FOLIA PARASITOLOGICA 40: 1 - 7, 1993

A coccidian (Apicomplexa: Eimeriidae) with extracytoplasmally located stages in the kidney tubules of golden carp (*Carassius auratus gibelio* L.) (Cyprinidae)

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Key words: coccidia, *Eimeria*, fish, fusion, host-parasite interaction

Abstract. Numerous coccidian stages were found in the kidney tubules of the golden carp (*Carassius auratus gibelio*). The merogonial and gamogonial stages were localized extracytoplasmally in the microvillous region of the epithelial cells. The host-parasite interface consisted of i) a large area where the parasite was separated from the host cytoplasm by the parasitophorous vacuole membrane only, and ii) a zone of multiple fusions of the host cell membrane investing the parasite to the neighbouring microvilli. The taxonomic status of the extracytoplasmic stages is not clear, however, their possible appurtenance to *Eimeria scardinii*, which was frequently found in the kidneys of golden carps in the same population, is discussed.

Several coccidia located extracytoplasmally have been recently described from various freshwater (Landsberg and Paperna 1987, Molnár and Rohde 1988, Molnár 1989, Jastrzebski and Komorowski 1990, Lukeš and Dyková 1990, Lukeš 1992) and marine fish (Lom and Dyková 1982, Daoudi et al. 1987, Kent et al. 1988). All of them were localized in the intestinal epithelium except for one example. Morrison and Poynton (1989 a,b) published an ultrastructural study on a coccidian extracytoplasmally located in kidney tubules which was, according to the presence of a sporocyst wall suture, placed into the genus *Goussia*.

In this paper we present ultrastructural data on another extracytoplasmic coccidian from the kidney tubules of a golden carp.

MATERIALS AND METHODS

Oocysts were found in 11 golden carps (*Carassius auratus gibelio* L.) from 32 specimens examined in July, September, and December 1989 and January 1990 at Pohořelice, South Moravia. Other developmental stages were only found in the histological material from a single specimen. Fifty fresh oocysts derived from kidneys were measured, while measurements of the merogonial and gamogonial stages were taken in semithin sections. Small pieces of kidney from selected specimens were fixed in 2 % osmic acid in 0.2 M cacodylate buffer at 4 °C for 1 hr, dehydrated, through propylenoxide embedded in Epon-Araldite resin, and sectioned on LKB Ultratome III. For light microscopy, the semithin sections (1-1.5 µm) were stained with 1% toluidine blue. For transmission electron microscopy, thin sections (0.5-0.7 µm) were stained either with uranyl acetate and lead citrate (Venable and Coggeshall 1965) or with Sato's lead stain solution (Hanaichi et al. 1986) and examined in a Philips 420 electron microscope.

RESULTS

Light microscopy

During the examination of fresh material from 32 golden carps, no merogonial and gamogonial stages were observed either in the kidneys or in other tissues examined. Developmental stages were only found in toluidine stained semithin sections in the kidneys from one specimen caught in December 1989.

All merogonial and gamogonial stages were localized either on the surface of the kidney epithelium or, less frequently, floating freely in the lumen of the tubules (Figs. 1-3).

