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SHORT COMMUNICATION

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Fine structural development of microgamonts of *Sarcocystis singaporensis* in *Python reticulatus*

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Abstract Three 4-month-old reticulated pythons (*Python reticulatus*), hatched from eggs laid by a newly caught female from Singapore Island, were fed on muscles of *Sarcocystis singaporensis*-infected *Rattus rattus* caught in Singapore. Snakes were sacrificed 5, 6 and 8 days later, the infected tissues were studied by transmission electron microscopy. The present communication summarizes findings on microgamont stages. Both premature and mature microgamonts were already present in the snake sacrificed 5 days post-feeding; young stages, however, were still common 8 days post-infection. Young microgamonts have characteristic, elongated nuclei, which round-up towards the time of microgamete emergence. Microgamonts complete their development within the mucosal epithelial layer; the infected apical epithelial cells undergo degeneration with the loss of the brush border.

Introduction

Zaman and Colley (1975) were the first to describe the developmental cycle of *Sarcocystis singaporensis*, in reticulated pythons (*Python reticulatus*) from Singapore Island, including the fine structure of the microgamont. The only other available account on snake *Sarcocystis* microgamont fine structure is that of *S. muriviperae*, described from both *Vipera palaestinae* and *Coluber jugularis* (Paperna and Finkelman 1996a). In this communication (one of three prepared on the endogenous infection process of *S. singaporensis* via timed experimental infections of its definitive host, the reticulated python) we present an ultrastructural study of microgamont development.

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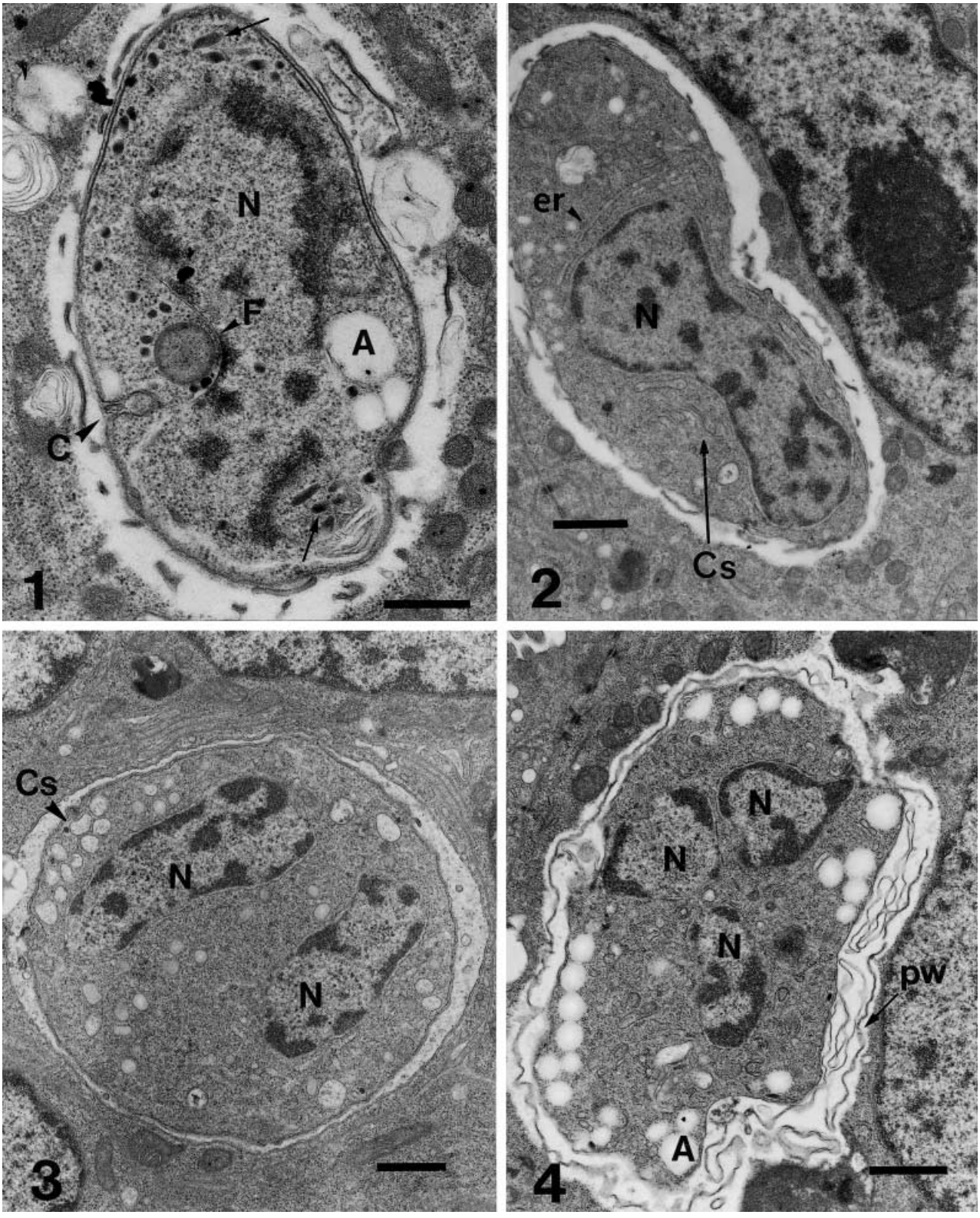
Materials and methods

