

Ultrastructure of sporogonial stages of *Eimeria kayarensis* Diouf and Toguebaye, 1994 (Apicomplexa, Coccidia)

Jacques Ngor DIOUF and Bhen Sikina TOGUEBAYE

*Laboratoire de Parasitologie, Département de Biologie Animale, Faculté des Sciences et
Techniques, Université Cheikh Anta Diop de Dakar, Dakar, Sénégal*
E-mail: parasito@refer.sn

The ultrastructure of sporogonial stages of Eimeria kayarensis (Coccidia, Eimeriidae) in the spiral valve of the brown ray, Raja miraletus L. (Pisces, Rajiformes), was studied. The oocysts had bilayered walls. Within the young oocysts were sporonts, surrounded by an electron dense wall with cytoplasm containing numerous amylopectin granules and some refractile bodies. The oocysts divided by plasmotomy into four sporoblasts. The young sporoblasts were enveloped by a unit electron dense wall that transformed into a thick sporocyst wall. The sporocysts were enveloped by a bilayered pellicle and possessed a Stieda body. Each mature sporocyst contained two sporozoites with numerous micronemes and two refractile bodies surrounded by amylopectin granules.

Key words: Apicomplexa, Coccidia, Eimeriidae, *Eimeria kayarensis*, Chondrichthyes, Rajidae, *Raja miraletus*, sporogony, ultrastructure, Senegal, eastern tropical Atlantic

INTRODUCTION

During the last ten years, several species of coccidia have been described in fishes from Africa (LANDSBERG & PAPERNA, 1987; PAPERNA, 1990; DIOUF & TOGUEBAYE, 1993, 1994, 1996). However, data on the ultrastructure are scarce. To our knowledge, data are available only for *Eimeria vanasi*, *E. spari*, *Goussia cichlidarum*, *G. clupearum*, *G. dakarensis*, *G. senegalensis* and *G. trachinoti* (KIM & PAPERNA, 1992, 1993; DIOUF & TOGUEBAYE, 1993, 1996).

Eimeria kayarensis is a coccidia that we described in the brown ray, *Raja miraletus*, from the coasts of Senegal (DIOUF & TOGUEBAYE, 1994). The oocysts were ellipsoidal ($16.7 \pm 1.1 \times 13.9 \pm 0.9 \mu\text{m}$) and the sporocysts were ovoid ($7.9 \pm 1.4 \times 6.0 \pm 1.2 \mu\text{m}$). Here we

present further findings describing ultrastructural features of the sporogonial stages.

MATERIAL AND METHODS

Twelve brown rays, *Raja miraletus* Linnaeus 1758 (Chondrichthyes: Rajidae), were collected from the coasts of Senegal. Five were infected by *Eimeria kayarensis*. Small fragments of the infected spiral valves of the rays were fixed at 4°C with 2.5% (v/v) glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) for 24 h, then post-fixed at 4°C with 1% osmium tetroxide in the same buffer for 1 h. After dehydration through a gradual ethanol series and propylene oxide, the cysts were embedded in Spurr resin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined using a JEOL 100 CXII electron microscope.

RESULTS

The earliest observed sporogonial stages were unsporulated oocysts containing the sporont (Fig. 1). Their cytoplasm contained numerous amylopectin granules and some refractile granules. The sporonts were surrounded by an electron-dense wall that became thicker than the wall of the oocyst. The oocysts divided simultaneously by invaginations of the cytoplasm (Fig. 2) into four sporoblasts (Fig. 3). The mature oocysts had a wall (about 40 nm) formed of two opaque layers separated by a translucent layer (Fig. 4).

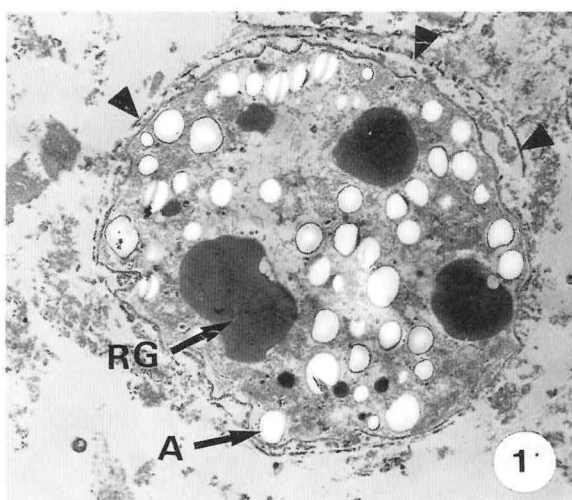


Fig. 1. An unsporulated oocyst whose wall (arrows) is starting to form. A: amylopectin granules; RG: refractile granules (x 6 800)