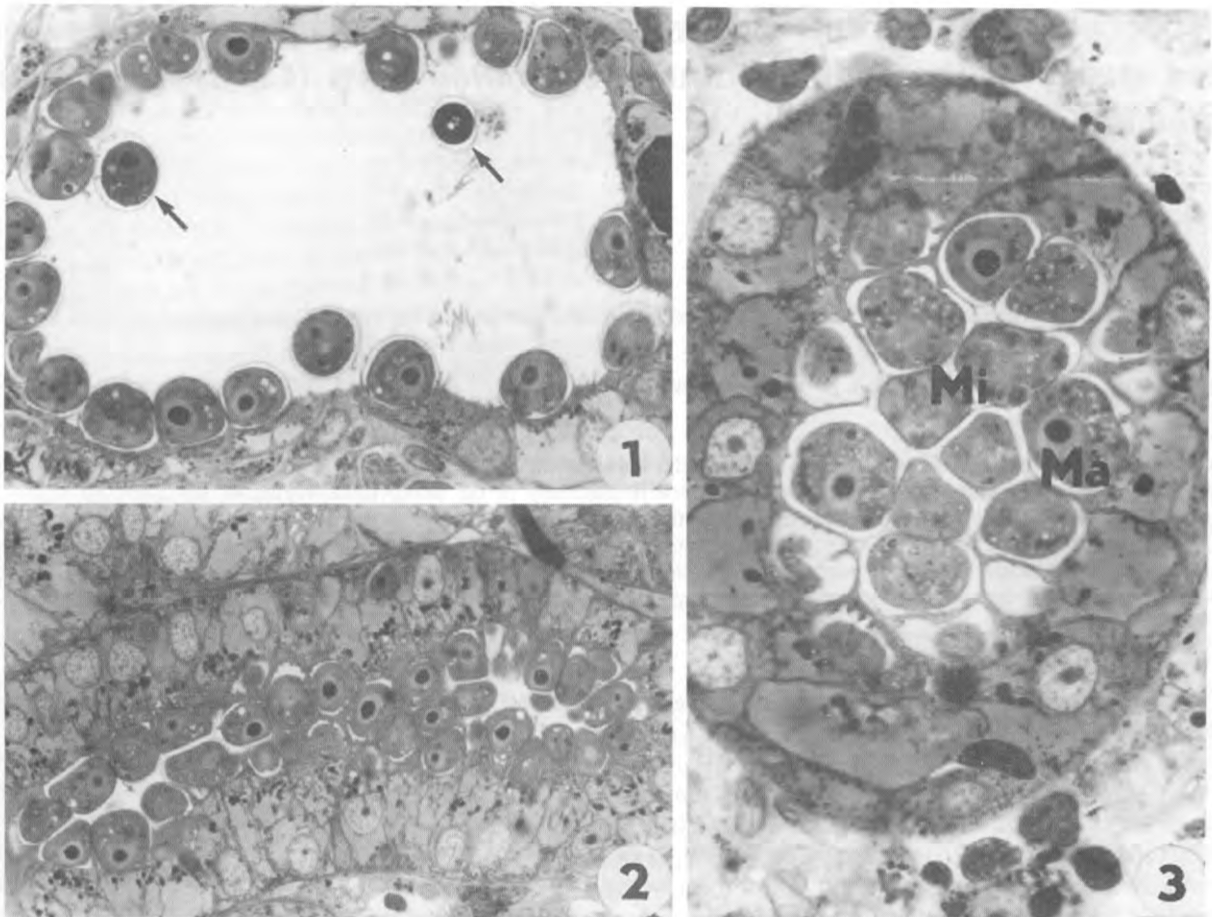
The infection was quite massive since in all processed pieces of the kidney, many kidney tubules were invaded by the coccidium. The lumen of some tubules was almost completely filled with developmental stages (Fig. 3), however, numerous tubules lying closely to the infected ones remained uninfected.

Spherical meronts, 5-8 µm in diameter (Figs. 1, 2), contained eccentrically located nucleus and small vacuoles in the dense cytoplasm. Meronts containing merozoites or mature merozoites could not be found.

Early microgamonts (10-15 µm x 8-13 µm) (Fig. 3) contained multiple small nuclei and less dense cytoplasm. Macrogamonts (15-20 µm x 11-17 µm) were characterized by a large nucleus with a prominent nucleolus and a cytoplasm filled with transparent and dense granules.

In the kidneys of 34 % of the fish examined, sporulated oocysts (Figs. 6, 7) and/or nodules containing the remnants of oocysts (Figs. 4, 5) were observed both in fresh and embedded samples collected in January, July, September, and December.

The oval oocysts (23.5-32.0 µm x 17.2-21.0 µm) have a smooth thin, single-layered oocyst wall and a prominent



Figs. 1-3. Light microscopy of extracytoplasmally located stages. **Fig. 1.** Merogonial stages within a large kidney tubule. Arrows indicate two stages free in the lumen (x 750). **Fig. 2.** Longitudinal section of a tubule filled with merogonial stages (x 540). **Fig. 3.** Cross section of a tubule obstructed by macrogametocytes (Ma) and microgametocytes (Mi)(x 900).

polar granule (1.8-3.2 µm x 1.5-2.3 µm) (Fig. 8). Four sporocysts were of irregular shape (15.8-18.5 µm x 5.8-7.3 µm) and each contained two irregular to oval sporozoites (7.2-9.5 µm x 3.8-5.5 µm). The thin sporocyst wall was hardly visible and in some oocysts it seemed to be absent (Fig. 7).

