The endogenous development, described by light and electron microscopy, of *Eimeria jamescooki* sp. n. (Apicomplexa: Eimeriidae) from the skink *Cryptoblepharus virgatus*

Ilan Paperna

Department of Animal Sciences, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot 76-100, Israel

Key words: Coccidia, *Eimeria jamescooki*, *Cryptoblepharus virgatus*, Australia, ultrastructure

Abstract. *Eimeria jamescooki* sp. n. was recovered from the skink *Cryptoblepharus virgatus* (Garman) found on the grounds of James Cook University, Townsville (type locality), North Queensland, Australia. Oocysts were 17.5–25.0 (22.1 ± 1.9) × 15–22.5 (17.7 ± 1.6) µm and sporocysts 6.25–10.0 (7.9 ± 1.15) × 3.75–6.25 (5.3 ± 1.0) µm in size. Endogenous stages are described from histological material examined by light microscope and by transmission electron microscope. Both merogony stages and gamonts of the presently described species were the Golgi “plaques” and an enclosure of tubuli. Mature macrogamonts and young oocysts were prematurely developed; some host cells contained two elongate macrogamonts. Unique to the skink species found in the cytoplasm of the anterior gut mucosal epithelium. Meront progeny were comprised of 10 to 21 microgamonts conformed in fine structure with that observed in other eimerians. Their sizes increased from 15.4 × 4.2 to 28 × 8.4 µm while dividing to over 70 nuclei, which formed a corresponding yield of microgametes.

Cannon’s publications (1967a, b) were the first comprehensive contributions on Australian lizard coccidia of the genera *Eimeria* and *Isospora*, preceded only by a description of *Eimeria molochis* from *Moloch horridus* by Bovee and Telford (1965). Subsequent contributions included a description of seven new species of *Isospora*, one from *Cryptoblepharus virgatus* (Finkelman and Paperna 1994a, b, and 2002), an account of the ultrastructure of these described *Isospora* (Paperna and Finkelman 1998), a report on the finding of a globidium (Paperna 1999), and a description of *Eimeria gastrosaure* from the stomach of a gecko (Paperna 1994). This latter coccidium, found in an unusual habitat for coccidians, was examined ultrastructurally (Paperna 1993) and exhibited several peculiarities which were not seen in *Eimeria* species inhabiting the intestinal epithelium of reptilian hosts (Paperna and Lainson 1999a). To date, the only other available fine structural account on *Eimeria* species of lizards which undergo endogenous development in the cytoplasm of the gut mucosal epithelial cells is that of *E. boveroi* (Paperna and Lainson 1999a). In the present communication, the endogenous development in the cytoplasm of the small intestinal epithelium of a new species of *Eimeria* from the Australian skink *Cryptoblepharus virgatus* (Garman) is described from material examined histologically and by transmission electron microscopy.

MATERIALS AND METHODS

Six specimens of *C. virgatus* were collected in August 1986, and 14 from August to October, 1988 in the gardens of James Cook University, Townsville, North Queensland. The skinks were identified by Dr. Steve Donnelan of the South Australian Museum, Adelaide and five preserved specimens have been deposited in the collection of this museum (QM J48419–48423). Collected skinks were left to defecate while confined in a glass container. For microscopic examination, freshly deposited faeces were macerated in tap water. Faeces of five skinks contained *Eimeria* sp. and were euthanized and their tissues fixed for histology; samples from two were fixed for electron microscopy. The non-infected skinks were released to their habitat.

For light microscopic (LM) histology, portions of the gut were fixed in 10% neutral buffered formalin and embedded in glycol methacrylate medium (GMA medium, Agar Scientific, Stansted, UK). Sections of GMA-embedded material were cut at 2.0 to 3.0 µm with a glass knife on a JB4 microtome. Some were stained with Meyer’s haemalum and eosin; others were post-fixed in aqueous Bouin’s solution for 20 min, washed in 70% ethanol until colourless and stained in Giemsa (10% in phosphate buffer, pH 7.4).

For transmission electron microscopy (TEM), portions of the intestine 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 same buffer, dehydrated
in a graded alcohol series and embedded in Agar 100° resin (Agar Scientific). Thin sections, cut on a Reichert Ultratome ultratome with a diamond knife, were stained on grids with uranyl acetate and lead citrate and examined in a JEOL 100CX TEM.

All measurements are reported in µm.

RESULTS

*Eimeria jamescooki* sp. n.

