longitudinal section of part of sporozoite showing rhoptries (Rh), portion of nucleus (N) and micronemes (Mc); scale bar = 2 μm. **12.** Oocyst showing surrounding encapsulating cells (arrows); scale bar = 2.5 μm. **13.** Degenerate sporocyst (S) within macrophage; scale bar = 2 μm

ance, some of which also showed small peripheral protrusions (Fig. 6). No intravacuolar tubules or intravacuolar folds were seen within the parasitophorous vacuole.

Sporulated oocysts occurred in encapsulated groups (Fig. 8). The oocyst wall was fine and membraneous, and the four enclosed sporocysts each had thick walls (Figs 9 and 10).

Sporocysts were relatively impervious to fixative and resin impregnation, so rendering their contents mostly opaque. However, when clearly visible, the sporocyst wall consisted of a broad, inner electron dense layer separated from an outer, narrow, dense layer by an electron lucent layer (Fig. 10); the outer dense layer also bore a fuzzy coat (Fig. 10). Two or three structures resembling the membraneous veils of some coccidian sporocysts were apparently connected to the slightly protruding dehiscence suture joining the two valves of the sporocyst wall (Fig. 10). However, not all sporocysts appeared to bear these structures and it was not clear whether they were actually veils. Where sporocysts were well impregnated with resin, their contained sporozoites showed typically apicomplexan structures, including a nucleus, and dense cytoplasm with abundant rhoptries and micronemes (Fig. 11).

Aggregates of sporulated oocysts were surrounded by inflammatory cells and these, contained in fibrous capsules, completely replaced some areas of normal splenic tissue (Figs 8, 9 and 12). There was evidence that the contents of many mature oocysts had been destroyed since the remains of eliminated sporocysts and sporozoites were observed within macrophages (Fig. 13).

Discussion

None of the *Eimeria* and *Goussia* species reported to infect the spleen of fishes (Dyková and Lom 1983, Davies and Ball 1993) has been examined ultrastructurally to any extent. In infected gudgeon examined here, gamogonic stages and sporulated oocysts of *G. metchnikovi* were present.

Microgamete development was typical of that seen in other fish coccidia (Lom and Dyková 1992, Davies and Ball 1993), including chromatin condensation within peripheral nuclei, nuclear constriction to create residual and DNA-containing microgamete nuclei, and the formation of perforatoria and associated flagella.

The parasitophorous vacuoles of the macrogametes of *G. metchnikovi* lacked intravacuolar folds and intravacuolar tubules which are common in mammalian and avian coccidia and are thought to be associated with the passage of nutrients (Scholtyseck *et al*. 1971). Macrogametes of *Goussia iroquoina* Molnár et Fernando, 1974, *Goussia laureleus* Molnár et Fernando, 1974 and *Eimeria variabilis* (Thélohan, 1893) Reichenow, 1921, fish coccidia infecting the intestine, have endogenous sporogony and thin-walled oocysts, but show no wall-forming bodies (Paterson and Desser 1981, 1984; Desser and Li 1984; Davies 1990). The small electron-dense bodies in the macrogamete cytoplasm of *G. metchinikovi* were too opaque to determine whether they were bounded by a membrane, but they did not appear similar to the wall-forming bodies type I of mammalian or avian coccidia (Scholtyseck 1973, Belli *et al.* 2006, Mai *et al.* 2009). No organelles were present resembling wall-forming bodies type II. Only sporulated oocysts were seen in our material and these were thin walled.

Lom (1971) and Lom and Dyková (1992) highlighted the membranous veil girdling the sporocyst along the suture of the two halves of the wall in *Goussia subepithelialis* Moroff et Fiebiger, 1905 in the intestine of *Cyprinus carpio*. Since then, similar girdles have been recorded around the sporocysts of *Goussia degiustii* Molnár et Fernando, 1974 from the spleen of *Campostoma anomalum* (see Paterson and Desser 1984), *Goussia gadi* Fiebiger, 1913 (see Odense and Logan 1976), *Goussia aculeati* Jastrzebski, 1984 (see Steinhagen *et al.* 1994) and *Goussia janae* Lukeš et Dyková, 1990 (see Jirků *et al.* 2002). Using the presence of veils as the criterion, Overstreet *et al*. (1984) created *Plagula* as a subgenus of *Goussia*, with *G*. (*Plagula*) *caseosa* as the type species, although this classification has been largely discontinued. We have attempted to view the veils of *G. metchnikovi* sporocysts by scanning electron microscopy (SEM), but without success (data unpublished). However, Jirků *et al*. (2002) reported that in *G. janae*, the fine veil of two loose membranes is retained in only a small proportion of sporocysts examined by SEM, and this may explain our lack of success in locating these.

Pellérdy and Molnár (1968), and Dyková and Lom (1981, 2007) reported an inflammatory reaction with concentrically arranged connective tissue encapsulating groups of *G. metchnikovi* oocysts in the spleen of gudgeon seen by light microscopy. Davies and Stewart (2000) also illustrated lipofuschin deposits around the oocysts by autofluorescence, although neither these deposits, nor the melanomacrophages common in fish spleens (Agius and Agbede 1984, Agius and Roberts 2003) were evident ultrastructurally. Molnár (1984) stated that *G. metchnikovi* can live in the host for a long time without causing any host reaction, and that the concentration of oocysts into islets could be regarded as the first sign of reaction. This demarcation was brought about by epithelial type reticulo-endothelial system macrophages. Later, a fibrotic capsule was formed by connective tissue around older oocyst masses. The shape of encapsulation in the infected spleens examined by us corresponded with this latter stage, following the demarcation stage by macrophages. This tissue reaction is not unique for *G. metchnikovi* infections. Paterson and Desser (1982) and Lom *et al*. (1989) showed fibrous capsules around oocysts *G. degiustii* in the spleen of *Notropis cornutus*, and Békési and Molnár (1991) depicted sporulated oocysts of *Calyptospora tucunarensis* surrounded by connective tissue capsules in the liver of *Cichla ocellaris*, as did Gestal and Azevedo (2006) in the liver of *Trisopterus luscus* infected by *G. lusca.*

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