The nodules were round having a thick wall formed by numerous concentric layers of fibroblasts. In most cases, inside these nodules, the remnants of sporulated oocysts, only rarely with discernible sporocysts, dense amorphous material, and melano-macrophages could be discerned (Fig. 5).

Electron microscopy

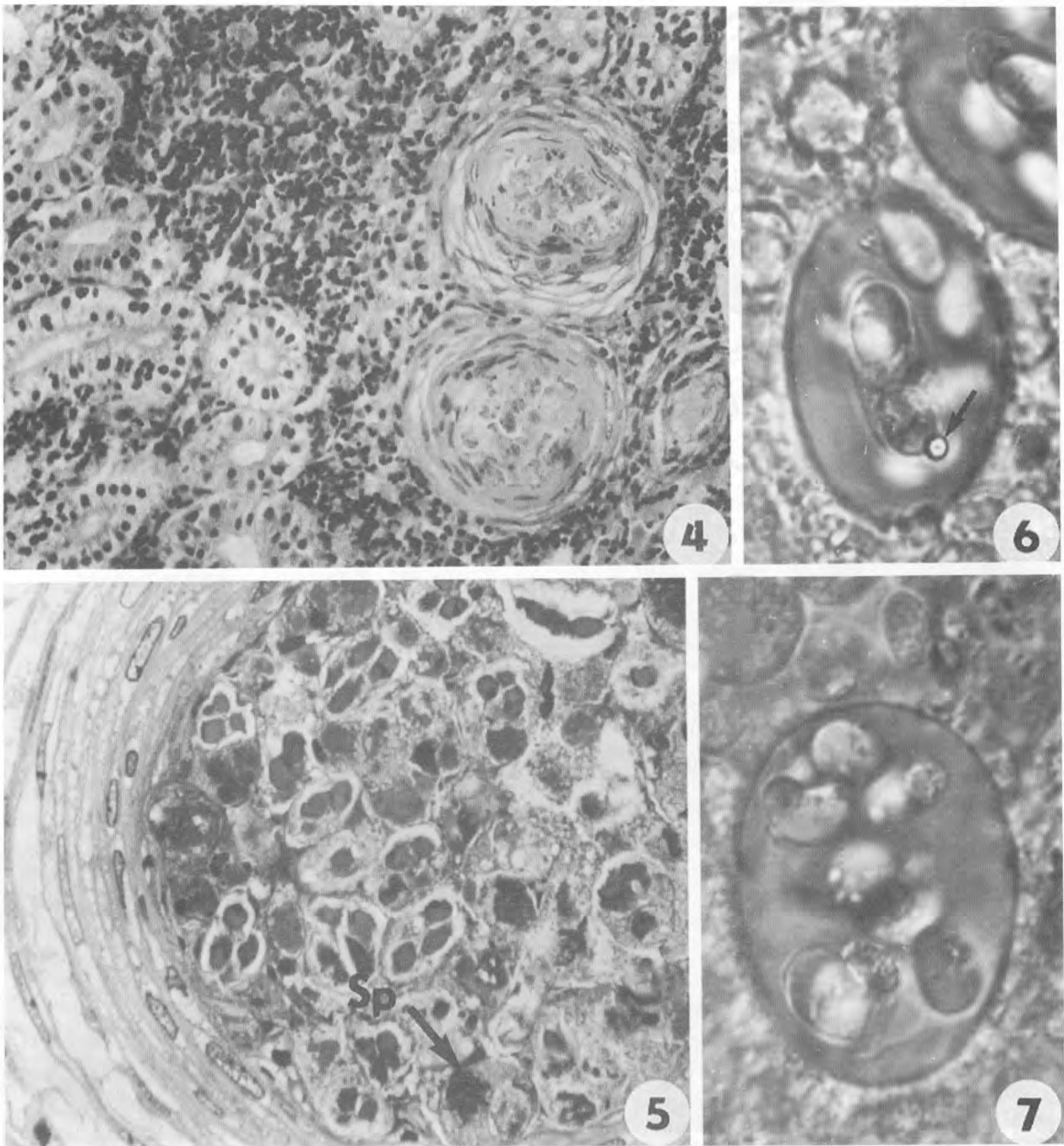
All intracellularly located developmental stages were found in an extracytoplasmic position in the apices of the epithelial cells (Figs. 11, 13, 15-18). They were separated from the lumen of the kidney tubule by two closely apposed unit membranes, from which the host cell cytoplasmic membrane was exterior to the parasitophorous vacuole membrane (Figs. 9a, 10). The parasitophorous vacuole of most stages was filled with a floccular amorphous material (Figs. 11, 15-18).