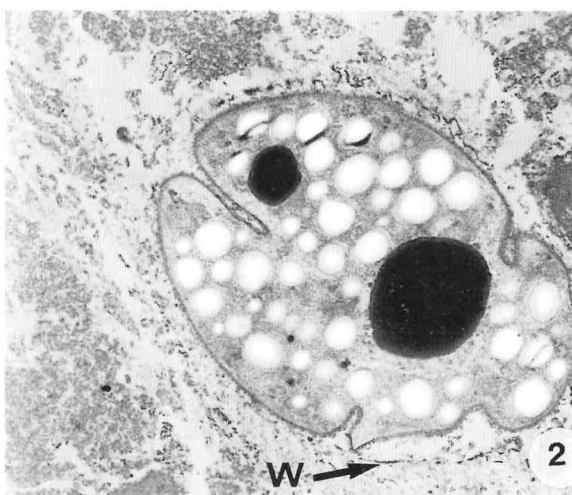


Fig. 2. Simultaneous formation of four sporoblasts by surface invaginations of the sporont. W: oocyst wall (x 5 800)



Fig. 3. Oocyst showing young sporoblasts (arrows) with relatively thin walls (x 4 800)



Fig. 4. Oocyst wall formed of two opaque layers separated by a lucent layer (x 100 000)

The sporocysts (Fig. 5) possessed a Stieda body (Fig. 6). The thin electron dense wall of the young sporoblasts transformed into the sporocyst wall (about 240 nm) consisting of

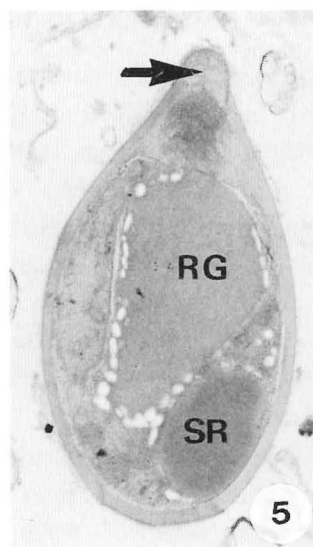


Fig. 5. Mature sporocyst showing the Stieda body (arrow), sporocyst residium (SR) and a refractile granule (RG; x 9 300)

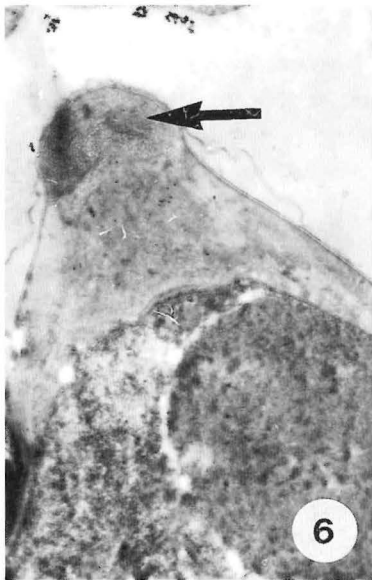


Fig. 6. An enlarged detail of the Stieda body (arrow; $\times 29\,000$)

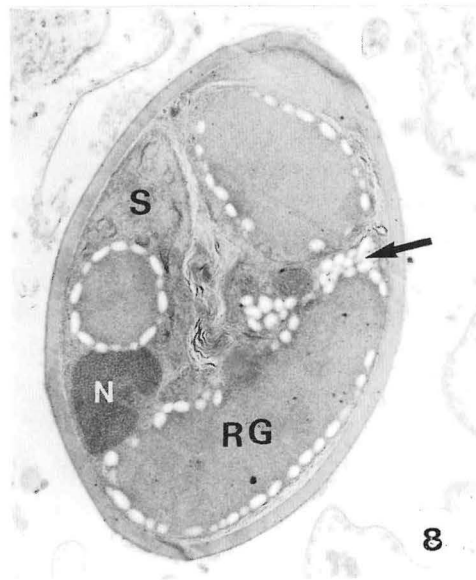


Fig. 8. Cross section of a mature sporocyst showing a sporozoite (S) with one nucleus (N) and refractile granules (RG) surrounded by amylopectin granules (arrow; $\times 11\,200$)

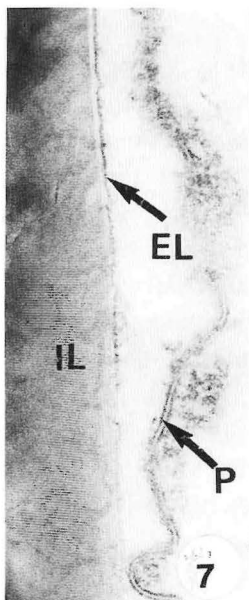


Fig. 7. The wall of mature sporocyst consisting of a thick transversally striated inner layer (IL), separated from an external layer (EL) by a thin lucent layer. P: bilayered pellicle ($\times 50\,000$)