**Type host:** *Cryptoblepharus virgatus* (Garman, 1901) (Reptilia, Sauria, Scincidae).

**Type locality:** James Cook University grounds, Townsville, Queensland, Australia.

**Prevalence in the collection site:** in 1 out of 6 (1986) and in 4 out of 14 (1989).

**Voucher material:** Histological slides No. 2958 (CP-8); No. 3092 (CP-17), TEM material (from CP-8), in author’s collection. Histological slide No. 2958-1 and a colour video print made out of this slide, in the collection of the Institute of Parasitology, Academy of Sciences of the Czech Republic, České Budějovice No. H-PA-070.

**Etymology:** The specific name refers to the type locality.

**Oocysts** Figs. 1–3

Sporulation endogenous: oocysts in freshly deposited faeces were fully sporulated. Sporulated oocysts ellipsoid, 17.5–25.0 (22.1 ± 1.9) × 15–22.5 (17.7 ± 1.6), n = 36, with colourless, smooth wall, and no micropyle or oocyst residuum. Shape index (length/width) 1.25, ranging from 1.16 to 1.25 among 5 individual oocysts. Sporocysts oval-shaped, 6.25–10.0 (7.9 ± 1.15) × 3.75–6.25 (5.3 ± 1.0), n = 21; with thickened apex, but lack Stieda or substieda body, with smooth wall and sporocyst residuum which was only vaguely seen.

**Endogenous stages** Fig. 4

Measurements of endogenous stages were taken from LM-examined histological sections of the anterior gut (n = 5). Meronts in cross section, with eight nuclei, were rounded, reaching 6.4 × 7 in size; divided meronts with their merozoite progenies (Fig. 4, z) were either round or (more often) elongate (7–14 × 2.8–4.2 in size); meront progeny were comprised of 10 to 21 merozoites. Both microgamonts and macrogamonts developed initially into oblong cells positioned longitudinally in the elongate mucosal epithelial cells, between the nucleus displaced to the basal end and the brush border (Fig. 4, ma, my). The young macrogamonts reached 9.8 × 1.4–2 in size, and the growing macrogamonts 14–23.6 × 4.2–5.6 in size, were identified by their vesiculate nucleus with large conspicuous nucleolus. They contained only a few eosinophilic or heterochromatic granules – the developing wall-forming bodies and variable quantities of amylopectin granules. Some host cells contained two longitudinally stretched macrogamonts (Fig. 4, ma). Mature macrogamonts and young oocysts ranged from 14 × 7 to 21 × 11 in size, contained canaliculi and two types of small dense granules, type 1 wall-forming bodies (WFB1) and large, round, conspicuously eosinophilic (and even more distinctly phloxinophilic) type 2 wall-forming bodies (WFB2). One young oocyst was seen surrounded by microgametes (not shown). Mature oocysts (16–20 × 11–14 in size; n = 5) still located in the epithelium became filled with amylopectin granules; they contained only a few, two to four, very large WFB2 (Fig. 4, o). Oocysts released to the gut lumen were 17–20 × 8.4–10 in size (n = 5, shape index 1.2–1.5); they contained only residues of WFB2, displaced to the walls.
Premature microgamonts with their nuclei (up to 70 in number) in marginal positions reached 15.4 × 4.2 in size (Fig. 4, my). Prior to differentiation they reached up to 28 × 8.4 in size, rounded up, and became infolded. Mature microgamonts bearing microgametes were 14.8–21 × 9.8–12.6 in size; the proximal end of some mature microgametes remained narrow and non-differentiated (Fig. 4, mi). After maturation, the cytoplasmic contents of the microgamont became depleted.

**Fine structure of endogenous stages**  
Figs. 5–14

Young meronts with a single nucleus were lodged in a parasitophorous vacuole and bounded by a single unit membrane. Their nucleus chromatin was either diffuse (Fig. 5) or with marginal condensed aggregates (Fig. 6). Their cytoplasm contained micronemes, small electron-dense spheres, a few larger electron-dense bodies (possibly cross-sectioned rhoptries), some lipid vacuoles, and endoplasmic reticulum (ER). In one meront, elongate mitochondria were seen aligned to the body wall (Fig. 5), and in another, an adnuclear body (para-golgi) was seen adjacent to the nucleus (Fig. 6). The latter trophozoite contained a conspicuous micropore. Seven merozoites were seen in a section of a dividing meront (Fig. 7). The pellicle-bound merozoites contained a nucleus with aggregated chromatin, rhoptries, micronemes, Golgi elements, large mitochondrion, food vacuoles with the flocculent contents and electron-lucent spheres, probably amylopectin granules.