The host-parasite interface was represented both by a single large area of contact (Fig. 12) and by multiple fusions of the host cell membrane investing the parasite to the surrounding microvilli (Figs. 9a, b) (see Discussion). Thus an intricate network of interconnections was formed (Fig. 9a).

The earliest stages found were small oval meronts (Figs. 11, 13). They contained a centrally located nucleus with a prominent dense nucleolus, usually one large lipid granule, numerous ribosomes, peripherally located mitochondria and small vacuoles. The vacuoles were filled with round dense precipitates which indicated the presence of hydroxyapatite (Fig. 14). The meront cytoplasm was surrounded by a trilaminar pellicle (Figs. 8, 9). The merogonial division was absent in our material.

The gamogonial development started with large oval meronts which were typified by the presence of a persistent conoid with the remnants of micronemes, several amylopectin and lipid granules, endoplasmic reticulum, and Golgi apparatus (Fig. 15).

Young microgamonts had, on their periphery, numerous nuclei with irregularly arranged heterochromatin and small lipid and amylopectin granules (Fig. 16). Further stages of microgametogenesis including mature microgametes could not be found.



Figs. 4-7. Light microscopy of oocysts and accompanying fibrillar nodules. **Fig. 4.** Two nodules in a histological section of an infected kidney (x 230). **Fig. 5.** The periphery of nodule with a thick wall formed by the concentric layers of fibroblasts. They envelope tightly packed sporonts (Sp) and sporulated oocysts (x 800). **Figs. 6 and 7.** Sporulated oocysts in a fresh smear of an infected kidney (both x 1500).

Early macrogamonts were especially characterized by multiple arrays of endoplasmic reticulum and large centrally located nucleus with a single dense nucleolus (Fig. 17). As macrogametogenesis proceeded, the number of lipid granules remained almost the same. However, the number and dimensions of amylopectin granules increased significantly in mature macrogamonts as is visible in Fig. 18.

DISCUSSION

Although the developmental stages were only found in one fish and their taxonomic position is not clear, we would suggest that the results presented herein are noteworthy especially because of the peculiar organization of the host-parasite interface.

Fish coccidia differ from the coccidia in other vertebrates by the tendency of a high number of species to use