Young, about 4-month-old, 60- to 70-cm-long, 80- to 90-g reticulated pythons, reared from eggs laid by a newly caught female from Singapore Island, were force-fed with infected *Rattus rattus* muscles removed from free-ranging rats, naturally infected with *S. singaporensis* and trapped in the forested area around Singapore Zoological Gardens (Paperna and Martelli 2000). Infection with *S. singaporensis* was confirmed by histological examination. Snakes were sacrificed with chloroform 5, 6 and 8 days after feeding. The gut was removed and sections along the digestive tract were examined by light microscope by squashing a small, fresh piece of gut tissue between slides. Small pieces from the loci in the gut found to host an infection were fixed for electron microscopy.

For transmission electron microscopy, gut segments were fixed in 2.5% glutaraldehyde in cacodylate buffer (0.1 M, pH 7.4) for 24 h at 4 °C, rinsed repeatedly in the same buffer, post-fixed in 1.0% osmium tetroxide in the same buffer for 1 h and, after rinsing in the buffer, dehydrated in graded ethyl alcohols and embedded in Agar 100 medium (Agar Scientific, UK). Thin sections, cut on a Reichert Ultracut microtome with a diamond knife, were stained on grids with uranyl acetate and lead citrate and were examined with a Jeol 100CX transmission electron microscope.

Results

Both premature and mature microgamonts were already present in the snake sacrificed 5 days post-feeding; microgamonts of all stages were still common in the snakes sacrificed on days 6 and 8 post-feeding. The parasitophorous vacuole (PV) lodging the pellicle-bound microgamonts was bordered by a double membrane, sometimes seen to be either breaking or sloughing off, possibly due to processing faults (Figs. 1, 2), as it was otherwise intact (Fig. 3). Where sloughing off occurred, additional membrane whorls filled the PV lumen, suggesting repetitive formation of boundary membranes