Young macrogamonts (Fig. 8), recognised by their large nucleus with a conspicuous nucleolus, contained micronemes, mitochondria, one of which was accompanied by a conspicuous ER cisterna, one lipid vacuole and a few bodies with an electron-dense core.

Both immature and mature macrogamonts, lay in a parasitophorous vacuole (Figs. 9–11), and were bounded by a single membrane. The host cell cytoplasm adjacent to the parasitophorous vacuole contained numerous ER cisternae and tubuli, including vesicles with granular contents (Fig. 10). These elements were less conspicuous in the more mature stages. Immature macrogamonts were usually oblong (Fig. 9). Their nucleus characteristically contained a large nucleolus; mitochondria assembled into a continuous layer beneath the cell boundary (Figs. 9, 10). The cytoplasm, densely loaded with ribosomes, was traversed by numerous cisternae of ER. Golgi elements were enclosed within
cytoplasmic enclaves ("plaques") of finer substance (Figs. 9, 10). An enclosure of winding tubuli, cisternae, and membrane-bounded inclusions of electron-dense to electron-lucent substance (= cross-sections of tubules?) was also observed. This enclosure also contained a fragment of canaliculi (Fig. 10). The immature macrogamonts (Figs. 9, 10) contained several lipid vacuoles, a few aggregates of canaliculi, but still very few small amylopectin granules. These macrogamonts already exhibited numerous electron-dense type 1 wall-forming bodies (WFB1; Figs. 9, 10); cisternae bordered by rough ER with fine granular content or with opaque substance of low to medium electron density seemed to be the anlagen of type 2 wall-forming bodies (WFB2; Fig. 9).

Mature macrogamonts and young oocysts contained many elongate or round (due to a processing fault) amylopectin granules (Figs. 11); some young oocysts contained large aggregates of canaliculi (Fig. 12). The cytoplasm of the mature macrogamont (Figs. 11) and the young oocyst (Fig. 12) also contained numerous WFB1, a few, large WFB2 enclosed within expanded cisternae, sometimes in association with Golgi elements, and also a number of rough ER-enclosed vesicles, with medium-electron-dense "opaque" contents – seemingly extracted WFB2. ER elements, mitochondria, and Golgi elements were traced in the cytoplasm between the above listed organelles.

Premature microgamonts (Fig. 13) contained nuclei with scattered chromatin masses. A more differentiated stage (Fig. 14), nuclei positioned beneath the cell wall were each accompanied by a centriole, mitochondria and Golgi elements, not always in a position adjoining the nuclei. A dense ER network filled the centre of the microgamonts, accompanied by a few amylopectin granules, inclusions with coarse granules, and small vacuoles filled with flocculent particles (Fig. 14). Mature microgamonts were seen only in LM.

**DISCUSSION**

On the precedent that described *Eimeria* species from reptilian hosts have so far demonstrated restricted host specificity and zoogeographical affinities, I shall limit my taxonomic discussion to species from Australian skinks. Of the three species of *Eimeria* described by Cannon (1967b) from skinks, which develop in the gut mucosa, *E. ablephari* from *Ablepharus boutoni*, *E. lampropholis* from *Lampropholis gurchenoti* and *E. leiopismatis* from *Leiopisma challengeri*, only the dimensions of oocysts (21–25.4 × 15.9–19.2; mean: 23.1 × 17.7) and sporocysts (8.1–10.3 × 5.8–7.6; mean 8.9 × 6.6) of *E. ablephari* approximate those of *E. jamescooki*, albeit with somewhat higher means. Another species, described by Cannon (1967b) as *Eimeria egerniae*, which infects the gall bladder of *Egeria whitii*, was transferred to the genus *Choleoeimeria* (see Paperna and Landsberg 1989b). Also overlapping in dimensions are oocysts and sporocysts of an as yet unpublished species of *Eimeria* from *Carlia rhomboidalis* (17.5–22.5 × 12.5–17.5 and 6.25–8.75 for oocysts and sporocysts, respectively). Oocyst shape indices of individual oocysts of *E. jamescooki* (1.16–1.25) are lower than that of *E. ablephari* (~1.3, extrapolated from data in Cannon 1967b). The shape index of *Eimeria* oocysts from *C. rhomboidalis* ranges from 1.3 to 1.55 (Paperna, unpublished). The oblong immature macrogamonts seen in both histological and ultrathin sections of the presently described species are not mentioned in the description of *E. ablephari*, or other species studied by Cannon (1967b), but they have been noted in *E. boveroi* (Lainson and Paperna 1999). Developing macrogamonts appear to assume the shape of the elongate host epithelial cells of the anterior gut mucosa.