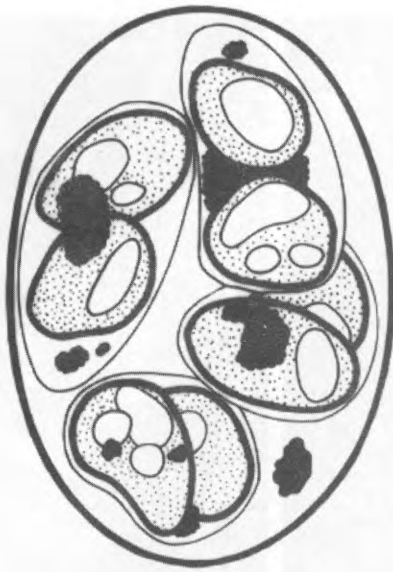
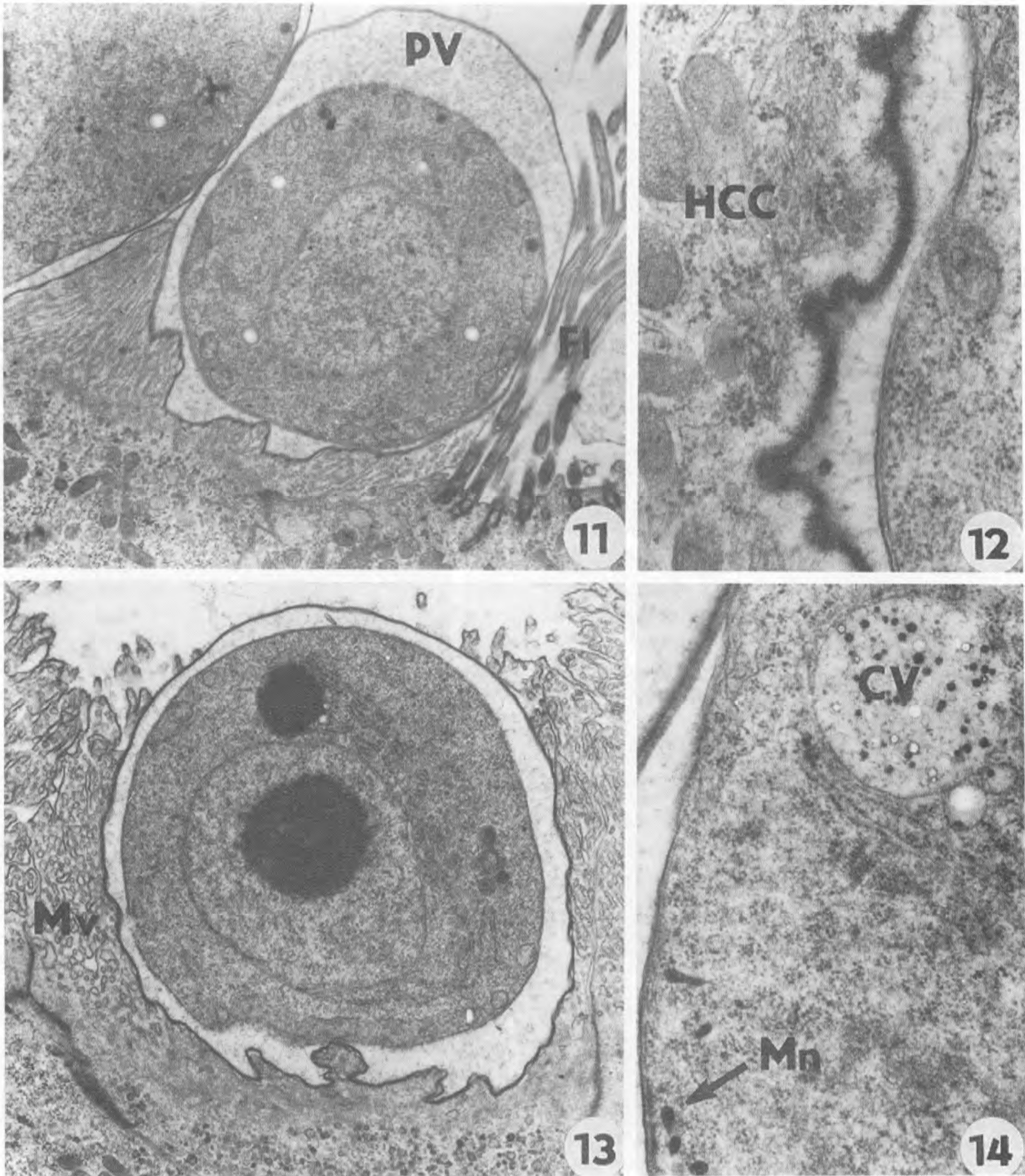


Fig. 8. Diagrammatic representation of a sporulated oocyst.

extraintestinal sites, including the kidney, for endogenous development. According to Dyková and Lom (1983), oocysts from at least 17 species were identified in the kidneys of various fish. However, with the exception of *Goussia spraguei* (Morrison and Poynton 1989 a,b), there are no other data on the developmental stages of kidney coccidia.