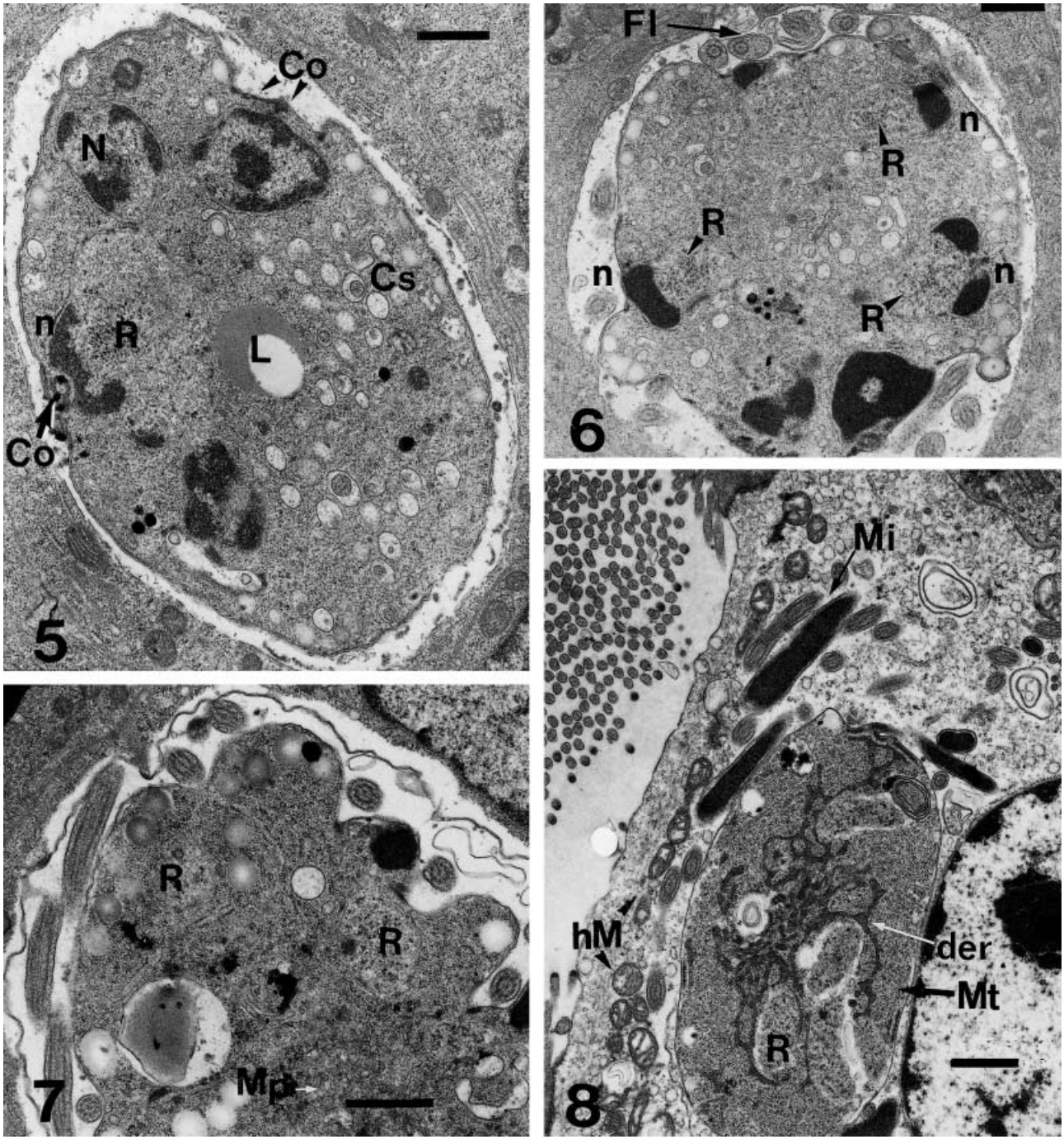
Figs. 1–4 Young microgamont of *Sarcocystis singaporensis* in gut epithelium of *Python reticulatus*

Fig. 1 Young microgamont, with an elongated nucleus (*N*) micronemes (*arrows*) cytostome (*C*) and food vacuole (*F*)

Fig. 2 Single- nucleate microgamonts with extensive endoplasmic reticulum (*er*), a few cisternae, but with no micronemes

Fig. 3 Microgamont with two elongated nuclei (*N*) and numerous cisternae (Cs); note intact parasitophorous vacuole lining

Fig. 4 Microgamont showing three nuclei (*N*), cytoplasm with many amylopectin granules (*A*) and the PV lumen filled with membrane whorls (*pw*). *Bar* 1 μm