Paperna and Landsberg (1989b) noted the absence of Stieda and substieda bodies in sporocysts of coccidia from reptiles of the genus *Choleoeimeria* undergoing endogenous development in gall bladders and species of *Acroeimeria* developing beneath the brush border of the gut epithelium. This feature, characteristic of sporocysts of avian and mammalian eimerian species, seems to be absent also among sporocysts of species of *Eimeria* of saurian reptiles that develop inside the gut mucosal cells (see Lainson and Paperna 1999, Lainson 2002). Stieda body or Stieda-like body has been, however, reported to occur in sporocysts of some species of *Eimeria* infecting snakes and terrapins (Vetterling and Widmer 1968, Upton and McAllister 1988, 1990, McAllister et al. 1990).

The only other published report on the ultrastructure of *Eimeria* developing within the cytoplasm of the gut mucosal cells of reptilian hosts is that of *E. boveroi* from the gecko *Hemidactylus mabouia* (see Paperna and Lainson 1999a). These intracytoplasmic species, as well as the epicytoplasmic *Acroeimeria* spp. (see Paperna 1989, Paperna and Lainson 1999b) and the species...
found in the gall bladder epithelium (*Choleoeimeria* spp., see Paperna and Landsberg 1989a, Paperna and Lainson 2000) share a common range of cytoplasmic organelles. Nevertheless, unique, to the presently described species, are the Golgi “plaques” and the enclosure of tubuli. The latter could have been either a generation centre or a clearing site for wasted organelles and other structures. The spheres with electron-dense cores seen in young macrogamonts appear to be the future WFB1. The expanded cisternae containing lower-density substance seen in premature macrogamonts are most likely anlagens (precursors) of WFB2. The rough ER-enclosed vesicles with “opaque” contents seen in zygotes (early oocysts?) are, on the other hand, expired or extracted WFB2. In *E. boveroi*, the ER cisternae lodging the WFB2 grossly expanded (Paperna and Lainson 1999a). In the present study, these cisternae only slightly enlarged. Gradual condensation of the WFB2 material and a variable degree of expansion of ER cisternae have been noted in several other eimeriid species (such as *E. papillata* from the house mouse, see Chobotar et al. 1980). Another feature seen in *E. 

---

**Figs. 9–12.** *Eimeria jamescooki* sp. n., transmission electron micrographs. **Fig. 9.** Immature elongate macrogamont with a nucleus (N), Golgi “plaque” (G), ER network (er), canaliculi (c), mitochondria (m), lipid vacuole (L), WFB1 (w1), and anlagen of WFB2 (aw). **Fig. 10.** Enlarged view of mid-zone of an immature macrogamont showing the “tubuli enclosure” (E), which also contains canaliculi (c), and also showing the nucleus (N), a Golgi “plaque” (G), mitochondria (m), WFB1 (w1) and cisterna with fine granular contents (open arrow). The parasitophorous vacuole is aligned by ER cisternae (cs), and vesicles with fine granular contents (v). **Fig. 11.** Mature macrogamont (zygote?) with conspicuously large WFB2 (w2), accompanied with defunct WFB2 (ew), WFB1 (w1) and many amylopectin granules (A). **Fig. 12.** Young oocyst showing large canaliculi (c), many amylopectin granules (A), WFB1 (w1), WFB2 in expanded cisterna (w2) in association with a Golgi “plaque” (bold arrow) and opaque (exhausted) WFB2 (ew). Scale bars = 1 µm.
boveroi and lacking in the presently described species, is the inflated, indented cisternae aligning the host’s cytoplasmic rims of the parasitophorous vacuole. In *E. jamescooki*, ER elements aggregate at the rims of the parasitophorous vacuole but fail to demonstrate such details, possibly due to inferior processing quality.

**REFERENCES**


**Acknowledgements.** I wish to thank Prof. D.B. Copman of the Graduate School of Tropical Veterinary Science, James Cook University, Queensland, for hosting me in his laboratory and for obtaining on my behalf the required permits from Queensland National Parks and Wildlife Service, and to Dr. S. Donnelan of the South Australian Museum, Adelaide, for identifying the skinks.

Received 22 February 2002 Accepted 2 October 2002