During the examination of golden carps, we regularly found in their kidney both sporulated oocysts and their remnants encapsulated in small nodules. The oocysts resembled those of *Eimeria scardinii*, as observed in the kidneys of roach and rudd by Athanassopoulou-Raptopoulou and Vlemmas (1986). However, the sporocyst walls were hardly visible in the sporulated oocysts examined by us, and in some oocysts they seemed to be absent. It cannot be excluded that the golden carp represents a host in which the sporulation of *E. scardinii* is aberrant. This is supported by frequent encapsulation and degradation of sporulated oocysts. Unfortunately, there is no direct evidence that the extracytoplasmally

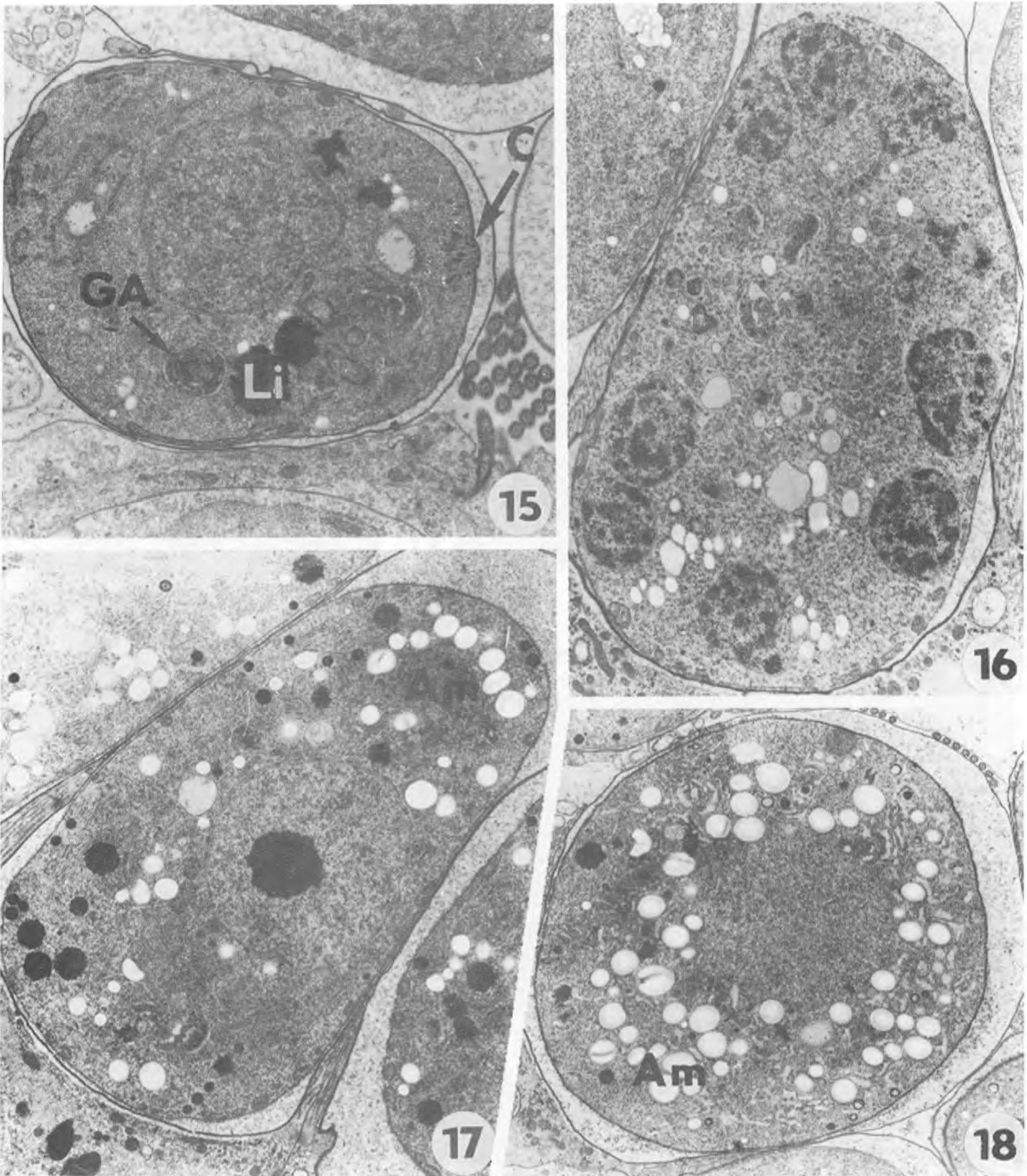
Figs. 9-10. Ultrastructure of the host-parasite interface. **Fig. 9a.** The host-parasite interface of a merogonial stage. Arrows indicate fusions of microvilli to the host cell cytoplasmic membrane enclosing the parasite. PV-parasitophorous vacuole; Pe-pellicle (x 120000). **Fig. 9b.** Detail of the fusions (arrows) of two microvilli (Mv) to the host cell cytoplasmic membrane. PV-parasitophorous vacuole (x 155000). **Fig. 10.** The host cell cytoplasmic membrane (HCM) and the underlying parasitophorous vacuole membrane (PVM) separating the parasite from the lumen of the tubule (LT). Pe-pellicle (x 155000).