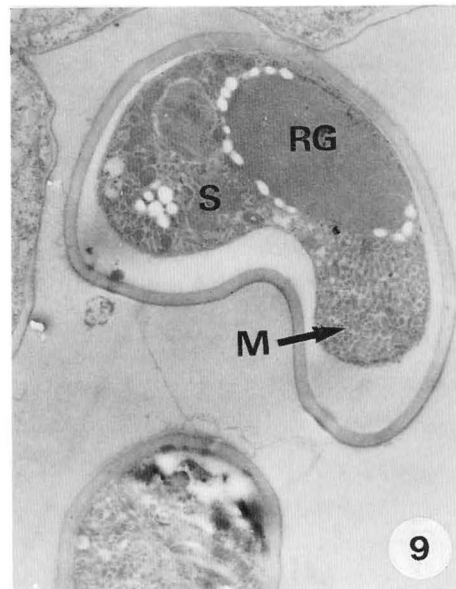


Fig. 9. Cross section of a mature sporocyst showing a sporozoite (S) with numerous micronemes (M). RG: refractile granule ($\times 9\,700$)

three layers (Fig. 7). The inner layer was thick, transversally striated and separated from the thin opaque external layer by a thin lucent layer. The sporocysts were enveloped by a bilayered

pellicle of about 20 nm. They contained two uninucleate sporozoites with two refractile bodies surrounded by granules of amylopectin (Fig. 8) and numerous micronemes (Fig. 9).

CONCLUSION

Data on the ultrastructure of *Eimeria* of fish indicate that the sporogonial stages resemble *E. kayarensis* (DESSER & LI, 1984; MORRISON & HAWKINS, 1984; DAVIES, 1990; MORRISON, 1991; KIM & PAPERNA, 1992; ALVAREZ-PELLITERO *et al.*, 1997). In *E. kayarensis*, four sporoblasts are formed by simultaneous plasmotomy of the sporont. Similar division of the sporont has been described for *Goussia carpelli* (LOM *et al.*, 1991). In contrast, sporoblasts in *E. laureleus* are formed by successive divisions of the sporont

(DESSER & LI, 1984) and in *G. spraguei* by simultaneous multiple divisions and successive divisions of the sporont (MORRISON & POINTON, 1989). Dense bodies have been described in the sporonts of *E. funduli*, *E. variabilis*, *G. clupearum*, *G. cichlidarum* and *G. zarnowskii* (HAWKINS *et al.*, 1983; MORRISON & HAWKINS, 1984; PAPERNA *et al.*, 1986; DAVIES, 1990; JASTRZEBSKI & KOMOROWSKI, 1990). According to DAVIES (1990), these dense bodies play no role in the formation of the oocyst wall. Rather, the wall derives from the plasma membrane of the zygote and the endoplasmic reticulum.

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Ultrastruktura sporogonija *Eimeria kayarensis* Diouf i Toguebaye, 1994 (Apicomplexa, Coccidia)

Jacques Ngor DIOUF i Bhen Sikina TOGUEBAYE

Laboratorij za parazitologiju, Odjel za zoologiju, Fakultet znanosti i tehnike, Sveučilište u
Dakaru Cheikh Anta Diop, Dakar, Senegal
E-mail: parasito@refer.sn

SAŽETAK

Proučavana je ultrastruktura sporogonijevih stadija kod vrste *Eimeria kayarensis* Diouf & Toguebaye, 1994 (Coccidia, Eimeriidae) u spiralnom naboru raže modropjege, *Raja miraletus* (Pisces, Rajiformes). Oocite su imale dvoslojnu stijenku. Unutar mladih oocita nalazili su se sporonti (raniji razvojni stadiji spora), okruženi gustom elektronskom stijenkom s citoplazmom koja je sadržavala brojna zrnca amilopektina i nekoliko refraktibilnih tjelešaca. Dijelile su se plazmotomijom u četiri sporoblasta. Mladi sporoblasti su bili omotani gustom elektronskom stijenkom koja se zatim transformirala u debelu stijenku. Sporociste su imale dvoslojnu opnu i posjedovale Stieda tjelelo. Svaka zrela sporocista je sadržavala dva sporozoida s brojnim mikronemama i dva refraktibilna tjelešca, koja su bila okružena amilopektinskim zrcima.

Ključne riječi: Apicomplexa, Coccidia, Eimeriidae, *Eimeria kayarensis*, Chondrichthyes, Rajidae, *Raja miraletus*, sporogony, ultrastruktura, Senegal, istočni tropski Atlantik