(Fig. 4). The youngest microgamonts had one large, elongated nucleus with peripherally aggregated chromatin (Figs. 1, 2); some (Fig. 1) retained a fair number of micronemes and had a distinct cytostome extending from the pellicular wall. Nearby, a large spherical food vacuole appeared to have been derived from this cytostome (Fig. 1). There were a few amylopectin bodies, traces of microfibrils and variable amounts of endoplasmic reticulum (ER), tubuli and cisternae (Fig. 2) . The cytoplasm of binucleate microgamonts contained numerous cisternae but lacked amylopectin. The two nuclei seemed to be splits of an elongated nucleus

Figs. 5–8 Growing and mature *S. singaporensis* microgamonts
Fig. 5 Microgamont in cross section, showing five differentiating nuclei, one already separated into microgamete (*n*) and residual (*R*) nucleoplasm. Centriole and osmiophilic plaques (*Co*) form near the nuclei. *L* Lipid vacuole
Fig. 6 Mature microgamont with formed, flagellated microgametes (*Fl*), microgamete nucleoplasms (*n*) and nuclei residues (*R*)
Fig. 7 Periphery of a mature microgamont. *Mp* Mitochondrial plaque, *R* nuclei residues
Fig. 8 Aged microgamont (*Mt*) with aberrant ER (*der*) and nuclei residues (*R*) inside a degenerated apical epithelial cell, which has defunct mitochondria (*hM*), lacks a brush-border and is filled with microgametes (*Mi*). *Bar* 1 μm

(Fig. 3). Microgamont cytoplasm, which in cross section showed three or four nuclei (Fig. 4), contained many amylopectin bodies. These were lacking in a subsequent stage, when only cisternae appeared in large numbers, some with conspicuous inner-membrane-bound vesicles.

These microgamonts also contained a large lipid vacuole (Fig. 5). Centrioles and osmiophilic plaques made a rudimentary or full appearance in microgamonts, showing sections of three or more nuclei. They occurred near the nuclei which had rounded-up and shifted their chromatin to their periphery. Microgamonts formed a few or moderate number of microgametes (Figs. 6, 7). The nuclei had separated into an electron-dense microgamete nucleoplasm, leaving behind a granular residual nucleus (Fig. 6). The mature microgamont's cytoplasm initially retained the same range of organelles (notably vesicles) as the premature one, but became gradually filled with residual nuclei. One showed a mitochondrial plaque (Fig. 7). Microgamonts completed their differentiation in the mucosal epithelial layer. Host cells of the premature and some of the differentiated microgamonts remained seemingly functional (Figs. 6, 7). A fully differentiated microgamont accompanied by numerous mature microgametes was seen located within a seemingly degenerating apical epithelial cell which lacked a brush border and had loose vesiculated cytoplasm, defunct mitochondria and multilaminate whorls. The microgamont's dense cytoplasm contained residual nuclei and a dense tubular (ER) network filled with electron-dense substance (Fig. 8).

Discussion

Microgamonts of *Sarcocystis* spp. yield a small number of microgametes relative to the numbers produced in *Eimeria* spp. (Scholtyseck et al. 1972). In the differentiating microgamonts before the first and even second divisions, the nucleus elongates, expands and ramifies in a manner similar to the nuclei of *Sarcocystis* meronts undergoing division in the intermediate rodent host (Pachedo and Fayer 1977; Paperna and Finkelman 1996b). This has also been seen in microgamonts of *S. muriviperae* (Paperna and Finkelman 1996a), and *Sarcocystis* spp. grown in tissue culture by Vetterling et al. (1973) and Becker et al. (1979). The expanded nucleus then splits into daughter nuclei which differentiate into a microgamete nucleus and a residuum. In *S. suihominis*, the ramified nuclear segments remain interconnected by the nuclear residuum, while differentiating into microgametes (Becker et al. 1979; Mehlhorn and Heydorn 1979). Microgamont development occurs within the apical mucosal epithelial cell. The final stage of microgamont differentiation coincides with its host-cell's degeneration and loss of brush-border. This has not been observed in any of the above quoted reports.

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