Figs. 11-14. Ultrastructure of merogonial stages. **Fig. 11.** Early meront localized closely to a cluster of tubular flagella (Fl). PV-parasitophorous vacuole (x 7000). **Fig. 12.** The area of contact between the meront and the host cell. HCC-host cell cytoplasm (x 33000). **Fig. 13.** Growing meront in the microvillar region (Mv) of the kidney epithelial cell (x 8500). **Fig. 14.** Large merogonial cytoplasmic vacuole (CV) with small dense precipitates in its lumen. Mn-peripherally located remnants of micronemes (x 23000).

located merogonial and gamogonial stages found in winter in one specimen belong to this species, except for the fact that no other coccidia were found in the golden carp population. Although the appurtenance of the developmental stages to *E. scardinii* is therefore quite probable, we prefer to postpone the decision on their taxonomic status till a more detailed study is performed.

The ultrastructure of the merogonial stages did not differ from that of piscine extracytoplasmic coccidia (Molnár and Baska 1986, Jastrzebski and Komorowski 1990, Lukeš 1992). In macrogamonts, organelles reminiscent of the wall-forming bodies of coccidia from terrestrial vertebrates were lacking. Although such organelles have been observed in macrogamonts from several fish coccidia (Paterson and Desser 1981, Hawkins et al. 1983, Paperna et al. 1986)



Figs. 15-18. Ultrastructure of gamogonial stages. **Fig. 15.** Early macrogametocyte with a persisting peripherally located conoid (C). GA-Golgi apparatus. Li-lipid granules (x 7400). **Fig. 16.** Early microgametocyte with nuclei migrating to the periphery (x 5600). **Fig. 17.** Macrogametocyte containing abundant ribosomes, lipid and amylopectin (Am) granules (x 4300). **Fig. 18.** More developed macrogametocyte with the abundant amylopectin granules (Am) in its cytoplasm (x 3600).

their participation in the formation of the oocyst wall has hitherto not been observed (Steinhagen et al. 1990). The presence of hydroxyapatite granules of the same form and location as they occur in *Goussia janae* (Lukeš and Starý 1992) should be noted.

As regards the location in the host cell, the coccidium clearly belongs to the group of extracytoplasmally located species. All coccidia with this extraordinary type of intracellular location have been observed in the intestine of various freshwater and marine fish (see Lukeš 1992), except for *Goussia spraguei* which parasitize the kidney tubules of *Gadus morhua* and *Melanogrammus aeglefinus* (Morrison and Poynton 1989 a, b). The only observation of another extracytoplasmally located coccidium in histological sections of the kidney was made in our laboratory using white bream (*Blicca bjoerkna*) (Lukeš and Dyková, unpubl.)

Although in the light microscope the location of stages studied by us seemed to be the same as it was seen in *Goussia spraguei*, the ultrastructural study revealed significant differences. The parasitophorous vacuole of the developmental stages of *G. spraguei* protruded into the epithelial cytoplasm through more or less deep invaginations which were filled with dense granular material (Morrison and Poynton 1989 a,b). Moreover, no fusions to the microvilli surrounding these stages can be identified from published microphotographs.

In our material, invaginations into the host cell cytoplasm were lacking. However, multiple fusions of the epithelial membrane stretched around the parasite to the surrounding microvilli could regularly be observed. This type of fusion has been detected in extracytoplasmally located *Goussia pannonica* (Molnár, 1989) and *G. janae* Lukeš et Dyková, 1990 in the intestine of fish (Lukeš 1992, Lukeš and Starý, 1992) and *Haemogregarina balli* (Paterson et Desser, 1976) in the intestine of leeches (Siddall and Desser 1990). The most probable explanation for this interesting phenomenon is the enlargement of the covering membranes accompanied by the establishment of many contact areas with the host cytoplasm, which is responsible for the nutrition of the parasite.

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Received 21 July 1992

Accepted 12 October 1992